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Diseases of the Respiratory System

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Principles of Respiratory Insufficiency

The principal function of the respiratory system is gas exchange in which oxygen is transferred from the environment to the blood and carbon dioxide is moved in the opposite direction. Other important functions include a role in thermoregulation in most species; in acid-base regulation in concert with the kidney; in functioning as an endocrine organ (e.g., angiotensin-converting enzyme); in the metabolism of metabolically active substances, including eicosanoids and nitric oxide; and in the immune response to inhaled immunogens and pathogens. Capillaries in the lungs of the farm animal species and horses also possess intravascular macrophages, which are important as a reticuloendothelial organ in the processing of antigens—an action achieved by similar cells in the liver of dogs, cats, and humans. Interference with these functions can occur in a number of ways and can have a variety of manifestations that are apparent during disease. The most readily apparent failure of the respiratory system is failure of gas exchange with resultant hypoxemia and hypercapnia. However, failure of other functions of the respiratory system can also result in clinically apparent disease.

Failure of gas exchange, and the resultant hypoxia and hypercapnia, is responsible for most of the clinical signs of respiratory disease and for respiratory failure, the terminal event of fatal cases. Death as a result of respiratory failure is attributable to hypoxia. An understanding of **hypoxia, hypercapnia,** and **respiratory failure** is essential to the study of clinical respiratory disease.

DEFINITIONS

A number of terms are used to describe the function of the respiratory tract, or abnormalities that arise because of a variety of diseases. Many of these terms are described in more detail in the text that follows, but a brief definition of each is provided here:

- Hypoxia is a broad term meaning diminished availability of oxygen to tissues.
- Hypoxemia is deficient oxygenation of blood, usually assessed by measurement of blood oxygen tension, or by measurement of blood hemoglobin saturation and hemoglobin concentration, and subsequent calculation of blood oxygen content.
- Hypercapnia is an abnormally high carbon dioxide tension in blood.
- P_aO_2 is the oxygen tension (partial pressure) in arterial blood.
- P_AO_2 is the oxygen partial pressure in alveolar gas.
- P_aCO_2 is the carbon dioxide tension (partial pressure) in arterial blood.
- P_ACO_2 is the carbon dioxide partial pressure in alveolar air.
- C_aO_2 is the arterial oxygen content (milliliters of O_2 per 100 mL of blood).
- P_vO_2 is the oxygen tension (partial pressure) in venous blood.
- P_vCO_2 is the carbon dioxide tension in venous blood.
- C_vO_2 is the venous oxygen content (milliliters of O_2 per 100 mL of blood).
- Respiratory failure is the inability of an animal to maintain arterial blood oxygenation and carbon dioxide tension within the normal range.
- Dyspnea refers to signs of respiratory distress in animals (in humans it describes the sensation of air hunger, which is a symptom and not a sign).
- Polypnea is an excessively high rate of breathing.
- Tachypnea is an excessively high rate of breathing, with the implication that the breathing is shallow.
- Hyperpnea is an increased minute ventilation.

HYPOXIA

Failure of the tissues to receive an adequate supply of oxygen occurs in a number of ways, and the differences are clinically relevant because they are associated with failure of different organ systems and different diseases, and they have fundamentally different pathophysiologic mechanisms

Hypoxic (or Hypoxemic) Hypoxia

Hypoxic (or hypoxemic) hypoxia occurs when there is inadequate oxygenation of blood (hypoxemia) and is usually associated with disease of the respiratory tract or other causes of hypoventilation. Situations in which there is inadequate oxygenation of blood in the lungs include hypoventilation, ventilation-perfusion mismatches, diffusion impairment, low inspired oxygen tension, and extrapulmonary right-to-left shunting.

Hypoventilation occurs in animals with depressed consciousness, such as occurs with general anesthesia and heavy sedation, or in newborns, in which the central respiratory drive is suppressed. **Airway obstruction**

caused by the presence of foreign bodies in the airway, luminal obstruction by masses such as retropharyngeal abscesses in horses with strangles, laryngeal spasm, or bronchoconstriction can cause inadequate alveolar ventilation and hypoxemia. Diseases that prevent adequate inflation of lungs cause alveolar hypoventilation and the consequent hypoxemia. These diseases include pneumothorax, pleural effusion, or respiratory muscle weakness, such as can occur with botulism, tick paralysis, tetanus, strychnine poisoning, or severe white muscle disease.

Ventilation-perfusion ($\dot{V}/\dot{Q}$) mismatches occur when the distribution of blood flow in the lungs does not match the distribution of alveolar ventilation, with the result that areas of lung that are well ventilated are not adequately perfused and those areas that are well perfused by blood are not well ventilated. Ventilation-perfusion mismatches are the most important cause of hypoxemia in many lung diseases, including pneumonia.

Diffusion impairment occurs when there is decreased transfer of oxygen from alveolar air that has a normal P_AO_2 to red blood cells in alveolar capillaries because of increased distance of diffusion through the alveolar membranes, such as might occur with pulmonary edema; decreased surface area available for diffusion, such as occurs with positional atelectasis or pulmonary embolism; or decreased transit time of red cells through the alveolar capillaries, such as occurs in horses during heavy exercise.

Low inspired oxygen tension occurs naturally only in animals at high altitude. It can also occur during anesthesia if there are defects in the ventilator causing low oxygen tension in the gases delivered to the animal.

Extrapulmonary right-to-left shunting occurs most commonly as a vascular defect (see Ch. 10).

The actual cause of hypoxemia in an individual animal or disease is often multifactorial and not simply a result of one of the mechanisms described previously. For instance, cows placed in dorsal recumbency during general anesthesia become hypoxemic because of compression of the thorax by the abdominal viscera, thereby causing hypoventilation and compression atelectasis with diffusion impairment, ventilation-perfusion mismatching, and reduced cardiac output because of reduced venous return.

Anemic Hypoxia

Anemic hypoxia occurs when there is a deficiency of hemoglobin per unit volume of blood (anemia). The percentage saturation of the available hemoglobin and the oxygen tension of arterial blood are normal but as a result of the low hemoglobin concentration the oxygen-carrying capacity of the blood is reduced. Anemia resulting from any cause has these characteristics. The decrease in oxygen-carrying capacity caused by a 50% reduction in hemoglobin concentration from normal values (from 20 down to 10 g/dL) is much greater than the decrease that results from a 50% reduction in arterial oxygen tension from normal (e.g., a reduction from 100 to 50 mm Hg).

Alteration of hemoglobin to pigments, such as methemoglobin or carboxyhemoglobin, that are not capable of carrying oxygen has the same effect on oxygen content as anemia. Thus in poisoning caused by nitrite, in which hemoglobin is converted to methemoglobin, and in that caused by carbon monoxide, when the hemoglobin is converted to carboxyhemoglobin, there is hypoxia as a result of inadequate oxygenation of blood.

Circulatory Hypoxia

Circulatory hypoxia occurs as a result of inadequate delivery of oxygen to tissue because of inadequate perfusion of tissues by blood. The blood is usually adequately oxygenated but blood flow rate to tissues is not, and therefore the rate at which it delivers oxygen to tissue is less than the amount of oxygen required to support the metabolic function of that tissue. In other words, the rate of delivery of oxygen to tissue does not match the metabolic requirements of that tissue. A common cause of this is low cardiac output, such as occurs with congestive heart failure or hypovolemic shock. It also occurs with local interruption to arterial flow, such as the thrombotic emboli of thromboembolic colic of horses or compression of vessels, such as in right displacement and torsion of the abomasum.

Histotoxic Anoxia

Histotoxic anoxia occurs when oxygen delivery to tissue is adequate because both oxygen content of arterial blood and blood flow are appropriate, but the tissue is unable to utilize oxygen. Cyanide poisoning is the only common cause of this form of anoxia.

Consequences of Hypoxia

Consequences of inadequate delivery of oxygen include changes in almost all body systems. The central nervous system and heart are most susceptible to the immediate and acute effects of hypoxia, whereas clinical signs related to hypoxic damage to the gastrointestinal tract and kidneys are somewhat delayed. Central nervous system hypoxia is evident as mild changes in mentation, such as depression, progressing through decreased alertness to coma and death. Cardiac changes include a reduction in the force and efficiency of contraction as a result of impaired myocardial contractility, and an increased susceptibility to arrhythmia. The kidney, gut, and liver are all metabolically active tissues and therefore susceptible to hypoxia. Renal function is reduced during hypoxia, with the renal medulla being most sensitive to decreases in oxygen delivery. Signs of gastrointestinal dysfunction during hypoxia include ileus, abdominal pain, and abdominal distension as a result of accumulation of gas and liquid in the gastrointestinal tract. Liver dysfunction can be evident as decreases in blood glucose concentration and increases in serum activity of liver-derived enzymes (alkaline phosphatase, gamma-glutamyl transpeptidase, sorbitol (inositol) dehydrogenase), and metabolites (bile acids, bilirubin).

Some metabolically active tissues, when deprived of oxygen, use anaerobic metabolism to sustain energy supply for short periods of time (depending on the tissue, but the brain cannot survive without oxygen for more than 2-3 minutes). Use of anaerobic glycolysis for energy causes metabolic acidosis. Animals in respiratory failure therefore often have a mixed acid-base disturbance characterized by metabolic and respiratory acidosis.

COMPENSATORY MECHANISMS

Compensation of respiratory insufficiency occurs as both short-term and long-term events. **Short-term** compensatory mechanisms for low arterial oxygen tension or oxygen delivery to tissues occur within seconds to minutes and include respiratory, cardiovascular, and behavioral responses. Stimulation of respiratory centers in the medulla oblongata by low arterial oxygen tension (P_aO_2) and high arterial carbon dioxide tension (P_aCO_2) causes an increase in respiratory minute volume mediated by an increase in tidal volume and respiratory frequency. Both low oxygen tension and high carbon dioxide tension in arterial blood, together or separately, are potent stimulators of these events. Inadequate tissue oxygenation also stimulates an increase in cardiac output, mainly as a result of increased heart rate and to a lesser extent by an increase in stroke volume. Splenic contraction, in those species such as the horse in which the spleen is an important reservoir of red blood cells, increases both blood volume and hemoglobin concentration, thereby increasing the oxygen-carrying capacity of blood. Hypoxemia also causes animals to attempt to decrease their oxygen requirement by decreasing physical activity, including moving and eating.

Longer-term compensatory mechanisms include an increase in erythropoietin secretion by the kidney with subsequent increases in bone marrow production of red blood cells and an increase in hemoglobin concentration in blood. This polycythemia increases the oxygen-carrying capacity of blood. Severe polycythemia, such as occurs with congenital cardiac anomalies causing chronic right-to-left shunting, increases the viscosity of blood and impairs tissue perfusion, increases the workload of the heart and the risk of thromboembolism. Longer-term

compensatory mechanisms also include changes in ventilatory pattern, such as in horses with heaves, and behavior.

CARBON DIOXIDE RETENTION (HYPERCAPNIA)

Respiratory insufficiency results in decreased elimination of carbon dioxide and its accumulation in blood and tissues. Animals breathing room air that are hypercapnic are always hypoxemic. Increasing the oxygen tension of inspired air can alleviate the hypoxemia but, by reducing hypoxic stimulation of the respiratory center, can cause further increments in arterial P_{CO_2}.

Acute hypercapnia causes a respiratory acidosis that reduces both blood and cerebrospinal fluid pH. The clinical signs of acute hypercapnia are initial anxiety followed by central nervous system depression and eventual coma and death. These clinical abnormalities are attributable to declines in the pH of cerebrospinal fluid (CSF), a consequence of the ease with which carbon dioxide crosses the blood–brain barrier. Decreases in CSF pH are greater for respiratory acidosis than for a similar degree of metabolic acidosis. Severe hypercapnia also causes peripheral vasodilation, which can contribute to arterial hypotension, and cardiac arrhythmia. The acid-base effects of chronic hypercapnia are compensated by renal mechanisms that return the arterial and CSF pH to almost normal and therefore do not cause more than mild clinical disease in most instances. So long as oxygen delivery to tissue is maintained, animals can tolerate quite high arterial carbon dioxide tensions for a number of days or longer; this is referred to as "permissive hypercapnia" and is sometimes an alternative to artificial or mechanical ventilation of animals with respiratory insufficiency.

RESPIRATORY FAILURE

Respiratory movements are involuntary and are stimulated and modified by the respiratory centers in the medulla. The centers appear, at least in some species, to have spontaneous activity that is modified by afferent impulses to higher centers, including the cerebral cortex and the heat-regulating center in the hypothalamus, from the stretch receptors in the lungs via the pulmonary vagus nerves, and from the chemoreceptors in the carotid bodies. The activity of the center is also regulated directly by the pH and oxygen and carbon dioxide tensions of the cranial arterial blood supply. Stimulation of almost all afferent nerves may also cause reflex change in respiration, with stimulation of pain fibers being particularly effective.

Respiratory failure is the terminal stage of respiratory insufficiency in which the activity of the respiratory centers diminishes to the point where movements of respiratory muscles cease. Respiratory failure can be paralytic, dyspneic or asphyxial, or tachypneic, depending on the primary disease.

The respiratory failure that occurs in animals with pneumonia, pulmonary edema, and upper respiratory tract obstruction is caused by combinations of hypoventilation, ventilation-perfusion mismatch, and diffusion impairment, which leads to hypercapnia and hypoxemia. Hypercapnia and hypoxia stimulate the respiratory center, and there is a potent respiratory drive evident as markedly increased respiratory rate and effort. As the disease progresses these changes become more marked until death occurs as a result of central nervous system or cardiac failure. Animals that die of the central nervous system effects of respiratory failure typically have dyspnea followed by periods of gasping and apnea just before death.

Paralytic respiratory failure is caused by depression of the respiratory centers or paralysis of the muscles of respiration. Depression of the respiratory center occurs with poisoning by respiratory center depressants, such as general anesthetics, or damage to the respiratory center, such as might occur with brainstem injury. Paralysis of respiratory muscles occurs in disease such as botulism, tetanus, strychnine poisoning, white-muscle disease, severe hypocalcemia, and tick paralysis. The signs of paralytic respiratory failure are a gradual or abrupt cessation of respiratory movements without preceding signs of increased respiratory effort or dyspnea. The animal is often unconscious, or unable to move, during the later stages of the disease.

The differentiation of these types of failure is of some importance in determining the type of treatment necessary. In the paralytic form of respiratory failure, the optimal treatment is mechanical ventilation, along with removal of the inciting cause. Administration of respiratory stimulants is seldom effective as sole therapy. The more complex pathogenesis of respiratory failure in most diseases requires a therapeutic approach that removes each of the underlying defects. In most cases this is achieved by treating the inciting disease, for example, administering antimicrobials to an animal with pneumonia or furosemide to an animal with pulmonary edema, in addition to supportive care, including, potentially, nasal or pharyngeal insufflation with oxygen or mechanical ventilation.

Principal Manifestations of Respiratory Insufficiency

Respiratory disease is evident as one or more of a variety of signs detectable on clinical examination. The signs vary with the etiology of the disease and its anatomic location. Diseases that impair ventilation or gas exchange have hypoxemia and hypercapnia as prominent life-threatening abnormalities. Infectious and inflammatory diseases can cause prominent clinical abnormalities as a result of a systemic inflammatory response and toxemia. The toxemia may be so severe (e.g., in calf diphtheria, aspiration pneumonia and equine pleuritis) as to cause death, even though oxygen and carbon dioxide exchange are not greatly impaired. The common signs of respiratory disease are as follows:

- Abnormalities in the rate, depth, or ease of breathing
- Lethargy or exercise intolerance
- Abnormal posture
- Abnormal lung sounds
- Abnormal respiratory noises
- Coughing
- Cyanosis
- Nasal discharge
- Epistaxis and hemoptysis.

ABNORMALITIES IN RATE, DEPTH, AND EASE OF BREATHING

Polypnea is a rate of breathing that is faster than observed in clinically normal animals of the same species, breed, age, sex, and reproductive status in a similar environment.

Tachypnea also describes an increased rate of breathing, although with the implication that breathing is shallow (i.e., of a reduced tidal volume).

Hyperpnea is an abnormal increase in the rate and depth of breathing (an abnormally high minute volume), but the breathing is not labored and is not associated with signs from which one could infer represent distress on the part of the animal (i.e., the animal is not dyspneic). This assessment requires measurement of minute ventilation or arterial blood gas tensions.

Dyspnea is a term borrowed from human medicine, in which it refers to the *sensation* of shortness of breath or air hunger. It is used in veterinary medicine to describe labored or difficult breathing in animals that also display some signs of distress, such as anxious expression, unusual posture or stance, or unusual behavior.

Dyspnea is a physiologic occurrence after strenuous exercise and is abnormal only when it occurs at rest or with little exercise. It is usually caused by hypoxia with or without hypercapnia, arising most commonly from diseases of the respiratory tract. In pulmonary dyspnea one other factor may be of contributory importance; there may be an abnormally sensitive Hering–Breuer reflex. This is most likely to occur when there is inflammation or congestion of the lungs or pleura. Rapid, shallow breathing results.

Expiratory dyspnea is prolonged and forceful expiration, usually associated with

diffuse or advanced obstructive lower airway disease. The dyspnea of pulmonary emphysema is characteristically expiratory in form and is caused by anoxic anoxia and the need for forced expiration to achieve successful expulsion of the tidal air. It is commonly accompanied by an **audible expiratory grunt** in ruminants but less so in pigs and almost never in horses.

Inspiratory dyspnea is prolonged and forceful inspiration as a result of obstruction of the extrathoracic airways, such as with laryngeal obstruction or collapse of the cervical trachea. It may also be associated with abnormalities that restrict thoracic expansion, such as restrictive lung diseases and space-occupying lesions of the thorax. It is accompanied by a stridor or loud harsh sound on inspiration when the cause is obstruction of the extrathoracic airways, such as is typical of laryngeal or tracheal disease.

Open-mouth breathing is labored breathing with the mouth held open, commonly with the tongue protruded in ruminants and most commonly associated with advanced pulmonary disease or obstruction of the nasal cavities.

DISEASES CAUSING DYSPNEA AT REST OR LACK OF EXERCISE TOLERANCE

Dyspnea, along with hypoxemia and hypercapnia, are the clinical and laboratory findings most likely to attract attention to the possible presence of disease in the respiratory system. It is most important, when attempting to differentiate diseases that cause dyspnea, to include diseases of systems other than the respiratory system that can result in dyspnea. Dyspnea at rest is usually, but not always, caused by respiratory tract disease, whereas exercise intolerance can be caused by disease in the respiratory, cardiovascular, musculoskeletal, and other body systems.

Respiratory Tract Disease

Respiratory tract diseases interfere with normal gas transfer, through the mechanisms discussed previously. Characteristics of respiratory disease that lead to dyspnea or lack of exercise tolerance include the following:

- **Flooding of alveoli with inflammatory cells** and/or protein-rich fluid—pneumonia and pulmonary edema
- **Atelectasis** (collapsed alveoli and small airways)—pleural effusion, hemothorax, hydrothorax, pneumothorax, chylothorax, pyothorax, prolonged recumbency of large animals, and diaphragmatic hernia
- **Airway obstruction**—nasal obstruction, pharyngeal/laryngeal obstruction, tracheal/bronchial obstruction, bronchoconstriction, and bronchiolar obstruction.

Cardiovascular Disease

Cardiovascular disease causes inadequate perfusion of tissues including the lungs. There is reduced oxygen delivery to tissues, even in the presence of normal arterial oxygenation.

- **Cardiac disease**. Cardiac dyspnea results from heart failure and is multifactorial. In animals with dyspnea attributable to cardiac disease, there are other readily evident signs of heart failure.
- **Peripheral circulatory failure**—usually as a result of hypovolemic shock, although shock associated with toxemia, including endotoxemia, can cause dyspnea. There are always other prominent signs of disease.

Diseases of the Blood

Diseases of the blood cause inadequate delivery of oxygen to tissues because of anemia or presence of hemoglobin that is unable to carry oxygen.

- **Anemia**—an abnormally low concentration of hemoglobin
- **Altered hemoglobin**—methemoglobinemia (e.g., in nitrite poisoning of cattle, red maple toxicosis of horses), Carboxyhemoglobinemia

Nervous System Diseases

Diseases of the nervous system affect respiratory function by one of several mechanisms:

- **Paralysis of respiratory muscles** occurs in tick paralysis or botulism. Tetanic spasm of respiratory muscles, such as in tetanus or strychnine toxicosis, also impairs or prevents alveolar ventilation. Both flaccid and tetanic paralysis cause hypercapnia and hypoxemia and, in extreme situations, death by suffocation.
- **Paralysis of the respiratory center**, as in poisoning by nicotine sulfate, or overall central nervous system depression, causes hypoventilation because of impaired ventilatory drive.
- **Stimulation of the respiratory center**, so-called neurogenic dyspnea, occurs as a result of stimulation of the center by a small irritative lesion, such as in animals with encephalitis, or administration of drugs, such as lobeline, that increase sensitivity of the respiratory center to hypoxemia or hypercapnia.

Musculoskeletal Diseases

- **Muscle diseases**—diseases of the respiratory muscles can impair ventilation. These include white-muscle disease in lambs, calves, and foals, and some congenital diseases (such as glycogen branching-enzyme deficiency in foals).
- **Fatigue**—animals with primary severe respiratory disease can develop fatigue of the respiratory muscles (intercostal, diaphragm, accessory muscles of respiration), which can further impair ventilation.
- **Trauma**—fractured ribs can impair ventilation both because of the pain of breathing and because of mechanical disruption to respiration (flail chest).

General Systemic States

Tachypnea can occur in a number of systemic states in which there is no lesion of the respiratory tract or nervous system. These include:

- **Pain**—such as in horses with colic
- **Hyperthermia**—as can occur with intense or strenuous exercise
- **Acidosis**—as a metabolic disturbance associated with any of a number of diseases but notably gastrointestinal disease that causes excessive loss of cationic electrolytes in feces

Environmental Causes

- Low inspired oxygen tension, such as in animals at high altitude
- Exposure to toxic gases.

Miscellaneous Poisons

A number of poisons cause dyspnea as a prominent sign, but in most cases the pathogenesis has not been identified. These poisons include the following:

- Farm chemicals, including metaldehyde and dinitrophenols (probable mechanism is stimulation of respiratory center)
- Organophosphates and carbamates (probable mechanism is alteration of pulmonary epithelium), urea (probably effective as ammonia poisoning)
- Nicotine depressing the respiratory center
- Poisonous plants, including *fast-death* factor of algae and the weeds *Albizia, Helenium, Eupatorium, Ipomoea, Taxus* spp., and *Laburnum* and ironwood (*Erythrophleum* spp.), all appear to act at least in part by central stimulation.

ABNORMAL POSTURE

Animals with respiratory disease, and especially those in respiratory distress, often adopt an unusual posture and are rarely recumbent except in the terminal stages of the disease. Animals in severe respiratory distress will stand with the head and neck

held low and extended. Animals, except horses, will often have open-mouthed breathing. Horses, except in extreme and unusual circumstances, are unable to breathe through the mouth because of the anatomic arrangement of the soft palate, which effectively provides an airtight barrier between the oropharynx and nasopharynx. Cattle with severe respiratory distress and open-mouthed breathing will often drool large quantities of saliva—probably a consequence of decreased frequency of swallowing as the animal labors to breathe.

The positioning of the legs is often abnormal. Severely affected animals, and those with pleuritic pain (horses or cattle with pleuritis) or severe respiratory distress, will usually stand with elbows (humeroradial joint) abducted. The animals are reluctant to move but when forced to do so can react violently. They are resistant to diagnostic or therapeutic interventions that interfere even transiently with their ability to breathe.

NORMAL AND ABNORMAL BREATH SOUNDS

Auscultation of the lungs and air passages is the most critical of the physical examinations made of the respiratory system. The examination should be performed in as quiet an environment as possible, although it is often difficult to achieve a silent listening environment in large animal practice. The animal should be adequately restrained so that the examiner can concentrate on the lung sounds, and should not be sedated or anesthetized because of the depression in lung sounds that can occur in these instances. To be effective and diagnostically reliable, auscultation must be systematic. Both the upper and lower parts of the respiratory tract must be examined in every case. It is preferable to begin the examination by auscultating the larynx, the trachea, and the area of the tracheal bifurcation to assess the rate of airflow and the volume of air sound to be heard over the lungs.

GENERATION OF BREATH SOUNDS

The animal must be breathing to generate lung sounds. The lung sounds are generated by movement of air in the large and midsized airways, including the trachea and bronchi. The greater the velocity of air in the airways, the louder the noise, explaining the loud sounds that are generated in the trachea. Air movement in the bronchioles, terminal airways, and alveoli is silent because of the large combined cross-sectional area of these airways and consequent low velocity of air movement and laminar character of the airflow. Sound is generated by turbulent airflow and the degree of turbulence is affected by the velocity of airflow and the diameter of the airway. This sound is then transmitted through the lung and chest wall to the surface of the thorax, where it can be detected by use of a stethoscope.

Quiet breath sounds can be a result of low tidal volume with resultant low velocity of airflow or impaired transmission of sounds to the surface of the chest. Sound is transmitted most readily through dense liquids such as water. Most tissue, except fat, is approximately 70% water and transmits sounds readily. Sound is reflected at the interface of two media of markedly different densities—such as air and tissue—and less sound is transmitted. Thus in the normal lung there is marked attenuation (softening) of breath sounds because of the extensive air–tissue interfaces. This is evident by comparing the intensity of breath sounds heard over the trachea to those heard over the chest wall. However, lung sounds are more readily transmitted when areas of the lung do not contain air, such as occurs with atelectasis, pulmonary edema or infiltration of lung by inflammatory exudates. Sounds generated in the large airways are more readily transmitted through this consolidated tissue and are evident at the chest wall as louder bronchial breath sounds. The presence of bronchial breath sounds that are audible on the chest surface is dependent on the presence of a patent bronchus with airflow to generate the lung sounds and of tissue that readily transmits the sounds generated in the bronchus. Lung sounds will not be heard if they are not generated (as a result of lack of airflow in bronchi) or are muffled by extensive accumulations of fluid or fat between the lung and the chest wall. Lung sounds are reduced in animals with airflow of low velocity in large airways, such as occurs in animals with low tidal volumes, or in which there is obliteration of the bronchial lumen by fluid or tissue. Low tidal volumes occur in animals at rest or in those in which there is rapid but shallow (low tidal volume) breathing. Obliteration of the bronchial lumen occurs in many diseases, including pneumonia.

REBREATHING ("BAGGING") EXAMINATION

Detection of abnormal lungs sounds is optimized by increasing the animal's tidal volume, and thereby the velocity of airflow in large airways. An expeditious means of temporarily increasing the animal's tidal volume is to occlude the nostrils for a brief period (30-60 seconds). When the animal is again allowed to breathe, it will take several large, deep breaths, during which lung sounds can be auscultated. However, the increase in tidal volume is transient and does not permit time for detailed auscultation of the chest. A preferred technique is to place an airtight bag over the animal's muzzle such that all the air that it inhales is contained within the bag. The volume of air in the bag should exceed the anticipated stimulated tidal volume of the animal. As a rule of thumb, the volume of the bag should be sufficient to allow the animal a tidal volume of 10 to 15 mL of air per kilogram of body weight (BW). A 500-kg horse or cow therefore needs a bag that contains 10 L of air. Hyperventilation is stimulated by an increase in carbon dioxide content of inspired air with subsequent hypercapnia and stimulation of the respiratory center. A more refined technique has the animal inhaling gas that is 5% carbon dioxide and 95% oxygen, thereby preventing hypoxemia as a result of the examination. Rebreathing examinations (or "bagging") are not indicated if abnormal lung sounds are detected on initial examination because the results of the rebreathing examination will not add any additional information. Animals in respiratory distress should not be subjected to a rebreathing examination because it might worsen the hypoxemia or hypercapnia already present and is inhumane. Rebreathing examinations are indicated when respiratory disease is suspected but initial auscultation of the thorax does not reveal abnormal lung sounds.

INTERPRETATION OF BREATH SOUNDS

Terminology used to describe normal and abnormal lung sounds is now well established and should be used consistently so that it is a useful diagnostic aid. Associations between abnormal respiratory sounds and diseases and abnormalities of respiratory function are well established. Correct identification of lung sounds, and consistency in terms used to describe them, therefore permits greater diagnostic accuracy and provides the ability to accurately and precisely describe diseases. The identification and clinical significance of respiratory sounds are summarized in Table 12-1. The clinician must carefully auscultate both the upper respiratory tract (larynx, trachea) and the entire aspects of both lung fields and interpret the sounds that are audible or not audible. The **variables that must be interpreted** include the following:

- **The nature of the sounds** (increased or decreased breath sounds, crackles or wheezes)
- **The timing of the sounds in the respiratory cycle**
- **Their anatomic location.**

The questions that should be asked are as follows:

- Are breath sounds audible?
- Are the breath sounds of normal intensity?
- Are the breath sounds normal or abnormal?
- If abnormal sounds are present, what are they (crackles, wheezes, stridor, stertor, etc.; see Table 12-1)?
- Are breath sounds audible over all lung fields?

Interpretation of these variables should indicate the nature of the lesion. Examples are summarized in Table 12-1. Lung sounds can

Table 12-1 Identification and clinical significance of breath sounds

Sounds	Acoustic characteristics	Significance and examples
Normal breath sounds	Soft blowing sounds, longer and louder on inspiration than on expiration, audible over the trachea and lungs.	Normal respiratory tract
Increased audibility of breath sounds	Mild to moderate increase in loudness of breath sounds audible on inspiration and expiration over the trachea and lungs.	Any factor that increases respiratory rate or depth of respirations, including fever, excitement, exercise, high environmental temperatures, lung disease. Harsh loud breath sounds are audible over the lungs with any disease resulting in collapse or filling of alveoli and leaving bronchial lumina open; pulmonary consolidation and atelectasis.
Decreased audibility of breath sounds	Decreased audibility of breath sounds on inspiration and/or expiration over the lungs.	Obese animal, pleural effusion, space-occupying mass of lung or pleural cavity, pneumothorax, diaphragmatic hernia, occlusive (lung) airway disease as in bronchial lumen filled with exudate.
Crackles	Short duration, interrupted, nonmusical breath sounds. Coarse crackles are loud and most commonly heard over large airways in animals with pulmonary disease and may be heard during inspiration and expiration. Fine crackles are of short duration, less intense, and higher pitched.	Coarse crackles are caused by air bubbling through, and causing vibrations in, secretions in large airways. Fine crackles are caused by sudden explosive popping open of a series of airways closed during expiration. May be detected in early or late inspiration. Suggest the presence of secretions and exudate in airways and edematous bronchial mucosa as in exudative bronchopneumonia, tracheobronchitis, aspiration pneumonia, and obstructive pulmonary disease. Loud crackles may be audible in animals with interstitial pulmonary emphysema.
Wheezes	Continuous musical-type squeaking and whistling sounds audible over the lungs.	Narrowing of large airways; expiratory polyphonic wheezing common in equine reactive airway disease bronchopneumonia, any species; inspiratory monophonic wheezing occurs when upper extrathoracic airways are constricted, such as in laryngeal disease.
Pleuritic friction sounds	"Sandpaper-like" sound; grating; sound close to the surface; on inspiration and expiration; tend to be jerky and not influenced by coughing.	Pleuritis; diminish or disappear with pleural effusion.
Stridor	A harsh, high-pitched sound on inspiration audible with or without stethoscope over the larynx and trachea.	Obstruction of upper airways, especially the larynx (as a result of edema, laryngitis, paralysis of vocal cord); prime example is calf diphtheria or retropharyngeal abscessation in strangles in horses or tracheal collapse in horses.
Stertor	Snoring sound (low-pitched, coarse, and raspy) audible without a stethoscope on inspiration and expiration over the pharyngeal and laryngeal areas.	Partial obstruction of the upper respiratory tract, commonly attributable to abnormalities of soft palate and nasopharynx.
Expiratory grunting	Loud grunting on expiration, which is usually forced against a closed glottis with sudden release, audible on auscultation of the thorax, over the trachea, and often audible without the aid of a stethoscope.	Severe diffuse pulmonary emphysema; pleuropneumonia and pericarditis; extensive consolidation; in acute pleurisy and peritonitis; a groan indicating pain may occur.
Transmitted upper respiratory tract breath sounds	Abnormal tracheal breath sounds (crackles and wheezes) audible by auscultation over the extrathoracic trachea during inspiration.	Indicates presence of abnormalities of the upper respiratory tract (larynx, nasopharynx, nasal cavities, and upper trachea), resulting in accumulation of respiratory secretions causing constriction of airways. Laryngitis is an excellent example.
Extraneous sounds heard on auscultation of respiratory tract		
Crepitations in subcutaneous tissues	Loud superficial crackling sounds induced by movement of stethoscope over the skin.	Subcutaneous emphysema from pulmonary emphysema in cattle; trauma to any part of respiratory tract that results in penetration of airway, allowing accumulation of air subcutaneously; gas-forming bacteria in subcutaneous tissues.
Peristaltic sounds	Gurgling, grating, rumbling, squishing sounds audible over the lungs.	Gastrointestinal sounds transmitted from the abdomen: ruminal sounds in cattle; stomach and intestinal sounds in horse. Does not indicate diaphragmatic hernia unless other evidence such as an absence of breath sounds is present.

be divided into normal breath sounds and abnormal breath sounds.

Breath sounds are produced by air movement through the tracheobronchial tree. The terms *bronchial sounds* and *vesicular sounds* are not anatomically accurate or based on physiologic principles and should not be used. The term *breath sounds* should be used. These are the sounds that are audible clearly over the trachea and that are attenuated over the lungs. Breath sounds are of normal, increased, or decreased intensity. Abnormally loud or soft breath sounds can be attributed to either changes in sound production in the airways by changes in flow rate or altered transmission of sound through various normal or abnormal tissues or fluids in the thorax, as discussed previously.

Normal Breath Sounds

Normal breath sounds vary in quality depending on where the stethoscope is placed over the respiratory tract. They are loudest over the trachea and base of the lung

and quietest over the diaphragmatic lobes of the lung. Normal breath sounds are louder on inspiration than on expiration because inspiration is active with more rapid airflow, whereas expiration is passive in normal animals and associated with lower rates of airflow. Breath sounds may be barely audible in obese animals or in the noisy surroundings common in field conditions.

Increased loudness of breath sounds is heard in normal animals with increased respiratory rate and depth of respiration. This can occur for physiologic reasons such as exercise, excitement or a high environmental temperature. They can also occur in abnormal states such as fever, acidosis, or pulmonary congestion in early pneumonia or myocardial disease.

Decreased loudness or an almost complete absence of breath sounds occurs in pleural effusion or pneumothorax because of almost complete reflection of the breath sounds at the pleural surface as a result of the mismatching of the acoustic properties of the pleural tissues and fluids. **Space-occupying masses** between the lung and the thoracic wall also cause a relative absence of breath sounds over the site as do areas of lung that are not ventilated, such as a pulmonary abscess. Thoracic auscultation is of limited value in detecting localized areas of consolidation in the lungs of calves or foals, with ultrasonographic examination having much greater sensitivity. The sensitivity of auscultation to detect lung consolidation, detected by ultrasonographic examination, in calves is only 6%.[1] Computer-aided lung auscultation has a reasonable sensitivity for detection of bovine respiratory disease in feedlot cattle (93%) compared with clinical examination by a veterinarian.[2]

Increased loudness of breath sounds occurs in some instances and can have diagnostic importance. The normal breath sounds heard over the trachea may sound abnormally loud over the lungs because of changes in the transmission properties of the respiratory system. This is because when sound waves pass through structures of different physical properties, the amount of sound transmitted depends on the matching of acoustic properties of the different structures. Consolidation results in less reflection of sound at the thoracic wall and consequently more transmission to the stethoscope. Thus in consolidation, the breath sounds are much louder than normal. These are harsh breath sounds that approximate those heard over the trachea. They are audible on inspiration and expiration but become louder on expiration in abnormal states such as consolidation or atelectasis. Any disease in which the bronchial lumen remains open and the surrounding lung tissue has been replaced by cells, exudate, or tissues (consolidation) that transmit sound without reflection will result in increased bronchial sounds.

Abnormal Breath Sounds

Abnormal breath sounds include **crackles** and **wheezes**. Crackles are discontinuous sounds and wheezes are continuous sounds.

Crackles are abnormal lung sounds described as clicking, popping or bubbling sounds. They are caused by airways that remain closed for a portion of inspiration and then suddenly open. The crackling is caused by the sudden equalization of pressure between the proximal and distal part of the airway. Crackles may thus be caused by the presence of exudate and secretions in the airways, and edematous bronchial mucosa. Crackling lung sounds are also audible in cattle with interstitial pulmonary emphysema. Crackling sounds may move their point of maximum intensity following coughing, presumably as a result of movement of exudate.

Wheezes are continuous whistling, squeaking sounds caused by vibrations of airways or air passing through a narrowed airway. They can be characterized as monophonic (single tone) or polyphonic (multiple tones) and by the timing of their occurrence in the respiratory cycle. *Inspiratory* wheezing suggests obstruction of the upper airways, usually extrathoracic. *Expiratory* wheezing usually indicates intrathoracic airway obstruction, such as bronchoconstriction, with or without distal airways that are narrowed because of tenacious exudate.

Pleuritic friction sounds are a combination of continuous and discontinuous sounds produced by the rubbing together of inflamed parietal and visceral pleura. The sound is loud, coarse, and usually not influenced by coughing. Pleuritic friction sounds are not common, and their absence does not preclude the presence of pleuritis, particularly in the horse. Pleuritic friction rubs may also occur in cattle with severe diffuse pulmonary emphysema as the relatively dry parietal and visceral surfaces rub together during the respiratory cycle.

Absence of lung sounds occurs when the breath sounds are reflected at the interface between the lung and thoracic wall by the presence of a medium such as a space-occupying mass, fluid, or air. The common causes of the "silent lung" include pleural effusion; space-occupying masses of the thorax; large pulmonary abscess; complete destruction of a lobe of lung including the terminal airways, such as can occur with bronchial lumen occlusion by a foreign body or tumor; and diaphragmatic hernia.

Extraneous sounds are miscellaneous unexpected sounds that are occasionally audible over the thorax and include peristaltic sounds, skin and hair sounds caused by the stethoscope, crepitating sounds as a result of subcutaneous emphysema, and muscular contractions. Subcutaneous emphysema occurs in diseases in which there is leakage of air from the lungs or airways into the subcutaneous space. This occurs with bullous lung disease in cattle, rib fractures and pneumothorax, and after percutaneous tracheal aspirate in animals that cough. Coughing in these animals causes air to be forced out of the trachea through the hole through which the tracheal aspirate was obtained. This occurs in the period of coughing when intratracheal pressures are markedly increased just before the opening of the glottis.

RESPIRATORY NOISES

Respiration may be accompanied by audible noises that indicate certain normal or abnormal occurrences in the respiratory tract such as **sneezing**, **snorting**, **stridor**, **stertor** or **snoring**, **wheezing**, **roaring**, **expiratory grunting,** and **snuffling**, **bubbling,** and **rattling sounds**.

Sneezing is a sudden, involuntary, noisy expiration through the nasal cavities caused reflexively by irritation of the nasal mucosae. Sneezing occurs in rhinitis and obstruction of the nasal cavities and digital manipulation and examination of the nasal mucosae.

Snorting is a forceful expiration of air through the nostrils as in a sneeze, but a snort is a voluntary act used by horses and cattle as a device to intimidate potential predators.

Stridor is an inspiratory stenotic sound originating from a reduction in the caliber of the larynx, as occurs in laryngeal edema and abscess.

Stertor or snoring is a deep guttural sound on inspiration originating from vibrations of pharyngeal mucosa. Snoring is often intermittent, depending on the animal's posture. For example, a fat young bull will often snore when he is dozing half asleep, with his head hung down, but the snore will disappear when he is alert and his head is held up in a more normal position. Stertor can occur during expiration in horses with dorsal displacement of the soft palate.

Wheezing is a high-pitched sound made by air flowing through a narrow lumen, such as a stenotic or inflamed nasal cavity.

Roaring may occur during exercise and is caused by air passing through a larynx with a reduced lumen (e.g., laryngeal hemiplegia in horses).

Expiratory grunting is a clearly audible grunting noise synchronous with expiration. It is most common in cattle with diffuse pulmonary disease. A painful grunt may occur in painful diseases of the thorax such as fibrinous pleuritis and is unassociated with inspiration or expiration.

Snuffling, **bubbling,** or **rattling sounds** may be audible over the trachea or base of the lungs when there is an accumulation of secretion, or exudate, in the nasal cavities, larynx, or trachea. These are most clearly audible on inspiration.

COUGHING

A cough is an explosive expiration of air from the lungs. It is initiated by reflex stimulation of the cough center in the medulla oblongata by irritation of sensory receptors in one of various organs, especially the respiratory tract. The stimulus may originate in the pharynx, larynx, trachea, or bronchi. Coughing may also be initiated by irritation of the esophagus, as in choking. The act of coughing consists of several stages:

- Deep inspiration followed by closure of the arytenoid cartilages (glottis)
- Compression of the air in the lungs and large increase in pressure in the thorax and airways by a forced expiratory effort against a closed glottis
- A sudden relaxation of the arytenoid adductor muscles, resulting in opening of the larynx and abrupt, vigorous, and forced expiration. Coughing in horses is associated with transient dorsal displacement of the soft palate so that material in the airways caudal to the larynx is expelled through the mouth.
- The sudden opening of the glottis allows an explosive expiration, during which the linear velocity attains a speed of several hundred kilometers per hour. The intrathoracic airways collapse after opening of the glottis during the forced expiration, whereas the extrathoracic airways are momentarily dilated.

The purpose of coughing is to remove the excess mucus, inflammatory products or foreign material from the respiratory tract distal to the larynx. An example of where impaired ability to cough reduces the capacity to clear tracheal respiratory secretions is in horses after surgical correction for recurrent laryngeal neuropathy. In this instance one side of the glottis is fixed open, thereby preventing the horse from achieving high flow rates normal associated with the explosive stage of coughing. This markedly reduces expectoration of material in the trachea.

Coughing indicates the existence of primary or secondary respiratory disease.

Coughing can be assessed according to several characteristics. Coughing is infrequent in the early stages of respiratory tract disease but can become frequent as the degree of inflammation in the larynx, trachea and bronchi becomes more severe. Assessment of the severity of coughing, at least in horses, requires prolonged observation (preferably for an hour). Coughing is a fairly specific but not very sensitive indicator of pulmonary inflammation. If coughing is detected, it is quite likely that the animal has inflammation of the airways, whereas failure to detect coughing does not reliably rule out the presence of clinically significant airway inflammation. The severity of coughing in horses is closely linked to the severity of inflammation and accumulation of mucopus in the airways. Racehorses that cough are 10 times more likely to have more than 20% neutrophils in a tracheal aspirate and more than 100 times more likely to have more than 80% neutrophils. The frequency of coughing correlates well with maximal changes in pleural pressure, extent of mucus accumulation, and proportion of neutrophils in bronchoalveolar lavage fluid of horses with heaves (recurrent airway obstruction). Coughing is therefore a specific indicator of the presence of respiratory inflammation.

The frequency of coughing is an indicator of the severity of lung disease in horses and presumably in other species. Horses that cough more than four times per hour have increased likelihood of mucus accumulation and higher pleural pressure changes during breathing than do horses that cough fewer than four times per hour. Use of **cough sound analysis** enables detection of outbreaks of respiratory disease in pigs and housed calves. Automated sound analysis systems can differentiate sounds of coughing from other sounds in the barn or piggery.[3-5]

A cough cannot be induced in normal adult cattle and horses by manual manipulation of the larynx or trachea. If a cough can be induced in an adult horse by manual manipulation of the larynx or trachea, then this indicates airway inflammation and is a reason for further examination of the respiratory tract.

The most common causes of coughing in farm animals are diseases of the larynx, trachea, bronchi and lungs, which are presented under the headings of diseases of those parts of the respiratory tract later in this chapter.

CYANOSIS

Cyanosis is a bluish discoloration of the skin, conjunctivae, and visible mucosae caused by an increase in the absolute amount of reduced hemoglobin in the blood. It can occur only when the hemoglobin concentration of the blood is normal or nearly so, and when there is incomplete oxygenation of the hemoglobin. Cyanosis is apparent when the concentration of deoxygenated hemoglobin in blood is greater than 5 g/dL (50 g/L). Cyanosis does not occur in anemic animals. The bluish discoloration should disappear when pressure is exerted on the skin or mucosa. In most cases, the oral mucous membranes are examined for evidence of cyanosis, although the skin of the pinna and the urogenital mucous membranes will suffice. Examination of vaginal mucosa is preferred in horses that have severe congestion of the oral and nasal mucosa as a result of disease affecting the head, such as cellulitis or bilateral jugular thrombophlebitis. Artificial lighting and skin pigmentation affect the ability to detect cyanosis.

Methemoglobinemia is accompanied by discoloration of the skin and mucosae but the color is more brown than blue and cannot be accurately described as cyanosis.

Cyanosis is classified as central or peripheral. **Central cyanosis** is present when arterial oxygen saturation is below normal with concentration of deoxygenated hemoglobin exceeding 4 to 5 g/dL. **Peripheral cyanosis** occurs when there is localized desaturation of blood despite arterial oxygen saturation being normal. This usually occurs because there is diminished blood flow to tissue, with a resulting increase in oxygen extraction by the ischemic tissues and low end-capillary and venous hemoglobin saturation.

Central cyanosis is caused by diseases include the following:

- Congenital cardiac diseases that cause right-to-left shunting
- Pulmonary diseases that cause hypoemia—cyanosis is not usually marked in pulmonary disease unless the degree of ventilation-perfusion mismatch is severe.
- Upper airway obstruction causing hypoxemia—cyanosis is common and is a sign of life-threatening disease in severe cases of laryngeal obstruction, as occurs in severe laryngitis in calves with necrotic laryngitis or horses with bilateral laryngeal paralysis (lead poisoning, after tracheal intubation during anesthesia, idiopathic).
- Abnormalities in hemoglobin function.

Peripheral causes of cyanosis include the following:

- Arterial obstruction, such as is seen in horses with aortoiliac thrombosis ("saddle thrombus") or thrombosis of distal limbs (such as can occur with severe septicemia)
- Venous obstruction
- Severe vasoconstriction

Central cyanosis is characterized by decreased arterial oxygen saturation as a result of right-to-left shunting of blood or impaired pulmonary function. Central cyanosis resulting from congenital heart disease or pulmonary disease characteristically worsens during exercise. Central cyanosis usually becomes apparent at a mean capillary concentration of 4 to 5 g/dL reduced hemoglobin (or 0.5 g/dL methemoglobin). Because it is the *absolute* quantity of reduced hemoglobin in the blood that is responsible for cyanosis, the higher the total hemoglobin content, the greater the tendency toward cyanosis. Thus cyanosis is detectable in patients with marked polycythemia at higher levels of arterial oxygen saturation than in patients with normal hematocrit values, and cyanosis may be absent in patients with anemia despite marked arterial desaturation. Patients

with congenital heart disease often have a history of cyanosis that is intensified during exertion because of the lower saturation of blood returning to the right side of the heart and the augmented right-to-left shunt. The inspiration of pure oxygen (100% F_iO_2) will not resolve central cyanosis when a right-to-left shunt is present, but it can resolve when primary lung disease or polycythemia is causing the cyanosis.

Peripheral cyanosis is caused by obstruction of blood flow to an area. This can occur as a result of arterial or venous obstruction, although it is usually more severe when arterial blood flow is obstructed. Obstruction of arterial blood flow also causes the limb to be cold and muscle and nerve function in the ischemic area to be impaired. Cyanosis can also occur as a result of cutaneous vasoconstriction attributable to low cardiac output or exposure to cold air or water. It usually indicates stasis of blood flow in the periphery. If peripheral cyanosis is localized to an extremity, arterial or venous obstruction should be suspected. Peripheral cyanosis resulting from vasoconstriction is usually relieved by warming the affected area.

Heart failure can cause cyanosis that is restricted to the extremities, probably because of reduced blood flow to extremities during this disease and the consequent markedly lower end-capillary oxygen content. Blood in the venous end of the capillaries, and in the venous bed draining these tissues, is therefore deoxygenated and cyanosis is observed. Although this type of cyanosis has a peripheral distribution, its underlying cause is central.

NASAL DISCHARGE

Excessive or abnormal nasal discharge is usually an indication of respiratory tract disease. Nasal discharges are common in all the farm animal species. Cattle can remove some or all of the nasal discharge by licking with their tongue, whereas horses do not remove any.

Origin

The nasal discharge is usually obvious but the determination of its origin and significance can be difficult and elusive. The history should determine the duration of the nasal discharge and if it has been **unilateral or bilateral.**

Nasal discharges may originate from lesions in the nasal cavities, congenital defects of the hard palate such as cleft palate in the newborn, paranasal sinuses, guttural pouch in the horse, pharynx, larynx, trachea, and lungs. Diseases of the esophagus and stomach that cause dysphagia and regurgitation or vomiting can also cause a nasal discharge stained with feed material.

The origin of a nasal discharge is sometimes determined by close inspection of the external nares and the visible aspects of the nasal cavities using a pointed source of light. Some important infectious diseases of the respiratory tract characterized by lesions of the nasal mucosae can be identified by examination of the external nares for the origin of a nasal discharge. If the source of the discharge is not apparent on this examination, then more detailed investigation is warranted.

Examination

The characteristics of the discharge are noted carefully by inspection. It may be copious, serous, mucoid, purulent, caseous, streaked with blood, and foul smelling (ozena), or it may contain feed particles.

- A copious bilateral serous nasal discharge is characteristic of early inflammation of the nasal cavities such as in viral rhinitis.
- A bilateral mucoid discharge suggests inflammation of a few days' duration.
- A bilateral purulent discharge can indicate inflammation in the upper or lower respiratory tract.
- A copious bilateral caseous discharge suggests an allergic or bacterial rhinitis.
- Foul-smelling nasal discharges are usually associated with necrosis of tissues anywhere in the nasal cavities, the guttural pouch in the horse, or severe necrotic and gangrenous pneumonia.
- A bilateral foul-smelling discharge containing feed particles suggests dysphagia, regurgitation, or vomiting.
- In most cases, a chronic unilateral nasal discharge suggests a lesion of one nasal cavity.
- A bilateral nasal discharge suggests a lesion posterior to the nasal system.

Examination of **the paranasal sinuses** for evidence of pain and facial deformity will assist in the diagnosis of sinusitis. Percussion is useful in identifying paranasal sinuses that are filled with fluid or tissue because sinuses affected in this way do not produce a resonant sound when the skin overlying the sinus is tapped. The pharynx and larynx of cattle can be examined through the oral cavity, whereas a **flexible endoscope** is necessary for close examination of the upper and lower respiratory tract of horses or cattle of almost any age to determine the origin of a nasal discharge. The examination should include both nares, the region of the opening of the nasomaxillary sinus (this opening cannot be seen), the nasopharynx (in horses) or the pharynx (in other species), the guttural pouches in horses, the larynx, and the trachea, preferably to the level of the carina, although this might not be possible in large animals or when short endoscopes are used.

Radiography of the structures of the head and pharynx is also useful to locate lesions of the nasal cavities and paranasal sinuses that might be the origin of a nasal discharge.

Nasal Discharge and Location of Lesion

There is not necessarily a correlation between the characteristics of a nasal discharge and the nature of any pulmonary lesions. In exudative pneumonias in cattle, mucopus is produced and is moved up the trachea and into the pharynx by the mucociliary mechanism or by coughing. Some of it is then swallowed, and some may be deposited in the nasal cavities and moved forward to the external nares by ciliary action. In the horse, with its long soft palate, most purulent material from the lungs will be deposited in the nasal cavities and appear as a nasal discharge.

Sampling of Nasal Discharge

When infectious disease is suspected, nasal swabs can be collected and submitted for microbiological examination. Nasal swabs are useful only when a specific etiologic agent is suspected and demonstration of its presence will confirm the cause of the disease. Examples of this include strangles (*Streptococcus equi*), influenza (equine or porcine), infectious bovine rhinotracheitis, and *Mycoplasma bovis*. Submission of nasal swabs for culture yields mixed flora and the results are impossible to interpret, with the exception noted previously. Organisms cultured from nasal or nasopharyngeal swabs are not representative of those cultured from lungs in individual animals but might be somewhat useful in herd outbreaks of disease. Culture of transtracheal aspirates or, in cattle but not horses, bronchoalveolar lavage fluid is representative of organisms causing pulmonary disease. Cytologic examination of the nasal discharge can reveal exfoliated cells in the case of nasal tumors or eosinophils when allergic rhinitis is present.

EPISTAXIS AND HEMOPTYSIS

- **Epistaxis** (blood from the nostril) is in most instances a result of disease of the mucosae of the upper respiratory tract but it may originate anywhere in the upper or lower respiratory tract. Epistaxis occurring during or within several hours of intense exercise by horses is caused by exercise-induced pulmonary hemorrhage.
- **Hemoptysis** is the coughing up of blood. The blood usually originates from hemorrhage in the lower respiratory tract. The presence of hemoptysis is difficult to detect in animals. Hemoptysis occurs in horses, which is perhaps unexpected given the anatomic separation of the nasopharynx and oropharynx.

Pulmonary hemorrhage, particularly in the horse, may be manifested as epistaxis.

Pulmonary hemorrhage in cattle is commonly manifested as hemoptysis and epistaxis. These are described in more detail later in this chapter.

A small amount of serosanguineous fluid in the nostrils, as occurs in equine infectious anemia and infectious equine pneumonia, does not represent epistaxis, which must also be differentiated from the passage of blood-stained froth caused by acute pulmonary edema. In this instance the bubbles in the froth are very small in size, and passage of the froth is accompanied by severe dyspnea, coughing, and auscultatory evidence of pulmonary edema.

THORACIC PAIN

Spontaneous pain, evidenced by grunting with each respiratory cycle, usually indicates pleural pain, such as from a fractured rib, torn intercostal muscle, or traumatic injury, including hematoma of the pleura, or pleurisy. A similar grunt may be obtained by deep palpation or gentle thumping over the affected area of the thoracic wall with a closed fist or a percussion hammer. Pain as a result of a chronic deep-seated lesion cannot be detected in this way. The use of a pole under the sternum, as described under "Traumatic Reticuloperitonitis," provides a useful alternative.

Special Examination of the Respiratory System

In addition to the routine clinical examination of the respiratory tract, there are a number of diagnostic techniques that can be used to aid in making a specific diagnosis, providing a reliable prognosis and formulating the most rational treatment. These techniques are being used more commonly by species specialists, particularly on valuable animals. Most equine practices have flexible endoscopes for the examination of the upper respiratory tract of horses. Medical imaging using thoracic radiography and ultrasonography of animals with suspected lung disease is now common, and the laboratory evaluation of respiratory tract secretions and exudates are commonplace. Almost all of these techniques increase the costs of making a diagnosis, and it is therefore important to consider whether the additional diagnostic testing will improve the final outcome of the case. Techniques for advanced evaluation of the respiratory system include the following:

- Auscultation and percussion of the thorax
- Endoscopy of the upper airways, guttural pouch (in Equidae), trachea, bronchi, and larger bronchioles
- Invasive endoscopic examination of the sinuses using rigid endoscopes
- Pleuroscopy using either rigid or flexible endoscopes
- Radiographic examination of the skull, pharynx, larynx, guttural pouch (in Equidae), trachea, and thorax
- Computed tomographic and magnetic resonance imaging
- Scintigraphic examination of respiratory function
- Ultrasonographic examination of the soft tissue of the pharynx and larynx, and thorax
- Collection and evaluation of the following respiratory tract secretions:
  - Nasal
  - Paranasal sinus
  - Guttural pouch
  - Pharyngeal
  - Tracheobronchial (tracheal aspirates, bronchoalveolar lavage)
  - Pleural (thoracocentesis)
- Pulmonary function testing, including measurement of tidal and minute volumes, pleural pressure, forced expiratory volume, flow-volume loops, forced oscillometry, and CO_2 breathing
- Arterial blood gas analysis
- Venous blood gas analysis
- Blood lactate concentration
- Pulse oximetry
- Collection and analysis of exhaled breath condensate
- Lung biopsy
- Respiratory sound spectrum analysis
- Exercise testing.

AUSCULTATION AND PERCUSSION

The techniques of auscultation and percussion used in examination of the thorax are discussed in Chapter 1, and references on percussion of the thorax are available in earlier editions of this text. Percussion of the thorax is a useful means of determining lung margins and therefore of detecting the presence of overinflation, as occurs with heaves in horses, or areas of consolidation. Consolidation is evident as a loss of resonance, and detection of this abnormality can reveal the presence of excessive pleural fluid or pulmonary consolidation. There is excellent agreement in the assessment of lung margins determined by percussion and by ultrasonographic examination. Percussion is therefore a valuable diagnostic tool, especially when ultrasonographic examination is not available.

ENDOSCOPIC EXAMINATION OF THE AIRWAYS (RHINOLARYNGOSCOPY, TRACHEOBRONCHOSCOPY)

Horses

Flexible endoscopes allow examination of the upper respiratory tract of horses, including the nasal cavities, nasopharynx, auditory tube diverticula (guttural pouches), palatal arch, epiglottis, larynx, trachea, and major bronchi. For examination to the level of the rostral trachea an endoscope of 1 m in length is suitable. However, an endoscope of 1.5 m in length is useful for examining to the level of the thoracic inlet. The endoscope is usually less than 1.5 cm in diameter. Endoscopic examinations are tolerated by most horses with the minimum of restraint (application of a nose or ear twitch). Sedation should be avoided if a purpose of the examination is to determine the functional integrity of the pharynx and larynx. Sedation depresses laryngeal function and impairs assessment of the symmetry and abductor function of the arytenoid cartilages. Sedated horses are more likely to displace the soft palate and to fail to return it to its normal position.

Rhinolaryngoscopic examination of horses should include a careful examination of the ventral and middle meatuses, turbinates, region of the nasomaxillary sinus opening (this cannot be visualized directly but discharge from it can be detected), ethmoidal turbinates, nasopharynx, soft palate, guttural pouches, dorsal pharyngeal recess, epiglottis, and larynx. The endoscope should be used to examine both left and right nasal cavities and ethmoid turbinates. Both guttural pouches should be examined. Passage of the endoscope into the guttural pouch is best achieved by passing the endoscope through the ipsilateral nasal cavity. The guttural pouch is then entered by first introducing a thin, stiff tube, such as an endoscopic biopsy instrument, through the biopsy port of the endoscope into the guttural pouch. The endoscope is then rotated so that the entrance to the guttural pouch is opened, and the endoscope is carefully advanced into the pouch. An alternative technique involves insertion of a stiff catheter, such as a Chambers mare uterine catheter, into the guttural pouch such that the entrance is dilated to enable passage of the endoscope.

Many disorders of the equine pharynx and larynx manifest only during strenuous exercise because of the high pressures generated in the airways by the large minute ventilation of exercising horses.[6] Pressures in the pharynx and larynx that are of similar magnitude to those occurring during intense exercise can be induced in resting horses by 60 seconds of **nasal occlusion**. The respiratory efforts of horses during nasal occlusion can therefore be used to simulate those during exercise, thereby permitting detection of disorders of the pharynx (displacement of the soft palate) and larynx (mild laryngeal hemiplegia) that would not otherwise be apparent in a resting horse. Rhinolaryngoscopic examination can also be performed on horses running on a treadmill (see following "Exercise Testing" section) or, by use of dynamic endoscopy, in horses running over ground.[7]

Bronchoscopic examination requires an endoscope that is at least 2 m in length and

less than 1.5 cm in diameter. Horses must be sedated for bronchoscopic examination (a combination of xylazine, 0.25-0.5 mg/kg intravenously [IV], and butorphanol, 1 mg per 40 kg IV, works well). Instillation of lidocaine (20 mL of 2% lidocaine diluted with 40 mL of isotonic saline or similar) minimizes coughing. The lidocaine is instilled into the trachea through the biopsy channel of the endoscope. The airways are examined in a systematic fashion and results are recorded using a system that has been described for identifying the major airways. Lobar bronchi are identified on the basis of the side of the bronchial tree on which they are found and the order in which they originate from the primary bronchus. On the right side, RB1, RB2, and RB3 refer to the right cranial lobar bronchus and subsequent right bronchi, respectively. On the left side, LB1 and LB2 refer to the left cranial lobar bronchus and the left caudal lobar bronchus, respectively. Segmental bronchi are identified by consecutive numbers in the order of origin from the lobar bronchus. The direction of the segmental bronchus is denoted by the capital letters D (dorsal), V (ventral), L (lateral), M (medial), R (rostral), and C (caudal). Subsegmental bronchi are identified in the order of origin from the segmental bronchi, using lowercase letters.

Cattle

The nasopharynx, pharynx, and larynx of cattle can be examined by endoscopy, and this should be done without sedation if possible. Xylazine is not recommended because it commonly interferes with normal laryngeal function. Acepromazine is recommended if sedation is necessary.

The anatomy of the proximal portion of the respiratory tract of cattle differs from that of horses. The nasal septum does not completely separate the left and right aspects of the nasopharynx. In cattle, the nasal septum tapers caudodorsally, allowing both ethmoturbinates to be observed from one side. The pharyngeal septum is contiguous with the nasal septum and merges with the caudodorsal wall of the pharynx. The nasopharyngeal openings of the auditory tubes are visible. The appearance of the vocal cords is similar to that observed in the horse. Cattle do not have a laryngeal saccule, and a laryngeal ventricle is not visible rostral to the vocal cords. During endoscopy, the arytenoid cartilages are maintained in fully abducted position. Constriction of the pharynx during swallowing is accompanied by rostroventral movement of the pharyngeal septum, completely occluding the nasopharynx, which differs from the situation in the horse.

ENDOSCOPY OF PARANASAL SINUSES

The paranasal sinuses of the mature horse can be examined with a 4-mm arthroscope while standing and sedated or under general anesthesia. The procedure is technically challenging and is usually performed by surgeons experienced in the use of arthroscopic equipment inserted through portals created by trephining holes in the sinus. The side to be examined is determined by physical, radiographic, and rhinoscopic examination of the animal. Endoscopic examination is indicated in animals in which diagnosis of the disease requires collection of tissue from the sinus. Therapeutic interventions that can be performed during endoscopic examination of the paranasal sinuses include lavage, removal of accretions of inflammatory material, drainage of cysts, and creation or enlargement of drainage holes.

PLEUROSCOPY

Pleuroscopy using a rigid or flexible endoscope enables direct visual inspection of the pleural cavity for the diagnosis of pleural disease. The procedure is well tolerated in healthy horses and cattle.[8,9] The technique is particularly valuable in diagnosis of diseases of the thorax that extend to the pleural surface and do not exfoliate large quantities of cells, thereby making diagnosis by examination of fluid obtained by pleurocentesis unlikely. The procedure is useful in collection of tissue samples, such as from suspected thoracic neoplasia,[10-12] or in therapeutic procedures including relief of pleural adhesions and resection of lung sections, and in collection of large biopsies samples from the lung.[13]

The procedure is performed in standing, sedated horses restrained in stocks. Strict aseptic technique is used. The portal for insertion of the endoscope is at the level of the 8th to 12th intercostal space, with optimal examination of intrathoracic structures obtained via the 10th or 12th intercostal space. Either a rigid endoscope (10-mm diameter, 57-cm length) or flexible endoscope (10-mm diameter, 1-m length) can be used. The endoscope is inserted through a small incision in the intercostal space made under local anesthesia. The ipsilateral lung is partially collapsed by induced pneumothorax to permit visualization of intrathoracic structures. The mediastinum is intact in most horses. Inadequate collapse of the lung increases the likelihood of it being damaged during the procedure. The lung is reinflated by removal of air in the pleural space at the end of the procedure. Potential complications of the procedure include pneumothorax, hemothorax, damage to intrathoracic structures, and infection.

RADIOGRAPHY

Radiography of the head, neck, and thorax is valuable in the diagnosis of diseases of the respiratory tract of animals. Examination is hindered by the large size of adult horses and cattle, the need for specialized high-capacity equipment for obtaining radiographs, and the need for adequate restraint. Radiographic examination of adult animals in the field using portable radiographic units is very limited. However, large practices with fixed radiographic units capable of generating sufficient voltage and amperage can obtain diagnostic radiographs of the thorax of adult horses and cattle. Exposure values for radiographs of the thorax of adult horses and cattle are in the range of 110 kV and 40 mAs for caudodorsal regions to 150 kV and 70 mAs for cranioventral regions.[14] Diagnostic films of smaller animals, including adult sheep and goats and foals and calves, can be obtained using portable units capable of generating 80 to 100 kVp and 15 to 20 mA.

Examination of the thorax of large animals is restricted to lateral radiographs because the large amount of tissue prevents adequate exposure for ventrodorsal views. Multiple films (usually four overlapping views) are required for complete examination of the thorax, and the exposure needed for optimal-quality films varies among anatomic sites.[14] Localization of focal lesions can be achieved by examining sets of radiographs that include images collected with the horse or cow standing first with one side to the plate and then with the other side toward the plate. The lesion will appear larger in views obtained with the lesion closer to the source of x-rays. The radiographic anatomy of the horse has been described.[14]

Interpretation of thoracic radiographs of horses has classically used an approach using terms such as bronchial, alveolar, interstitial, and vascular patterns. An alternative approach that is recently suggested, and which we recommend, is depicted in Figure 12-1. The process involves identification of areas of opacity, their extent and characteristics of the borders of the opacity (poorly defined vs. discrete), and the number and location of opacities. A differential diagnosis can then be established for each pattern of abnormalities (Box 12-1).[14]

Radiographs of **calves and foals** can be recorded with them standing or recumbent. Images obtained with the foal or calf in lateral recumbency with the forelimbs pulled forward permit optimal examination of the cranial thorax. However, calves or foals that are recumbent for prolonged periods of time (e.g., > 30 min) can develop atelectasis of the down lung that can mimic pneumonia radiographically. Ventrodorsal views assist with localizing lesions in foals and calves. Radiographic evidence of lung disease is common in ill neonatal foals (74% having such lesions in one study), and is not related to clinical evidence of respiratory disease or dyspnea. The characteristics of lung lesions detected in neonatal foals are associated with likelihood of survival. Guidelines for recognition of pulmonary patterns in foals have been proposed (Table 12-2), and these guidelines are likely to be useful aids for interpretation and

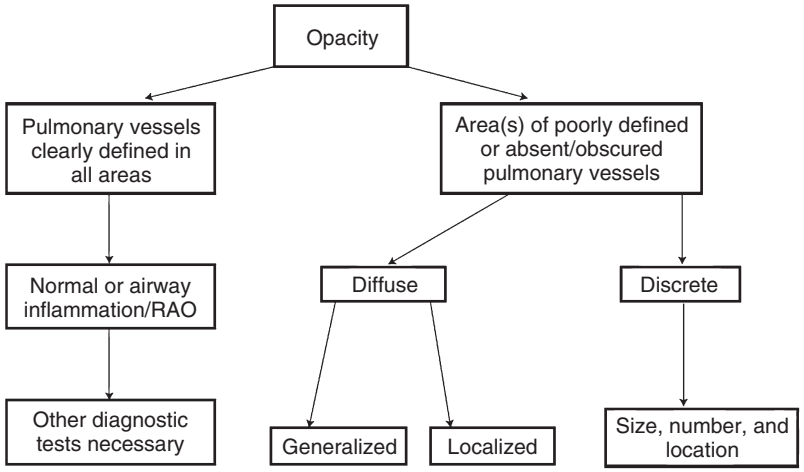

Fig. 12-1 Decision tree for assessing thoracic radiographs of horses. RAO is recurrent airway obstruction or heaves. (Reproduced from Dunkel et al. 2013.[14])

Box 12-1 Differential diagnoses for patterns of abnormalities detected on radiographic examination of the thorax of horses

No abnormalities detected (but other clinical signs of respiratory disease are present)
- Inflammatory airway disease
- Heaves
- Summer pasture-associated recurrent airway obstruction
- Upper respiratory tract disease

Diffuse, localized opacities
- Caudodorsal lung
  - Exercise-induced pulmonary hemorrhage
  - Iatrogenic following bronchoalveolar lavage (transient)
  - Focal pneumonia
- Cranioventral
  - Infectious bronchopneumonia
  - Aspiration pneumonia
  - Pleuropneumonia

Diffuse, generalized opacities
- Interstitial pneumonia
- Pulmonary fibrosis
- Pulmonary edema
- Acute respiratory distress syndrome

One or multiple discrete opacities
- Single
  - Neoplasia
  - Pulmonary abscess
  - Bacterial or fungal granuloma
  - Foreign body
- Multiple
  - Pulmonary abscesses
  - Neoplasia
  - Disseminated fungal (e.g., *aspergillus* sp.), bacterial (*Rhodococcus equi*), or parasitic
  - Equine multinodular pulmonary fibrosis
  - Eosinophilic interstitial pneumonia
  - Idiopathic
- Other
  - Bronchiectasis
  - Tracheal stenosis
  - Bullae
  - Increased vascular pattern
  - Pneumothorax
  - Pneumomediastinum
  - Diaphragmatic hernia
  - Megaesophagus
  - Foreign body
  - Pleural fluid (pleuritic, hemothorax, chylothorax)

description of pulmonary patterns in neonates of other species.

Indications for thoracic radiography in horses (and likely in other large animal species) include the following:[14]

- Mild respiratory disease that is unresponsive to treatment
- Severe respiratory distress
- Thoracic trauma
- Signs of respiratory disease and weight loss or recurrent colic
- Suspicion of infectious or aspiration pneumonia
- Suspected thoracic or mediastinal mass
- Esophageal disease
- Suspected diaphragmatic hernia

Radiography can assist in the recognition and differentiation of atelectasis and consolidation, interstitial and exudative pneumonias, the alveolar pattern of pulmonary disease, neoplasms, pleural effusions, pneumothorax, hydropericardium, and space-occupying lesions of the thorax. Cardiomegaly, abnormalities of the cranial mediastinum, fractures of ribs, and diaphragmatic hernia can also be detected. Thoracic radiography is not as sensitive as is ultrasonography for detecting pulmonary lesions in foals with *Rhodococcus equi* pneumonia, with fewer than 50% of affected foals detected by radiographic examination.[15]

Many pulmonary diseases do not have lesions that are readily detected on radiographic examination. Failure to detect abnormalities on radiographic examination of the thorax does not eliminate pulmonary disease. Furthermore, radiographically detectable signs of lung disease can persist after the animal has clinical and clinicopathologic signs of recovery or improvement.

Bronchography utilizing contrast agents is of value in determining the patency of the trachea and bronchi, but general anesthesia is required to overcome the coughing stimulated by the passage of the tracheal catheter. Using a fluoroscope to determine the location of the catheter tip, the contrast agent can be deposited in each dependent lobe in turn. This technique is used infrequently.

Radiographic examination of the trachea can reveal the presence of abnormalities in shape, such as occur with tracheal collapse, or the presence of foreign bodies or exudate.

Radiographic examination of the **head** can identify diseases of the paranasal sinuses, ethmoids and pharynx. Radiographic examination is useful in defining diseases of the guttural pouches and in detecting retropharyngeal abscesses or abnormalities, such as the presence of foreign bodies.

MAGNETIC RESONANCE IMAGING

The utility of magnetic resonance imaging (MRI) in large-animal medicine is constrained by the size of the imaging bore on MRI units which limits the size of the animal, or anatomic region, that can be imaged. MRI is useful in diagnosis of diseases of the head of horses and other large animals,[16] and the anatomy as visualized on MRI of the head of horses and pigs has been reported.[17] The lack of units suitable for examination of large animals precludes routine use of this imaging modality.

COMPUTED TOMOGRAPHY

Examination of the lung through **computed tomographic** (CT) is very sensitive and specific for lung disease in companion animals and is technically feasible in calves,[18] foals,[19,20] and small ruminants.[21] The technique is useful in the diagnosis of mediastinal disease in foals and correlates well with postmortem estimates of the volume of consolidated lung in experimentally induced pneumonia in calves.[22] CT is likely to be useful in evaluation of extent, severity and progression of lung disease in calves.

The **CT** anatomy of the head of horses and foals has been described including detailed anatomy of the guttural pouches and paranasal sinuses.[23,24] CT imaging of the nasal cavities and paranasal sinuses of horses is useful in the detection of diseases of these

Table 12-2 Guidelines for radiographic pulmonary pattern recognition in foals

Alveolar lung pattern (Vessels not visualized. There is displacement of air from the distal air spaces of the lung leading to a relatively homogeneous increase in soft tissue opacity. Formation of air bronchograms is usually associated with the pattern but is not always present)	
Absent	The pulmonary vessels are easily seen
Minimal alveolar component (< 10%)	No visualization of vessels in < 10% of the lung field. Usually occurs in conjunction with a moderate or severe interstitial lung pattern
Focal (> 10% to 30%)	No visualization of vessels in 11–30% of lung fields. Air bronchograms might or might not be present within < 30% of lung fields
Localized (> 30% to 50%)	No visualization of vessels in 31–50% of lung fields. Air bronchograms might or might not be present within < 50% of lung fields
Extensive (≥ 50%)	No visualization of vessels in ≥ 50% of lung fields. Air bronchograms might or might not be present throughout the entire section of lung field
Interstitial lung pattern (Characterization of the non-air-containing elements of the lungs including blood vessels and bronchi)	
Normal	Clear visualization of vessels. Borders are well defined
Mild increase	The pulmonary vessels appear slightly ill defined (hazy borders with loss of visualization of the fine vascular structures). Mildly lacy appearance to lung field
Moderate increase	The vessels are ill defined, resulting in moderately lacy appearance and increased opacity of the lung field
Marked increase	Significantly increased opacity; vessel borders are barely recognizable
Bronchial pattern (Characterized by alterations in bronchial wall thickness and density, or in bronchial lumen diameter. Note that periobronchial cuffing is a feature of interstitial not bronchial pattern)	
Normal	Bronchial structures seen in cross section appear as small, thin-walled hollow rings between paired vessels. The bronchial walls are barely distinguishable when viewed side-on and are not clearly visualized at the periphery of the lung field
Moderate increase	A few thickened bronchial walls evident in cross section ("doughnuts") at the periphery of the lung fields. Longitudinal sections appear as tram lines reaching two-thirds of the way to the lung periphery
Marked increase	Extensive bronchial thickening might be observed, extending far into the periphery of the visible lung field

From: Bedenice D et al. J Vet Intern Med 2003; 17:876.

structures and of the teeth, pharynx, larynx, and guttural pouches.[25,26] For example, CT of the head of horses with suspected ethmoidal hematoma provides information that influences the treatment of approximately two-thirds of cases, including identification of bilateral disease and the extent of involvement of the paranasal sinuses (and in particular the sphenopalatine sinus). CT is recommended for patients in which the lesion cannot be viewed endoscopically, when sinus involvement or multifocal disease are suspected, or when the lesion has been unresponsive to treatment.[27]

The technique is technically feasible in ruminants and pigs, including detection of otitis media, pulmonary and pharyngeal abscesses, and congenital pulmonary anomalies in calves.[28-30] CT is useful in pigs for quantitation of the extent of atrophic rhinitis and evaluation of pneumonia, in addition to assessment of body composition.[31,32]

SCINTIGRAPHY (NUCLEAR IMAGING)

The basis of pulmonary scintigraphy is detection at the body surface of radiation emitted from the lungs after injection or inhalation of radioactive substances. The technique has been described in both horses and calves. The technique has limited diagnostic usefulness in large animals because of the need for availability of appropriate isotopes and detection equipment.[33] Furthermore, the large size of adult cattle and horses limits the sensitivity of the technique. The technique has been used to determine the distribution of pharmaceuticals administered by aerosolization and the presence of ventilation-perfusion mismatches. Alveolar clearance can be detected using scintigraphic examination. Currently pulmonary scintigraphy is largely a research tool.

ULTRASONOGRAPHY

Ultrasonographic examination of the thorax of farm animals and horses is a very useful diagnostic tool. Ultrasonographic examination of the thorax provides diagnostic information that is not obtained by radiographic examination and is more sensitive than radiography in detecting pulmonary abscesses in foals and is more useful than auscultation in detecting consolidation in lungs of preweaned calves.[1,15,34] The widespread availability of portable ultrasound units and the ability to image parts of the thorax using ultrasound probes intended for examination of the reproductive tract of mares and cows makes this a potentially valuable diagnostic aid for both field and hospital-based practitioners. Furthermore, the absence of radiation exposure and the "real-time" nature of images obtained by ultrasonography aid in frequent assessment and monitoring of abnormalities and performance of diagnostic or therapeutic procedures such as thoracocentesis or aspiration of masses.

There are limitations to imaging imposed by aerated lung and the bones of the ribcage. Examination of the thorax is limited by the presence of ribs and aerated lungs because the sound waves used to create ultrasound images are reflected from these surfaces. Ultrasonography cannot reveal lesions of the lungs that are not confluent with the visceral pleura. Imaging windows are restricted to the intercostal spaces, but this impediment can be overcome by scanning through adjacent intercostal spaces and angling of the ultrasound beam.

Ultrasonographic examination of the thorax should be performed in a consistent manner that ensures thorough examination of the thorax. Preferences for the pattern of examination differ somewhat among examiners, but one common and successful technique is to scan each intercostal space from dorsal to ventral starting at the 17th intercostal space in horses and the 12th intercostal space in cattle. The ultrasound probe is slowly moved from dorsal to ventral while the examiner studies the images. When one scanning of one intercostal space is completed, the probe is moved to the most dorsal aspect of the next intercostal space, and the examination is repeated. Each side of the chest is

examined in this manner. This consistent and thorough examination ensures that no important or localized abnormalities are missed. The examination is performed in adult horses and cattle with the animal standing. The rostral thorax is scanned by pulling the ipsilateral forelimb forward. This is more readily achieved in horses than in cattle. Thorough examination of the rostral thorax might require placing the animal in lateral recumbency. Calves and foals can be examined either standing or in lateral recumbency.

Ultrasound examination of the thorax is particularly useful for detecting diseases of the pleura, pleural space, or lung surface. This is in addition to the well-documented utility of ultrasonographic examination of the heart and great vessels (see Ch. 11). The normal ultrasonographic anatomy of the thorax of cattle, horses, and calves has been determined. The following is a partial list of disorders or abnormalities detectable by percutaneous ultrasonographic examination of the thorax of farm animals or horses (excluding cardiac abnormalities):

- Excess pleural fluid
- Characteristics of pleural fluid (flocculent, bubbles, fibrin)
- Extent of pleural fluid accumulation
- Localized areas of pleural fluid accumulation
- Nonaerated lung (atelectatic, consolidated)
- Pulmonary abscesses (must be confluent with visceral pleura)
- Intrathoracic masses (thymic lymphoma, cranial thoracic mass, gastric squamous-cell carcinoma)
- Pleural roughening ("comet-tail" lesions)
- Pneumothorax
- Pulmonary hematoma
- Exercise-induced pulmonary hemorrhage
- Hemothorax
- Diaphragmatic hernia
- Fractured ribs (especially in neonates).

Ultrasonographic examination is more sensitive and specific than radiographic examination in detecting the presence of pleural fluid and is particularly useful in the diagnosis and management of pleuritis in horses and cattle and pneumonia in calves. The extent of pulmonary lesions detected at necropsy correlates closely with the results of ultrasonographic examination of calves with pasteurellosis. Ultrasonographic examination is useful in diagnosis of thoracic diseases of cattle. Ultrasonography can identify pulmonary lesions in horses with infectious viral pneumonia. Ultrasonography is useful in identifying the presence of pleural fluid and guiding thoracocentesis to sample and drain this fluid.

Ultrasonographic examination of the larynx and associated structures is useful in identifying recurrent laryngeal neuropathy in horses, arytenoid chondritis, and dynamic laryngeal collapse.[35-37] Sensitivity and specificity for ultrasonographic examination of the larynx for detection of recurrent laryngeal neuropathy was 71% to 79% and 86% to 91%, respectively, compared with dynamic endoscopy.[38]

LABORATORY EVALUATION OF RESPIRATORY SECRETIONS

SAMPLING RESPIRATORY SECRETIONS

When an inflammatory disease process of the respiratory tract is suspected, the collection of samples of secretions and exudate for microbiological and cytologic examination can be considered. The objective is to obtain a sample uncontaminated with environmental flora, which are common in the upper respiratory tract, and to isolate the pathogen(s) or demonstrate inflammatory cells which may be associated with the lesion. This can be done by the following methods:

- Swabbing the nasal cavities or the pharynx
- Collection of fluid from the paranasal sinus
- Collection of fluid from the guttural pouch of Equidae
- Transtracheal aspirate
- Tracheal lavage
- Bronchoalveolar lavage
- Thoracocentesis

NASAL SWAB

A swab of the nasal cavities is a reliable method for the evaluation of the secretions associated with disease of the upper respiratory tract such as infectious bovine rhinotracheitis. However, when attempting to assess the health status of the lungs the nasal swab can be unsatisfactory because microbiological examination usually yields a large population of mixed flora, consisting of pathogens and nonpathogens, which is difficult to interpret. Examination of nasal swabs is only useful when seeking to detect specific pathogens (*Strep. equi,* influenza) and when the diagnostic tests are directed toward detecting these agents.

NASOPHARYNGEAL SWABS

For more reliable results and to lessen the contamination that occurs with nasal cavity samples, swabs of the laryngeal–pharyngeal area can be collected. A swab in a long covered sheath, of the type used for collecting cervical swabs from mares, is easily passed through the nasal cavities to the pharyngeal area. Important differences exist between the microbial isolates from nasopharyngeal swabs and those from lung tissues, which makes nasal swabs unreliable for diagnosis. For example, at the individual animal level, nasopharyngeal swabs and bronchoalveolar lavage show only moderate agreement; at the group or herd level, the isolation rates of various organisms are similar.

For isolation of viruses associated with disease of the upper respiratory tract, nasal swabs are satisfactory provided a copious amount of nasal discharge is collected, and the swabs are kept moist during transport to the laboratory. Nasal swabs sometimes contain an insufficient amount of secretion, and certain viral pathogens can become inactivated in transit.

NASAL LAVAGE

When larger quantities of nasal discharge are required for research purposes, nasal washings are usually collected, with the simplest technique being irrigation of the nasal cavities and collection into an open dish. From these samples, it is possible to isolate bacteria and viruses and identify immunoglobulins. The development of immunofluorescent and enzyme-linked immunosorbent assay (ELISA) tests for agents of infectious disease has provided reliable systems for the diagnosis of a variety of virus diseases in the early stages of infection. A technique and apparatus are available that obtain much better samples than the conventional cotton-wool swab provides. A vacuum pump aspirates epithelial cells and secretion from the nasal passage and pharynx. Cell smears are then prepared for microscopic examination and the mucus, and cells are used for conventional microbiological isolation.

PARANASAL SINUS FLUID

Fluid can be collected from the frontal and paranasal sinuses of most of the domestic large animals. Indications for collection of fluid include the presence or suspected presence of disease of the paranasal sinus. Medications can be administered and infected sinuses lavaged using this approach. Absolute contraindications are few but include failure to be able to adequately restrain the animal.

Demonstration of fluid in the paranasal sinuses is aided by radiographic examination of the skull. Fluid is collected by percutaneous centesis of the frontal or maxillary sinus and submitted for cytologic and bacteriologic examination (Gram stain, culture). The procedure begins with restraint of the animal, which can include the induction of moderate sedation by administration of alpha-2 agonists and narcotics, or in cattle restraint in a head gate with the head secured with a halter. Next, the area over the centesis site is prepared aseptically and the skin and subcutaneous tissues are anesthetized with local anesthetic. A stab incision (< 1cm) is made in the skin and subcutaneous tissues. A hole is then drilled into the sinus using a Jacob's chuck with a Steinmann pin (2- to 4-mm diameter). Only a short (5-mm) length of the Steinmann pin should be exposed by the chuck. The hole is drilled by applying steady pressure and making alternating clockwise and counterclockwise

movements with the chuck. Entry into the sinus cavity is evident as a sudden release of tension and easy passage of the Steinmann pin. The pin is then withdrawn, and sterile polyethylene tubing is inserted into the sinus cavity. Fluid can be aspirated at this time or, if none is forthcoming, 10 to 20 mL of sterile 0.9% saline or similar fluid can be instilled to the sinus cavity. Some of this fluid may run out the nostril if the animal's muzzle is lower than the sinus.

Complications include injury to adjacent structures, including the infraorbital nerve (trigeminal nerve), nasolacrimal duct or parotid salivary duct near its entrance to the oral cavity at the level of the upper cheek teeth. Hemorrhage is usually minor and self-limiting. Subcutaneous emphysema resolves within days. Cellulitis is a risk, especially for animals with septic processes in the paranasal sinuses. Prophylactic administration of antibiotics should be considered in these cases.

GUTTURAL POUCH FLUID

Indications for collection of fluid from the guttural pouches of equids include bacteriologic or polymerase chain reaction (PCR) examination to determine whether the horse is infected by *S. equi* (the etiologic agent of strangles) or to investigate the suspected presence of other inflammatory or neoplastic disease. The preferred method of collection is during endoscopic examination of the guttural pouch. During this examination, fluid can be collected through a polyethylene tube inserted through the biopsy port of the endoscope. Fluid collected in this manner is potentially contaminated by organisms in the upper respiratory tract, and results of bacteriologic examination should be interpreted with caution. Usually, bacteriologic examination is for the presence of *S. equi* and demonstration of its presence is all that is required for a diagnosis of infection. Fluid can also be obtained from the guttural pouch by blind passage of a firm catheter, such as a Chambers mare catheter or 10 French dog urinary catheter, into the guttural pouch. This procedure requires some skill, and there is always the uncertainty that one might not have actually manipulated the catheter into the guttural pouch. A third technique involves percutaneous puncture of the guttural pouch just posterior to the ramus of the mandible and ventral to the ear. This technique has the potential to yield fluid that is uncontaminated by organisms from the upper respiratory tract, but it carries with it a high risk of injury to the important vascular and neural structures in and around the guttural pouch (internal and external carotid arteries, pharyngeal branch of the vagus nerve, hypoglossal nerve, and others). Percutaneous sampling of guttural pouch fluid should not be undertaken without careful consideration of the risks and benefits of the procedure.

TRACHEOBRONCHIAL SECRETIONS

The collection and evaluation of tracheobronchial secretions is a useful method for assessing lower airway disease and is widely used in the determination of the etiology of infectious pneumonia (viral, mycoplasmal, fungal, and parasitic) or the severity of disease (bronchoalveolar lavage fluid cytology in horses with heaves, exercise-induced pulmonary hemorrhage in athletic horses). It is also used as a tool in evaluating the respiratory health of intensively housed animals, such as in piggeries. Cytologic examination of recovered fluid can provide valuable information about the severity, extent, and etiology of disease of the lower airway. There are two methods of sampling tracheobronchial secretions—aspiration of tracheal fluid or lavage of bronchioles and distal airways. Each sampling method yields fluid of differing characteristics and source, and interpretation of the results of examination of these fluids depends on their source and the method of collection.

Comparison of Tracheal Aspirates and Bronchoalveolar Lavage Fluid

Examination of tracheal aspirates and bronchoalveolar lavage fluid yields different, but often complementary, information about the lower respiratory tract. The differences between tracheal aspirates and bronchoalveolar lavage fluid arise because cell populations, and types of cell, differ markedly among segments of airways. There is no correlation between cytologic features of tracheal aspirates and bronchoalveolar lavage fluid of horses, and this is probably the case in other species. Tracheal aspirates are representative of cell and bacterial populations of the large conducting airways (trachea and mainstem bronchi), which can originate in both the large and small conducting airways and the alveoli. Secretions of more distal airways can be modified during rostrad movement, such that fluid in a tracheal aspirate is not representative of processes deeper within the lung. Furthermore, disease localized to one region of the lung can alter tracheal fluid. Examination of tracheal aspirates is useful for detecting inflammation of the large airways and for isolation of microorganisms causing disease in these structures.[39,40] There is no good evidence that findings on examination of tracheal aspirates correlate with abnormalities in pulmonary function, although they can correlate with exercise performance (racing).[41,42] Tracheal aspirates do not accurately reflect lesions in the lungs of horses, but presence of excess mucus, detected on endoscopic examination, is associated with impaired performance, whereas presence of excess neutrophils is not.[41]

Bronchoalveolar lavage is useful for sampling secretions in the more distal airways. It provides a sample of secretions that have not been contaminated by upper respiratory tract organisms or secretions before collection, and the sample is therefore assumed to be more representative of small airway and, to a lesser extent, pulmonary parenchymal and alveolar secretions or exudates. Bronchoalveolar lavage is useful in the detection of widespread lung disease but not necessarily in the detection of localized disease. Tracheal aspirates, because they in theory represent a composite sample of secretions from all regions of the lung, are likely to be more sensitive in detecting focal disease, such as a pulmonary abscess. Bronchoalveolar lavage fluid composition correlates well with pulmonary function in horses.

There is little agreement in cytologic examination of tracheal aspirates and bronchoalveolar lavage fluid of sick and healthy horses, and this difference probably exists in other species. Typically, the proportion of cells that are neutrophils is much higher in tracheal aspirates than in bronchoalveolar lavage fluid of both horses with heaves and normal horses. Mast cells are detected more frequently, and eosinophils less frequently, in bronchoalveolar lavage fluid than in tracheal fluid of normal horses.

Tracheal Aspirates

Indications for collection of tracheal aspirates include the need for microbiological and cytologic assessment of tracheal fluids. The primary indication is collection of samples for microbiological diagnosis of infectious respiratory disease.[39,43-45] Other indications include detection and characterization of inflammation of the conducting airways. **Contraindications** include severe respiratory distress, although this is not an absolute contraindication, inability to adequately restrain the animal, and severe, spontaneous coughing. Percutaneous tracheal aspirate collection performed in animals with severe coughing can result in development of severe subcutaneous emphysema as a result of the high intratracheal pressures associated with the early phase of coughing. Most animals in which percutaneous tracheal aspirates are collected subsequently have radiographic evidence of pneumomediastinum.

Tracheal aspirates can be collected either by percutaneous puncture of the trachea or through an endoscope passed through the upper airways. The advantage of percutaneous collection of tracheal aspirates is that there is minimal risk of contamination of the sample by upper respiratory tract or oropharyngeal secretions. Microbiological examination of the samples is therefore likely to accurately reflect microbes present in tracheal fluid. Collection of tracheal aspirates through an endoscope markedly increases the risk of contamination of the sample with oropharyngeal fluids, and it compromises the diagnostic utility of culture of the sample. This disadvantage is partially alleviated by the use of guarded catheters inserted through the endoscope. The disadvantage of percutaneous collection of tracheal fluid is that it is invasive, and there is a risk of localized

cellulitis and emphysema at the site of puncture. Endoscopic collection is relatively noninvasive and readily accomplished.

Percutaneous Transtracheal Aspiration

Percutaneous transtracheal aspiration is a practical method that has been used extensively in the horse and is adaptable to cattle, sheep and goats. For the horse, a 60-cm no. 240 to 280 polyethylene tube is passed through a 9- to 14-gauge needle inserted into the trachea between two rings. Commercially prepared kits for performing tracheal aspirates in horses are available that include all catheters and needles required. An alternative to polyethylene tubing is to use an 8 to 10 French male dog urinary catheter inserted through an appropriately sized cannula. The site for insertion of the needle or cannula is at the junction of the proximal and middle one-third of the ventral neck. The horse is usually sedated before insertion of the needle or cannula. The skin site is prepared aseptically, and a short stab incision is made after the area has been anesthetized. The cannula is removed to avoid cutting the tube, and the tube is pushed in as far as the thoracic inlet. Fluid typically pools in the trachea at the thoracic inlet in horses with lung disease (the tracheal lake or pool), and it is this fluid that is aspirated. Thirty to 50 mL of sterile saline (not bacteriostatic saline) is rapidly infused. The catheter or tubing should be rotated until tension is felt on aspiration by a syringe. Fluid is aspirated and submitted for cytologic, microbiological, or other examination.

Complications such as subcutaneous emphysema, pneumomediastinum, and cellulitis can occur, which necessitates care and asepsis during the procedure. Sudden movement of the cannula during insertion of the tubing may cause part of the tube to be cut off and to fall into the bronchi, but without exception this is immediately coughed up through the nose or mouth.

Endoscopic Sampling of Tracheal Secretions

The flexible fiberoptic endoscope can be used to obtain tracheal lavage samples and at the same time visualize the state of the airways. The process is as for rhinolaryngoscopic examination with the addition of passage of a catheter through the biopsy port of the endoscope. Tracheal fluid is then visualized and aspirated through the catheter. The clinical advantages of the endoscopic collection include noninvasiveness, visual inspection of the airways, guidance of the catheter, and speed. The use of an endoscope with a guarded tracheal swab minimizes contamination by oropharyngeal secretions but does not eliminate it.

Assessment of Results

Microbiological examination can yield any one or more of a variety of bacteria, depending on the species examined, the animal's age, and its clinical condition. Tracheal aspirates of normal animals rarely yield any bacterial growth on culture. Growth of unusual organisms or known oropharyngeal commensal bacteria from samples obtained by endoscopic examination should not be given undue clinical significance as they probably result from contamination of the tracheal aspirate during collection. *Pseudomonas* spp. and anaerobes isolated from tracheal aspirates collected by endoscopy are almost always contaminants and of no clinical significance. The extent of contamination of tracheal aspirate samples by oropharyngeal bacteria can be estimated from the number of squamous epithelial cells in the sample. There is an apparent approximate linear relationship between the number of squamous cells per milliliter of fluid and the number of colony-forming bacterial units in tracheal aspirates. Samples containing over approximately 10 squamous epithelial cells per milliliter of tracheal aspirate had markedly greater bacterial contamination. Examination of Gram-stained smears of tracheal fluid is specific but not very sensitive for detection of bacteria, compared with culture. In other words, if examination of a Gram-stained smear of tracheal fluid reveals bacteria, then the sample is likely to yield bacteria on culture, whereas failure to detect bacteria predicts poorly the likelihood of growth of bacteria on culture of the sample. This indicates that examination of Gram-stained samples of tracheal fluid does not reliably predict bacterial isolation, and if an infectious etiology is suspected, the fluid should be cultured. Results of the microbiological examination of the tracheal fluid should be consistent with the animal's clinical condition and expected isolates.

Cytologic examination of tracheal fluid is an important diagnostic tool. Various stains are available to aid identification of cell types and numbers in tracheal aspirates. Neutrophils, macrophages, lymphocytes, and epithelial cells are readily identified on the basis of their classical morphology and staining using fast Romanowsky stain (Diff-Quik), but this stain is not suitable for identifying mast cells in equine tracheal fluid and probably that of other species. Leishman's stain is useful to identify mast cells. Clinically normal horses typically have fewer than 20% to 30% of cells as neutrophils with the majority of remaining cells being macrophages, lymphocytes and epithelial cells. Animals with inflammation of the airways typically have increased cell counts and proportion of neutrophils and large amounts of mucus. Horses with inflammatory airway disease such as heaves typically have more than 20% of the cells as neutrophils (see following "Heaves" section), and those with infectious pneumonia often have 50% to 90% of cells as neutrophils. Exercise markedly increases the proportion of neutrophils in tracheal fluid collected within 1 hour of the horse completing intense exercise.[46] The presence of eosinophils is considered abnormal and is consistent with parasite migration (*Parascaris equorum* in foals, *Dictyocaulus viviparus* in calves). The presence of hemosiderin-laden macrophages is evidence of prior pulmonary hemorrhage.

Bronchoalveolar Lavage

Bronchoalveolar lavage provides a sample of secretions and cells of the distal airways and alveoli, referred to as bronchoalveolar lavage fluid. It is a widely used procedure in horses and, to a lesser extent, cattle and calves, sheep, camelids,[44] and pigs. The procedure can be performed on foals, either sedated or anesthetized with improved fluid recovery in the latter.[43] Analyses performed on bronchoalveolar lavage fluid include measurement of cell number and concentrations of various acute-phase proteins, analysis of type of immune proteins and surfactant, culture (usually in pigs and cattle), and use of PCR to detect specific pathogens (e.g., the causative agent of ovine pulmonary adenocarcinoma).[47,48] It is a relatively noninvasive procedure that allows cytologic and biochemical evaluations of the lower airways and alveoli, which are useful diagnostic aids when evaluating animals with lung disease. Although fiberoptic bronchoscopy and tracheal aspirates permit assessment of the major bronchi and upper airways, bronchoalveolar lavage offers an extension of the diagnostic potential by sampling the terminal airways and alveolar spaces.

The primary **indication** for collection of bronchoalveolar lavage fluid is acute or chronic lung disease. This includes both infectious and noninfectious diseases, although interpretation of samples collected by passage of the collection tube through the nostrils or mouth is complicated by the inevitable contamination of the sample by oropharyngeal commensal bacteria. Despite this shortcoming, the technique has been used to detect pneumonia associated with *Mycoplasma* sp. in cattle. **Contraindications** are few, with respiratory distress being an obvious one. **Complications** of bronchoalveolar lavage are also few, and include a mild neutrophilia in lavaged sections of lungs and changes in phagocytic function of pulmonary macrophages, and microbial content, for several days after the procedure. Transient bronchial collapse can occur during the procedure in horses and is an indication of airway inflammation.[49]

A shortcoming of bronchoalveolar lavage is that it lavages only a small region of the lung, with the risk that focal lung disease is not detected. There is clear evidence that important differences can exist in bronchoalveolar lavage fluid from left and right lungs and that the ideal technique involves collection of fluid from both lungs.[50] This is best exemplified in pneumonia in horses, in

which bronchoalveolar lavage fluid from pneumonic horses can contain large numbers and a high proportion of neutrophils or can be normal, depending on the lung or area of lung lavaged. Therefore the bronchoalveolar lavage procedure is a very specific but not very sensitive test for pneumonia in horses. Abnormal lavage fluid is helpful diagnostically, whereas normal results do not exclude the presence of foci of pulmonary disease. The lavage samples may be normal in horses affected with pneumonia or pleuropneumonia, and because of these false-negative results, this is not the best diagnostic technique to evaluate a horse with pneumonia. In contrast, the tracheobronchial aspirates are more sensitive and most horses with pneumonia have cytologic abnormalities.

Endoscopic Bronchoalveolar Lavage

Endoscopic bronchoalveolar lavage has the advantage of permitting visual examination of the airways during the procedure and selection of the region of the lung to be lavaged. This technique does require access to sophisticated endoscopic equipment. The technique described here for horses can be modified for use in other species.

Horses for bronchoalveolar lavage should be appropriately restrained. Sedation is usually essential and is achieved by administration of alpha-2-agonists. Coadministration of narcotics is recommended by some authorities to reduce the frequency and severity of coughing. Butorphanol tartrate 10 mg for a 400-kg horse is recommended, although this drug is not as effective as intratracheal lidocaine at reducing the frequency or severity of coughing when combined with detomidine for collection of bronchoalveolar lavage fluid. Effective suppression of coughing during collection of bronchoalveolar lavage fluid can be achieved by instillation of lidocaine (60 mL of a 0.7% solution—made by diluting 20 mL of 2% lidocaine solution by addition of 40 mL of isotonic saline). The lidocaine solution is administered as the endoscope enters the rostral trachea. A twitch can be applied to the nares. The endoscope must be at least 2 m in length and the external diameter should be 10 to 15 mm. Endoscopes of 10-mm diameter will pass to about the fifth-generation bronchi, whereas endoscopes of larger diameter will not pass quite as far into the lung. The endoscope is passed until it wedges, and then 300 mL of warmed (to reduced bronchospasm) isotonic saline is introduced in 5 × 60 mL aliquots. Air is infused after the last aliquot to ensure that all fluid is instilled. After the horse has taken between one and three breaths, the fluid is withdrawn and the aliquots are mixed. There is no difference in the cytologic composition of the first and subsequent aliquots.

Blind Bronchoalveolar Lavage

Commercial bronchoalveolar lavage tubes are available for use in horses, and are suitable for use in adult cattle and calves. The tubes are made of silicone and are therefore considerably more pliable than stomach tubes (which are not suitable for this procedure). The tubes are 2 m in length and have an external diameter of about 8 mm. The horse is restrained and sedated as for endoscopic bronchoalveolar lavage, and the tube is passed through one nostril into the trachea. The tube is then advanced until it wedges, evident as no further insertion of the tube with mild pressure. Continued vigorous attempts to pass the tube can result in the tube flexing in the pharynx and a loop of the tube entering the mouth. After the tube wedges, the cuff on the tube is inflated to prevent leakage of fluid around it, 300 mL of warm isotonic saline is instilled, the tube is flushed with air, and fluid is aspirated. The fluid should be foamy and, if cell counts are high, slightly cloudy.

Bronchoalveolar lavage can be performed in conscious **sheep** by insertion of 1.7-mm external diameter polyethylene tubing through a cannula inserted percutaneously in the trachea. The tubing is inserted until resistance is detected (about 40-45 cm in an adult sheep) and the lung is lavaged with 30 mL of sterile isotonic saline.

Laboratory Assessment of Tracheobronchial Secretions

A problem with comparison of cell counts of bronchoalveolar lavage fluid reported by different authors is the use of inconsistent quantities of fluid to perform the lavage. The use of different volumes alters the extent of dilution of the fluid. There is a need for uniformity in technique. An approach to this problem has been to measure substances in the bronchoalveolar lavage fluid that can provide an indication of the extent of dilution of the sample. Both endogenous (urea, albumin) and exogenous (inulin, methylene blue) markers have been used. Dilution factors using urea concentration in plasma and in bronchoalveolar lavage fluid appear to be useful. The assumption is that urea concentrations in bronchial and alveolar secretions will be identical to that in plasma. The formula for correcting for dilution that occurs during collection of bronchoalveolar lavage fluid is:

$$\textit{Dilution factor} = \textit{Urea concentration in bronchoalveolar lavage fluid} / \textit{Urea concentration in plasma}$$

where urea concentration in bronchoalveolar lavage fluid and in plasma is expressed in the same units. The volume of the pulmonary epithelial lining fluid can then be calculated:

$$\textit{Pulmonary epithelial lining fluid volume} = \textit{Dilution factor} \times \textit{Volume of bronchoalveolar lavage fluid}$$

Samples for cytology are submitted for preparation involving centrifugation of the sample to concentrate cells for preparation of slides for staining and microscopic examination. At least for samples from horses, examination of smears made directly from the sample, without centrifugation, is diagnostically useful. As for tracheal fluid, the proportion of mast cells in equine bronchoalveolar lavage fluid is underestimated if cells are stained with fast Romanowsky stain (Diff-Quik). Ideally, five fields are examined for each slide, rather than simply counting 400 cells, to ensure that the cell proportions are accurately reported, particularly for mast cells.[51]

Diagnostic Value

The aspirates from normal animals contain ciliated columnar epithelial cells, mononuclear cells, and a few neutrophils with some mucus. Bronchoalveolar lavage fluid samples can be collected at 24-hour intervals without affecting the composition of the fluid, whereas collection as soon as 2 hours can result in a neutrophilic response.[45] The concentration of the cells depends on the volume of fluid infused and the disease status of the animal. Representative values for various species are listed in Table 12-3. The general pattern is that animals with inflammatory airway disease, either infectious or noninfectious, have a higher proportion of neutrophils than do disease-free animals. However, ranges of normal values vary considerably depending on the species, the age of the animal, and its management (primarily housing conditions). Care should be taken not to overinterpret findings on examination of tracheal aspirates or bronchoalveolar lavage fluid. Although there is good correlation between microbiological results and cell counts in bronchoalveolar lavage fluid of calves with pneumonia and Thoroughbred racehorses with inflammatory airway disease, this association might not hold for all diseases or species.

There is the potential for a seasonal effect on bronchoalveolar lavage fluid composition, with mastocytosis occurring more commonly in the antipodean spring and neutrophilia and eosinophilia more common in the summer.[52] Aged horses have a higher percentage of lymphocytes and lower proportion of macrophages than do younger horses.[53] The clinical importance of this finding is unclear.

Thoracocentesis (Pleurocentesis)

Paracentesis of the pleural cavity is of value when the presence of pleural fluid is suspected and, in the absence of ultrasonographic examination, needs to be confirmed, and when sampling of pleural fluid for cytologic and bacteriologic examination is indicated. The primary indication for sampling pleural fluid is the presence of excess pleural fluid. Sampling of pleural fluid is usually accompanied by therapeutic drainage, in which case the cannula used for sampling is

Table 12-3 Representative results of cytology of bronchoalveolar lavage fluid of cattle, sheep, pigs, and horses

Species	Disease status	Volume infused (mL)	Total nucleated cell count (cells × 10^9/L)	Neutrophil (%)	Macrophages (%)	Lymphocytes (%)	Eosinophils (mast cells) (%)
Weaner pigs	Normal	15–30	0.7 ± 0.2	2.0 ± 1.2	95.6 ± 2.7	1.7 ± 1.2	NR
Weaner pigs	Respiratory disease	15–30	0.9 ± 0.3	7.0 ± 4.2	87.9 ± 5.9	3.7 ± 2.0	NR
Adult sheep	Normal, pastured	30	NR	6.9 ± 5.8	81.1 ± 15.3	10.8 ± 15.8	1.2 ± 2.7
Adult sheep	Normal, housed	30	NR	21.8 ± 23.4	57.6 ± 19.6	16.1 ± 12.6	4.5 ± 9.5
Adult sheep	Respiratory disease	30	NR	26.8 ± 16.8	55.4 ± 20.9	11.6 ± 11.1	6.2 ± 8.6
Calves (2–3 months old)	Normal	240	NR	12 ± 10	86 ± 10	2 ± 1	0
Calves (2–3 months old)	Parasitic (*Dictyocaulus viviparus*) pneumonia	240	NR	20 ± 20	20 ± 10	2 ± 1	70 ± 10
Cattle (6–10 months old)	Healthy	180–240	1.4 ± 0.3	< 5	80–85	10	NR
Calves (2 months old)	Healthy	180	NR	9.1 ± 11.6 (all but one < 12)	90.7 ± 11.6	NR	NR
Horses (yearling)	Healthy, at pasture	300	85 ± 10.2 cells/μL	3.6 ± 0.8	39.5 ± 2.6	42.8 ± 2.4	0.8 ± 0.4 (mast cells 8.3 ± 1.7)
Horses (yearling)	Healthy, stabled	300	74.5 ± 7.8 cells/μL	13.2 ± 3.0	40.1 ± 2.7	39.1 ± 2.3	0.6 ± 0.2 (mast cells 4.1 ± 1.3)
Horses (adults)	Healthy	300	182 ± 035	8.9 ± 1.2	45 ± 2.8	43 ± 2.7	< 1
Standardbred racehorses	Healthy	300	153.2 ± 17.1	3.8 ± 0.3	64.8 ± 4.6	28.3 ± 2.9	1.2 ± 0.8 (mast cells 0.3)
Standardbred racehorses	Inflammatory airway disease	300	366 ± 16.8 cells/μL	10.4 ± 1.1	48.4 ± 1.9	36.0 ± 1.9	3.8 ± 1.5 (mast cells 1.8 ± 1.5)
Adult horses	Heaves	300	860 ± 324 cells/μL	60.3 ± 12.4	14.6 ± 4.8	22.7 ± 10.1	(mast cells 0.8 ± 0.6)
Adult horses	Remission from heaves (at pasture)	300	85 ± 15 cells/μL	17.7 ± 5.4	38.9 ± 9.1	42.4 ± 8.9	3.0 ± 0.8
Adult horses*	Mild heaves	250	253 (80–414)	17 (7–67)	28 (10–47)	43 (19–71)	0 (0–1) 1 (0–3)
Adult horses*	Moderate heaves	250	255 (117–3564)	17 (12–92)	19 (3–33)	43 (6–60)	1 (0–32) 1 (0–4)
Adult horses*	Severe heaves	250	286 (98–913)	25 (9–85)	34 (6–49)	31 (7–68)	0 (0–1) 1 (0–1)

Values are mean ± SD or median and range (*); NR, not reported. See Radostits et al. Veterinary Medicine 10th edition. 2006. Page 488. Table 10-3 for references.

larger than if only collection of pleural fluid is desired. Contraindications are minimal, especially if the procedure can be performed under ultrasonographic guidance. The principal contraindication is the inability to restrain an unruly animal because this increases the risk of laceration of the lung or a coronary vessel, or cardiac puncture. Complications include hemorrhage from lacerated intercostal or pleural vessels, pneumothorax secondary to laceration of the lung or introduction of air through the cannula, cardiac puncture and sudden death, irritation of the myocardium and ventricular arrhythmia (premature ventricular contractions), or coronary artery laceration and subsequent cardiac tamponade and death. There is a risk of cellulitis at the site of centesis, especially if indwelling cannulas are maintained for more than a day.

The procedure is performed with the animal standing. Sedation or systemic analgesia is usually not needed, unless it is medically indicated or the animal is not easily restrained. The equipment for sampling of pleural fluid from adult horses or cattle is a blunt 10- to 15-cm cannula of approximately

3 mm in diameter (such as a bovine teat cannula) or a 7.5-cm spinal needle. The blunt-tipped cannula is preferred because use of it reduces the risk of laceration of vital structures. A three-way stopcock or similar device should be attached to the hub of the needle or cannula and closed to prevent aspiration of air when the pleural cavity is entered. The site for centesis is best identified by ultrasonographic examination of the thorax or, if that is not available, by percussion and auscultation of the chest to identify the fluid level. A commonly used site is the seventh intercostal space on the left side and the sixth intercostal space on the right side. The skin should be clipped of hair and aseptically prepared. The region can be anesthetized with approximately 10 mL of 2% lidocaine, mepiricaine, or a similar product. The cannula or needle should be introduced over the rib and then directed cranial to the rib (the intercostal vessels and nerves course along the caudal edge of the rib). If a cannula is used, then a slight "popping" sensation is felt as the cannula perforates the parietal pleura. A syringe is attached to the cannula or needle and fluid is aspirated from the pleural space.

Collected fluid should be examined visually. Normal pleural fluid, which is present in small quantities in normal animals, is clear and slightly yellow. Abnormal fluid can be bloody, thick, and yellow, suggestive of purulent material, or flocculent. The material should be smelled—a foul odor is usually present when the pleural fluid is infected by anaerobic bacteria and is a sign of a poor prognosis. Cytologic examination should be performed, including white cell count and measurement of total protein concentration. Ancillary measurements on pleural fluid include pH, P_{CO_2}, P_{O_2}, bicarbonate, glucose, and lactate. Sterile pleural fluid has a pH, P_{O_2} and P_{CO_2} and lactate, glucose, and bicarbonate concentrations similar to those of venous blood. Infected pleural fluid is acidic, is hypercarbic, and has an increased concentration of lactate and decreased concentrations of bicarbonate and glucose compared with venous blood. Pleural fluid should be cultured for aerobic and anaerobic bacteria and mycoplasmas. Antimicrobial susceptibility should be determined for isolated organisms. Fungal cultures are rarely indicated.

Ultrasound-guided needle puncture of a suspected lung abscess to determine the species of bacteria present is sometimes practiced, but there is the risk that infection will be spread to the pleura by this technique. This technique is not recommended as a routine procedure because microbiological examination of tracheal aspirates will probably yield the offending bacteria.

PULMONARY FUNCTION TESTS

Pulmonary function tests provide quantitative assessment of pulmonary ventilatory function through measurement of expired and inspired gas volumes, intrathoracic pressures, and derivations of these variables—sometimes referred to as pulmonary mechanics. The techniques are widely used in research into pulmonary diseases, especially heaves in horses, and have been adapted for use in ruminants. A relatively simple assessment of pulmonary function is measurement of **pleural pressure changes** during respiration. This can be achieved by either insertion of a blunt cannula through the intercostal space or passage of a balloon catheter into the thoracic esophagus. The pressure changes during respiration are then recorded and the maximal pressure change between inspiration and expiration is calculated. The pressure change is closely correlated with airway resistance to airflow and is an excellent indicator of the severity of bronchoconstrictive diseases.

More complex measurements are made by application of an airtight face mask containing a flow meter to the animal. Combined with measures of airway pressure, airflow during tidal breathing yields measures of tidal volume, minute volume, respiratory rate, pulmonary resistance, and pulmonary dynamic compliance. Measurements made with the animal at rest are relatively insensitive to small changes in pulmonary function, and the sensitivity of these tests to detect heaves is low. The sensitivity of changes in maximal pleural pressure and resistance of the lower airways are 44% and 22%, respectively. The sensitivity of the test can be increased by measuring these variables during exercise. Measurement of pulmonary mechanics in horses with heaves is reproducible over both short (hours) and long (months) periods of time, indicating the usefulness of these techniques for monitoring of disease progression and response to therapy.

Measurement of **flow–volume loops** has been performed for both stationary and exercising horses. A number of variables are derived from these measures and used as indicators of pulmonary function. However, the large variability in these measures in stationary horses (16%-32%) severely limits the utility of this test to detect mild or subclinical respiratory disease. Similarly, flow–volume loops in exercising horses with obstructive lung disease of moderate severity do not differ markedly from those of the same horses when they do not have lung disease. Flow–volume loops have limited use in evaluation of lung function in animals.

Other tests of pulmonary function include the nitrogen dilution test and the single-breath diagram for CO_2. For the **nitrogen dilution test** concentrations of nitrogen in exhaled air are measured while the animal breathes 100% oxygen. A number of variables are calculated from the decay curve of nitrogen concentration in exhaled air, including the functional residual capacity. There are clinically significant differences between animals with normal respiratory function and those with pulmonary disease. However, this test is not readily adapted for routine clinical use. Volumetric capnography is the graphic examination of expired breath CO_2 concentrations versus expired volume to create a **single-breath diagram for CO_2**. The results are divided into phase I, which represents the relatively carbon-dioxide-free air from the proximal or orad conducting airways, phase II, which is the transitional phase, and phase III, which is the carbon-dioxide-rich air from the alveoli. Measures of pulmonary function obtained include estimates of dead space ratio, physiologic dead space volume and alveolar efficiency. The clinical utility of this test and its ability to detect mild or subclinical disease in animals have not been demonstrated.

Impulse oscillometry offers the potential of being a potentially clinically useful test of respiratory function in horses, pigs, and cattle.[54-57] The test measures impedance of the respiratory system and provides estimates of respiratory resistance and reactance. The technique has the advantage of being more sensitive to changes in pulmonary function than measurement of pleural pressure changes, is minimally influenced by respiratory rate and tidal volume and is relatively easier to perform than more complex measures of respiratory mechanics. The test involves fitting an airtight facemask containing a pneumotachograph for measurement of respiratory volumes and tubing to a horse. The tubing is attached to a loudspeaker, which is used to generated square-wave signals containing harmonics between 0 and 10 Hz. Information from the system is analyzed using a computer program and indices of pulmonary resistance and reactance are determined. The forced oscillation technique in feedlot cattle with naturally occurring shipping fever indicates the presence of a large increase in pulmonary resistance and a decrease in dynamic compliance with obstructive lung disease located mainly at the level of large airways but also in small airways. The test is more sensitive than conventional techniques in detecting partial upper airway obstruction, heaves, and inflammatory airway disease in horses, and in pigs with lung disease.[54-56] Impulse oscillometry can also be used to monitor response to therapy.[56]

The sensitivity of these tests can be increased by provocative tests in which animals are administered agents, such as histamine or methacholine, that cause bronchoconstriction in animals with reactive airways.

Measurement of **forced expiratory flow–volume curves** and forced vital capacity in horses is a sensitive indicator of bronchoconstriction. The test involves the heavily sedated horse having a nasotracheal tube inserted. The nasotracheal tube is then attached to a large vacuum reservoir and a valve is opened abruptly. The maximum rate of forced

expiratory airflow is measured and various variables indicative of pulmonary function are calculated, including forced expiratory volume in 1 second (FEV_1). The clinical utility of this test of pulmonary function is limited by the extensive instrumentation of the animal and the need for sophisticated electronics.

ARTERIAL BLOOD GAS ANALYSIS

Measurement of P_aO_2, P_aCO_2, and arterial oxygen content (C_aO_2) provides valuable information about pulmonary function and oxygen delivery to tissues. The arterial oxygen tension and arterial oxygen content are not equivalent. The arterial oxygen tension (P_aO_2) is a measure of the partial pressure of oxygen in arterial blood determined by the amount of oxygen dissolved in the blood (not the amount bound to hemoglobin) and the temperature of the blood—it is not a direct measure of arterial oxygen content. Arterial oxygen content is the amount of oxygen per unit of blood and includes both dissolved oxygen and that bound to hemoglobin. The oxygen tension can be viewed as the driving force for diffusion of oxygen from capillaries into mitochondria (in which the oxygen tension is about 2 mm Hg), whereas the oxygen content is the amount of oxygen delivered to tissue. Both are important measures of pulmonary function and oxygen delivery to tissue.

Measurement of **oxygen tension** in blood is achieved by analysis of an appropriately collected sample of arterial blood using a blood gas analyzer (oxygen electrode). Instruments designed for medical or veterinary clinical use measure pH, PO_2, and PCO_2 at a temperature of 37° C (98.6 F). Depending on the software included with the instrument, various derived values are also reported, including bicarbonate concentration, base excess, and oxygen saturation. It is important to understand that **oxygen saturation** reported by blood gas instruments is a *calculated* value and might not be correct. Oxygen saturation is *measured* by a cooximeter, which is different from a blood gas machine, and the amount of oxygen carried by hemoglobin is then calculated from this value, with the assumption that each gram of hemoglobin, when fully saturated, carries approximately 1.34 to 1.39 mL of oxygen. The total **oxygen content** of blood is calculated by adding the amount carried by hemoglobin to the amount of oxygen dissolved in the aqueous phase of the blood. The formula is

$$O_2\ content = (S_aO_2 \times 1.34 \times [Hb]) + (0.003 \times P_aO_2)$$

where O_2 content is in mL/100 mL, S_aO_2 is the arterial oxygen saturation (%), 1.34 is the amount of oxygen carried by fully saturated hemoglobin (mL/g), Hbwsa is the concentration of hemoglobin in blood (g/100 mL), 0.003 is the amount of oxygen dissolved in the aqueous phase of 100 mL of blood for each 1-mm Hg increase in PO_2, and P_aO_2 is the oxygen tension in arterial blood. The appropriate substitutions can be made to calculate the oxygen content of venous blood.

The oxygen content of arterial blood is the critical factor (with cardiac output) in determining **oxygen delivery** to tissues. However, measurement of arterial oxygen content is not as readily accomplished as measurement of arterial oxygen tension. Therefore in animals with normal hemoglobin concentration and function the arterial oxygen tension is used as a surrogate measure of arterial oxygen content. In doing so, it must be recognized that the extent of hemoglobin saturation with oxygen is dependent on both the affinity of hemoglobin for oxygen and the oxygen tension of the blood. The oxygen tension/percentage saturation relationship is sigmoidal, with 50% saturation occurring at about 30 mm Hg in most species (there are minor variations) and 80% saturation at a PO_2 of 45 to 55 mm Hg. The sigmoidal shape of the oxygen–hemoglobin saturation curve has important clinical consequences. Small decrements in P_aO_2 from normal values (usually 95-105 mm Hg in animals breathing ambient air at sea level) have a minimal effect on oxygen content of blood. Many modern blood gas analyzers have software that calculates oxygen content of blood, but it must be recognized that these calculations often use an assumed, not measured, hemoglobin concentration (usually 15 g/dL) and values for the human SO_2–PO_2 relationship. These assumed values may not be correct for animals, and one should always check the assumptions used to calculate oxygen content of blood before accepting and acting on those values. Direct measurement of blood oxygen content is restricted to research laboratories—indirect estimates gained from oxygen saturation and hemoglobin concentration are usually sufficiently accurate for clinical use.

The oxygen tension in blood is proportional to the amount of oxygen dissolved in the aqueous phase of the blood and the temperature of the blood. For a given amount of oxygen dissolved in blood, the tension varies according to the temperature of the animal. Almost all blood gas analyzers measure the PO_2 at 37° C (98.6 F). If the animal's body temperature is markedly different from that, then the reported PO_2 can be erroneous. For instance, the P_aO_2 of a horse with a body temperature of 40°C (104.0 F) measured using an analyzer with a temperature of 37° C (98.6 F) would be 80 mm Hg (the PCO_2 would be 35 mm Hg). If the P_aO_2 was adjusted for the difference between the horse's body temperature and that of the analyzer, then the reported P_aO_2 would be 100 mm Hg (and the P_aCO_2 would be 44 mm Hg). Failure to make the appropriate temperature corrections can result in errors of 6% to 7% per °C (3% to 4% per F). When interpreting blood gas values, attention should be paid to the temperature of the animal and consideration given to adjusting gas tension values according to the animal's body temperature. This is probably only clinically important when there are extreme deviations from normal temperature and oxygen tension. Most blood gas analyzers include software that makes the appropriate corrections.

The arterial oxygen tension is determined in the alveolus by the alveolar oxygen tension and the alveolar–arterial difference. The alveolar oxygen tension (P_AO_2) can be calculated from the following equation:

$$P_AO_2 = F_iO_2(P_B - PH_2O) - (P_aCO_2/RQ)$$

where F_iO_2 is the inspired oxygen fraction (21% for ambient air), P_B is the barometric pressure (760 mm Hg at sea level), PH_2O is the partial pressure of water vapor in the alveolar air (47 mm Hg at 37° C, 98.6 F), and RQ is the respiratory quotient (usually assumed to be 0.8 for resting animals). The alveolar–arterial PO_2 difference (A – a PO_2) is calculated as

$$A - a\ PO_2 = P_AO_2 - P_aO_2$$

The A–a PO_2 difference has clinical significance in that it is an indicator of pulmonary function that is somewhat independent of inspired oxygen fraction and is therefore useful in animals being supplemented with oxygen (there is a small increase in the A–a difference with marked increases in F_iO_2). Increases in A–a PO_2 difference are indicative of ventilation-perfusion mismatches, with the A–a PO_2 difference increasing with worsening ventilation-perfusion abnormalities.

Normal Values

Values obtained from clinically normal animals breathing room air at sea level vary slightly between species, with most animals having an arterial P_aO_2 of 95 to 105 mm Hg and a P_aCO_2 of 35 to 45 mm Hg. Oxygen saturation in clinically normal animals breathing air at sea level is above 98% and oxygen content of arterial blood is 16 to 24 mL/dL of blood (this depends on the hemoglobin concentration in blood). The difference in oxygen content of arterial and mixed venous blood is usually 4 to 8 mL/dL of blood. Values can be influenced substantially by changes in physiologic state (exercise, hyperpnea), positioning, pulmonary disease, and altitude (Table 12-4). Positioning of the animal can be important, especially in neonatal foals, in which the compliant chest wall can impair ventilation in laterally recumbent foals—foals have lower arterial oxygen tension when in lateral recumbency than when in sternal recumbency.

Collection of Arterial Blood Gas Samples

Arterial samples can be collected from any of the appropriate peripheral arteries, which

Table 12-4 Changes in blood gas tensions in various disease states compared with values in normal animals breathing air at sea level

Arterial oxygen tension (P_aO_2, mm Hg)	Arterial carbon dioxide tension (P_aCO_2, mm Hg)	Alveolar–arterial oxygen difference (mm hg)	Physiologic state or disease
↑	↓	↔	Hyperventilation (excitement, panting)
↔ or ↓	↓	↔	Low inspired O_2 (altitude)
↓	↑	↔	Hypoventilation
↓	↔	↑	Diffusion impairment (rarely encountered)
↓	↔ or ↑	↑	Ventilation-perfusion mismatch. ↑ P_aCO_2 with this disorder is uncommon
↓	↑	↑	Strenuous exercise by horses

↑, above value in normal animal breathing ambient air at sea level; ↓, below value in normal animal breathing ambient air at sea level; ↔, unchanged from value in normal animal breathing ambient air at sea level.

vary depending on species. An arterial sample is representative of aortic blood in almost all instances. Samples can be collected from the carotid, transverse facial, metacarpal, and metatarsal arteries in horses and foals, and from the carotid, radial, and coccygeal arteries in cattle and calves. Minimally invasive arterial access is difficult in pigs.

Samples should be collected in glass, in which the dead space has been filled with heparin solution, and stored at 0°C until analyzed.[58] Typically, a 3-mL plastic syringe containing approximately 0.1 mL of sodium heparin and attached to a 22- to 25-gauge needle is used. All air should be expelled from the syringe before collection of the sample, and care should be taken to not introduce air into the syringe until blood gas tensions are measured. Air in the syringe will increase the measured oxygen tension of blood from normal animals. The sample should be measured as soon after collection as possible (within minutes). If immediate analysis is not available, the sample should be stored in iced water until analysis to prevent consumption of oxygen, production of carbon dioxide and a decrease in pH.[58] Storage of arterial samples in plastic syringes in iced water can increase the oxygen tension from 100 mm Hg to 109 mm Hg in as little as 30 minutes. This does not occur when samples are stored in glass syringes in iced water. The pH_a and P_aCO_2 are not affected by the type of syringe.

Blood samples stored in plastic tubes (vacutainer) are not suitable for measurement of oxygen and carbon dioxide tensions, but measurements of total carbon dioxide and bicarbonate concentrations and base excess are reliable.[59]

VENOUS BLOOD GAS ANALYSIS

Measurement of gas tensions in venous blood is of limited value in assessing pulmonary function because of the extensive and variable effects of passage through the capillary beds on gas tensions. However, measurement of venous oxygen tension, saturation, or content can be useful in assessment of the adequacy of oxygen delivery to tissue. The oxygen tension, saturation and content of venous blood depends on the extent of oxygenation of arterial blood, the blood flow to the tissues, the metabolic rate of the tissues drained by the veins from which blood is sampled, and the transit time of blood through capillaries. The multiplicity of these factors means that determining the precise reasons for abnormalities in venous blood gas tensions is not possible. However, some generalizations can be made about venous oxygen tension, saturation and content.

In normal, resting animals, oxygen delivery to tissues exceeds oxygen needs (demand) of the tissue, with the result that venous blood draining these tissues is only partially desaturated. Hence, venous blood from the pulmonary artery (mixed venous blood) has oxygen tension, saturation, and content of approximately 35 to 45 mm Hg, 80% to 90%, and 12 to 18 mL/100 mL, respectively (the latter depending on hemoglobin concentration in addition to hemoglobin saturation). However, in situations in which oxygen delivery to tissue is decreased to levels that only just meet or do not meet the oxygen needs of tissue, there is extraction of a greater proportion of the oxygen in blood, and venous oxygen tension, saturation, and content decline, and the arterial–venous difference in oxygen content increases. Reasons for oxygen delivery to tissue not meeting the oxygen needs of that tissue are decreased perfusion of tissue, such as can occur with shock or circulatory failure, anemia, or decreased P_aO_2. Additionally, tissues with a high metabolic rate, such as exercising muscle, have high oxygen demands that can outstrip delivery.

Ideally, whole-body assessment of oxygen delivery by measurement of venous blood gas tensions is best achieved by examination of mixed venous blood. Mixed venous blood represents an admixture of blood draining all tissues and is collected from the pulmonary artery (although samples collected from the right ventricle or atrium are also appropriate in most instances). Although this blood is optimal for assessment of oxygen delivery to tissue, collection of mixed venous samples is not routine because of the need for catheterization of the pulmonary artery. Samples from peripheral veins are therefore used, but care should be taken when interpreting these values as venous blood gas tensions can vary considerably among veins. For animals with normal circulatory status, blood gas tensions in jugular vein blood are likely to be reasonable estimates of mixed venous gas tensions. However, if circulatory function is not normal, then samples from peripheral veins may not be indicative of values in mixed venous blood.

Samples for venous blood gas analysis should be collected into syringes in which the dead space is filled with sodium or lithium heparin solution. The volume of heparin should not be more than 2% of the amount of blood. Samples should be processed promptly. If samples cannot be processed within an hour, they should be stored in iced water. Samples stored in iced water for 24 hours have values that are minimally different from those before storage, whereas samples stored at 25°C (77 F) change markedly in 2 to 3 hours.

PULSE OXIMETRY

Pulse oximeters are devices for measurement of blood oxygen saturation that attach to skin or mucous membranes and sense the absorption spectrum of light by hemoglobin (the same principle is used in bench top cooximeters) in the underlying tissues. The devices are widely used for noninvasive monitoring of oxygenation in humans and have been adopted for use in animals. However, important challenges to their use exist in animals, not least of which is the presence of hair and densely pigmented skin in most farm animals. The devices have important deficiencies when used in foals and adult horses, but those applied to the ear, lip, or tongue of

foals have good sensitivity and specificity for detecting arterial So_2 of less than 90 mm Hg (12 kPa).[60] The devices consistently underestimate arterial So_2 at low saturations. Care should be taken when using these devices to monitor arterial hemoglobin saturation in animals.

BLOOD LACTATE CONCENTRATION

Measurement of blood lactate concentration is useful in assessing the adequacy of oxygen delivery to tissues and is now provided by point-of-care units, some of which have been validated for use in horses and calves.[61,62] Hypoxia causes a shift to anaerobic metabolism and the production of lactate. Lactate production is related to the severity and duration of hypoxia, with more severe hypoxia resulting in greater accumulation of lactate in tissues and its subsequent diffusion or transport into blood. Hypoxia also reduces the rate of removal of lactate from blood. The combination of increased production and decreased removal causes lactate to accumulate in blood. Measurement of blood lactate concentrations (which are usually lower than plasma lactate concentrations) is gaining increasing clinical usefulness as point-of-care analyzers become more readily available and testing more affordable.

Samples for measurement of blood lactate can be collected into syringes containing heparin solution (as used for measurement of blood gas tensions) if the sample is to be analyzed within 30 minutes. Samples should be stored in iced water until analysis. Prolonged storage at room temperature results in increases in blood lactate concentration. If sample collection is anticipated to be delayed, then samples should be collected into evacuated tubes containing sodium fluoride and potassium ethylenediamine tetraacetic acid (EDTA)—the sodium fluoride inhibits glycolysis. However, plasma lactate concentrations collected in these tubes are approximately 10% lower than in samples collected into tubes containing heparin, probably because of the osmotic effect of sodium fluoride/potassium EDTA on red cells. Samples for clinical analysis should be collected into syringes containing a heparin solution and analyzed within 30 minutes of collection. Measurement of blood or plasma lactate concentrations can be made using point-of-care analyzers, although these can yield results that differ markedly from traditional analyzers, especially in animals with extreme values for hematocrit (severe anemia or polycythemia). Ideally, blood and plasma lactate concentrations should be measured only on analyzers that have been validated for the species and clinical situation being studied.

Blood lactate and plasma lactate concentrations are not equal, with blood lactate concentration being lower because of the dilutional effect of red blood cells, which have a lower lactate concentration than plasma. However, most clinical assessments are based on blood lactate concentrations. Mixed venous or arterial blood lactate concentrations in most farm animal species are less than 2 mmol/L in normal, healthy animals. Tissue hypoxia, in addition to other conditions such as toxemia and septic shock, can increase blood lactate concentration. Blood lactate concentrations between 2 and 4 mmol/L should be interpreted with caution, whereas values above 4 mmol/L are indicative of clinically important disruption of oxygen transport and cellular metabolism. Repeated measurements over time can be useful for assessing progression of disease or efficacy of treatment. For instance, plasma lactate concentrations above 4 mmol/L in cattle with pneumonia are predictive of death within 24 hours.

COLLECTION AND ANALYSIS OF EXHALED BREATH CONDENSATE

Collection and analysis of exhaled breath condensate have use primarily in research studies at the current time and are not likely to be sensitive or specific markers of specific disease states, although they might provide a means of assessing disease severity or pathogenesis.[63] Breath condensate is collected and analyzed for markers of pulmonary or systemic disease, including pH and markers of oxidative stress.[64] The pH of breath condensate is affected by the design of the collection device and condensation surface temperature, precluding comparison of data between studies using different methodologies.[65] Examples of use of analysis of breath condensate include that induction of pneumonia in calves by infection with *Pasteurella multocida* increases the concentration of leukotriene B_4 in breath condensate, horses with heaves have higher concentrations of hydrogen peroxide than normal horses, probably a result of the airway neutrophilia in affected animals, and altered oxidative stress state in foals with *Rhodococcus equi* pneumonia.[64]

LUNG BIOPSY

Percutaneous biopsy of the lung is useful in confirming diagnosis of lung disease by providing tissue for histologic and microbiological examination in cattle, sheep, and horses. The technique is most useful by providing a histologic diagnosis of diffuse lung diseases or, when used with ultrasonographic guidance or performed by thoracoscopy, for focal disease. Biopsy provided a diagnosis in ~80% of 65 horses with clinical evidence of diffuse lung disease.[66]

Biopsy is usually percutaneous but can also be performed during thoracoscopic examination.[13,67] Indications for the procedure include the presence of diseases of the lungs in which a diagnosis cannot be arrived at through other forms of examination, including tracheal aspiration or bronchoalveolar lavage. It can also be used for assessing the severity of histologic changes and response to therapy. The procedure is best suited for widespread diseases of the lung, but it can be used for diseases that produce focal lesions if the biopsy is performed with ultrasonographic guidance. Contraindications include abnormalities in clotting function, pneumothorax, and severe respiratory distress. The danger in performing lung biopsy in animals in severe respiratory distress is that complications of biopsy, such as pneumothorax, hemothorax, or hemorrhage into airways, could further impair lung function and cause the death of the animal.

Complications include pneumothorax, hemothorax, hemorrhage into airways with subsequent hemoptysis or epistaxis, pulmonary hematoma, and dissemination of infection from infected lung to the pleural space. The risk of complications increases as the number of attempts at biopsy increase.[66] Pneumothorax, which is usually not clinically apparent, occurs in some horses in which the procedure is performed.[68] Coughing and epistaxis occur in about 20% and 10% of horses, respectively. Life-threatening hemorrhage occurs uncommonly (≈ 2% of cases). Bleeding into the airways, detected by tracheobronchoscopic examination, occurred in 16 of 50 horses after use of the manually discharged biopsy needle and in 5 of 50 horses after use of the automatically discharged needle.[68] Two of 60 cows collapsed immediately after the procedure, but subsequently stood and recovered. The remaining cows had no clinical abnormalities detected after biopsy, although necropsy examination 24 hours later revealed small lesions in the pulmonary parenchyma at the site of biopsy. One of 10 healthy sheep had coughing and bloody nasal discharge after lung biopsy.

The procedure is performed in adult horses and cattle using a 14-gauge biopsy needle, either manually operated or one that discharges automatically. Such instruments yield tissue in over 95% of attempts in cattle. The area for examination is best determined by radiographic or ultrasonographic examination of the thorax. A common site for biopsy is at the junction of the dorsal and middle thirds of the thorax at the 9th intercostal space in cattle and sheep and the 13th intercostal space in horses. The procedure is best performed with the animal standing. The skin over the area should be clipped of hair and aseptically prepared and local anesthesia induced by injection of 2% lidocaine or a similar compound into the intercostal space. A 0.5-cm incision is made through the skin, and the biopsy instrument is advanced through the caudal intercostal space (intercostal vessels and nerves course along the

caudal aspect of the ribs) and into the lung perpendicular to the skin surface. The instrument is advanced approximately 2 cm into the lung, and tissue is collected at the end of inspiration. The procedure is repeated as necessary for collection of samples for histologic and microbiological examination. The skin incision is closed with a single suture if necessary. The animal is then monitored closely for 12 to 24 hours for signs of coughing, epistaxis, hemoptysis, fever, or respiratory distress. Hemorrhage into the airways is usually evident, often within minutes of completing the procedure, by the animal coughing. Hemorrhage into the airways is often evident as hemoptysis, even in horses. Respiratory distress can be caused by pneumothorax, hemothorax, or hemorrhage into airways. Treatment includes percutaneous aspiration of pleural air, administration of oxygen by insufflation or, in extreme instances, mechanical ventilation.

An alternative technique in cattle involves collection of lung tissue through the right cranioventral intercostal 2 space using a manual or automated 12- or 14-gauge biopsy needle.[69] Lung was successfully harvested from 56% of feedlot steers with chronic bovine respiratory disease and had the same pathologic diagnosis as that obtained by necropsy examination in 75% of animals. One animal of 34 had fatal complications.

RESPIRATORY SOUND SPECTRUM ANALYSIS

Analysis of respiratory sounds has utility in the diagnosis of disorders of the upper respiratory tract of horses. Respiratory sounds can be detected by a small microphone near the horse's nostril with the recording made by a tape recorder or similar device worn on the saddle or girth strap. Studies can be performed with horses running on either a treadmill or outside over ground.[70] Dorsal displacement of the soft palate produces broad-frequency expiratory noises with rapid periodicity (rattling), whereas dynamic unilateral collapse of the arytenoid causes an increase in inspiratory broad band high-frequency noise. The technique correctly identifies more than 90% of horses with dynamic collapse of the left arytenoid cartilage ("roarers").

EXERCISE TESTING

Exercise testing for assessment of respiratory tract function is essentially limited to horses, in which it is the gold standard for diagnosis of dynamic upper respiratory disease in horses.[71] Tests available for use on horses running on a treadmill include endoscopic examination of the upper airway, respiratory noise analysis, blood gas analysis, and measurement of respiratory mechanics. The most important of these in a clinical setting is videoendoscopy during exercise to detect dysfunction of the upper airway of horses. With the exception of recurrent laryngeal neuropathy, there is only poor correlation between results of endoscopic examination performed in standing horses compared with results of dynamic endoscopic examination during exercise.[6,71,72] Endoscopy of standing horses has very limited capacity to detect disorders that occur only during exercise, and some disorders of the upper respiratory tract, such as progressive weakness of the laryngeal abductor muscles, axial deviation of the aryepiglottic fold, and epiglottic retroversion, can only be diagnosed by endoscopic examination performed during strenuous exercise.[71,73] Another finding is the presence of multiple upper airway abnormalities in a high proportion of horses examined during exercise.[7,71,74,75]

Although testing has historically been conducted on a high-speed treadmill, a recent advance has been the development of endoscopes and recording systems that allow horses to be examined while exercising in the field (overground or dynamic endoscopy).[7,73-83] Such overground endoscopy systems comprise an endoscope, water pump, endoscope control unit (for manipulating the tip of the endoscope), and a recording/transmitting unit carried on the horse or the rider (Fig. 12-2) weighing up to 2.5 kg.[77,78] The source of light are light emitting diodes in the end of the endoscope. Preferred is a recording unit that simultaneously transmits the image in real time, usually using Bluetooth or similar technology, to an observer situated a short distance (up to 220 m depending on the unit) from the horse.[77] The development of overground endoscopy has enabled more widespread use of examination of the upper airway of horses during exercise and the refinement of understanding of dynamic obstructive disorders of the upper airway.

Overground endoscopy has the great advantage that it enables examination of the horse while it is performing wearing its usual tack, ridden by its usual rider, and performing its customary exercise.[83] Overground endoscopy performed on Standardbred racehorses does not appear to impair race time in qualifying races, and allows detection of important abnormalities associated with poor performance.[81] Examination using overground endoscopy allows the effect of gait and head position on upper airway function to be assessed and the relative importance of one or more abnormalities to be determined.[7,77,84] A disadvantage is that the intensity of exercise might not be as easily controlled by the veterinarian, or that exercise intensity that mimics that of actual competition in racehorses is not achieved, although exercise protocols to ensure the consistency of exercise are available.[83]

Principles of Treatment and Control of Respiratory Tract Disease

TREATMENT OF RESPIRATORY DISEASE

Treatment of diseases of the lower respiratory tract depends on the cause of the disease. However, the common principles are as follows:

Fig. 12-2 Horse wearing equipment for overground endoscopy, including endoscope and water pump and control, recording, and telemetry units. (Reproduced with permission van Erck 2011.[7])

- Ensure adequate oxygenation of blood and excretion of carbon dioxide.
- Relieve pulmonary inflammation.
- Effectively treat infectious causes of respiratory disease.
- Relieve bronchoconstriction.
- Provide supportive care to minimize demands for respiratory gas transport.

Respiratory Gas Transport

Cause of acute death in animals with respiratory disease is usually failure of transport of respiratory gases with subsequent hypoxemia and hypercapnia. Treatment of failure of oxygenation of blood and excretion of carbon dioxide can be achieved through administration of supplemental oxygen or mechanical ventilation. The reasons for failure of respiratory gas transport were discussed previously, and these should be considered when therapy of an animal with respiratory disease and hypoxemia with or without hypercarbia is planned. Animals with hypercarbia and hypoxemia are probably hypoventilating, and consideration should be given to increasing the animal's minute ventilation through relief of airway obstruction (e.g., by foreign bodies or bronchoconstriction), improvement in function of the respiratory muscles (restore hydration, maintain normal blood concentrations of electrolytes, including calcium), and positional adjustments (foals have better respiratory function when in sternal recumbency). Artificial ventilation should be considered, but it is impractical for long-term treatment in animals other than those housed in referral centers. Ventilation-perfusion abnormalities cause hypoxemia with normal to only slightly elevated P_aCO_2 in most affected animals. Oxygen therapy can be useful in ameliorating or attenuating the hypoxemia as a result of ventilation-perfusion abnormalities.

OXYGEN THERAPY

The principal treatment for hypoxemia caused by diseases of the lungs is the administration of oxygen. Oxygen therapy is not often used in large animals in field situations, but the use of a portable oxygen cylinder may find a place in tiding animals over a period of critical hypoxia until inflammatory lesions of the lungs subside. It has been used most often in valuable calves and foals. Oxygen therapy must be given continuously, requires constant or frequent attendance on the animal, and can be expensive. Supplemental oxygen is usually administered through a nasal cannula with the tip placed in the nasopharynx, through a mask, or through a cannula inserted percutaneously in the trachea. The use of an oxygen tent is impractical.

Oxygen therapy is useful only when hypoxemia is attributable to failure of oxygen transport in the respiratory system. It is of no value when the hypoxia is a result of toxins that interfere with oxygen metabolism in tissues (e.g., cyanide). Oxygen therapy will only minimally increase oxygen transport in animals with anemia, abnormal hemoglobin (methemoglobinemia), or cardiovascular shock. Cases of pneumonia, pleurisy, and edema and congestion of the lungs are most likely to benefit from provision of supplemental oxygen.

Oxygen should be delivered through a system that includes a humidifier so the insufflated gas is humidified and therefore drying of the respiratory mucosa is minimized.

Oxygen is often administered to **newborn animals**, either during resuscitation after birth or in those animals with respiratory disease. The value of supplemental oxygen in increasing P_aO_2 has been examined in foals, but the recommendations probably apply to newborns of other species as well. Both a face mask and nasopharyngeal tube are effective in increasing P_aO_2 when oxygen is administered at 10 L/min. The ability to elevate arterial oxygen increases with age from birth to 7 days of age because of the existence of right-to-left shunts in the newborn foal. Maximal changes in arterial oxygen tension occur within 2 minutes of the start of supplementation. In normal foals a flow rate of 4 L/min increases arterial oxygen tension, but responses in sick foals are often attenuated as a result of positional effects on gas exchange (recumbency) and other causes of hypoventilation.

Nasal insufflation improves arterial oxygen tensions and acid-base status in healthy foals[85] and in mild to moderately affected foals but might not be sufficient for oxygenation of foals with severe impairment of gas exchange. The efficacy of nasal insufflation of oxygen through intranasal catheters in foals depends on the rate of oxygen administration and whether catheters are inserted in one or both nostrils. When arterial oxygen tensions (P_aO_2) and inspired oxygen fraction (F_iO_2) are measured in arterial blood collected from the metatarsal artery and thoracic trachea, respectively, of healthy, standing, 5- to 7-day-old foals, insufflation results in significant increases in F_iO_2 and arterial oxygen tension (Table 12-5). Unilateral administration of oxygen at flow rate of 50 mL O_2 per kg body weight per minute resulted in an increase in F_iO_2 from 18% to 23% and in arterial oxygen tension from 93 to 136 mm Hg. Bilateral administration and increased rates of flow up to 200 mL/kg/min resulted in further increases.[85] It is important to note that this study was done in healthy, standing foals and that the effect of oxygen insufflation on P_aO_2 could be attenuated by recumbency and lung disease. However, F_iO_2 is unlikely to be affected by these variables and this information will allow calculation of the P_aO_2:F_iO_2 ratio in foals as a way of detecting lung injury. Intranasal catheters are also difficult to maintain in active sucking foals and require the use of higher oxygen flow rates to achieve beneficial effects. Flow rates in foals with lung injury should be adjusted based on repeated measurement of arterial oxygen tension with the aim of maintaining P_aO_2 at ≥ 100 mm Hg and S_aO_2 at greater than 90%.

A **transtracheal oxygen delivery system** has been used in foals with pneumonia and rapidly progressive dyspnea and hypoxemia despite intranasal oxygen therapy. A catheter is inserted into the midcervical trachea and directly distally in the tracheal lumen for approximately 25 cm. The catheter is attached to about 6 m of oxygen tubing and suspended above the foal, allowing it to move around the stall and suck the mare for up to 6 days without dislodging the catheter. This system was more effective than nasal insufflation in increasing arterial oxygen tension, probably because the catheter tip is in the distal trachea and bypasses a significant length of dead space that would not be oxygenated were the oxygen delivered into the nasopharynx.

In foals with neonatal respiratory distress, signs of respiratory failure may be evident at birth or several hours after birth. Tachypnea, shallow and paradoxical respiration, an expiratory grunt with accentuated abdominal effort, and cyanosis are all common. Management of foals with respiratory distress includes oxygen therapy, but when the distress is severe, oxygen insufflation alone is insufficient to improve the P_aO_2, which is usually 45 to 60 mm Hg (6.0-8.0 kPa). The atelectasis and alveolar hypoventilation worsen, resulting in progressive hypoxemia and respiratory acidosis, which requires ventilatory assistance by the use of continuous positive airway pressure.

In cattle and adult horses, the nasal tube must be inserted to the nasopharynx because passage short of this causes excessive waste of oxygen. The length of tube inserted should equal the distance from the nostril to a point one-third of the way from the lateral canthus of the eye to the base of the ear. Insertion of a nebulizer in the system permits the simultaneous administration of antibiotics and moisture to prevent drying of the pharyngeal mucosa. The volume of oxygen used should be about 10 to 20 mL of oxygen per min per kg of body weight. Repeated measurement of arterial oxygen tension, if available, is useful for determining the flow rate. Arterial oxygen tension responds to changes in the rate of administration of oxygen within several minutes.

Oxygen toxicity is a risk in animals breathing pure oxygen for periods exceeding 1 to 2 days, but this rarely occurs in veterinary medicine because supplementation with oxygen does not result in the animal breathing pure oxygen (except for animals under general anesthesia). Oxygen toxicosis

Table 12-5 Effects of unilateral or bilateral nasal insufflation of oxygen at flow rates of 50 mL O2 per kg bodyweight per minute to 5- to 7-day-old healthy foals on inspired oxygen tension (measured in the thoracic trachea), arterial oxygen tension and measures of acid : base balance. (Reproduced from Wong et al.2010.[85])

		Oxygen delivery							
		Unilateral (mL/kg/min)				Bilateral (mL/kg/min)			
Variable	*Baseline*	*50*	*100*	*150*	*200*	*50*	*100*	*150*	*200*
F_iO_2 (%)	18.0 ± 0.7[a]	23.0 ± 1.4[b]	30.9 ± 2.1[b]	44.2 ± 5.8[b,c,d]	52.6 ± 8.3[b,d,e]	30.9 ± 2.6[b]	48.7 ± 6.2[b,c]	56.4 ± 3.4[b,e]	74.6 ± 4.2[b]
pHa	7.435 ± 0.02[a]	7.415 ± 0.02	7.417 ± 0.01	7.418 ± 0.01	7.411 ± 0.02[b]	7.422 ± 0.01	7.412 ± 0.02	7.422 ± 0.02	7.426 ± 0.02
P_aO_2 (mm Hg)	92.5 ± 8.2[a]	135.9 ± 13.2[b]	175.2 ± 14.6[b]	219.6 ± 31.9[b,e,f]	269.7 ± 40.8[b,d,f]	174.3 ± 26.8[b]	261.2 ± 38.3[b,c,e]	307.8 ± 41.0[b,c,d]	374.2 ± 58.2[b]
P_aCO_2 (mm Hg)	47.7 ± 2.8[a]	49.7 ± 2.4	50.5 ± 2.3[b]	50.1 ± 2.8	51.3 ± 3.1[b]	49.8 ± 1.8	51.0 ± 2.2[b]	49.8 ± 2.9	48.6 ± 3.6
$P_{ET}CO_2$ (mm Hg)	53.9 ± 3.3	52.6 ± 4.9	52.8 ± 7.9	53.9 ± 7.9	54.6 ± 5.6	55.6 ± 2.8	55.3 ± 6.0	55.2 ± 5.1	55.3 ± 4.8
Bicarbonate (mmol/L)	31.4 ± 2.7	30.7 ± 1.3	31.4 ± 1.2	31.2 ± 1.3	31.4 ± 1.2	30.8 ± 1.9	31.5 ± 1.4	31.4 ± 2.0	30.8 ± 2.2
TCO_2 (mmol/L)	32.3 ± 2.7	32.0 ± 1.5	32.8 ± 1.3	32.6 ± 1.3	32.8 ± 1.3	32.2 ± 2.0	32.9 ± 1.4	32.8 ± 2.1	32.2 ± 2.3
S_aO_2 (%)	96.7 ± 0.7[a]	98.5 ± 0.3[b]	99.2 ± 0.1[b]	99.4 ± 0.2[b]	99.6 ± 0.1[b]	99.1 ± 0.3[b]	99.6 ± 0.1[b]	99.7 ± 0.1[b]	99.8 ± 0.1[b]
P_aO_2; F_iO_2 ratio	514 ± 39	594 ± 73[g,h]	569 ± 61	502 ± 75[g]	517 ± 58	563 ± 55	540 ± 73	547 ± 81	501 ± 57[h]

[a,b]Within a row, mean baseline value and values at individual oxygen flow rates that have different superscript letters differ significantly ($P < 0.05$). [c-f]Within a row, F_iO_2 or P_aO_2 at individual oxygen flow rates that have different superscript letters differ significantly ($P \leq 0.02$ and ≤ 0.03 respectively). [g,h]Within a row, mean ratio values at individual oxygen flow rates that have different superscript letters differ significantly ($P < 0.05$).

can be prevented by limiting the F_iO_2 to less than 60%.[85]

RESPIRATORY STIMULANTS

Use of respiratory stimulants, including doxapram, picrotoxin, leptazol (Metrazol), lobeline, theophylline, nikethamide (Coramine), caffeine, and amphetamine sulfate, has been advocated in animals with hypoxemia resulting from respiratory disease. In many of these animals, and especially in adults, there is already maximal stimulation of the respiratory center, and administration of drugs such as caffeine or doxapram is at best useless and at worst harmful, in that they can increase oxygen demand, in particular myocardial oxygen demand, thus exacerbating any oxygen deficit.

The situation appears to be different in neonates, in which the depression of respiration is a result of diminished central control, as is the case in foals with neonatal encephalopathy and in premature calves. Doxapram (constant rate infusion of 0.02-0.05 mg/kg/h IV) is more effective than caffeine (loading dose of 7.5-12 mg/kg followed by maintenance dose of 2.5-5 mg/kg PO q24 h) in reducing arterial carbon dioxide tension neonatal foals with respiratory acidosis ($P_aCO_2 \geq 55$ mm Hg and pH < 7.35) secondary to neonatal encephalopathy. There was no difference in survival rates although the number of animals (eight in each group) was likely too low to detect important effects on survival.[86] Similarly, in healthy newborn calves doxapram (40 mg IV) increased respiratory rate, peak inspiratory and expiratory flow rates, minute volume and P_aO_2, and reduced P_aCO_2 within minutes of administration, although the effect lasted less than 90 minutes.[87,88] Administration of doxapram (40 mg IV), atropine or caffeine to neonatal calves with naturally occurring asphyxia resulted in improvement in arterial blood gas values with all treatments with the greatest effect, and lowest death rate, among doxapram-treated calves.[89]

Doxapram appears to be useful in stimulating respiration in foals with pharmacologic depression of the respiratory center by general anesthetics.[90]

MECHANICAL VENTILATION

Short-term mechanical ventilation can be achieved in neonates and small adults by use of a nasotracheal tube and a hand-operated bellows, which is usually in the form of a resilient bag equipped with a one-way valve. The animal's trachea is intubated and the bag is connected and squeezed to supply a tidal volume of approximately 5 to 10 mL/kg BW at a rate of approximately 20 breaths per minute. Commercial bags (Ambubag) are available in a variety of sizes suitable for neonates and small ruminants. There is a simple device for respiratory resuscitation of newborn calves and lambs consisting of a mouthpiece, a nonreturn valve, a flange and an oral tube. Ventilation of larger animals requires use of compressed gases and appropriate valving systems, including a Hudson demand valve. In an emergency situation, artificial ventilation of neonates and small ruminants can be achieved by mouth-to-nose ventilation by the veterinarian. This should be done only with an awareness of the risks of disease transmission (e.g., a weak newborn calf could be infected by *Brucella* sp. or *Leptospira* sp.).

Prolonged mechanical ventilation is an activity requiring special equipment and expertise. It is indicated for the treatment of diseases of neonates, and perhaps adults, that cause hypoxemia and hypercarbia. There is usually a significant component of hypoventilation in these diseases and this is a prime indication for use of mechanical ventilation. An excellent example is the use of mechanical ventilation to treat foals with botulism. In experienced hands, this technique is effective. Because of the highly technical and demanding requirements for mechanical ventilation, the interested reader is referred to more detailed sources for descriptions of the methodology.

ANTI-INFLAMMATORY THERAPY

Many infectious and noninfectious diseases of the lower respiratory tract have inflammation as a major component of the tissue response to the initial insult. Primarily inflammatory diseases include heave and inflammatory airway disease of horses. Inflammation is an important component of pneumonia and some of the allergic or toxic lung diseases. Suppression of the inflammatory response is indicated when the inflammatory response is exacerbating clinical signs of the disease through obliteration of alveoli (inflammatory atelectasis), blockage of airways by inflammatory exudates and infiltration of bronchial walls, and bronchoconstriction as a consequence of inflammation increasing airway reactivity.

Administration of antiinflammatory drugs is indicated as the definitive therapy in noninfectious inflammatory airway diseases (with control achieved by environmental controls; see following discussion). Care must be taken that suppression of the inflammatory response does not impair innate and adaptive immune responses to infectious agents.

Antiinflammatory drugs used in the treatment of diseases of the respiratory tract include glucocorticoids and nonsteroidal antiinflammatory drugs (NSAIDs), with other agents such as leukotriene antagonists, interferon, and cromolyn sodium used in particular situations.

Nonsteroidal antiinflammatory drugs are useful in the treatment of infectious respiratory disease of cattle and horses, and likely other species. The drugs act by inhibiting the inflammatory response induced by the infecting organism and tissue necrosis. Meloxicam (0.5 mg/kg subcutaneously, once), when administered with tetracycline, improves weight gain and reduces the size of lesions in lungs of cattle with bovine respiratory disease complex over those of animals treated with tetracycline alone. NSAIDs also improve the clinical signs of cattle with respiratory disease. Use of these drugs is routine in horses with pneumonia or pleuritis.

Glucocorticoids are administered for control of inflammation in a variety of inflammatory lung diseases but notably heaves of horses and interstitial pneumonia of foals. Treatment can be administered orally, by intravenous or intramuscular injection, or by inhalation. Oral, intramuscular, or intravenous administration results in systemic effects of the agents. Inhalation of glucocorticoids provides therapy directed to the site of the disease and minimizes, but does not always prevent the systemic effects of the drugs. Drugs for inhalation are usually human preparations of fluticasone, beclomethasone, and flunisolide that are available as metered-dose inhalers. The compounds are administered through a mask adapted so that a large proportion of the drug is inhaled. Antiinflammatory responses in the airways are pronounced and result in marked improvement in respiratory function in horses with heaves (see Heaves, Recurrent airway obstruction).

IMMUNOMODULATORS

Interferon is used for the treatment of inflammatory airway disease in racehorses and feedlot cattle with respiratory disease. A dose of 50 to 150 IU of interferon-alpha administered orally once daily for 5 days reduced signs of airway inflammation in young Standardbred racehorses. Immune stimulation by injection of a suspension of *Propionibacterium acnes* has been investigated for treatment of chronic inflammatory airway disease in horses. The compound enhances expression of interferon-gamma and NK-lysin in peripheral blood mononuclear cells, increases the proportion of CD4 cells in peripheral blood and increases phagocytic activity of cells in peripheral blood. Similar changes were detected in bronchoalveolar lavage fluid. The effect on respiratory disease has yet to be definitively determined.

ANTIMICROBIAL THERAPY

Bacterial infections of the respiratory tract of all species are treated with antimicrobial agents given parenterally or, less commonly, orally. Individual treatment is usually necessary, and the duration of treatment will depend on the causative agent and the severity when treatment was begun. In outbreaks of infectious respiratory disease, the use of mass medication of the feed and water supplies may be advisable for the treatment of subacute cases and for convalescent therapy. The response to mass medication will depend on the total amount of the drug ingested by the animal and this is a reflection of the appetite or thirst of the animal, the palatability of the drug, and its concentration in the feed or water. The choice of drug used will depend on its cost, previous experience on similar cases, and the results of drug sensitivity tests if available. The individual treatment of all in-contact animals in an affected group may be useful in controlling an outbreak of respiratory disease such as shipping fever in feedlot cattle.

Selection of antimicrobials is based on the principles detailed in Chapter 6. Briefly, antimicrobials for treatment of bacterial respiratory disease should be active against the causative agent, should be able to achieve therapeutic concentrations in diseased lung, and should be convenient to administer. The antimicrobials should be affordable and, if used in animals intended as human food, must be approved for use in such animals.

Antimicrobials for treatment of lung disease are preferably those that achieve therapeutic concentrations in diseased lung tissue after administration of conventional doses. This has been convincingly demonstrated for the macrolide (azithromycin, erythromycin, clarithromycin), triamilide (tulathromycin), and fluoroquinolone (danofloxacin, enrofloxacin) antimicrobials, and fluorfenicol in a variety of species. The beta-lactam antimicrobials (penicillin, ceftiofur) are effective in treatment of pneumonia in horses, pigs, and ruminants despite having chemical properties that do not favor their accumulation in lung tissue.

Routes of administration include oral (either individually or in medicated feed or water), parenteral (subcutaneous, intramuscular, intravenous), or by inhalation. Intratracheal administration of antimicrobials to animals with respiratory disease is not an effective means of achieving therapeutic drug concentrations in diseased tissue. **Aerosolization and inhalation** of antimicrobials has the theoretic advantage of targeting therapy to the lungs and minimizing systemic exposure to the drug. However, although administration by inhalation achieves good concentrations of drug in bronchial lining fluid,[91-93] it does not penetrate unventilated regions of the lungs, in which case parenteral or oral administration of antimicrobials is indicated. Gentamicin, marbofloxacin, ceftiofur, and defquinome all achieve high concentrations in pulmonary epithelial lining fluid when administered to horses.[91-94] Aerosol administration of gentamicin to normal horses results in gentamicin concentrations in bronchial lavage fluid 12 times that achieved after intravenous administration. Aerosolized ceftiofur sodium (1 mg/kg) is superior to intramuscular administration in treatment of calves with *Pasteurella (Mannheimia) haemolytica.*

BRONCHODILATOR DRUGS

Bronchoconstriction is an important component of the increased airway resistance present in many animals with disease of the lower respiratory tract. Administration of bronchodilators can relieve respiratory distress and improve arterial blood oxygenation. Bronchodilatory drugs are beta-2-agonists (clenbuterol, albuterol/salbutamol, terbutaline), parasympatholytic drugs (ipratropium, atropine), and methylxanthines (aminophylline, theophylline).

The **indication** for the use of bronchodilators is relief of bronchoconstriction. Bronchoconstriction is an important component of the pathophysiology of many diseases of the lungs and airways. Bronchodilators are used extensively in horses with heaves and inflammatory airway disease and less so in animals with infectious diseases. **Contraindications** are few, but caution should be exercised when using these drugs in animals that are severely hypoxemic because the beta-2-agonists can transiently worsen gas exchange by increasing perfusion of nonventilated sections of the lung, and in pregnant animals, in which the tocolytic effect of the beta-2-agonists can delay parturition. The use of beta-2-adrenergic agonist bronchodilator drugs in food animals is not permitted in most countries because of the risk of contamination of foodstuffs intended for consumption by people. This is particularly the case with clenbuterol, a drug approved in many countries for use in horses that is administered to cattle illicitly as a growth promoter. People can be poisoned by clenbuterol in tissues of treated cattle.

The **beta-2-adrenergic agonists** are potent and effective bronchodilators that can be administered orally, intravenously, or by inhalation. These drugs also enhance mucociliary clearance of material from the lungs. Most administration is oral or by inhalation. Use of these drugs is restricted to horses, and the drugs are discussed in the section on heaves.

Parasympatholytic (anticholinergic) drugs relieve vagally mediated bronchoconstriction. Again, their use is restricted to horses. These drugs can cause tachycardia and gastrointestinal dysfunction, including ileus.

The **methylxanthines** are used in horses and have been investigated for use in cattle with respiratory disease. Their use in horses is mainly of historical interest because the availability of the more efficacious beta-2-adrenergic agonists and parasympatholytic drugs has superseded the use of methylxanthines. The use of theophylline in feedlot cattle with respiratory disease in field conditions is associated with accumulation of toxic concentrations in blood and an excessive mortality rate.

MUCOLYTICS, MUCOKINETIC, AND ANTITUSSIVE DRUGS

Many groups of drugs are used in the therapy of respiratory diseases with the objective of improving **mucokinesis** or **effective mucociliary clearance**. Mucokinetic agents have been divided into six groups according to their mode of action:

- Diluents, surface acting agents, and mucolytics are supposed to reduce the viscosity of the respiratory secretions.
- Bronchomucotropic agents, formerly called expectorants, are supposed to increase the production of a less viscous mucus.
- Other agents, such as beta-adrenergic agonists and methylxanthine derivatives, promote more effective clearance of mucus and act as ciliary augmentors or bronchodilators.

The aim of mucokinetic agents is to decrease the viscosity of the respiratory secretions, but in some animals with respiratory disease the excessive secretions are of low viscosity and the use of a mucolytic agent in such cases would further decrease mucokinesis. There is little or no evidence that administration of mucolytic or mucokinetic agents, with the possible exception of clenbuterol and dembrexine, relieves signs of respiratory disease or hastens recovery.

Inflammation of the lower respiratory tract results in production of mucus and immigration of inflammatory cells. This accumulation of material is cleared by rostral movement into the pharynx, where it is discharged through the nostrils or swallowed. Clearance is by the mucociliary apparatus or coughing. **Mucolytics** are agents that alter the constituents of mucoid or purulent respiratory secretions and make them less viscous. Bromhexine is a popular mucolytic with horse owners. It is said to reduce the viscosity of airway mucus and increase mucus production, although its clinical efficacy has not been determined. It may be of some value in cattle to increase mucociliary clearance. Dembrexine alters the carbohydrate side chains of mucin and improves its flow properties and is reported to decrease coughing and hasten recovery in horses with respiratory disease.

Hyperhydration, the administration of large quantities of fluids intravenously, has been suggested as being useful in the treatment of horses with accumulation of excessive amounts of mucus or mucopus in the lower airways. However, experimental trials have demonstrated that this approach is not effective in horses with heaves.

Bronchomucotropic agents (expectorants) are administered with the intention of augmenting the volume of respiratory secretions by stimulating the mucus-producing cells and glands. Formerly called expectorants, they are supposed to increase the production of a less viscous mucus. These compounds include the iodides and ammonium and glycerol guaiacolate, which are commonly found in cough mixtures. These are commonly used in farm animals, especially horses, although their efficacy is unknown.

Coughing is a common sign in animals with respiratory disease, and it is an important pulmonary defense mechanism, allowing the expulsion of mucus and foreign bodies. **Antitussive (cough suppressant)** drugs are infrequently used in large-animal medicine. These drugs should only be used when definitive therapy has been implemented for the underlying disease. Control of the underlying disease will in almost all instances resolve the coughing. It is not appropriate to use antitussive agents (butorphanol, codeine, diphenhydramine) to suppress a cough when the underlying cause is unknown or untreated.

SURFACTANT

Surfactant is critical to normal alveolar function, and a lack of this complex phospholipid results in progressive alveolar collapse. Composition of surfactant from lungs of neonatal foals differs from that of adult horses, with that from foals having a lower protein concentration and higher surface tension.[95] Lack of surfactant is an important cause of respiratory disease in newborn animals, with those born prematurely being at increased risk. Attempts have been made to prevent acute respiratory disease in premature newborn foals, such as those delivered by caesarian section because of maternal disease, but the results have been disappointing.

SURGERY

Many conditions of the upper respiratory tract of horses are amenable to surgical correction. Tracheostomy is often used in the emergency or urgent relief of acute upper airway obstruction and in the removal of large amounts of tracheal debris, such as occurs in animals with smoke inhalation. Drainage of excessive or infected pleural fluid can be therapeutic in animals with pleuritis.

GENERAL NURSING CARE

Animals with respiratory disease should have minimal or no enforced activity, and environmental stressors should be minimized. One of the most important aspects of the treatment of respiratory tract disease in farm animals is the provision of a comfortable, well-ventilated environment during and after the disease episode. Affected animals should be placed in a draft-free area that is adequately ventilated and supplied with an abundance of bedding for comfort and warmth, particularly during convalescence. Feed and water should be readily available and dusty feeds avoided.

CONTROL OF RESPIRATORY DISEASE

Infectious diseases of the respiratory tract of farm animals are caused by a combination of infectious agents and predisposing causes such as inclement weather, the stress of weaning or transportation, and poorly ventilated housing, each of which can weaken the defense mechanisms of the animal. Prevention and control of these diseases include the following tactics:

- Minimizing exposure to inciting agents (infectious or physical)
- Maximizing innate resistance by ensuring that the animals are in excellent general health through attention to nutrition, housing, and animal welfare
- Maximizing adaptive resistance by the administration of effective vaccines such that maximal resistance is produced to coincide with the time of greatest risk of the disease

IMPORTANCE OF DIAGNOSIS

For some complex respiratory diseases of food animals, it is becoming increasingly more difficult to obtain a definitive etiologic diagnosis because some of the common diseases appear to be caused by multiple infections rather than a single one. Most of the infective agents that cause respiratory disease are ubiquitous in the environment and are present as normal residents in the nasal cavities of normal animals. This often creates difficulty with the interpretation of the microbiological findings in outbreaks of respiratory disease because the infectious agents can commonly be isolated from both sick and well animals. Thus there may be no well-defined cause-and-effect relationship, and the predisposing causes begin to assume major importance in any control program.

MANAGEMENT TECHNIQUES

Most of the common respiratory diseases occur at certain times under certain

conditions, and successful control will depend on the use of management techniques before the disease is likely to occur. For example, in beef cattle, pneumonic pasteurellosis can be kept to a minimum with the use of certain management procedures that minimize stress at weaning. The incidence of pneumonia can be minimized in young bulls destined for a performance testing station if they are weaned well in advance of movement to the test center. In North America, bovine respiratory disease is most common in feedlots where young cattle from several different backgrounds have been mingled after having been transported long distances. Outbreaks of equine respiratory disease occur in young horses that are assembled at the racetrack for training or at horse shows.

HOUSING FACILITIES

The quality of air in housing facilities is a critical determinant of the respiratory health of most species, including humans who work in these facilities.[96] Poor air quality, such as high particulate concentrations, persistently high humidity, bacterial and fungal growth, and excessive concentrations of ammonia, predispose to infectious and noninfectious diseases in animals housed in the barns.[96-99]

The incidence of pulmonary inflammation, excessive mucus in airways and coughing (heaves) in horses is much higher in those that are housed in barns that are dusty and not ventilated compared with horses kept outdoors.[96,100,101] Bad stabling management as a major cause of coughing in horses was described almost 200 years ago, but there is still a major emphasis on the clinical management of chronic coughing in housed horses using a wide spectrum of antibiotics, expectorants, and other drugs. Attention to barn design such that concentration of small particulates in the air is minimized will improve the respiratory health of horses housed in the barn.[101,102]

In pigs, enzootic pneumonia is widespread, but the effects of the pneumonia can be maintained at an insignificant level with adequate housing, ventilation, and nutrition. Too much emphasis has been placed on the attempted eradication of *Mycoplasma* spp., which is extremely difficult, and insufficient emphasis on building design and ventilation methods.

VACCINES

Vaccines are available for the immunization of farm animals against some of the common infectious diseases of the respiratory tract. Their advantages and disadvantages are discussed under each specific disease. The general principles underlying use of vaccines for control of respiratory disease are as follows:

- The disease must be caused by a disease that is infectious.
- There must be an effective vaccine suitable for use in the species and age group of animals at most risk of the disease. Ideally, this will be known from published, appropriately designed trials testing the vaccine in a group of animals identical to those in which the vaccine will be used in practice.
- The vaccine must be administered to animals in such a manner (route, timing, frequency) to optimize the immunization (adaptive immunity).
- The timing of the vaccination program should be such that maximal resistance to the anticipated diseases is achieved at the time of greatest risk of the disease.
- Vaccination should be part of an ongoing program of disease control and should not be regarded as a panacea with which to rectify other shortcomings in management of the animals.

ENVIRONMENTAL CONTROL

In effect, the principles of control and prevention of airborne respiratory disease are based largely on keeping the levels of pathogens in the air at a low level. This can be accomplished by a combination of the following practices:

- The use of filtered-air positive-pressure ventilation systems
- The removal of affected animals from the group
- Increasing the ventilation rate of the building unit
- Subdivision of the unit into small units, each with its own ventilation system
- A continual disinfection system where appropriate and practicable
- The provision of supplemental heat so that during cold weather the ventilation can be maintained and animals will not huddle together to keep warm and thereby increase the exposure rate of infection
- The use of vaccines for specific diseases of the respiratory tract
- Effective dust control

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Diseases of the Upper Respiratory Tract

RHINITIS

Rhinitis (inflammation of the nasal mucosa) is characterized clinically by sneezing, wheezing, and stertor during inspiration and a nasal discharge that can be serous, mucoid, or purulent in consistency depending on the cause.

ETIOLOGY

Rhinitis usually occurs in conjunction with inflammation of other parts of the respiratory tract. It is present as a minor lesion in most bacterial and viral pneumonias, but the diseases listed are those in which it occurs as an obvious and important part of the syndrome.

Cattle

- Catarrhal rhinitis in infectious bovine rhinotracheitis; adenoviruses 1, 2, and 3; and respiratory syncytial virus infections
- Ulcerative/erosive rhinitis in bovine malignant catarrh, mucosal disease, rinderpest
- *Actinobacillus lignieresii* can cause outbreaks of respiratory disease in adult cattle characterized by stertorous breathing, nasal discharge, and excessive salivation. Lesions included thickening and ulceration of the nasal planum, turbinates, and paranasal sinuses. Treatment with oxytetracycline was associated with resolution of the disease.[1]
- Nasal schistosomiasis
- Nasal mycosis
- Infection by *Pseudalleschia boydii* species complex[2]
- Nasal actinomycosis
- Rhinosporidiosis caused by fungi and atopic rhinitis
- Familial allergic rhinitis and allergic nasal granuloma[3]
- Bovine nasal eosinophilic granuloma attributable to *Nocardia* sp.

Horses

- Glanders, strangles, and epizootic lymphangitis
- Infections with the viruses of equine viral rhinopneumonitis (herpesvirus-1), equine herpesvirus-3,[4] equine viral arteritis, influenza H3N8 equine rhinovirus, parainfluenza virus, reovirus, adenovirus
- Chronic rhinitis claimed to be caused by dust in dusty stables, and acute rhinitis occurring after inhalation of smoke and fumes
- Nasal granulomas as a result of chronic infections with *Pseudoallescheria boydii* and *Aspergillus, Conidiobolus,* and *Mucoraceous* fungi
- Equine grass sickness (dysautonomia) in the chronic form causes rhinitis sicca[5]

Sheep and Goats

- Melioidosis, bluetongue; rarely, contagious ecthyma and sheep pox
- *Oestrus ovis* and *Elaeophora schneideri* infestations
- Allergic rhinitis
- Purulent rhinitis and otitis associated with *P. aeruginosa* in sheep showered with contaminated wash
- Infection by *Conidiobolus* spp. and *Pythium* spp.[6-8]
- Nasal polyps in sheep[9]

Pigs

- Atrophic rhinitis, inclusion-body rhinitis, swine influenza, some outbreaks of Aujeszky's disease

PATHOGENESIS

Rhinitis is of minor importance as a disease process except in severe cases when it causes obstruction of the passage of air through the nasal cavities. Its major importance is as an indication of the presence of some specific diseases. The type of lesion produced is important. The erosive and ulcerative lesions of rinderpest, bovine malignant catarrh, and mucosal disease; the ulcerative lesions of glanders, melioidosis, and epizootic lymphangitis; and the granular rhinitis of the anterior nares in allergic rhinitis all have diagnostic significance.

In atrophic rhinitis of pigs, the destruction of the turbinate bones and distortion of the face appear to be a form of devitalization and atrophy of bone caused by a primary inflammatory rhinitis. Secondary bacterial invasion of facial tissue of swine appears to be the basis of necrotic rhinitis.

CLINICAL FINDINGS

The primary clinical finding in rhinitis is a nasal discharge, which is usually serous initially but soon becomes mucoid and, in bacterial infections, purulent. Erythema, erosion, or ulceration may be visible on inspection. The inflammation may be unilateral or bilateral. Sneezing is characteristic in the early acute stages, and this is followed in the later stages by snorting and the expulsion of large amounts of mucopurulent discharge. A chronic unilateral purulent nasal discharge lasting several weeks or months in horses suggests nasal granulomas associated with mycotic infections.

"Summer Snuffles"

"Summer snuffles" of cattle presents a characteristic syndrome involving several animals in a herd. Cases occur in the spring and autumn when the pasture is in flower and warm, moist environmental conditions prevail. The disease may be most common in Channel Island breeds. There is a sudden onset of dyspnea with a profuse nasal discharge of thick, orange to yellow material that varies from a mucopurulent to caseous consistency. Sneezing, irritation, and obstruction are severe. The irritation may cause the animal to shake its head, rub its nose along the ground, or poke its muzzle repeatedly into hedges and bushes. Sticks and twigs may be pushed up into the nostrils as a result and cause laceration and bleeding. Stertorous, difficult respiration accompanied by mouth breathing may be evident when both nostrils are obstructed. In the most severe cases, a distinct pseudomembrane is formed that is later snorted out as a complete nasal cast. In the chronic stages, multiple proliferative nonerosive nodules 2 to 8 mm in diameter and 4 mm high with marked mucosal edema are visible in the anterior nares.

Familial Allergic Rhinitis

In familial allergic rhinitis in cattle, the clinical signs begin in the spring and last until late fall. Affected animals exhibit episodes of violent sneezing and extreme pruritus manifested by rubbing their nostrils on the ground, trees, and other inanimate objects and frequently scratching the nares with their hindfeet. Dyspnea and loud snoring sounds are common, and affected animals frequently clean their nostrils with their tongues. The external nares contain a thick mucoid discharge, and the nasal mucosa is

edematous and hyperemic. The clinical abnormalities resolve during the winter months. All affected animals are positive to intradermal skin testing for a wide variety of allergens.

Mycotic Rhinitis

Mycotic rhinitis in the horse is characterized by noisy respirations, circumferential narrowing of both nasal passages, and thickening of the nasal septum. The nasal conchae and turbinates may be roughened and edematous, and the ventral meati decreased in size bilaterally. The nasal discharge may be unilateral or bilateral. Endoscopically, granulomas may be found in almost any location in the nasal cavities and extending to the soft palate and into the maxillary sinus. The disease is discussed in detail in this chapter.

Endoscopic Examination

Endoscopic examination is useful for the visual inspection of lesions affecting the nasal mucosae of horses and cattle that are not visible externally. Radiographic or computed tomographic imaging can be used to detect atrophic rhinitis, although use of these techniques on a wide scale is clearly not practical.

CLINICAL PATHOLOGY

Examination of nasal swabs of scrapings for bacteria, inclusion bodies, or fungi may aid in diagnosis. Discharges in allergic rhinitis usually contain many more eosinophils than normal. Nasal mucosal biopsy specimens are useful for microbiological and histopathologic examination.

NECROPSY FINDINGS

Rhinitis is not a fatal condition, although animals may die of specific diseases in which rhinitis is a prominent lesion.

DIFFERENTIAL DIAGNOSIS

Rhinitis is readily recognizable clinically. Differentiation of the specific diseases listed previously under "Etiology," is discussed under their respective headings.

Allergic rhinitis in cattle must be differentiated from maduromycosis, rhinosporidiosis, and infection with the pasture mite (*Tyrophagus palmarum*). The differential diagnosis may be difficult if allergic rhinitis occurs secondary to some of these infections.

Rhinitis in the horse must be differentiated from inflammation of the facial sinuses or guttural pouches in which the nasal discharge is usually purulent and persistent and often unilateral, and there is an absence of signs of nasal irritation. A malodorous nasal discharge, frontal bone distortion, draining tracts at the poll, and neurologic abnormalities are common in cattle with chronic frontal sinusitis as a complication of dehorning.

TREATMENT

Specific treatment aimed at control of individual causative agents is described under each disease. Thick tenacious exudate that is causing nasal obstruction may be removed gently and the nasal cavities irrigated with saline. A nasal decongestant sprayed up into the nostrils may provide some relief. Newborn piglets with inclusion-body rhinitis may be affected with severe inspiratory dyspnea and mouth breathing that interferes with sucking. The removal of the exudate from each nostril followed by irrigation with a mixture of saline and antimicrobials will provide relief and minimize the development of a secondary bacterial rhinitis. Animals affected with allergic rhinitis should be taken off the pasture for about a week and treated with antihistamine preparations.

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NASAL DISCHARGE

Nasal obstruction occurs commonly in cattle and sheep. The disease is usually chronic and occurs as a result of the following:

- In sheep, infestation with *Oestrus ovis*
- In cattle, most often enzootic nasal granuloma, acute obstruction or the allergic condition "summer snuffles." Cystic enlargement of the ventral nasal conchae in cattle can cause unilateral or bilateral nasal obstruction.

Minor occurrences include the following:

- Large mucus-filled polyps developing in the posterior nares of cattle and sheep and causing unilateral or bilateral obstruction
- Granulomatous lesions caused by a fungus, *Rhinosporidium* sp., and by the blood fluke, *Schistosoma nasalis*
- A chronic pyogranuloma as a result of *Coccidioides immitis* infection has occurred in the horse.
- Foreign bodies may enter the cavities when cattle rub their muzzles in bushes in an attempt to relieve the irritation of acute allergic rhinitis.
- Nasal amyloidosis occurs rarely in mature horses and is characterized clinically by stertorous breathing and raised, firm, nonpainful, nodular swellings on the rostral nasal septum and floor of the nasal cavity. Affected horses do not have any other illness, and surgical removal of the lesions is recommended
- Infestation of the nasopharynx of horses by *Gasterophilus pecorum* causes obstruction of the upper airway.

Neoplasms

Neoplasms of the olfactory mucosa are not common but do occur, particularly in sheep, goats, and cattle, where the incidence in individual flocks and herds may be sufficiently high to suggest an infectious cause. The lesions are usually situated just in front of the ethmoid bone, are usually unilateral but may be bilateral, and have the appearance of adenocarcinomas of moderate malignancy. In cattle, the disease is commonest in 6- to 9-year-olds and may be sufficiently extensive to cause bulging of the facial bones. The tumors are adenocarcinomas arising from the ethmoidal mucosa, and they metastasize in lungs and lymph nodes. Clinical signs include nasal discharge, often bloody; mouth breathing; and assumption of a stretched-neck posture. There is evidence to suggest that a virus may be associated. A similar syndrome is observed in cattle with other nasal tumors such as osteoma.

Neoplasia that obstructs the nasal cavity occurs in horses with squamous-cell carcinoma or adenocarcinoma of the sinus or nasal cavity or ethmoids,[11] angiosarcoma, and a variety of other rare tumors. Epidermal inclusions cysts of the nasal diverticulum of horses can cause obstruction of the nasal cavity, but are not neoplasms. Cysts of the paranasal sinuses can cause marked facial deformity and obstruction to air passages.

Enzootic Nasal Adenocarcinoma

Enzootic nasal adenocarcinoma is a contagious disease that occurs in sheep and goats, in which it is associated with a virus—enzootic nasal tumor virus.[1] The putative etiologic agent is a beta-retrovirus, with different strains occurring in sheep and goats. The disease can be reproduced by inoculation of 14-day-old lambs with the virus.[2] The clinical findings include a persistent serous, mucous, or mucopurulent nasal discharge and stridor.[3] Affected sheep and goats progressively develop anorexia, dyspnea, and mouth breathing, and most die within 90 days after the onset of signs. The tumors originate unilaterally or occasionally bilaterally in the olfactory mucosa of the ethmoid turbinates.[3] They are locally invasive but not metastatic. Histologically, the tumors are classified as adenomas or, more frequently, adenocarcinomas. Budding and extracellular retrovirus-like particles have been observed ultrastructurally in enzootic nasal tumors of goats. A reverse-transcription polymerase chain reaction (RT-PCR) can detect the virus in healthy and affected sheep, although its clinical utility in allowing control of the infection has not been determined.[4]

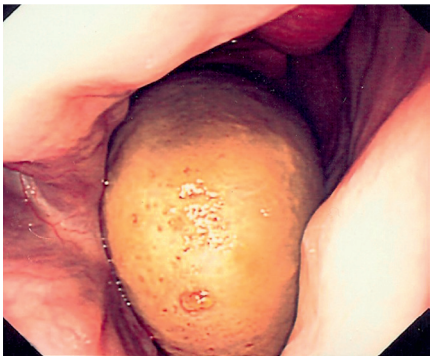

Fig. 12-3 Endoscopic view of a progressive ethmoidal hematoma in a horse. (Reproduced with permission.[12])

Progressive Ethmoidal Hematomas in Equids

Ethmoidal hematomas are non-neoplastic tumors that are encapsulated, usually expanding, insidious, potentially distorting and obstructing lesions of the nasal cavities that occur in horses.[5,6] The etiology is unknown but a viral etiology (papilloma virus) should be considered. Chronic unilateral nasal discharge is common, and lesions are usually advanced at the time of diagnosis. There is stertorous breathing and upper airway obstruction in later stages of the disease. The nasal discharge is serous or mucoid and intermittently sanguineous, sanguinopurulent, and usually unrelated to exercise. The tumor arises in the ethmoids and can invade paranasal sinuses, and especially the sphenopalatine sinus, and is bilateral in approximately 50% of horses.[7] Diagnosis is made by endoscopy and radiography (Figs. 12-3 and 12-4). Computed tomographic examination yields information additional that obtained by radiographic examination (Fig. 12-5) and is useful in determining treatment modality and approach and prognosis. Magnetic resonance imaging (MRI) yields similar information to that obtained by computed tomography (CT) examination.[8]

Surgical removal is possible and successful in some cases. Surgical removal is challenging and often associated with clinically important hemorrhage and the need for intraoperative or postoperative blood transfusion.[9] Surgical removal of the tumor can be achieved in some horses during a standing procedure, but the long-term success has yet to be determined.[10]

Multiple intralesional injection of formalin (1-100 mL of 10% neutral buffered formalin injected at 10-day intervals) through an endoscope can cure the tumor, but there is the risk of serious adverse effects if the ethmoidal hematoma penetrates the cribriform plate. The procedure involves the injection of a sufficient volume of 10% neutral buffered formalin to distend the lesion. The formalin is injected via an endoscope once every 10 days until the lesion resolves by sloughing. Between 1 and 20 injections will be required. The combination of intralesional injection of formalin and laser ablation resulted in severe neurologic complications in one horse with ethmoidal adenocarcinoma treated in this way.[11] Similar adverse effects can occur with use of these treatments in horses with ethmoidal hematomas.

However, the prognosis for long-term resolution of the tumor is poor because of high rates of recurrence.

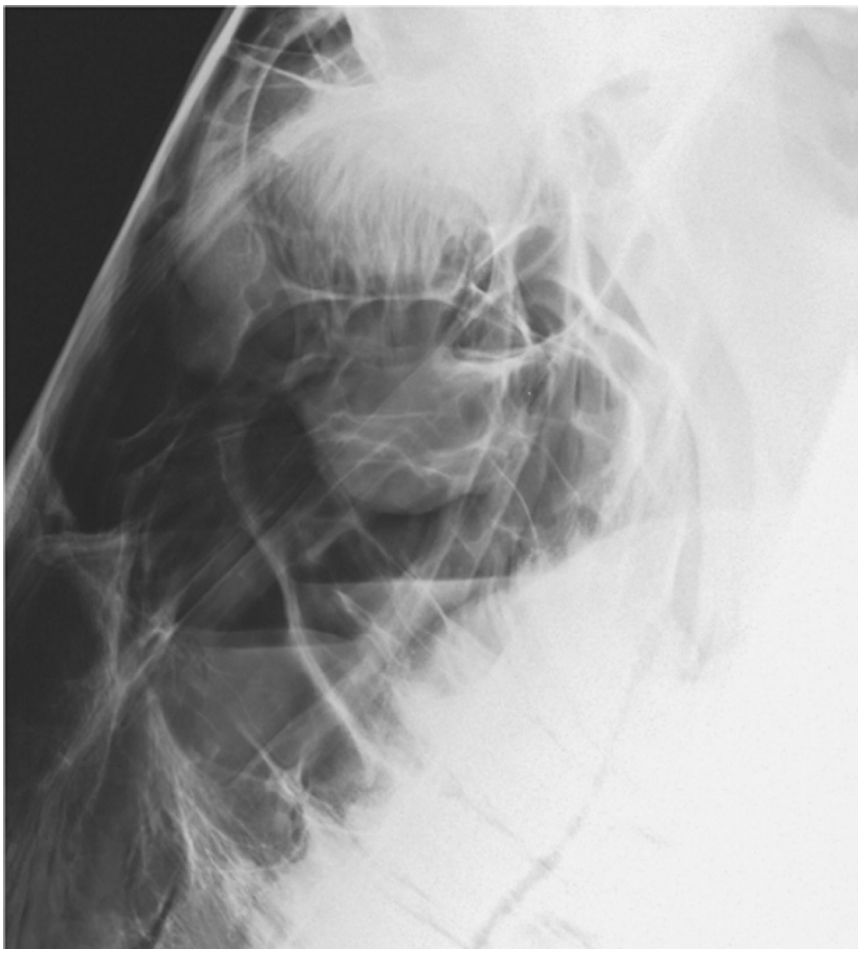

Fig. 12-4 Lateral radiograph of the head of a horse with progressive ethmoidal hematoma (black arrow) and related hemorrhage into the paranasal sinuses (white lines). (Reproduced with permission.[12])

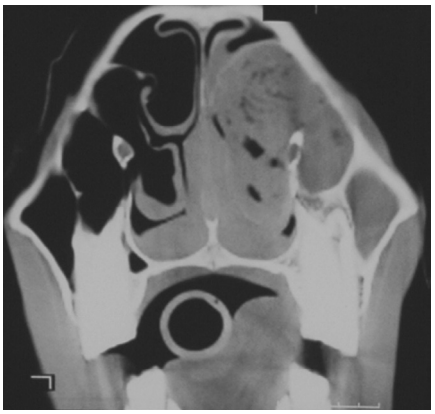

Fig. 12-5 Computed tomographic image of the head of a horse with advanced ethmoidal hematoma revealing extension into the paranasal sinuses (white arrow). (Reproduced with permission.[12])

CLINICAL FINDINGS

In cattle, sheep, and pigs there is severe inspiratory dyspnea when both cavities are blocked. The animals may show great distress and anxiety and breathe in gasps through the mouth. Obstruction is usually not complete and a loud, wheezing sound occurs with each inspiration. A nasal discharge is usually present but varies from a small amount of blood-stained serous discharge when there is a foreign body present to large quantities of purulent exudate in allergic rhinitis. Shaking of the head and snorting are also common signs. If the obstruction is unilateral, the distress is not so marked, and the difference in breath streams between the two nostrils can be detected by holding the hands in front of the nose. The magnitude of the air currents from each nostril on expiration can be assessed with the aid of a piece of cotton thread (watching the degree of deflection). The passage of a

stomach tube through each nasal cavity may reveal evidence of a space-occupying lesion. Endoscopic examination is frequently diagnostic.

TREATMENT

Treatment must be directed at the primary cause of the obstruction. Removal of foreign bodies can usually be effected with the aid of long forceps, although strong traction is often necessary when the obstructions have been in position for a few days.

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EPISTAXIS AND HEMOPTYSIS

Epistaxis is bleeding from the nostrils regardless of the origin of the hemorrhage, and hemoptysis is the coughing up of blood, with the hemorrhage usually originating in the lungs. Both epistaxis and hemoptysis are important clinical signs in cattle and horses. The bleeding can be in the form of a small volume of blood-stained serous discharge coming from one or both nares, or it can be a large volume of whole blood coming from both nostrils and sometimes the mouth. Pulmonary hemorrhage as a cause of epistaxis or hemoptysis is dealt with under that heading ("Pulmonary Hemorrhage" section in this chapter). The first and most important decision is to determine the anatomic location of the lesion causing bleeding.

ETIOLOGY

Epistaxis occurs commonly in the horse and may be caused by lesions in the nasal cavity, nasopharynx, auditory tube diverticulum (guttural pouch), or lungs (see Table 12-12 in the section on exercise-induced pulmonary hemorrhage in horses). Epistaxis in horses can also be a result of trauma, progressive ethmoidal hematoma, foreign bodies lodged in the respiratory tract, hemorrhagic diatheses, sinusitis, nasal amyloidosis, polyps, and bleeding from the nasolacrimal duct.[1] **Exercise-induced pulmonary hemorrhage** is described under that heading later in this chapter.

Hemorrhagic lesions of the nasal cavity, nasopharynx, and guttural pouch in the horse usually cause unilateral epistaxis of varying degree depending on the severity of the lesions. **Pulmonary lesions** in the horse resulting in hemorrhage into the lumen of the bronchi also result in epistaxis. Blood originating from the lungs of the horse is discharged most commonly from the nostrils and not the mouth because of the anatomy of the horse's soft palate.

Bleeding from lesions of the upper respiratory tract of horses usually occurs spontaneously while the horse is at rest. One of the commonest causes of unilateral epistaxis in the horse is guttural pouch mycosis with erosion of the internal carotid artery.[2]

Other less common causes of nasal bleeding include hemorrhagic polyps of the mucosa of the nasal cavity or paranasal sinuses, and ethmoidal hematoma (see "Progressive ethmoidal hematoma" in the section on Nasal discharge in this chapter.). Another cause, most uncommonly, is a parasitic arteritis of the internal carotid artery as it courses around the guttural pouch. Pseudoaneurysm of the palatine artery causes unilateral epistaxis.[3]

Mild epistaxis is a common finding in horses and cattle with severe thrombocytopenia.[4]

Erosions of the nasal mucosa in glanders, granulomatous disease such as cryptococcal sinusitis,[5] neoplastic diseases, and trauma as a result of passage of a nasal tube or endoscope, or from physical trauma externally, are other obvious causes. Trauma to the head and skull fractures can result in epistaxis.[6] Rupture of the longus capitus muscle and fracture of the basisphenoid bone in horses that rear and fall backward and strike the poll causes epistaxis, among other signs.[7]

A case of fibrous dysplasia in the ventral meatus of a horse with epistaxis is recorded. Congestive heart failure and purpura hemorrhagica can cause mild epistaxis in horses.

Neoplasia, and notably hemangiosarcoma, of the upper or lower respiratory tract can cause epistaxis. Osteoma of the nasal bones causes nasal obstruction and epistaxis in cattle.[8,9]

Envenomation of horses by rattlesnakes in the western United States causes a clinical syndrome that includes swelling of the head, dyspnea, and epistaxis.

Poisoning by bracken fern or moldy sweet clover is a common cause of spontaneous epistaxis in cattle.[10] The epistaxis can be bilateral, and hemorrhages of other visible and subcutaneous mucous membranes are common. An enzootic ethmoidal tumor has been described in cattle in Brazil and was at one time a disease of some importance in Sweden. The lesion occupies the nasal cavities, causes epistaxis, and can invade paranasal sinuses.

In hemoptysis in horses, the blood flows along the horizontal trachea and pools in the larynx until the swallowing reflex is stimulated and swallowing occurs; or coughing is stimulated and blood is expelled through the mouth and nostrils. The origin of the hemorrhage is usually in the lungs, and in cattle the usual cause is a pulmonary arterial aneurysm and thromboembolism from a posterior vena caval thrombosis (see "Caudal Vena Cava Syndrome"). Recurrent attacks of hemoptysis with anemia and abnormal lung sounds usually culminate in an acute intrapulmonary hemorrhage and rapid death.

The origin of the hemorrhage in epistaxis and hemoptysis may be obvious, as in traumatic injury to the turbinates during passage of a stomach tube intranasally or if a systemic disease with bleeding defects is present. In many other cases, however, the origin of the hemorrhage is not obvious, and special examination procedures may be required. Careful auscultation of the lungs for evidence of abnormal lung sounds associated with pulmonary diseases is necessary.

CLINICAL EXAMINATION

The nasal cavities should be examined visually with the aid of a strong, pointed source of light through the external nares. Only the first part of the nasal cavities can be examined directly but an assessment of the integrity of the nasal mucosa can usually be made. In epistaxis resulting from systemic disease or clotting defects, the blood on the nasal mucosa will usually not be clotted. When there has been recent traumatic injury to the nasal mucosa or erosion of a blood vessel by a space-occupying lesion such as tumor or nasal polyp, the blood will usually be found in clots in the external nares.

The nasal cavities should then be examined for any evidence of obstruction as set out in the previous section. When the blood originates from a pharyngeal lesion there are frequent swallowing movements and a short explosive cough, which may be accompanied by the expulsion of blood from the mouth. Hematologic examinations are indicated to assist in the diagnosis of systemic disease or clotting defects. Radiologic examinations of the head are indicated when space-occupying lesions are suspected.

Use of the flexible fiberoptic endoscope will permit a thorough examination of the nasal cavities, nasopharynx, guttural pouch and larynx, trachea, and major bronchi.

TREATMENT

Specific treatment of epistaxis and hemoptysis depends on the cause. Hemorrhage from traumatic injuries to the nasal mucosa does not usually require any specific treatment. Space-occupying lesions of the nasal mucosa might warrant surgical therapy. Epistaxis associated with guttural pouch mycosis usually requires surgical intervention. There is no successful treatment for the hemoptysis attributable to pulmonary aneurysm and posterior vena caval thrombosis in cattle. General supportive therapy is as for any spontaneous hemorrhage and includes rest, blood transfusions, and hematinics.

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PHARYNGITIS

Pharyngitis in all species is associated with infectious diseases of the upper airway. It is most studied in horses, probably because of the frequency of examination of the upper airway in this species. Pharyngitis in horses has many similarities to tonsillitis in children. The disorder in horses involves follicular lymphoid hyperplasia of the pharynx affecting both the pharyngeal tonsil and the extensive and diffuse lymphoid tissue in the walls and dorsal aspect of the pharynx. These tissues form the mucosal associated lymphoid tissues and are an important component of the normal immunologic response of horses. The condition occurs in a high proportion of Thoroughbred racehorses and is probably as common in other breeds of horse.[1] The condition is first detectable in 2- to 3-month-old foals and reaches its highest prevalence and greatest severity in yearlings and 2-year-old horses in race training. It is evident on endoscopic examination as diffuse, multiple, small, white nodules in the roof and walls of the pharynx. The nodules can be confluent, and there is often excessive mucus present in severely affected horses. The clinical significance of the condition is debated.[1-3] Affected racehorses do not have impaired race performance. Affected horses recover spontaneously as they age or after treatment with topical antiinflammatory drugs. The condition is probably a normal aging process and necessary for development of a competent immune system in young horses.

Infestation of the nasopharynx of horses by larvae of the bot fly *Gasterophilus pecorum* causes obstruction of the upper airway and a parasitic pharyngitis. Diagnosis is by visualization of the parasite during endoscopic examination.

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LARYNGITIS, TRACHEITIS, BRONCHITIS

Inflammation of the air passages usually involves all levels, and no attempt is made here to differentiate between inflammations of various parts of the tract. They are all characterized by one or more of cough, noisy inspiration, and some degree of inspiratory embarrassment.

ETIOLOGY

All infections of the upper respiratory tract cause inflammation, either acutely or as chronic diseases. In most diseases the laryngitis, tracheitis, and bronchitis form only a part of the syndrome, and the causes listed here are those diseases in which upper respiratory infection is a prominent feature.

Cattle

- Infectious bovine rhinotracheitis (bovine herpesvirus-1), calf diphtheria (necrotic laryngitis), *Histophilus somnus*
- Tracheal stenosis in feedlot cattle, "honker cattle," etiology unknown
- Necrotic laryngitis in calves[1]
- *Syngamus laryngeus* infests the larynx of cattle in the tropics
- Trauma, including balling gun–induced injury[2]

Sheep

- Chronic infection with *Actinomyces pyogenes*

Horses

- Equine herpesvirus 1, 2 or 5 (EVR), equine viral arteritis (EVA), equine viral influenza (EVI), strangles (*S. equi*)
- Idiopathic ulceration of the mucosa covering the arytenoid cartilages
- Lymphoid hyperplasia of the pharynx of horses—the disease is more common in younger horses and might be associated with reduced athletic capacity or increased propensity to palatal instability.[3,4]
- Bronchitis and tracheitis of horses, most evident in athletic horses, and characterized by accumulation of mucus in the trachea and increased proportion of neutrophils in tracheal or bronchoalveolar lavage fluid—the condition is associated with impaired athletic performance.[5-7]

Pigs

- Swine influenza
- Necrotic tracheitis (akin to "honker syndrome" in cattle) of uncertain etiology.[8]

PATHOGENESIS

Irritation of the mucosa causes frequent coughing, and swelling causes partial obstruction of the air passages, with resulting inspiratory dyspnea. Necrotic laryngitis in calves is associated with marked changes in pulmonary function, modifies tracheal dynamics, and disturbs the growth process by increasing the energetic cost of breathing; this can result in impaired feed intake and predisposition to secondary pulmonary infection and subsequent respiratory failure from progressive exhaustion.

CLINICAL FINDINGS

Coughing and inspiratory dyspnea with laryngeal roaring or stridor are the common clinical signs. In the early stages of acute infections, the cough is usually dry and nonproductive and is easily induced by grasping the trachea or larynx, or by exposure to cold air or dusty atmospheres. In acute laryngitis, the soft tissues around the larynx are usually enlarged and painful on palpation. In chronic infections, the cough may be less frequent and distressing and is usually dry and harsh. If the lesions cause much exudation or ulceration of the mucosa, as in bacterial tracheobronchitis secondary to infectious bovine rhinotracheitis in cattle, the cough is moist, and thick mucus, flecks of blood, and fibrin may be coughed up. The cough is very painful, and the animal makes attempts to suppress it. Fever and toxemia are common, and affected animals cannot eat or drink normally.

Inspiratory dyspnea varies with the degree of obstruction and is usually accompanied by a loud stridor and harsh breath sounds on each inspiration. These are best heard over the trachea, although they are quite audible over the base of the lung, being most distinct on inspiration. The respiratory movements are usually deeper than normal and the inspiratory phase more prolonged and forceful. Additional signs, indicative of the presence of a primary specific disease, may also be present.

Examination of the larynx is usually possible through the oral cavity using a cylindrical speculum of appropriate size and a bright, pointed source of light. This is done relatively easily in cattle, sheep, and pigs but is difficult in the horse. Lesions of the mucosae of the arytenoid cartilages and the vault of the larynx are usually visible if care and time are taken. In laryngitis, there is usually an excessive quantity of mucus, which may contain flecks of blood or pus in the pharynx. Palpation of the pharyngeal and laryngeal areas may reveal lesions not readily visible through a speculum. During opening of the larynx, lesions in the upper part of the trachea are sometimes visible. The use of a fiberoptic endoscope allows a detailed examination of the upper respiratory tract.

Inflammation or lesions of the larynx may be severe enough to cause marked inspiratory dyspnea and death from asphyxia. In calves and young cattle with diphtheria, the lesion may be large enough (or have a pedicle and act like a valve) to cause severe inspiratory dyspnea, cyanosis, anxiety, and rapid death. The excitement associated with loading for transportation to a clinic or of a clinical examination, particularly the oral examination of the larynx, can exaggerate

the dyspnea and necessitate an emergency tracheotomy.

Most cases of bacterial laryngitis will heal without obvious residual sign after several days of antimicrobial therapy. Some cases in cattle become chronic in spite of therapy as a result of the inflammation extending down into the arytenoid cartilages resulting in a chronic chondritis caused by a sequestrum similar to osteomyelitis. Abscess formation is another common cause of chronicity. Secondary bacterial infection of primary viral diseases, or extension of bacterial infections to the lungs, commonly results in pneumonia.

Tracheal stenosis in cattle is characterized by extensive edema and hemorrhage of the dorsal wall of the trachea, resulting in coughing (honking), dyspnea, and respiratory stertor. Complete occlusion of the trachea may occur. Affected animals may be found dead without any premonitory signs.

CLINICAL PATHOLOGY

Laboratory examinations may be of value in determining the presence of specific diseases.

NECROPSY FINDINGS

Upper respiratory infections are not usually fatal, but lesions vary from acute catarrhal inflammation to chronic granulomatous lesions depending on the duration and severity of the infection. When secondary bacterial invasion occurs, a diphtheritic pseudomembrane may be present and be accompanied by an accumulation of exudate and necrotic material at the tracheal bifurcation and in the dependent bronchi.

DIFFERENTIAL DIAGNOSIS

Inflammation of the larynx usually results in coughing and inspiratory dyspnea with a stertor and loud abnormal laryngeal sounds on auscultation over the trachea and over the base of the lungs on inspiration. Lesions of the larynx are usually visible by laryngoscopic examination; those of the trachea and major bronchi are not so obvious unless special endoscopic procedures are used. Every reasonable effort should be used to inspect the larynx and trachea. Obstruction of the nasal cavities and other parts of the upper respiratory tract may also be difficult to distinguish unless other signs are present.

TREATMENT

Most of the common viral infections of larynx, trachea, and major bronchi will resolve spontaneously if the affected animals are **rested**, not worked, and not exposed to inclement weather and dusty feeds. Secondary bacterial complications must be recognized and treated with the appropriate antimicrobial.

The bacterial infections can result in severe inflammation with necrosis and granulomatous lesions and must be treated with **antimicrobials**. Calves with calf diphtheria should be treated with a broad-spectrum antimicrobial daily for 3 to 5 days. Several days are usually required for the animal to return to normal. A broad-spectrum antimicrobial daily or more often for up to 3 weeks or more may be necessary for treatment of the chondritis.

NSAIDs such as flunixin meglumide may be used in an attempt to reduce the laryngeal edema associated with some severe cases of bacterial laryngitis in cattle.

Animals with severe lesions and marked inspiratory dyspnea may require a **tracheotomy** and insertion of a tracheotomy tube for several days until the lesion heals.[1,9] The tube must be removed, cleaned out, and replaced at least once daily because of the accumulation of dried mucus plugs, which interferes with respiration. The techniques of tracheotomy and permanent tracheostomy in the horse have been described. Surgical excision of chronic granulomatous lesions and abscesses of the larynx may be indicated following failure of long-term antimicrobial therapy, but postoperative complications of laryngeal and pharyngeal paralysis may occur. Laryngotomy as a treatment for chronic laryngeal obstruction in cattle with long-term survival of 58% has been described.

Tracheolaryngostomy of calves with chronic laryngeal obstruction as a result of necrobacillosis has been used with success.[1] Under general anesthesia and dorsal recumbency, an incision is made over the lower third of the thyroid and cricoid cartilages and the first two tracheal rings. The larynx is easily visualized and necrotic tissue removed using a curette. The edges of the cartilages are sutured closed. A wedge-shaped piece of the first two tracheal rings is removed to create a tracheostomy, which is allowed to close after about 1 week when the postoperative swelling has subsided with the aid of daily care of the surgical site and the possible use of flunixin meglumide. No tracheotomy tube is required.

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TRAUMATIC LARYNGOTRACHEITIS, TRACHEAL COMPRESSION, AND TRACHEAL COLLAPSE

Traumatic laryngotracheal injury can occur following endotracheal intubation used for general anesthesia. Nasotracheal intubation can result in mucosal injury to the nasal meatus, the arytenoid cartilages, the trachea, the dorsal pharyngeal recess, the vocal cords, and the entrance to the guttural pouches.[1] The laryngeal injury is attributed to the tube pressure on the arytenoid cartilages and vocal folds, and the tracheal damage is attributable to the pressure exerted by the inflated cuff on the tracheal mucosa.

Tracheal obstruction can be intramural, a result of extramural compression, or a result of tracheal collapse. Intramural obstruction of the trachea can be caused by space-occupying lesions such as foreign bodies, neoplastic lesions (e.g., granular cell tumors in horses—see "Neoplastic Diseases of the Respiratory Tract" at the end of this chapter), infections (granulomatous tracheitis, "honking" syndrome in pigs—see following discussion), trauma, or hemorrhage. Extramural compression can be caused by intrathoracic or extrathoracic lesions, including abscesses and granulomatous lesions, cranial mediastinal masses (abscess, neoplasia), or trauma.

Tracheal collapse occurs in calves, in mature cattle, in goats, and in horses, including Miniature horses[2] and foals. Dynamic collapse is a cause of exercise intolerance in racehorses that is evident only by endoscopic examination of the trachea during strenuous exercise. Restriction of the tracheal lumen and laxity of the dorsal tracheal membrane results in varying degrees of inspiratory dyspnea with stridor, coughing, and reduced exercise tolerance. Tracheal collapse in **American Miniature horses** is not uncommon.[2] The clinical signs of respiratory distress, tachypnea, inspiratory honking noises, and increased respiratory effort occur in adult horses (mean age 11, range 2-15 years) and are exacerbated by exercise, pregnancy, and eating. None of the affected horses in one case series had a history of trauma. Confirmation of the diagnosis is by endoscopic tracheobronchial examination or radiography (Fig. 12-6). The severity of tracheal collapse is graded: Grade 1 = minor protrusion of the dorsal tracheal membrane into the lumen with less than 25% reduction in airway diameter; Grade 2 = mild elongation and flattening of the tracheal rings with 50% reduction in airway diameter; Grade 3 = marked flattening of the tracheal rings and lengthening of the dorsal tracheal membrane with 75% reduction in airway diameter; and Grade 4 = severe flattening of tracheal rings with dorsal elevation of the ventral tracheal surface and an airway diameter less than 10% of normal.[2] The case-fatality rate is high (~80%). Tracheotomy for emergency treatment of severe disease is not useful unless the collapsed trachea is dilated by an endotracheal tube. Necropsy examination of four affected horses revealed chondromalacia. The cause of the condition is not known. Tracheal prostheses have been used for the treatment of tracheal collapse in calves and Miniature horses,[3-5] although given the

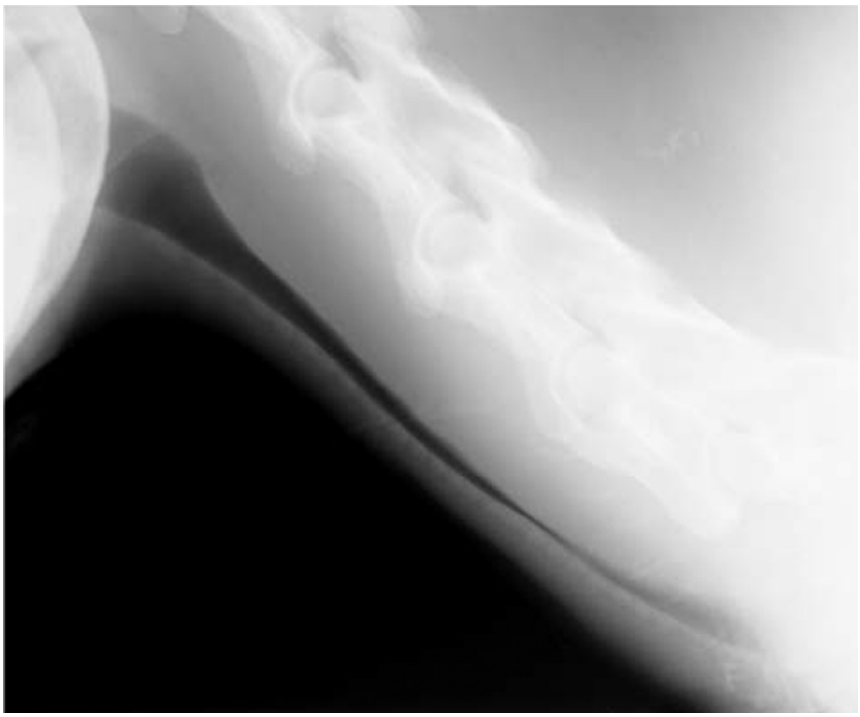

Fig. 12-6 Lateral radiograph of the cervical region of an American Miniature horse revealing extensive tracheal collapse. (Reproduced with permission.[2])

extent of collapse and high rate of complications associated with surgical repair, there is not an ideal method for alleviating the condition.[6]

A "honking" respiratory noise is common in affected calves when coughing spontaneously or when the trachea is palpated. Tracheal collapse in calves is associated with injuries associated with dystocia and clinical signs usually occur within a few weeks after birth. In some cases the trachea is compressed at the level of the thoracic inlet in association with callus formation of healing fractured ribs attributed to dystocia. In some cases in cattle, there is no history of dystocia or preexisting disease or previous manipulation of the trachea, and the overall lumen size may be reduced to less than 25% of normal. Auscultation of the thorax may reveal loud referred upper airway sounds. A similar syndrome is described in pigs with development of acute tracheal edema, hemorrhage, and tracheitis. Clinical signs are of sudden onset of respiratory distress, open-mouth breathing, loud inspiratory stridor, and death soon after clinical signs became apparent.[7] The lesion is edema, hemorrhage, and mixed cellular infiltration in the tunica adventitia, with lung lesions of mild to severe fibrinonecrotic or hemorrhagic pleuropneumonia. No infectious etiology has been identified.[7]

Tracheal obstruction and collapse can result from tracheitis associated with pneumonia in the horse, tracheal neoplasia, tracheal stricture, presence of foreign bodies in the trachea, and compression by masses external to the trachea. It is suggested that increased respiratory effort associated with pneumonia causes collapse of the soft tissue structures of the trachea, rather than collapse of the tracheal rings. Tracheal rupture as a result of blunt trauma in the horse may result in severe subcutaneous emphysema and pneumomediastinum. Conservative therapy is usually successful. Tracheal compression secondary to enlargement of the cranial mediastinal lymph nodes can also cause inspiratory dyspnea, and conservative treatment with antimicrobials is successful.

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Diseases of the Lung Parenchyma

PULMONARY CONGESTION AND EDEMA

Pulmonary congestion is caused by an increase in the amount of blood in the lungs as a result of engorgement of the pulmonary vascular bed. It is sometimes accompanied by pulmonary edema, and the temporal relationship between the two can be difficult to determine. The various stages of the vascular disturbance are characterized by respiratory compromise, the degree depending on the extent of interference with pulmonary gas exchange as a result of diffusion impairment across the alveolus, ventilation/perfusion mismatch in the lungs, and decreases in pulmonary blood flow.

ETIOLOGY

Pulmonary congestion and edema is a common terminal event in many diseases but is frequently overshadowed by other disturbances. Congestion that is clinically apparent can be primary when the basic lesion is in the lungs or secondary when it is in some other organ, most commonly the heart.

Pulmonary edema occurs because of imbalances in the Starling forces across the pulmonary capillary. From a clinical perspective, the common proximate causes of pulmonary edema are injury to the endothelium of the pulmonary capillary with subsequent leakage of protein-rich fluid into the interstitial spaces, elevated blood pressure in the alveolar capillaries, or, less commonly, low plasma oncotic pressure.

Damage to pulmonary vascular endothelium can occur in infectious diseases (e.g., African horse sickness) or intoxications (endotoxemia). Physical injury, including inhalation of excessively hot air or smoke, can damage the alveolar epithelium with secondary damage to capillary endothelium.[1] Elevated pulmonary capillary pressure occurs in left-sided heart failure (ruptured chordae tendineae of the mitral valve or congenital cardiac anomalies[2]) and during strenuous exercise by horses. Air embolism causes acute heart failure and pulmonary edema in horses.[3] Low plasma oncotic pressure occurs in diseases causing hypoproteinemia but is rarely a cause for pulmonary edema by itself, although it contributes to the pulmonary edema in hypoproteinemic animals administered large volumes of fluids intravenously.

Primary Pulmonary Congestion

- Early stages of most cases of pneumonia
- Inhalation of smoke and fumes
- Anaphylactic reactions
- Recumbency, especially in large animals
- Yew (*Taxus* sp.) intoxication
- Racehorses with acute severe exercise-induced pulmonary hemorrhage[4]

Secondary Pulmonary Congestion

- Congestive heart failure (cardiogenic pulmonary edema), including ruptured chordae tendineae of the mitral valve, ionophore toxicosis,[5] and left-sided heart failure

Pulmonary Edema

Pulmonary edema as a sequela to pulmonary capillary hypertension or pulmonary

microvascular damage occurs in the following situations:

- Acute anaphylaxis
- Acute pneumonia—*Pasteurella haemolytica* produces several virulence factors that induce direct or leukocyte-mediated pulmonary endothelial cell injury. Pulmonary edema is one of a number of abnormalities in cattle infected by *Theilieria annulata*.[6]
- Gram-negative sepsis in ruminants and pigs
- Congestive heart failure and acute heart failure (e.g., the myocardial form of enzootic muscular dystrophy in inherited myocardiopathy of Hereford calves); ruptured mitral valve or chordae tendonae
- Inhalation of smoke or manure gas
- Transient upper airway obstruction in the horse (negative-pressure pulmonary edema)[7]
- After general anesthesia in horses[8]
- Yew (*Taxus* sp.) intoxication
- Exercise-induced pulmonary edema in racehorses
- Fumonisin intoxication in pigs[9]
- Specific diseases, including: mulberry heart disease of swine; East Coast fever in cattle; the pulmonary form of African horse sickness; Hendra virus infection of horses; poisoning with organophosphates, alpha-naphthyl thiourea (ANTU), ionophore antibiotics (monensin, salinomycin), or 1080 (sodium fluoracetate);[10] plant poisonings by oleander, *Hymenoxis* spp., and *Phenosciadium* spp.
- Doxycycline intoxication of calves
- *Clostridium perfringens* type D epsilon toxin in calves and sheep
- The Barker syndrome in young pigs
- Semen embolism

PATHOGENESIS

In **pulmonary congestion**, ventilation is reduced, and oxygenation of the blood is impaired. Oxygenation is reduced by the decreased rate of blood flow through the pulmonary vascular bed. Hypoxemic anoxia develops and is the cause of most of the clinical signs that appear.

Hypoxemia occurs in **pulmonary edema** because of ventilation-perfusion abnormalities, diffusion abnormalities (although this is usually a minor contributor to the hypoxemia), and hypoventilation caused by the physical obstruction of airflow by fluid and foam in the airways. The edema is caused by damage to the capillary walls by toxins or anoxia or by transudation of fluid as a result of increased hydrostatic pressure in the capillaries. Filling of the alveoli, and in severe cases the bronchi, effectively prevents gaseous exchange.

Smoke inhalation in horses results in decreased oxygen content of inspired air and exposure of the respiratory tract tissues to various noxious gases. Following smoke inhalation, diffuse tracheobronchial mucosal sloughing occurs, which, if progressive, causes separation of the epithelium and development of pseudomembranous casts, which may cause partial or complete airway obstruction. Pulmonary edema is also extensive.

CLINICAL FINDINGS

All degrees of severity of pulmonary congestion and edema occur commonly in farm animals, and only the most severe form is described here. The depth of respiration is increased to the point of extreme dyspnea with the head extended, the nostrils flared, and mouth breathing. Breathing movements are greatly exaggerated and can be best described as heaving; there is marked abdominal and thoracic movement during inspiration and expiration. A typical stance is usually adopted, with the front legs spread wide apart, the elbows abducted, and the head hung low. The respiratory rate is usually increased, especially if there is hyperthermia, which occurs in acute anaphylaxis and after violent exercise and in the early stages of pneumonia. The heart rate is usually elevated (up to 100/min), and the nasal mucosa is bright red or cyanotic in terminal cases. **Radiography** reveals diffuse pulmonary opacity in animals with pulmonary edema.

In **acute pulmonary congestion**, there are harsh breath sounds, but no crackles are present on auscultation.

When **pulmonary edema** develops, loud breath sounds and crackles are audible over the ventral aspects of the lungs. In long-standing cases, there may be emphysema with crackles and wheezes of the dorsal parts of the lungs, especially if the lesion is caused by anaphylaxis.

Coughing is usually present, but the cough is soft and moist and is not painful. A slight to moderate serous nasal discharge occurs in the early stage of congestion, but in **severe pulmonary edema** this increases to a voluminous, frothy nasal discharge, which is often pink-colored as a result of blood.

The primary importance of pulmonary congestion is as an indicator of early pathologic changes in the lung or heart. Spontaneous recovery occurs quickly unless there is damage to alveolar epithelium or myocardial asthenia develops. Severe pulmonary edema has much greater significance and usually indicates a stage of irreversibility. Death in cases of pulmonary edema is accompanied by respiratory failure.

Smoke inhalation in horses is characterized by the following:[1]

- Polypnea and dyspnea
- Diffuse wheezes throughout the lungs
- Coughing
- A bronchointerstitial pattern radiographically
- The horse might expectorate large proteinaceous tracheobronchial casts.

The prognosis is good if affected animals can survive the initial stages of pulmonary damage and secondary organ involvement.

CLINICAL PATHOLOGY

Laboratory examinations are of value only in differentiating the causes of the congestion or edema. Bacteriologic examination of nasal swabs and a complete hematologic examination, looking particularly for the presence of eosinophilia, are the standard examinations that are carried out.

NECROPSY FINDINGS

In acute pulmonary congestion the lungs are dark red in color. Excessive quantities of venous blood exude from the cut surface. Similar but less marked changes occur in milder forms of congestion but are only seen in those animals that die of intercurrent disease. Histologically, the pulmonary capillaries are markedly engorged and some transudation and hemorrhage into alveoli is evident.

Macroscopic findings in pulmonary edema include swelling and loss of elasticity of the lungs, which pit on pressure. They are usually paler than normal. Excessive quantities of serous fluid exude from the cut surface of the lung. Histologically, there are accumulations of fluid in the alveoli and parenchyma.

DIFFERENTIAL DIAGNOSIS

The diagnosis of pulmonary congestion and edema is always difficult unless there is a history of a precipitating cause, such as an infectious disease, strenuous exercise, ingestion of toxicants, or inhalation of smoke or fumes. Pneumonia usually presents itself as an alternative diagnosis and a decision cannot be based entirely on the presence or absence of pyrexia. The best indication is usually the presence of toxemia, but this is not entirely dependable. Bacterial pneumonia is usually accompanied by some toxemia, but cases of viral pneumonia are often free of it. Response to antibacterial treatment is one of the best indications, the only variable being the tendency for congestion and edema of allergic origin to recover spontaneously. In many instances there will be doubt, and it is then advisable to treat the animal for both conditions.

TREATMENT

The principles of treatment of pulmonary congestion and edema are one or more of the following: reduction of pulmonary capillary pressure (by reduction either of pulmonary venous or pulmonary arterial pressure), alleviation of pulmonary microvascular damage,

and correction of low plasma oncotic pressure. The treatment of pulmonary congestion and edema must first be directed at correction of the primary cause as listed under etiology. Affected animals should be confined at rest in a clean, dry environment and exercise avoided.

Pulmonary capillary pressure can be reduced in animals with left-sided heart failure by reduction of cardiac preload, improvement in cardiac pump function, or a combination of these factors. These topics are dealt with in detail in Chapter 12. Briefly, preload can be reduced by administration of furosemide and pump function improved by administration of drugs that improve myocardial function (digoxin) or decrease afterload (arterial vasodilators). The usual first step is the administration of furosemide (1-2 mg/kg intravenously).

Alleviation of pulmonary microvascular damage is more difficult. Administration of antiinflammatory drugs, including NSAIDs or glucocorticoids, is indicated in animals in which microvascular damage is suspected. These drugs are used to treat, among other diseases, smoke inhalation of horses.

Plasma oncotic pressure can be increased by intravenous infusion of plasma (10-40 mL/kg) or synthetic colloids such as hetastarch. Administration of crystalloid solutions should be judicious and the amount of fluid administered must be monitored carefully to ensure that only sufficient fluids to meet the needs of the animal are given.

Oxygen should be administered to hypoxemic animals in conjunction with other specific treatments.

Special Diseases

When edema is attributable to **organophosphate poisoning,** prompt administration of atropine may reduce fluid transudation. In these cases, the animal is in considerable danger, and repeated injections may be necessary. Details of the recommended treatment regimen are given in the section on treatment of poisoning by organophosphorus compounds.

Epinephrine is recommended in **pulmonary edema resulting from anaphylaxis.** It will have an immediate pharmacologic effect, which may be followed by the use of a corticosteroid to maintain vascular integrity and to decrease permeability of pulmonary vessels. Antihistamines are commonly used in conjunction with epinephrine for the treatment of acute pulmonary edema resulting from anaphylaxis. However, recent studies of experimental anaphylaxis in cattle and horses have shown that the antihistamines may be of limited value because histamine and serotonin are of relatively limited significance as mediating substances. On the other hand, the kinins, prostaglandins, and slow-release substances may be more important.

Studies in cattle have found that antihistamines and 5-hydroxytryptamine (5-HT) antagonists failed to protect cattle in experimental hypersensitivity. Sodium meclofenamate has been more successful in antagonizing experimental anaphylaxis in cattle and horses. Acetylsalicylic acid was more effective than antihistamines or antiserotonin agents in providing symptomatic relief in experimental acute interstitial pneumonia of calves.

It is difficult, however, to extrapolate the results of these studies in which the drugs were usually given before or at the same time as the experimental disease was produced. There is a need for development of more effective antianaphylactic drugs for the treatment of acute anaphylaxis in farm animals, which invariably results in pulmonary edema and emphysema. Thus epinephrine is the drug of choice for the emergency treatment of pulmonary edema resulting from anaphylaxis.

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PULMONARY HYPERTENSION

Pulmonary hypertension is an increase in pulmonary arterial pressure above values in healthy animals. It is usually a result of structural or functional changes in the pulmonary vasculature and can result in heart failure. Primary pulmonary hypertension occurs in cattle with high-altitude disease (Brisket disease, see Chapter 10).[1,2] Chronic pulmonary hypertension results in right-sided congestive heart failure caused by right ventricular hypertrophy or **cor pulmonale**.[3]

Causes

Hypoxemia is a potent stimulus of pulmonary arterial pressure through increased pulmonary vascular resistance induced by pulmonary vasoconstriction. Pulmonary artery pressure can also increase in response to increases in cardiac output that are not matched by pulmonary vasodilation—the most extreme example of this being the large increase in pulmonary artery pressure of strenuously exercising horses. Alveolar hypoxia causes constriction of the precapillary pulmonary vessels, resulting in pulmonary hypertension. Conditions that can induce hypoxia include the following:

- Exposure to high altitude
- Respiratory impairment secondary to thoracic wall abnormalities
- Airway obstruction
- Pneumonia, including granulomatous pneumonia[4]
- Pulmonary edema
- Emphysema
- Pulmonary vascular disease
- Heaves (recurrent airway obstruction of horses)[5-9]

At high altitudes, the low inspired oxygen tension causes hypoxic pulmonary vasoconstriction and hypertension that are common causes of cor pulmonale (brisket disease) in cattle. Susceptible cattle can be identified by measurement of pulmonary artery pressure before clinical disease develops. This test is used to select bulls for use in high-altitude pastures. Cattle grazing pastures that contain locoweed have an increased incidence of brisket disease but the pathogenesis is unknown. Although uncommon, right-sided congestive heart failure and pulmonary hypertension can occur in cows at low altitudes with primary lung disease.

Pulmonary hypertension is a component of heaves in horses and results in abnormalities in myocardial function, which can progress to overt heart failure.[6,9] Progression to heart failure is uncommon, but should be considered in horses with severe or prolonged heaves. Similarly, heart function should be considered in horses with longstanding pneumonia.[4,8]

Pulmonary hypertension occurs in neonates and is a consequence of persistent fetal circulation. This is particularly a problem of cloned calves (see "Diseases of Cloned Offspring," Chapter 19).

An outbreak of pulmonary hypertension in a group of dairy calves 5 to 6 months of age has been described. Some affected calves died suddenly. Clinical findings included lethargy, anorexia, pale mucous membranes, tachypnea, tachycardia, weakness, engorged jugular veins, and loss of body condition. Right-sided cardiac catheterization revealed pulmonary hypertension. Necropsy findings revealed evidence of right-sided congestive heart failure and periarteritis and fibrosis of the pulmonary and bronchial arteries. Lesions were characterized by variable stages of vasculitis; the airways were free of pathologic changes. Ingestion of monocrotaline, a pyrrolizidine alkaloid, can cause similar pulmonary vascular lesions in rats, but no evidence of such ingestion was found in affected calves.

Pulmonary hypertension occurs secondary to left-sided heart disease in horses, although the hypertension has been mistakenly identified as the primary lesion.

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ATELECTASIS

Atelectasis is collapse of the alveoli as a result of failure of the alveoli to inflate or because of compression of the alveoli. Atelectasis is therefore classified as obstruction (resorption), compression, or contraction. **Obstruction atelectasis** occurs secondary to obstruction of the airways, with subsequent resorption of alveolar gases and collapse of the alveoli. This disease is usually caused by obstruction of small bronchioles by fluid and exudate. It is common in animals with pneumonia or aspiration of a foreign body. **Compression atelectasis** occurs when intrathoracic (intrapleural) pressure exceeds alveolar pressure, thereby deflating alveoli. This occurs when there is excessive pleural fluid or the animal has a pneumothorax. It also occurs in the dependent lung or portions of lung in recumbent animals and is evident on computed tomographic or radiographic examination of the lungs of foals (Fig. 12-7).[1,2] Compression atelectasis is the explanation for the large shunt fraction and hypoxemia that occurs in anesthetized horses, causing marked reduction in ventilation of the dependent lung.[3] Compression atelectasis and secondary bronchopneumonia can occur in horses kept in flotation tanks for up to several weeks for treatment of skeletal injuries. **Contraction atelectasis** occurs when there is compression of parts of the lung by fibrotic changes in the pleura. **Patchy atelectasis** occurs in the absence of surfactant, such as can occur in newborns. Failure of the lung to inflate, or development of atelectasis of the lungs of the newborn, usually those born prematurely, occurs because of lack of pulmonary surfactant. The disorder can progress to hyaline membrane disease. Affected newborn animals are severely dyspneic, hypoxemic, cyanotic, and weak, and they commonly die in a few hours.

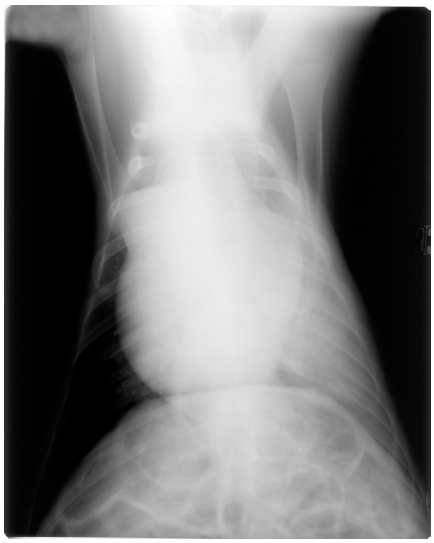

Fig. 12-7 Ventrodorsal radiograph of the chest of a 7-day-old foal immediately after ~30 minutes of enforced lateral recumbency. Note the consolidation of the previously dependent lung. Repeat radiographic examination 24 hours later, and without a period of recumbency, did not reveal lesions in the lungs.

The clinical signs of atelectasis are not apparent until there is extensive involvement of the lungs. Animals develop respiratory distress, tachypnea, tachycardia, and cyanosis. Blood gas analysis reveals hypoxemia, with or without hypercapnia. Thoracic radiographs reveal pulmonary consolidation. Ultrasonographic examination of the thorax demonstrates consolidated lung.

Atelectasis is reversible if the primary obstruction or compression is relieved.

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PULMONARY HEMORRHAGE

Pulmonary hemorrhage is uncommon in farm animals but does occur occasionally in cattle, and exercise-induced pulmonary hemorrhage (EIPH) occurs in 45% to 75% of racehorses (see "Exercise-induced pulmonary hemorrhage" elsewhere in this chapter). Pulmonary hemorrhage occurs with pulmonary abscesses, tumors, parasitic cysts (*Fascioloides magna*),[1] or foreign bodies. Tracheobronchoscopic, radiographic, and ultrasonographic examinations are useful in identifying the site and cause of the hemorrhage.

Cattle

In cattle the most common cause of epistaxis and hemoptysis secondary to pulmonary hemorrhage is erosion of pulmonary vessels adjacent to lesions of embolic pneumonia associated with vena caval thrombosis and hepatic abscessation (Fig. 12-8).[2] The onset of hemorrhage can be sudden, and affected animals hemorrhage profusely and die after a short course of less than 1 hour. Marked epistaxis and hemoptysis, severe dyspnea, muscular weakness and pallor of the mucous membranes are characteristic. In other cases, episodes of epistaxis and hemoptysis can occur over a period of several days or a few weeks along with a history of dyspnea.

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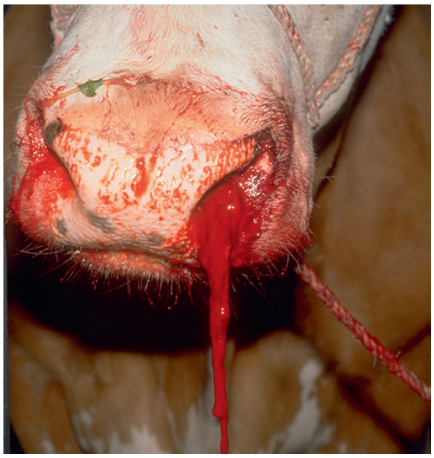

Fig. 12-8 Epistaxis and hemoptysis in a cow with pulmonary hemorrhage and vena caval thrombosis. (Reproduced with permission from Braun 2008.[2])

PULMONARY EMPHYSEMA

Pulmonary emphysema is distension of the lung caused by overdistension of alveoli with rupture of alveolar walls with or without escape of air into the interstitial spaces. Overinflation describes the situation in which there is enlargement of airspaces without tissue destruction. Pulmonary emphysema is always secondary to some primary lesion that effectively traps an excessive amount of air in the alveoli. It is a common clinicopathologic finding in many diseases of the lungs of all species and is characterized clinically by dyspnea, hyperpnea, poor exercise tolerance, and forced expiration.

ETIOLOGY

Pulmonary emphysema is an important lesion only in cattle, although occasional cases occur in pigs. Approximately 3.4% of cattle examined after slaughter in Tanzania had lesions of emphysema sufficiently severe to warrant condemning of the carcass.[1] The bovine lung is highly susceptible to the development of emphysema from many different causes, not all of them respiratory in origin. In those of respiratory origin, it is common to find pulmonary emphysema when the primary lesion in the lung causes trapping of air in alveoli or terminal bronchioles. Endotoxemia, for example, can result in diffuse alveolar damage resulting in pulmonary edema and emphysema. Some causes of emphysema are as follows.

Cattle

- Acute interstitial pneumonia
- Parasitic pneumonia with pulmonary edema in acute anaphylaxis
- Perforation of the lung by foreign body as in traumatic reticuloperitonitis

- Poisoning by the plants *Senecio quadridentatus,* rape, *Zieria arborescens,* and *Perilla frutescens* and the fungus *Periconia* spp. are recorded as causing pulmonary emphysema in cattle.
- Pulmonary abscess

Horses

- Bronchiolitis as a result of viral infection of the respiratory tract in young horses

All Species

- Secondary to bronchopneumonia
- Poisoning by oleander, *Bryophyllum pinnatum,* and moldy sweet potatoes
- Acute chemical injury—as in inhalation of welding fumes
- Chlorine gas poisoning
- Local or perifocal emphysema is also a common necropsy finding around local pulmonary lesions, especially atelectasis, often with no respiratory dysfunction. In calves and pigs, the emphysema is sometimes sufficiently extensive to kill the animal.
- Bullous emphysema is a rarely reported disorder of premature foals.[2]

PATHOGENESIS

Emphysema occurs because of destruction of the connective tissues of the lung, including the supporting and elastic tissue of the pulmonary parenchyma. Tissue damage resulting in emphysema in humans is caused by the action of proteases in the lung. Whether this occurs in the farm animal species is unknown but is a consideration. An initial lesion probably leads to an area of weakness from which emphysema spreads during coughing or exertion. In interstitial emphysema, there is the additional factor of distension of the connective tissue with air and compression collapse of the alveoli.

The development of interstitial emphysema depends largely upon the amount of interstitial tissue that is present and is most common in cattle and pigs. Whether there is simple overdistension of alveoli or whether their walls are also ruptured is very important in prognosis and treatment. Excellent recoveries occur in simple alveolar emphysema, especially those occurring acutely at pasture. This suggests that the lesion is functional and that the alveoli are not substantially damaged.

The **pathophysiologic consequences of emphysema** depend on the inefficiency of evacuation of pulmonary airspace and failure of normal gaseous exchange in the lungs. The elastic recoil of the tissue is diminished, and when the thorax subsides during expiration, incomplete evacuation occurs. Because of the increase in residual volume, the tidal volume must be increased to maintain normal gaseous exchange. Retention of carbon dioxide stimulates an increase in the depth of respiration, but maximum respiratory effort necessitated by exercise cannot be achieved. Anoxia develops and metabolism of all body tissues is reduced. The characteristic effect of emphysema is to produce an increase in expiratory effort necessitated by the failure of normal elastic recoil.

Interference with the pulmonary circulation results from collapse of much of the alveolar wall area and a consequent diminution of the capillary bed. The decreased negative pressure in the chest and the abnormally wide respiratory excursion also cause a general restriction of the rate of blood flow into the thorax. The combined effect of these factors may be sufficient to cause failure of the right ventricle, especially if there is a primary defect of the myocardium. Acidosis may also result because of the retention of carbon dioxide.

CLINICAL FINDINGS

Characteristically, diffuse pulmonary emphysema causes severe expiratory dyspnea with a grunt on expiration and loud crackling lung sounds on auscultation over the emphysematous lungs. In severe cases in cattle, the emphysema is commonly interstitial, and dissection of the mediastinum and fascial planes results in subcutaneous emphysema over the withers (Fig. 12-9). In severe cases in cattle, open-mouth breathing is common.

In cattle and pigs, the presence of pulmonary emphysema in pulmonary disease is often not detectable clinically.

CLINICAL PATHOLOGY

There is hypoxemia and, often, hypercapnia. Compensatory polycythemia may develop. There are no characteristic hematologic findings, but if there is a significant secondary bronchopneumonia, a leukocytosis and left shift may be evident. In the appropriate location, an examination of feces for lungworm larvae may be desirable. In cases suspected of having an allergic origin, swabs of nasal secretion may reveal a high proportion of eosinophils, and a hematologic examination may show eosinophilia.

NECROPSY FINDINGS

The lungs are distended and pale in color and may bear imprints of the ribs. In interstitial emphysema, the interalveolar septae are distended with air, which may spread to beneath the pleura, to the mediastinum, and under the parietal pleura. There may be evidence of congestive heart failure. On histopathologic examination, a bronchiolitis is present in most cases. This may be diffuse and apparently primary or originate by spread from a nearby pneumonia.

TREATMENT

The treatment of pulmonary emphysema will depend on the species affected, the cause of the emphysema, and the stage of the disease.

There is no known specific treatment for the pulmonary emphysema associated with acute interstitial pneumonia in cattle, which is discussed under that heading. The emphysema secondary to the infectious pneumonias will usually resolve spontaneously if the primary lesion of the lung is treated effectively. In valuable animals, the administration of oxygen may be warranted if the hypoxia is severe and life-threatening. Antihistamines, atropine, and corticosteroids

Fig. 12-9 Marked subcutaneous emphysema on the dorsal midline of a Holstein Friesian cow with acute respiratory disease. The cow's head is to the right. The skin can be depressed 5 cm with a forefinger, creating a crinkling feeling that is slightly painful to the cow. The emphysema gradually disappeared over a few days after antimicrobial treatment for bacterial pneumonia was implemented.

have been used for the treatment of pulmonary emphysema secondary to interstitial pneumonia in cattle, but their efficacy has been difficult to evaluate.

DIFFERENTIAL DIAGNOSIS

Acute emphysema in cattle is often accompanied by pulmonary edema with the presence of consolidation and crackles in the ventral parts of the lungs. It may be similar to acute pulmonary congestion and edema caused by anaphylaxis, but forced expiration is not a characteristic of these latter conditions.

Acute pneumonia in cattle or horses is characterized by fever and localization of abnormal respiratory sounds, which are not as marked nor as widely distributed as those of emphysema.

Chronic pneumonia is characterized by dyspnea, chronic toxemia, crackles and wheezes, and poor response to therapy.

Pneumothorax is accompanied by forced inspiration and an absence of normal breath sounds.

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PNEUMONIA

Pneumonia is inflammation of the pulmonary parenchyma, usually accompanied by inflammation of the bronchioles and often by pleuritis. It is manifested clinically by an increase in the respiratory rate, changes in the depth and character of respirations, coughing, abnormal breath sounds on auscultation, and, in most bacterial pneumonias, evidence of toxemia.

ETIOLOGY

Pneumonia may be associated with viruses, mycoplasmas, bacteria, or a combination of all three; fungi; metazoan parasites; and physical and chemical agents. Most of the pneumonias in animals are bronchogenic (inhalation) in origin, but some originate by the hematogenous route, such as pneumonia of foals and calves with septicemia. Mycoplasmal pneumonias can be devastating in cattle, goats, and pigs (Table 12-6). The pneumonias that occur in farm animals are grouped here according to species.

Cattle

- Pneumonic pasteurellosis (shipping fever)—*M. haemolytica, P. multocida* with or without parainfluenza-3 virus
- *Histophilus somnus* in feedlot cattle is not necessarily associated with the septicemic form of the disease. The role of the organism as a primary pathogen in acute bovine respiratory disease is uncertain.
- *Bibersteinia trehalosi* appears to be a component of the bovine respiratory disease complex, as it is in sheep.[1,2]
- Enzootic pneumonia of calves—bovine respiratory syncytial virus; bovine herpesvirus-1 (the IBR virus); parainfluenza-3; adenovirus-1, -2, and -3; rhinovirus; reovirus; and *Chlamydia* spp., *Mycoplasma* spp., *Pasteurella* spp.,[3] *Mannheimia* spp., *Trueperella* (formerly *Actinomyces* or *Arcanobacterium* or *Corynebacterium) pyogenes, Streptococcus* spp., *Bedsonia* sp., and *Actinobacillus actinoides*
- Corona virus infection in adult feedlot cattle[4,5]
- Pneumonia, mastitis, and arthritis in cattle associated with *Mycoplasma bovis*[6] and in calves with *Mycoplasma californicum* or *M. bovis*[7]
- Viral interstitial pneumonia in recently weaned beef calves associated with bovine respiratory syncytial virus; it may also occur in yearling and adult cattle.
- Contagious bovine pleuropneumonia—*Mycoplasma mycoides*
- Acute and chronic interstitial pneumonia associated with D,L-tryptophan, moldy hay, and other pneumotoxic agents
- Atypical interstitial pneumonia associated with ryegrass staggers in calves
- Massive infestation with pig ascarid larvae
- Lungworm pneumonia—*Dictyocaulus viviparus*
- *Klebsiella pneumoniae* infection in calves and nursing cows with mastitis associated with this organism
- Sporadically in tuberculosis associated with *M. bovis*
- *Fusobacterium necrophorus* as a complication of calf diphtheria, and sporadically in feedlot cattle
- There is a preliminary report of circovirus in adult cattle with pneumonia.

Table 12-6 Major pathogenic *Mycoplasma* spp. of ruminants, swine, and horses

Animal host/*mycoplasma* species	Disease
Bovine	
M. mycoides subsp. *mycoides SC*	Contagious bovine pleuropneumonia, CBPP
Mycoplasma sp. *bovine group 7*	Pneumonia and arthritis
M. bovis	Mastitis, pneumonia (calf), polyarthritis (calf) metritis, abortion, sterility
M. dispar	Pneumonia (calf)
M. californicum	Mastitis
M. canadense	Mastitis
M. bovocculi	Conjunctivitis
Ureaplasma diversens	Metritis, sterility, abortion
Mycoplasma (Eperythrozoon) wenyonii	Anemia
Sheep and goat	
M. capricolum subsp. *capripneumonia*	Contagious caprine pleuropneumonia
M. capricolum subsp. *capricolum*	Mastitis, arthritis
M. mycoides subsp. *capri*	Pneumonia, arthritis septicemia (goat)
M. mycoides subsp. *mycoides LC*	Pneumonia, mastitis, arthritis, septicemia (goat)
M. agalactiae	Infectious agalactia
M. ovipneumoniae	Pneumonia (lamb)
M. conjunctivae	Infectious keratoconjunctivitis (IKC) (sheep)
Pig	
M. hyopneumoniae	Enzootic pneumonia
M. hyorhinis	Pneumonia, arthritis
M. hyosynoviae	Arthritis
Mycoplasma (Eperythrozoon) suis	Anemia
Horse	
M. felis	Pleuritis
M. equirhinis	
M. equipharyngis	

- *Trueperella pyogenes* causes pneumonia[8] mastitis, abscesses, and lymphadenitis in cattle, goats, sheep, pigs, and horses
- Experimental infection with *Parachlamydia acanthamoebae* in calves[9]
- Mycotic pneumonia associated with *Mortierella wolfii* in adult cattle

Pigs

- Enzootic pneumonia—*Mycoplasma* sp. with *Pasteurella* sp. secondarily
- Pneumonic pasteurellosis—*P. multocida*
- Pleuropneumonia—*Actinobacillus pleuropneumoniae*
- Interstitial pneumonia—septicemic salmonellosis
- *Bordetella bronchiseptica, Salmonella choleraesuis*
- Influenza virus
- Porcine reproductive and respiratory syndrome virus
- *Haemophilus parasuis*
- *Actinobacillus pyogenes*
- Paramyxovirus causing respiratory and central nervous system disease in pigs
- Uncommonly, lungworm pneumonia
- Anthrax by inhalation, causing pulmonary anthrax

Horses

- Pleuropneumonia in mature horses as a result of aerobic and anaerobic bacteria—the aerobic bacteria most commonly isolated are alpha-hemolytic *Streptococcus* spp., *Pasteurella* spp., *Escherichia coli*, and *Enterobacter* spp. The anaerobic bacteria most frequently isolated are *Bacteroides* spp., *Prevotella* spp., *Fusobacterium* spp., and *Clostridium* spp.
- Newborn foals[10]—*Streptococcus* spp., *E. coli, Actinobacillus equuli,* and other agents causing septicemia in this age group
- In immunodeficient foals, and rarely adult horses,[11] pneumonia associated with adenovirus or *Pneumocystis jiroveci* (formerly *P. carinii*)
- Immunosuppression following corticosteroid therapy for other diseases
- Older foals—*R. equi,* equine herpesvirus-1 or 4 (EVR)
- Bronchointerstitial pneumonia in foals 1 to 8 months of age—etiology uncertain
- Eosinophilic pneumonia secondary to parasite migration (*Parascaris equorum*) or *Dictyocaulus arnfieldi* infection, or as part of the multisystemic eosinophilic syndrome in adult horses[12]
- Interstitial proliferative pneumonia in foals from 6 days to 6 months of age, and the adult form in horses 2 years of age and older
- *Nicoletella semolina* in adult horses
- *Bordetella bronchiseptica* in adult horses
- Glanders and epizootic lymphangitis (*Histomonas farcinicus*) usually include pneumonic lesions.
- *Paecilomyces* spp. in foals
- Pleuropneumonia associated with pulmonary hydatidosis in a horse
- As a sequela to strangles
- Interstitial pneumonia associated with equine infectious anemia virus infection[13]
- Rarely, as a sequel to equine viral arteritis or equine viral rhinopneumonitis in adult animals
- Equine influenza virus causes pneumonia in foals and adult horses[14,15]
- Equine multinodular pulmonary fibrosis (putatively caused by EHV-5 infection)[16]
- Equine rhinitis A virus infection (putative cause)[17]
- Mycotic pneumonia associated with *Emmonsia crescens* (adiaspiromycosis) in adult horses
- Pulmonary aspergillosis in adult horses with predisposing conditions (such as colitis) and in donkey foals[18]
- Strenuous exercise in very cold conditions can cause damage to the airways of horses (and probably other species).

Sheep

- Pneumonic pasteurellosis (*Mannheimia* spp.) as acute primary pneumonia in feedlot lambs, or secondary to parainfluenza-3 or *Chlamydia* sp. infection
- Newborn lambs—uncommonly *Streptococcus zooepidemicus, Salmonella abortusovis*
- Severe pneumonia as a result of *Mycoplasma* sp. in lambs—kageda in Iceland and Switzerland
- Clinically inapparent pneumonias without secondary infection—adenovirus, respiratory syncytial virus, reovirus, *Mycoplasma* spp. (including *M. ovipneumoniae, M. dispar*)
- *M. bovis* in sheep[19]
- *Corynebacterium pseudotuberculosis*—sporadic cases only
- Melioidosis (*Pseudomonas pseudomallei*)
- Lungworm (*Dictyocaulus filaria*)
- Ovine herpesvirus-2
- Progressive interstitial pneumonia (maedi) and pulmonary adenomatosis (jaagsiekte)
- Carbolic dip toxicosis

Goats

- Pleuropneumonia associated with *Mycoplasma* strain F 38 or *Mycoplasma capri,* a devastating disease
- Chronic interstitial pneumonia with cor pulmonale as a common sequela associated with a number of *Mycoplasma* spp., but *M. mycoides* var. *mycoides* appears to be the most commonly recorded.
- Parainfluenza type 3
- Contagious ecthyma virus
- Retrovirus infection

All Species

- Toxoplasmosis—rare, sporadic cases
- Systemic mycoses
- Aspiration pneumonia is dealt with as a separate entity.
- Sporadic secondary pneumonia associated with *Streptococcus* sp., *Corynebacterium* sp., *Dermatophilus* sp.
- Interstitial pneumonia, pulmonary consolidation and fibrosis by toxins in plants—*Eupatorium glandulosum* in horses, *Zieria arborescens* (stinkwood) in cattle, *Astragalus* spp. in all species

EPIDEMIOLOGY

In addition to the infectious agents that cause the pneumonia, there are risk factors that contribute to the susceptibility of the animal. Three **risk factors** interact in the pathogenesis of specific pneumonias:

- **Animal**
- **Environmental and management**
- **Pathogen**

These are of paramount importance in any consideration of pneumonia, and the details of the epidemiology of each specific pneumonia are presented with each specific disease in this book. As examples, some of the commonly recognized risk factors include the following:

- The weaning of beef calves in northern climates
- The long transportation of beef cattle to feedlots
- The collection and mixing of animals at auction marts where they might be deprived of feed and water for prolonged periods
- The transportation of Thoroughbred horses farther than 500 miles and viral respiratory tract disease or exposure to horses with respiratory tract disease
- Housing dairy calves in poorly ventilated, overcrowded barns
- Marked changes in weather

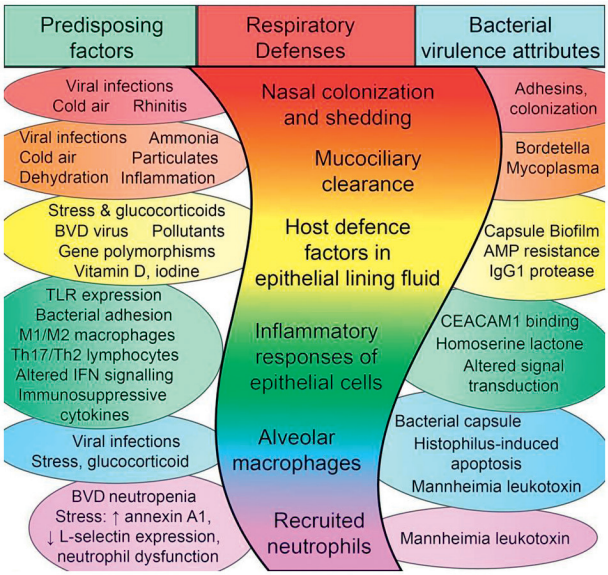

Fig. 12-10 Mechanisms of failure of the respiratory defenses. The center column illustrates the layered arrangement of the respiratory defenses. At left is a partial summary of causes and mechanisms whereby each of these respiratory defenses is comprised of factors that predispose to bacterial pneumonia. At right is a partial summary of how pathogens further contribute to failure of the lung defenses. BVDV, bovine viral diarrhea virus; IFN, interferon; TLF, toll-like receptor. (Data from: Couetil, L.L. (2014) In Hinchcliff KW, Kaneps AJ, and Geor RJ (eds): Equine Sports Medicine and Surgery: Basic and clinical sciences of the equine athlete, ed. 2. Elsevier Health Sciences. p 614.)

Susceptibility to pneumonia is determined by the animal's resistance to infection by agents that cause or predispose to pneumonia. Factors that impair innate resistance or adaptive resistance (immunity) increase the animal's susceptibility to pneumonia. For instance, shipping not only increases the risk of exposure of animals to pathogens to which they have not been exposed but also can impair innate resistance through damage to the respiratory tract by airborne irritants, dehydration, food deprivation, and the effects of stress.

PATHOGENESIS

Pulmonary Defense Mechanisms

Under normal conditions the major airways and the lung parenchyma prevent the entry of and neutralize or remove injurious agents, so that the lung contains very few, if any, organisms beyond the large airways. Many infections of the respiratory tract originate from aerosolized particles carrying infectious agents that arise external to or within the respiratory tract. To induce an infection by the aerosol route, an etiologic agent must be aerosolized, survive in the aerosol, be deposited at a vulnerable site in the respiratory tract of a susceptible host, and then multiply. Thus the pathogenesis of these respiratory infections is related to the deposition of particles and infectious agents within the respiratory tract.

Under normal conditions a complex of biochemical, physiologic, and immunologic defense mechanisms protect the respiratory tract from inhaled particles that could be injurious or infectious. The major defense mechanisms of the respiratory tract include (Fig. 12-10) the following:[20]

- Aerodynamic filtration by the nasal cavities
- Sneezing
- Local nasal antibody
- The laryngeal reflex
- The cough reflex
- Mucociliary transport mechanisms
- Alveolar macrophages
- Systemic and local antibody systems

Compromise of one or more of these defense mechanisms can result in infection, or proliferation, of infectious agents, leading to development of pneumonia. Factors that can compromise respiratory defense systems include stress, administration of glucocorticoids, viral infections, exposure to cold air by animals unaccustomed to it, or poor air quality.[20]

Respiratory Mucociliary Clearance

The mucociliary escalator has important functions in the lung's physical defenses against the constant challenge of inhaled pathogens. By various physical mechanisms, mucus traps and subsequently transports inhaled particles to the pharynx, where they are normally swallowed. Mucus also protects the airways by absorbing inhaled chemicals and gases, by humidifying the inspired air and by keeping the underlying mucosa hydrated. Mucus contains antibodies, especially IgA, which together with lactoferrin and lysozyme provide immunologic defense.

Airway secretions consist of two layers. An underlying liquid layer, known as the periciliary fluid, in which the cilia beat, originates largely from transepithelial osmosis. An overlying gel or mucus layer is composed of intertwined mucin strands. Airway mucus is secreted in small globules, which expand several hundredfold within seconds and are later drawn into strands and transported rostrally by ciliary activity.

The secretion of respiratory mucus is a protective mechanism by which inhaled particles touching the airway mucosa stimulate local mucus production, which then traps and transports the particle from the lung. Airway mucus is produced mainly by submucosal glands and goblet cells, also known as mucus-producing cells. Airway secretions also contain alveolar fluid, surfactant and alveolar cells, including macrophages, which are drawn into the mucociliary ladder by surface tension.

Airway mucus is a complex substance consisting of 95% water and a 5% combination of glycoproteins, proteoglycans, lipids, carbohydrates, and minerals. Mucin is the main nonaqueous component. Effective mucociliary clearance or mucokinesis can occur over a range of mucus viscosity, but very-low-viscosity mucus is poorly transported and tends to gravitate toward the alveoli, whereas excessively viscous mucus, which is also poorly transported, may lodge in the airways and become inspissated.

In **respiratory disease,** mucociliary clearance is impaired through disruption of effective ciliary activity or changes in the quantity or quality of the mucus or periciliary fluid, or all three factors. In viral pulmonary disease, ciliary activity can be disrupted because of temporary deciliation or lesions of the respiratory mucosa. The defective mucociliary clearance may also last for several weeks. In chronic obstructive pulmonary disease in the horse, metaplasia of ciliated epithelium to a nonciliated epithelium may occur in the smaller airways.

Changes in the quality of mucus are common in respiratory tract disease, especially increases in viscosity with pulmonary disease. The destruction of leukocytes and respiratory epithelial cells and the release of DNA increases the viscosity. Large increases in the glycoprotein content of mucus also occur, which affects the mucokinetic properties. Purulent respiratory secretions have reduced elasticity and together with the increased viscosity affect the mucociliary clearance. Acute inflammation also results in the production of serum proteins from the airway exudate, which alters the viscoelasticity of mucus and further reduces mucokinesis.

Yellow or green respiratory secretions are attributable to the enzyme myeloperoxidase, released from leukocytes in the static secretion, or to high numbers of eosinophils.

The quantity of mucus increases in most cases of respiratory disease as a result of stimulation of goblet cells and submucosal glands by inflammatory mediators. The abnormal production can also exacerbate the original pulmonary dysfunction. Tracheal mucociliary clearance can be assessed endoscopically, in vivo, by dropping dye or small markers on the tracheal mucosa and measuring their rate of transit visually or using radioactive particles detected by scintigraphy.

Large Particles in Upper Respiratory Tract

Large aerosolized particles that are inhaled are removed by the nasal cavities and only small ones are able to get into the lung. In the upper respiratory tract, essentially 100% of particles more than 10 µm in diameter and 80% of particles of the 5 µm size are removed by gravitational settling on mucosal surfaces. Particles deposited between the posterior two-thirds of the nasal cavity and the nasopharynx and from the larynx to the terminal bronchioles land on airways lined by mucus-covered, ciliated epithelium and are removed by means of the mucociliary transport mechanism. The nasopharyngeal and tracheobronchial portions of the ciliated airways transport mucus toward the pharynx, where it can be eliminated by swallowing. The cilia beat most effectively in mucus at a certain elasticity, viscosity, and chemical composition. Anything that interferes with the secretion and maintenance of normal mucus will interfere with the clearance of particles from the upper respiratory tract. The damaging effect of viruses on mucociliary clearance has been demonstrated in laboratory animals and in humans.

Mycoplasma pneumoniae infection slows tracheobronchial clearance for as long as 1 year, suggesting a possible explanation for the predisposition to bacterial pneumonia commonly observed after these infections. Viral diseases of the upper respiratory tract of farm animals are common and a similar interference in the mucociliary transport mechanism may explain the occurrence of secondary bacterial pneumonia.

Cough Reflex

The cough reflex provides an important mechanism by which excess secretions and inflammatory exudates from the lungs and major airways can be removed and disposed of by expectoration or swallowing. In animals with relatively normal lungs, coughing represents a very effective means of expelling inhaled foreign bodies, or excessive or abnormal respiratory secretions, down to the level of the fourth- or fifth-generation bronchi. If the airways become deciliated, the cough reflex is the main and only mucus-clearance mechanism remaining. The cough reflex is valuable for transporting the increased secretions present in equine pulmonary disease, and antitussive agents should therefore not be used in horses.

In the presence of severe tracheitis and pneumonia, coughing may result in retrograde movement of infected material to the terminal respiratory bronchioles and actually promote spread of the infection to distal parts of the lung. Any process that causes airway obstruction can predispose the lung to secondary bacterial infections. Experimental obstruction of the bronchi supplying a lobe of lung in sheep allows the development of secondary bacterial pneumonia. It has been postulated that damage to small airways following viral infections may allow the accumulation of exudate and cellular debris, which may facilitate secondary bacterial infections.

Small Particles Into Lower Respiratory Tract

Particles of 1 to 2 µm in size settle in the lungs through the action of gravity in the alveolar spaces, and particles below 0 to 2 µm settle through diffusion of air. The alveolar macrophage plays a major role in clearing inhaled particles from the lung. Under normal conditions, bacteria that gain entry into the alveoli are cleared quickly and effectively in a matter of hours. Experimental parainfluenza-3 (PI-3) virus infection has the greatest adverse effect on the pulmonary clearance of *M. haemolytica* administered by intranasal aerosol on the seventh day following viral infection. The effect on pulmonary clearance is much less when the bacteria are given on the 3rd or 11th day following the initial viral infection.

The presence of preexisting antibody to *M. haemolytica* eliminates the effect of the viral infection on pulmonary clearance. Thus there is some evidence that in domestic animals, lung clearance mechanism may be affected by a concurrent viral infection. This may have major implications in the control of some of the common infectious respiratory diseases of farm animals.

Species Susceptibility

The anatomic and physiologic features of the respiratory system of cattle may predispose them to the development of pulmonary lesions much more than other farm animal species. Cattle have a small physiologic gaseous exchange capacity and greater resultant basal ventilatory activity. The small gaseous exchange capacity may predispose cattle to low bronchiolar or alveolar oxygen levels during exposure to high altitudes and during periods of active physical or metabolic activity. During these times, low oxygen tension or hypoxia may slow mucociliary and alveolar macrophage activity and decrease pulmonary clearance rates. The basal ventilatory activity is comparatively greater than other mammals, which results in the inspired air becoming progressively more contaminated with infectious, allergenic, or noxious substances.

The bovine lung also has a higher degree of compartmentalization than other species. This may predispose to airway hypoxia peripheral to airways that become occluded. This results in reduced phagocytic activity and the retention or multiplication of infectious agents. In addition, because of the low numbers of alveolar macrophages in the bovine lung, the pulmonary clearance mechanism may not be as effective as in other species. There is also a low level or atypical bioactivity of lysozyme in bovine respiratory mucus, which may make cattle more susceptible to infection of the respiratory tract than other species.

Development of Pneumonia

The process by which pneumonia develops varies with the causative agent and its virulence and with the portal by which it is introduced into the lung.

Bacteria are introduced largely by way of the respiratory passages and cause a primary bronchiolitis that spreads to involve surrounding pulmonary parenchyma. The reaction of the lung tissue may be in the form of an acute fibrinous process as in pasteurellosis and contagious bovine pleuropneumonia, a necrotizing lesion as in infection with *F. necrophorum,* or as a more chronic caseous or granulomatous lesion in mycobacterial or mycotic infections. Spread of the lesion through the lung occurs by extension but also by passage of infective material along bronchioles and lymphatics. Spread along the air passages is facilitated by the normal movements of the bronchiolar epithelium and by coughing. Bronchiectasis and pulmonary abscesses are complications and common causes of failure to respond to therapy. Hematogenous infection by bacteria results in a varying number of septic foci, which may enlarge to form lung abscesses. Pneumonia occurs when these abscesses rupture into air passages and spread as a secondary bronchopneumonia.

Viral infections are also introduced chiefly by inhalation and cause a primary bronchiolitis, but there is an absence of the acute inflammatory reaction that occurs in bacterial pneumonia. Spread to the alveoli causes enlargement and proliferation of the alveolar epithelial cells and the development of alveolar edema. Consolidation of the affected tissue results but again there is an absence of acute inflammation and tissue necrosis so that toxemia is not a characteristic development. Histologically, the reaction is manifested by enlargement and proliferation of the alveolar epithelium, alveolar edema, thickening of the interstitial tissue, and lymphocytic aggregations around the alveoli, blood vessels, and bronchioles. This interstitial type of reaction is characteristic of viral pneumonias.

The **pathophysiology** of all pneumonias, regardless of the way in which lesions develop, is based on interference with

gaseous exchange between the alveolar air and the blood. Anoxia and hypercapnia develop, which results in polypnea, dyspnea, or tachypnea. Consolidation results in louder than normal breath sounds, especially over the anteroventral aspects of the lungs, unless a pleural effusion is present to muffle the sounds. In bacterial pneumonias, there is the added effect of toxins produced by the bacteria and necrotic tissue; the accumulation of inflammatory exudate in the bronchi is manifested by abnormal lung sounds such as crackles and wheezes on auscultation. Interstitial pneumonia results in consolidation of pulmonary parenchyma without involvement of the bronchi, and on auscultation loud breath sounds predominate in the early stages.

Extension of the pneumonia to the visceral surface of the pleura results in pleuritis, pleuropneumonia, pleural effusion, and thoracic pain. Fibrinous pleuritis is a common complication of pneumonic pasteurellosis in cattle. Pleuritis and pleural effusion secondary to pneumonia and pulmonary abscess are commonly recognized in adult horses with the pleuropneumonia complex associated with aerobic and anaerobic bacteria. Anaerobic bacterial pleuropneumonia in the horse is accompanied by a putrid odor of the breath, the sputum, or the pleural fluid. It is suggested that most anaerobic bacterial pulmonary infections in the horse are the result of aspiration of oropharyngeal contents, and they are most commonly located in the right lung because of the proximity of the right mainstem bronchus. Some horses with pleuropneumonia may develop acute hemorrhagic pulmonary infarction and necrotizing pneumonia.

Restriction of gaseous exchange occurs because of the obliteration of alveolar spaces and obstruction of air passages. In the stage before blood flow through the affected part ceases, the reduction in oxygenation of the blood is made more severe by failure of part of the circulating blood to come into contact with oxygen. Cyanosis is most likely to develop at this stage and to be less pronounced when hepatization is complete and blood flow through the part ceases. An additional factor in the production of anoxia is the shallow breathing that occurs. Pleuritic pain causes reduction in the respiratory excursion of the chest wall, but when no pleurisy is present the explanation of the shallow breathing probably lies in the increased sensitivity of the Hering–Breuer reflex. Retention of carbon dioxide with resulting acidosis is most likely to occur in the early stages of pneumonia because of this shallow breathing.

CLINICAL FINDINGS

- **Rapid, shallow breathing** is the cardinal sign of early pneumonia.
- **Dyspnea** occurs in the later stages when much of the lung tissue is nonfunctional.
- **Polypnea** may be quite marked with only minor pneumonic lesions; the rapidity of the respiration is an inaccurate guide to the degree of pulmonary involvement.
- **Coughing** is another important sign, with the type of cough varying with the nature of the lesion.

Bacterial bronchopneumonia is usually accompanied by a moist and painful cough. In viral interstitial pneumonia, the coughing is frequent, dry, and hacking, often in paroxysms. Auscultation of the thorax before and after coughing may reveal coarse crackling sounds suggestive of exudate in the airways. Cyanosis is not a common sign and occurs only when large areas of the lung are affected. A nasal discharge may or may not be present, depending on the amount of exudate present in the bronchioles and whether or not there is accompanying inflammation of the upper respiratory tract. The odor of the breath may be informative: it may have an odor of decay when there is a large accumulation of inspissated pus present in the air passages; or it may be putrid, especially in horses affected with anaerobic bacterial pleuropneumonia.

In **acute bacterial bronchopneumonia**, toxemia, anorexia, depression, tachycardia, and a reluctance to lie down are common. In the advanced stages, severe dyspnea with an expiratory grunt are common.

In **viral interstitial pneumonia**, affected animals are usually not toxemic, but they may have a fever and be inappetent or anorexic. However, some cases of viral interstitial pneumonia can be diffuse and severe and cause severe respiratory distress, failure to respond to therapy, and death within a few days. A severe **bronchointerstitial pneumonia of foals** aged 1 to 2 months of age has been described. The disease was characterized clinically by sudden onset of fever and increasingly severe dyspnea with respiratory distress and no response to treatment. In **acute interstitial pneumonia of cattle,** exemplified by the acute disease seen in mature cattle moved on to a lush pasture within the previous 10 days, some animals may be found dead. Other affected animals are severely dyspneic and anxious, commonly mouth breathe and grunt with each expiration, and, if forced to walk, may collapse and die of asphyctic respiratory failure.

Auscultation of the lungs is a valuable aid to diagnosis. The stage of development and the nature of the lesion can be determined, and the area of lung tissue affected can be outlined. In the early congestive stages of bronchopneumonia and interstitial pneumonia, the breath sounds are increased, especially over the anteroventral aspects of the lungs. Crackles develop in bronchopneumonia as bronchiolar exudation increases, but in uncomplicated interstitial pneumonia, clear, harsh breath sounds are audible. In viral interstitial pneumonia, wheezes may be audible because of the presence of bronchiolitis. When complete consolidation occurs in either form, loud breath sounds are the most obvious sound audible over the affected lung, but crackles may be heard at the periphery of the affected area in bronchopneumonia. Consolidation also causes increased audibility of the heart sounds. When pleurisy is also present, a pleuritic friction rub may be audible in the early stages and muffling of the breath sounds over the ventral aspects of the lungs in the late exudative stages. If a pleural effusion is present, percussion of the thorax will reveal dullness of the ventral aspects, and a fluid line can usually be outlined. Consolidation can be detected also by percussion of the thorax.

In **chronic bronchopneumonia in cattle**, there is chronic toxemia, rough hair coat, and a gaunt appearance. The respiratory and heart rates are above normal, and there is usually a moderate persistent fever. However, the temperature may have returned to within a normal range even though the animal continues to have chronic incurable pneumonia. The depth of breathing is increased and both inspiration and expiration are prolonged. A grunt on expiration and open-mouth breathing indicate advanced pulmonary disease. A copious bilateral mucopurulent nasal discharge and a chronic moist productive cough are common. On auscultation of the lungs, loud breath sounds are usually audible over the ventral half of the lungs, and crackles and wheezes are commonly audible over the entire lung fields but are most pronounced over the ventral half.

With adequate treatment in the early stages, bacterial pneumonia usually responds favorably in 24 hours, but viral pneumonia may not respond at all or may relapse after an apparent initial beneficial response. The transient response may be attributable to control of the secondary bacterial invaders. In some bacterial pneumonias, relapses also occur that are a result of either reinfection or persistence of the infection in necrotic foci that are inaccessible to antimicrobials. The final outcome depends on the susceptibility of the causative agent to the treatments available and the severity of the lesions when treatment is undertaken. Pleurisy is a common complication of pneumonia and rarely occurs independently of it, and it is described later under that heading.

Pneumonia and pleuritis in horses are described separately (see following "Equine Pleuropneumonia" section).

Congestive heart failure or cor pulmonale may occur in some animals that survive a chronic pneumonia for several weeks or months.

Medical Imaging

Thoracic radiography and ultrasonography are now commonly performed in veterinary teaching hospitals and specialty clinics and are discussed earlier in this chapter. They can

provide considerable diagnostic assistance in assessing the severity of the lesion and explaining certain clinical manifestations that may be difficult to interpret. Ultrasonography is a useful diagnostic aid in cattle and horses with anaerobic bacterial pleuropneumonia and pulmonary abscessation. Gas echoes within pleural or abscess fluid were found to be a sensitive and specific indicator of anaerobic infection, as was a putrid breath or pleural fluid.

In cattle with pleuropneumonia, ultrasonographic examination of both sides of the thorax may reveal accumulations of anechogenic and hypoechogenic fluid in the pleural space in the ventral aspect of the thorax.[21] In cattle, pleural effusion associated with pleuritis is usually unilateral because the pleural sacs do not communicate. Bilateral pleural effusion may indicate either bilateral pulmonary disease or a noninflammatory cause such as right-sided congestive heart failure or hypoproteinemia.

CLINICAL PATHOLOGY

Respiratory Secretions

The laboratory examination of the exudates and secretions of the respiratory tract is the most common diagnostic procedure performed when presented with cases of pneumonia. Nasal swabs, tracheobronchial aspirates, and bronchoalveolar lavage samples can be submitted for isolation of viruses, bacteria, and fungi; cytologic examination; and determination of **antimicrobial sensitivity.** Tracheobronchial aspirates are considered more reliable for the cytologic examination of pulmonary secretions in horses with suspected pneumonia or pleuropneumonia. Bronchoalveolar lavage samples may be normal in horses affected with pneumonia or pleuropneumonia. In suspected cases of pleuropneumonia, the collection and culture of pleural fluid is a valuable aid to diagnosis, and both anaerobic and aerobic bacteria must be considered.

Thoracocentesis

When pleural effusion is suspected, thoracocentesis can be used to obtain pleural fluid for analysis.

Hematology

Hematologic examination can indicate if the infection is bacterial or viral in nature and its severity. The hematocrit will be elevated in severely toxemic animals that are not drinking water. Severe bacterial bronchopneumonia and pleuritis is characterized by marked changes in the leukon. Serum fibrinogen or serum amyloid A concentrations are markedly elevated in horses with pleuropneumonia and pleuritic, or other inflammatory, lung disease.[22] Some limited studies indicate that the measurement of acute-phase proteins in bovine respiratory disease may be a valuable diagnostic and prognostic aid.

Serology

When viral interstitial pneumonia is suspected, acute and convalescent sera are recommended for viral neutralization titer evaluation. For specific diseases such as porcine pleuropneumonia, serum can be taken from a percentage of the herd and submitted for serotyping to determine which serotype is most prevalent in the herd.

Fecal Samples

When lungworm pneumonia is suspected, fecal samples can be submitted for detection of the larvae.

Necropsy

In outbreaks of respiratory disease wherein the diagnosis is uncertain, necropsy of selected early cases will often assist in making a diagnosis.

NECROPSY FINDINGS

Gross lesions are usually observed in the anterior and dependent parts of the lobes; even in fatal cases where much of the lung is destroyed, the dorsal parts of the lobes may be unaffected. The gross lesions vary a great deal depending on the type of pneumonia present. Bronchopneumonia is characterized by the presence of serofibrinous or purulent exudate in the bronchioles and lobular congestion or hepatization.

In the more severe, fibrinous forms of pneumonia, there is gelatinous exudation in the interlobular septae and an acute pleurisy, with shreds of fibrin present between the lobes.

In interstitial pneumonia, the bronchioles are clean, and the affected lung is sunken and dark red in color, and it has a granular appearance under the pleura and on the cut surface. There is often an apparent firm thickening of the interlobular septae. These differences are readily detected on histologic examination.

In chronic bronchopneumonia of cattle, there is consolidation, fibrosis, fibrinous pleuritis, interstitial and bullous emphysema, bronchi filled with exudate, bronchiectasis, and pulmonary abscessation.

Lesions typical of the specific infections listed under etiology are described under the headings of the specific diseases.

TREATMENT

Antimicrobial Therapy

In specific bacterial infections as listed previously, isolation of affected animals and careful surveillance of the remainder of the group to detect cases in the early stages should accompany the administration of specific antimicrobials to affected animals. The choice of antimicrobial will depend on the tentative diagnosis, the experience with the drug in previous cases and the results of drug sensitivity tests. The common bacterial pneumonias of all species will usually recover quickly (24-72 hours) if treated with an adequate dose of the drug of choice early in the course of the disease. Animals with severe pneumonia will require daily treatment for several days until recovery occurs. Those with bacterial pneumonia and toxemia must be treated early on an individual basis. Each case should be identified and carefully monitored for failure to recover, and an assessment should be made. Clinical field trials to evaluate different antimicrobials for the treatment of acute bovine respiratory disease occurring under natural conditions are becoming more common and more meaningful, particularly under commercial feedlot conditions.

DIFFERENTIAL DIAGNOSIS

There are two major difficulties in the clinical diagnosis of pneumonia. The first is to decide that the animal has pneumonia; the second is to determine the nature of the pneumonia and its cause. The suspected cause will influence the prognosis, the clinical management, and, more particularly in infectious pneumonias, the kind of antimicrobial therapy used.

There are two kinds of errors made in the clinical diagnosis of pneumonia. One is that the pneumonia is not detected clinically because the abnormal lung sounds are apparently not obvious. The other is to make a diagnosis of pneumonia because of the presence of dyspnea that is attributable to disease in some other body system.

- In **bacterial pneumonia** the major clinical findings are polypnea in the early stages and dyspnea later, abnormal lung sounds, and fever and toxemia.
- In **viral interstitial pneumonia** uncomplicated by secondary bacterial pneumonia, there is no toxemia. Pulmonary edema and congestion, embolism of the pulmonary artery, and emphysema are often mistaken for pneumonia but can usually be differentiated by the absence of fever and toxemia, on the basis of the history and on auscultation findings.
- **Diseases of other body systems** can cause polypnea and dyspnea. Congestive heart failure, the terminal stages of anemia, poisoning by agents such as hydrocyanic acid, hyperthermia, and acidosis are accompanied by respiratory embarrassment but not by the abnormal sounds typical of pulmonary involvement.

If pneumonia is present, the next step is to determine the nature and cause of the pneumonia. All the practical laboratory aids described earlier should be used when necessary. This is of particular importance when outbreaks of pneumonia are encountered, in which case necropsy examination of selected cases is indicated. In single routine cases of pneumonia, the cause is usually not determined. However, the age

and class of the animal, the history and epidemiologic findings, and the clinical findings can usually be correlated and a presumptive etiologic diagnosis made.

Pleuritis is characterized by shallow, abdominal-type respiration; by pleuritic friction sounds when effusion is minimal; a muffling of lung sounds on auscultation; and the presence of dullness and a horizontal fluid line on acoustic percussion when there is sufficient pleural fluid present. Thoracocentesis or ultrasonographic examination reveals the presence of excessive pleural fluid.

In **pneumothorax** there is inspiratory dyspnea and on the affected side and the abnormalities include the following:

- Absence of breath sounds over the lobes but still audible sounds over the base of the lung
- Increase in the absolute intensity of the heart sounds
- Increased resonance on percussion.

Diseases of the upper respiratory tract such as laryngitis and tracheitis are accompanied by varying degrees of inspiratory dyspnea, which is often loud enough to be audible without a stethoscope. In less severe cases, auscultation of the midcervical trachea will reveal moist wheezing sounds on inspiration. These sounds are transmitted down into the lungs and are audible on auscultation of the thorax. These transmitted sounds must not be interpreted as attributable to pneumonia. In some cases of severe laryngitis and tracheitis, the inspiratory sounds audible over the trachea and lungs are markedly reduced because of almost total obliteration of these organs. In laryngitis and tracheitis, there is usually a more frequent cough than in pneumonia, and the cough can be readily stimulated by squeezing the larynx or trachea. In pneumonia the abnormal lung sounds are audible on both inspiration and expiration. Examination of the larynx through the oral cavity in cattle and with the aid of a rhinolaryngoscope in the horse will usually reveal the lesions.

Antimicrobial agents in a long-acting base may be used to provide therapy over a 4- to 6-day period instead of the daily administration of the shorter-acting preparations. However, the blood concentrations from the long-acting preparations are not as high as the shorter-acting preparations, and treatment with these compounds are not as effective in severely affected animals.

Selection of antimicrobials is based on the principles detailed in Chapter 6. Briefly, antimicrobials for treatment of bacterial respiratory disease should be active against the causative agent, should be able to achieve therapeutic concentrations in diseased lung, and should be convenient to administer. The antimicrobials should be affordable and, if used in animals intended as human food, must be approved for use in such animals.

Antimicrobials for treatment of lung disease are preferably those that achieve therapeutic concentrations in diseased lung tissue after administration of conventional doses. This has been convincingly demonstrated for the macrolide (azithromycin, erythromycin), triamilide (tulathromycin), and fluoroquinolone (danofloxacin, enrofloxacin) antimicrobials and fluorfenicol in a variety of species. The beta-lactam antimicrobials (penicillin, ceftiofur) are effective in treatment of pneumonia in horses, pigs, and ruminants despite having chemical properties that do not favor their accumulation in lung tissue.

Routes of administration include oral (either individually or in medicated feed or water), parenteral (subcutaneous, intramuscular, intravenous), or inhalational. Intratracheal administration of antimicrobials to animals with respiratory disease is not an effective means of achieving therapeutic drug concentrations in diseased tissue. **Aerosolization and inhalation** of antimicrobials has the theoretic advantage of targeting therapy to the lungs and minimizing systemic exposure to the drug. However, although administration by inhalation achieves good concentrations of drug in bronchial lining fluid, the drug does not penetrate unventilated regions of the lungs, in which case parenteral or oral administration of antimicrobials is indicated. Administration of gentamicin to horses and ceftiofur sodium to calves with pneumonia has been investigated. Aerosol administration of gentamicin to normal horses results in gentamicin concentrations in bronchial lavage fluid 12 times that achieved after intravenous administration. Aerosolized ceftiofur sodium (1 mg/kg) is superior to intramuscular administration in treatment of calves with *M. haemolytica.*

Treatment of parasitic lung disease, such as that caused by migrating larvae or lungworms, is by administration of appropriate anthelmintics such as ivermectin, moxidectin, or the benzimidazoles. Refer to the sections in this book that deal with these diseases for details of the specific treatments. Treatment of *P. jiroveci* pneumonia involves the administration of a sulfonamide–trimethoprim combination or dapsone (3 mg/kg orally every 24 hours).

The antimicrobials and other drugs recommended for the treatment of each specific pneumonia listed under etiology are presented with each specific disease elsewhere in the book. The common causes for failure to respond favorably to treatment for bacterial pneumonia include the following:

- **Advanced disease when treatment was undertaken**
- **Presence of pleuritis and pulmonary abscesses**
- **Drug-resistant bacteria**
- **Inadequate dosage of drug**
- **Presence of other lesions or diseases that do not respond to antimicrobials**

There is no specific treatment for the viral pneumonias, and although many of the *Mycoplasma* spp. are sensitive to antimicrobials in vitro, the pneumonias associated with them do not respond favorably to treatment. This could be attributable to the intracellular location of the *Mycoplasma* making them inaccessible to the drugs. Because viral and mycoplasmal pneumonias are commonly complicated by secondary bacterial infections, it is common practice to treat acute viral and mycoplasmal pneumonias with antimicrobials until recovery is apparent.

Intensive and prolonged therapy may be required for the treatment of diseases such as equine pleuropneumonia. It may include daily care and treatment in a veterinary clinic consisting of daily lavage of the pleural cavity, including thoracostomy to drain pulmonary abscesses, and intensive antimicrobial therapy and monitoring for several weeks.

Mass Medication

In outbreaks of pneumonia where many animals are affected and new cases occur each day for several days, the use of mass medication of the feed and/or water supplies should be considered. Outbreaks of pneumonia in swine herds, lamb feedlots, veal calf enterprises, and beef feedlots are usually ideal situations for mass medication through the feed or water. Mass medication may assist in the early treatment of subclinical pneumonia and is a labor-saving method of providing convalescent therapy to animals that have been treated individually. The major limitation of mass medication is the uncertainty that those animals that need the drug will actually get it in the amounts necessary to be effective. Total daily water intake by animals is a function of total dry matter intake and well-being, and water consumption is therefore markedly reduced in toxemic animals. The provision of a reliable concentration of the drug in the water supply on a 24-hour basis is also a problem. However, with careful calculation and monitoring, mass medication can be a valuable and economical method of treating large numbers of animals. The method of calculating the amount of antimicrobials to be added to feed or water supplies is presented in Chapter 7 on antimicrobial therapy.

When outbreaks of pneumonia occur and new cases are being recognized at the rate of 5% to 10% per day of the total in the group, all the remaining in-contact animals may be injected with an antimicrobial in a long-acting base. This may help to treat subclinical cases before they become clinical and thus control the outbreak.

Other Drugs

Nonsteroidal antiinflammatory drugs are useful in the treatment of infectious respiratory disease of cattle and horses, and likely other species. The drugs act by inhibiting

the inflammatory response induced by the infecting organism and tissue necrosis. Meloxicam (0.5 mg/kg subcutaneously, once), when administered with tetracycline, improves weight gain and reduces the size of lesions in lungs of cattle with bovine respiratory disease complex over those of animals treated with tetracycline alone. NSAIDs also improve the clinical signs of cattle with respiratory disease. Use of these drugs is routine in horses with pneumonia or pleuritis.

Corticosteroids have been used for their antiinflammatory effect in the treatment of acute pneumonia. However, there is no clinical evidence that they are beneficial, and they might be deleterious.

Bronchodilators have been investigated in the treatment of pneumonia in food animals. The **beta-2 adrenergic agonists** are potent and effective bronchodilators that can be administered orally, intravenously, or by inhalation. These drugs also enhance mucociliary clearance of material from the lungs. Most administration is orally or by inhalation. The use of beta-2 adrenergic agonist bronchodilator drugs in food animals is not permitted in most countries because of the risk of contamination of foodstuffs intended for consumption by people. This is particularly the case with clenbuterol, a drug approved in many countries for use in horses that is administered to cattle illicitly as a growth promoter. People can be poisoned by clenbuterol in tissues of treated cattle. Theophylline has been evaluated as a bronchodilator to relieve respiratory distress in cattle with pneumonia. When it was given orally at a dose of 28 mg/kg BW daily for 3 days, along with antimicrobial therapy, to calves with naturally acquired respiratory disease, the respiratory rate and rectal temperature decreased. However, some calves died, presumably from the accumulation of lethal concentrations of plasma theophylline. It is recommended that the drug should not be used unless plasma levels can be monitored.

Supportive Therapy and Housing

Affected animals should be housed in warm, well-ventilated, draft-free accommodation and provided with ample fresh water and light, nourishing food. During convalescence premature return to work or exposure to inclement weather should be avoided. If the animal does not eat, oral or parenteral force-feeding should be instituted. If fluids are given intravenously, care should be exercised over the speed with which they are administered. Injection at too rapid a rate may cause overloading of the right ventricle and death as a result of acute heart failure.

Supportive treatment might include the provision of oxygen, if it is available, especially in the critical stages when hypoxia is severe. In foals, the oxygen can be administered through an intranasal tube passed back to the nasopharynx and delivered at the rate of about 8 L/min for several hours. Oxygen therapy is detailed in the previous general section on treatment of respiratory disease.

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ACUTE RESPIRATORY DISTRESS SYNDROME

Acute respiratory distress syndrome is a well-recognized clinical syndrome of humans characterized by acute onset of hypoxemia and pulmonary infiltrates without increases in left atrial pressure (i.e., without evidence of cardiogenic pulmonary edema). Precipitating causes include both direct and indirect lung injury, including sepsis, multiple transfusions, trauma, near-drowning, smoke inhalation, pancreatitis, and more. The underlying lesion is diffuse alveolar capillary damage with secondary severe pulmonary edema. The disease occurs spontaneously in domestic animals, and although the spontaneous disease is not extensively documented, the disease produced experimentally as a model of the human disease is better described.

Acute respiratory distress syndrome (ARDS) in animals occurs in newborns and in adult animals. The disease in some newborn farm animals is related to lack of surfactant, but except for animals born prematurely, this is more the exception than the rule.[1] Most young animals and all adult animals with ARDS have some inciting acute lung injury that then progresses to ARDS. The causes can be infectious (e.g., influenza virus infection, leptospirosis,[2] porcine reproductive and respiratory syndrome virus infection[3]), physical (smoke inhalation or thoracic trauma[4]), toxic, or sepsis.[5-7]

The **pathophysiology** of the disease involves a common final pathway that results in damage to alveolar capillaries. The initial injury can be to either the endothelium of pulmonary capillaries or to alveolar epithelium. Damage to these structures leads to extravasation of protein-rich fluid and fibrin with subsequent deposition of hyaline membranes. The capillary injury is attributed to activated leukocytes (macrophages and neutrophils) and cytokines. Accumulation of hyaline membranes and ventilation/perfusion mismatches impair respiratory gas exchange and cause hypoxemia.

The **clinical signs** are characteristic of acute, progressive pneumonia. Animals are anxious, tachycardic, tachypneic and have crackles and wheezes on thoracic auscultation. Severely affected animals can be cyanotic. Thoracic radiographs reveal diffuse pulmonary infiltrates. Hematologic changes are characteristic of the inciting disease but usually include leukopenia. There is arterial hypoxemia.

Treatment includes administration of antiinflammatory drugs (NSAIDs with or without glucocorticoids), colloids, antimicrobials, and oxygen. The arterial blood gas response to oxygen therapy is often minimal in severely affected animals. If it is available, mechanical ventilation can be useful, although the prognosis is grave. Inhalation of nitric oxide is beneficial in some humans with the disease, and there are anecdotal reports that it has been used to treat foals with ARDS.

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ASPIRATION PNEUMONIA

Aspiration or inhalation pneumonia is a common and serious disease of farm animals caused by inhalation of ingesta, lipid, medications, meconium, or excessive dust. Cases occur after careless drenching, oral administration of medications,[1] or inadvertent intratracheal passage of an intended nasogastric tube during treatment for other illness, for example, administration of mineral oil to horses with colic. Even when care is taken these procedures are not without risk. Other causes include the feeding of calves and pigs on fluid feeds in inadequate troughing, with inhalation occurring in the struggle for food. Dipping of sheep and cattle when they are weak, or keeping their heads submerged for too long, also results in inhalation of fluid. Vomiting in ruminants can be followed by aspiration, especially in cattle with parturient paresis or during the passage of a stomach tube if the head is held high. Rupture of a pharyngeal abscess during palpation of the pharynx or passage of a nasal tube may cause sudden aspiration of infective material. Animals suffering from congenital defects[2]

and paralysis or obstruction of the larynx, pharynx, or esophagus can aspirate food or water when attempting to swallow. Esophageal obstruction is an important risk factor for aspiration pneumonia in horses (see following discussion), with 39 of 109 cases of esophageal obstruction developing aspiration pneumonia.[3] Bluetongue infection (BTV-12) of sheep causes myonecrosis of esophageal musculature and outbreaks of aspiration pneumonia.[4] Aspiration pneumonia is the consistent lesion of crude oil poisoning in cattle and probably results from vomiting or regurgitation.

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Lipid Pneumonia

Lipid pneumonia usually results from aspiration of mineral oil (liquid paraffin) administered for gastrointestinal disease.[1] Pneumonia is sometimes the result of inadvertent administration of the oil into the trachea through a misplaced stomach tube or inhalation during oral administration of oil. However, aspiration of oil can occur even when it is delivered into the stomach through a nasogastric tube, presumably because of regurgitation of oil either around the tube or after the tube has been removed. Administration of oil to sedated or severely depressed animals might increase the risk of aspiration.

Clinical signs include cough, tachypnea, tachycardia, pyrexia, respiratory distress, and abnormal lung sounds. Radiographs can reveal an alveolar and interstitial pattern. Examination of tracheal aspirates reveals a neutrophilic inflammation and the presence of lipid. Lipid can be readily identified by Sudan or oil red O staining of smears of the aspirate in acute cases. Necropsy examination reveals consolidated lungs. On cut section of these areas oil can be visible. Chronic cases have tissue necrosis and severe interstitial pneumonia. Lipid droplets can be identified in affected lung tissue after oil red O staining of sections. The presence and nature of the lipid can be demonstrated by thin-layer chromatography and gas chromatography. The prognosis for recovery is poor. Treatment is supportive and includes antiinflammatory drugs, antimicrobials, and oxygen. There is no specific treatment. Prevention includes careful insertion of nasogastric tubes, verification of their placement in the stomach, and not administering mineral oil to animals with a distended stomach or ones that are heavily sedated or severely depressed.

Esophageal Obstruction

Esophageal obstruction is a common and important cause of pneumonia in horses. Of 109 horses with esophageal obstruction, 39 had clinical signs of aspiration pneumonia.[2] Obstruction of the esophagus in horses, and in other species, leads to the accumulation of saliva and feed material in the esophagus oral to the obstruction. When the esophagus is full, this material accumulates in the pharynx, with subsequent aspiration into the trachea resulting in contamination of the trachea and lower airways with feed material and oropharyngeal bacteria. Feed material is irritant and also causes obstruction of the smaller airways. Pulmonary defense mechanisms are weakened or overwhelmed by the contamination, and infection and pneumonia result. The duration of esophageal obstruction is a good indicator of the risk of aspiration pneumonia, although the extent of contamination of the trachea with feed material is not. Affected horses are pyrexic, tachycardic, and toxemic. Lung sounds can include crackles and wheezes, but the only auscultatory abnormality can be decreased breath sounds in the ventral thorax. Radiography reveals a characteristic pattern of bronchopneumonia restricted, at least initially, to the cranioventral and caudoventral lung lobes in adult horses. Ultrasonography reveals comet tail lesions in the ventral lung fields and variable consolidation. Pleuritis is a not uncommon sequela to aspiration pneumonia. Examination of tracheal aspirates demonstrates neutrophilic inflammation with presence of degenerate neutrophils, bacteria that are both intracellular and extracellular, and plant material. Culture of tracheal aspirates yields one or more of a wide variety of bacteria, including *S. zooepidemicus, Pasteurella* sp., *Actinobacillus* sp., *E. coli,* and anaerobes. Treatment involves prompt relief of the esophageal obstruction and administration of broad-spectrum antimicrobials, such as a combination of penicillin, aminoglycoside, and metronidazole. The prognosis for recovery from aspiration pneumonia secondary to esophageal obstruction is guarded to fair, partly because the animal has to recover from two diseases—the pneumonia and the esophageal obstruction. Prevention of aspiration pneumonia in horses with esophageal obstruction includes prompt relief of the obstruction and administration of broad-spectrum antimicrobials.

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Meconium Aspiration Syndrome

Aspiration of meconium during parturition is associated with severe lung disease in newborns. Passage of meconium in utero, and subsequent aspiration by the fetus, is a sign of fetal distress. It is suggested that fetal distress results in expulsion of meconium into the amniotic fluid. This is followed by aspiration of contaminated amniotic fluid. The passage of meconium-contaminated amniotic fluid into the lungs may occur before birth when the fetus gasps for air in an attempt to correct hypoxemia or when the calf takes its first breath and aspirates meconium from the oropharynx. Normally, fetal aspiration of amniotic fluid does not occur because the inspiratory forces are insufficient to allow amniotic fluid to reach the lungs, and the lung liquid, a locally produced viscous material present in the trachea and lungs, constantly flows up the major airways to the oropharynx. The result is that the fetus is doubly challenged in that it must deal with both the cause of the fetal distress and the pneumonia induced by aspiration of meconium. Although meconium is sterile, it induces a severe inflammatory response in the lungs.

The **meconium aspiration syndrome** is best described in newborn calves,[1] although there are numerous reports of its experimental induction in piglets and lambs as a model of the human disease. In a series of calves under 2 weeks of age submitted to a diagnostic laboratory, 42.5% had evidence of meconium, squamous cells, or keratin in the lung. Diffuse alveolitis with exudation of neutrophils, macrophages, and multinucleated cells and obstruction of small airways with atelectasis were common.

Treatment of aspiration pneumonia in farm animals is not well described. Administration of antimicrobials is prudent. Antiinflammatory drugs are indicated. Pentoxifylline is used in human neonates with meconium aspiration, but there are no reports of its use for this purpose in farm animals.

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Dusty Feed

Although farm animals fed on dusty feeds inhale many dust particles and bacteria, which can be readily isolated from the lung, this form of infection rarely results in the development of pneumonia. Much of the dust is filtered out in the bronchial tree and does not reach the alveoli. However, this may be of importance in the production of the primary bronchiolitis that so often precedes alveolar emphysema in horses. The inhalation of feed particles in pigs in a very poorly ventilated environment has been demonstrated to cause foreign body pneumonia. Also, a dry, dusty atmosphere can be created in a piggery by overfrequent changing of wood shavings used as bedding, and this can lead to the production of foreign body pneumonia. Liquids and droplets penetrate to the depths of the alveoli and run freely into the dependent portions, and aspiration pneumonia often results.

DROWNING

Near drowning has been defined as survival following asphyxia and aspiration of water while submerged. Cases are rare in large

animals, but there is potential with the popularity of swimming as a method of exercising and training horses. There are cases of pneumonia after plunge dipping of sheep, which might be attributable to lung damage as a result of inhalation of dip water contaminated with bacteria.

The pulmonary responses to near drowning in sea water differ from that in fresh water.[1] Fresh water can inactivate pulmonary surfactant and lead to collapse of the alveolus with a loss of pulmonary compliance, and the resultant ventilation/perfusion mismatch coupled with alveolar damage can lead to severe hypoxemia. The inhalation of water may also carry bacteria and the risk of secondary bacterial aspiration pneumonia. Affected animals present with an elevated heart rate, tachypnea, and dyspnea. There is a decrease in normal airflow sounds on auscultation, which can occur in all areas of auscultation or be more pronounced in one lung, and rales or crackles may be heard in local areas. Consolidation may be detected with thoracic radiography. The mucous membranes may be congested, cyanotic, or muddy. Arterial blood gas analysis has shown a metabolic acidosis and hypoxemia. The response is typical of acute lung injury (acute respiratory distress syndrome).

Therapy has been based on experience with near drowning cases in humans; horses have been successfully treated by nasal insufflation of humidified oxygen, the correction of the base deficit with sodium bicarbonate and lactated Ringer's solutions administered intravenously, treatment with bronchodilators and nonsteroidal antiinflammatory drugs, and pulmonary infusion with a surfactant transplant from a recently euthanized horse. Antibacterials are given to cover the risk or the presence of a bacterial pneumonia, and the cover should include the possibility of infection with anaerobic organisms. Respiratory distress can be more severe when the animals are recumbent. Near drowning requires immediate and aggressive therapy, and the recovery can be prolonged.

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PULMONARY ABSCESS

The development of single or multiple abscesses in the lung causes a syndrome of chronic toxemia, cough, and emaciation. Abscesses can be solitary, multiple, military, or coalescing. Small solitary abscesses can be clinically silent, with clinical signs becoming more apparent at the extent of the lesions increases.

ETIOLOGY

Pulmonary abscesses can be part of a primary disease or arise secondarily to diseases in other parts of the body.

Primary Diseases

- *R. equi* pulmonary abscesses of foals
- *S. zooepidemicus* and *Actinobacillus* sp. in adult horses—one-third of infectious causes of abscesses in horses are polymicrobial, and anaerobic bacteria are isolated in 20% of cases
- Solitary abscess associated with strangles in horses, caseous lymphadenitis in sheep
- Tuberculosis
- Actinomycosis rarely occurs as granulomatous pulmonary lesions
- Aerogenous infections with "systemic" mycoses (e.g., coccidioidomycosis, aspergillosis, histoplasmosis, cryptococcosis, and moniliasis)
- *Helcococcus ovis* in horses and goats[1]
- *Mycoplasma bovis* in cattle

Secondary Diseases

- Sequestration of an infected focus of pneumonia (e.g., bovine pleuropneumonia or pleuropneumonia in horses)
- Pulmonary abscesses secondary to ovine estrosis
- Emboli from endocarditis, caudal or cranial vena caval thrombosis, metritis, mastitis, omphalophlebitis[2,3]
- Rumenitis is strongly associated with development of liver abscesses, which in turn are risk factors for lung abscesses in cattle. Of cattle with liver abscesses, 14% have severe lung lesions, and 28% have mild lung lesions.[4]
- Aspiration pneumonia from milk fever in cows, drenching accident in sheep—in which case the abscess is a manifestation of aspiration pneumonia.
- Penetration by foreign body, such as in traumatic reticuloperitonitis, inhalation of a foreign body, or unusual causes such as diaphragmatic hernia with ileal diverticulitis causing a lung abscess[5]

PATHOGENESIS

Pulmonary abscesses are present in many cases of pneumonia and are not recognizable as clinically distinct entities. In the absence of pneumonia, pulmonary abscess is usually a chronic disease, with clinical signs being produced by toxemia rather than by interference with respiration. However, when the spread is hematogenous and large numbers of small abscesses develop simultaneously, respiratory function can be compromised to the extent that it becomes clinically apparent. However, in more chronic cases the abscesses can reach a size sufficient to cause respiratory difficulty by obliteration of large areas of lung tissue. In rare cases, erosion of a pulmonary vessel occurs, resulting in pulmonary hemorrhage and hemoptysis.

In many cases there is a period of chronic illness of varying degree when the necrotic focus is walled off by connective tissue. Exposure to environmental stress or other infection can result in a sudden extension from the abscess to produce a fatal suppurative bronchopneumonia, pleurisy, or empyema.

CLINICAL FINDINGS

In typical cases there is dullness, anorexia, emaciation, and a fall in milk yield in cattle. The temperature is usually moderately elevated and fluctuating. Coughing is marked. The cough is short and harsh and usually not accompanied by signs of pain. Intermittent episodes of bilateral epistaxis and hemoptysis can occur and terminate in fatal pulmonary hemorrhage following erosion of an adjacent large pulmonary vessel. Respiratory signs are variable depending on the size of the lesions, and although there is usually some increase in the rate and depth, this may be so slight as to escape notice. When the abscesses are large (2-4 cm in diameter), careful auscultation and percussion will reveal the presence of a circumscribed area of dullness over which no breath sounds are audible. Crackles are often audible at the periphery of the lesion.

Multiple small abscesses may not be detectable on physical examination, but the dyspnea is usually more pronounced. There can be a purulent nasal discharge and fetid breath, but these are unusual unless bronchopneumonia has developed from extension of the abscess. Radiographic examination can be used to detect the presence of the abscess and give some information on its size and location. Ultrasonographic examination is sensitive and specific in detecting lung abscesses in foals and is useful in adult horses and other species.[6]

Most cases progress slowly, and many affected animals have to be euthanized because of chronic ill-health; others die of bronchopneumonia or emphysema. Persistent fever, tachycardia, and polypnea are common. A rare sequela is the development of hypertrophic pulmonary osteoarthropathy.

The clinical findings of *R. equi* pulmonary abscessation in young foals are presented under that disease.

Solitary lung abscesses are not uncommon in adult horses. Presenting signs are usually low-grade fever and depression. Most horses with lung abscesses cough. There is excessive mucopurulent material in the trachea, and examination of a tracheal aspirate reveals neutrophilic inflammation. Radiographic examination of the chest demonstrates the presence of one or more abscesses. Abscesses are in the caudal lung lobes in 60% of cases. Ultrasonography can be useful in detecting the abscess, provided

that it is confluent with the visceral pleura. The prognosis for life and for return to racing is excellent in horses that are treated appropriately.

CLINICAL PATHOLOGY

Examination of nasal or tracheal mucus may determine the causative bacteria, but the infection is usually mixed, and interpretation of the bacteriologic findings is difficult. Culture of tracheal aspirates yields growth of pathogenic bacteria in approximately 70% of samples from horses with lung abscesses. Hematologic examination may give an indication of the severity of the inflammatory process, but the usual leukocytosis and shift to the left might not be present when the lesion is well encapsulated. In lung abscesses in foals and adult horses, hyperfibrinogenemia and neutrophilic leukocytosis are common.

NECROPSY FINDINGS

An accumulation of necrotic material in a thick-walled fibrous capsule is usually present in the ventral border of a lung, surrounded by a zone of bronchopneumonia or pressure atelectasis. In sheep there is often an associated emphysema. In rare cases the abscess may be sufficiently large to virtually obliterate the lung. A well-encapsulated lesion may show evidence of recent rupture of the capsule and extension as an acute bronchopneumonia. Multiple small abscesses may be present when hematogenous spread has occurred.

DIFFERENTIAL DIAGNOSIS

The diagnosis might not be obvious when respiratory distress is minimal, and especially when multiple small abscesses are present. These cases present a syndrome of chronic toxemia, which may be mistaken for splenic or hepatic abscess. Differentiation between tuberculous lesions and nonspecific infections may require the use of the tuberculin test. Focal parasitic lesions, such as hydatid cysts, can cause a similar syndrome, but are not usually accompanied by toxemia or hematologic changes. Pulmonary neoplasms usually cause chronic respiratory disease, a progressive loss of weight, and lack of toxemia.

TREATMENT

Pulmonary abscesses secondary to pneumonia in cattle and pigs are usually not responsive to therapy. The daily administration of large doses of antimicrobials for several days can be attempted but is usually not effective, and slaughter for salvage or euthanasia is necessary. Treatment of pulmonary abscesses in adult horses by administration of broad-spectrum antimicrobials is usually effective. Most (>80%) racehorses with single abscesses return to racing.

Diagnosis and treatment of pulmonary abscess and bronchopleural fistula can be achieved by thoracoscopy or thoracotomy and partial pneumonectomy.[7] As noted previously, almost all horses with solitary pulmonary abscesses recover with antimicrobial therapy.

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Diseases of the Pleural Cavity and Diaphragm

HYDROTHORAX AND HEMOTHORAX

The accumulation of edematous transudate or whole blood in the pleural cavities is manifested by respiratory embarrassment caused by collapse of the ventral parts of the lungs.

ETIOLOGY

Hydrothorax and hemothorax occur as part of a number of diseases. Hemothorax can involve rupture of vessels or leakage of blood from abnormal tissues or result from prolonged clotting times. Hydrothorax is a result of excessive accumulation of transudate secondary to altered Starling's forces or to chylothorax.

Hydrothorax

- As part of a general edema resulting from congestive heart failure or hypoproteinemia
- As part of African horse sickness or bovine viral leukosis
- Chylous hydrothorax, very rarely as a result of ruptured thoracic duct
- Secondary to thoracic neoplasia
- Yellow wood (*Terminalia oblongata*) poisoning of sheep
- Dilated cardiomyopathy of Holstein Friesian cattle

Hemothorax

- Traumatic injury to thoracic wall, a particular case of which is rib fractures in newborn foals[1,2]
- Hemangiosarcoma of pleura[3]
- Lung biopsy
- Strenuous exercise (racing) by horses.—intrathoracic, but extrapulmonary, hemorrhage caused death in 6 of 143 Thoroughbred racehorses that died while racing.[4]
- Administration of phenylephrine (intravenously) to horses with left dorsal displacement (nephrosplenic entrapment) of the colon[5]
- Prolonged blood clotting times

PATHOGENESIS

Accumulation of fluid in the pleural cavities causes compression atelectasis of the ventral portions of the lungs, and the degree of atelectasis governs the severity of the resulting dyspnea. Compression of the atria by fluid may cause an increase in venous pressure in the great veins, decreased cardiac return, and reduced cardiac output. Extensive hemorrhage into the pleural space can cause hemorrhagic shock.

CLINICAL FINDINGS

Clinical signs depend on the evolution of the disease. Acute, severe hemothorax, such as occurs with penetrating injury, during racing, or associated with administration of phenylephrine, presents as sudden death or with signs of acute, severe hemorrhagic shock. Hemorrhage of lesser severity causes increased heart and respiratory rates, pale mucous membranes, and exercise intolerance.

Hydrothorax develops more slowly, and often there is an absence of systemic signs. There can be dyspnea, which usually develops gradually, and an absence of breath sounds, accompanied by dullness on percussion over the lower parts of the chest. In thin animals, the intercostal spaces might be observed to bulge. If sufficient fluid is present, it causes compression of the atria and engorgement of the jugular veins and increased amplitude of the jugular pulse. The cardiac embarrassment is not usually sufficiently severe to cause congestive heart failure, although this disease can already be present.

The accumulation of pleural fluid or blood is evident on radiographic or ultrasonographic examination of the thorax. Large quantities of blood in the pleural cavity have a characteristic swirling, turbulent appearance.

CLINICAL PATHOLOGY

Thoracocentesis will yield a flow of clear serous fluid in hydrothorax, or blood in recent cases of hemothorax. The fluid is bacteriologically negative, and total nucleated cell counts are low ($< 5 \times 10$/L, $< 5000 \times 10$/dL). The pH, P_{CO_2}, and lactate and glucose concentrations of pleural fluid in animals with hydrothorax are similar to those of blood.

NECROPSY FINDINGS

In animals that die of acute hemorrhagic shock resulting from hemothorax, the

pleural cavity is filled with blood, which usually has not clotted. Hydrothorax is not usually fatal but is a common accompaniment of other diseases, which are evidenced by their specific necropsy findings.

DIFFERENTIAL DIAGNOSIS

Hydrothorax and hemothorax can be differentiated from pleurisy by the absence of pain, toxemia, and fever and by the sterility of an aspirated fluid sample.

TREATMENT

Treatment of the primary condition is necessary. If the dyspnea is severe, aspiration of fluid from the pleural space causes a temporary improvement, but the fluid usually reaccumulates rapidly. Parenteral coagulants and blood transfusion are rational treatments in severe hemothorax.

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PLEURITIS (PLEURISY)

Pleuritis refers to inflammation of the parietal and visceral pleura. Inflammation of the pleura almost always results in accumulation of fluid in the pleural space. Pleuritis is characterized by varying degrees of toxemia, painful shallow breathing, pleural friction sounds, and dull areas on acoustic percussion of the thorax because of pleural effusion. Treatment is often difficult because of the diffuse nature of the inflammation.

ETIOLOGY

Pleuritis is almost always associated with diseases of the lungs. Pneumonia can progress to pleuritis, and pleuritis can cause consolidation and infection of the lungs. Primary pleuritis is usually caused by perforation of the pleural space, such as by a penetrating thoracic injury, and subsequent infection. Most commonly this occurs as a result of trauma, but it can occur in cattle with traumatic reticuloperitonitis and in any species after perforation of the thoracic esophagus.

Secondary pleuritis refers to that which develops from infectious lung disease subsequent to the following conditions. As with pneumonia, the classic triad of host–pathogen(s)–environment is present in development of pleuritic in any species. Risk factors (see following discussion) of crowding, temperature, housing, age, and weight all contribute to increased susceptibility to the disease when the animal is exposed to one or more potential pathogens. Etiologic agents associated with pleuritis or disease syndromes involving pleuritis for specific animals are as follows.

Pigs

- Glasser's disease
- Pleuropneumonia associated with *Actinobacillus* (*Haemophilus*) *pleuropneumoniae* and *Haemophilus influenzae suis*[1,2]

Cattle

- Secondary to *Mannheimia haemolytica* pneumonia in cattle, especially feedlot cattle, which can be related to a high percentage of fibrotic pleural lesions found in adult cattle examined at the abattoir
- Infection of calves by *Pasteurella multocida* type B[3]
- Tuberculosis
- Sporadic bovine encephalomyelitis
- Contagious bovine pleuropneumonia
- *Histophilus somni* infection

Sheep and Goats

- Pleuropneumonia associated with *Mycoplasma* spp., including *Mycoplasma mycoides* subsp. *mycoides* and *Haemophilus* spp.
- Caprine pleuropneumonia (*Mycoplasma capricolum* subsp. *capripneumoniae*)[4]
- *Streptococcus dysgalactiae* in ewes
- *Helcococcuss ovis* in sheep and goats[5,6]

Horses

The disease in horses is discussed separately in the next section. Rare causes of pleurisy and pleural effusion in horses include lymphosarcoma and equine infectious anemia. Mesothelioma of the pleura causing persistent dyspnea, pleural effusion, and death is also recorded in the horse. Thoracic hemangiosarcoma is recorded as a cause of chylothorax in the horse.

Other Causes

Sporadic and nonspecific diseases may be accompanied by pleurisy. Examples include septicemias as a result of *Pseudomonas aeruginosa* and bacteremia with localization causing a primary septic pleural effusion. In horses, the infection is usually *S. equi,* and the original disease is strangles. In goats, it is usually spread from a mycoplasmal pneumonia.

Perforation of the diaphragm occurs in **traumatic reticuloperitonitis** in cattle and goats. Spread into the pleural cavity can occur without actual penetration of the diaphragm because it enters via the lymphatics. Abomasopleural fistula secondary to abomasal ulceration can cause pleuritis in cattle.

Chronic pleuritis is an important cause of loss in commercial **piggeries**. The prevalence can be as low as 5.6% of pigs at slaughter in specific-pathogen-free piggeries and as high as 27% in conventional piggeries. In piggeries with a high incidence of pleuritis, 45% of lungs examined at slaughter had gross lesions of the chronic disease.[1] Examination of a larger number of animals (~4900) from 48 herds revealed a similar frequency of lesions of chronic pleuritic.[7] Risk factors for pleuritic in pigs include the following:[7] risk of a high pleuritic score was increased when the farrowing facilities were not disinfected (odds ratio [OR] = 2.7, 95% confidence interval [CI]:1.2-5.8, $p = 0.01$), when tail docking was performed later than 1.5 days after birth (OR = 2.6, 95% CI: 1.2-5.7, $p = 0.01$), when piglets were castrated at more than 14 days old (OR = 2.7, 95%CI: 1.1-6.8, $p = 0.03$), a temperature range of less than 5°C (41 F) for the ventilation control rate in the farrowing room (OR = 2.7, 95% CI: 1.2-5.9, $p = 0.01$), a mean temperature in the finishing room less than 23°C (OR = 3.0, 95% CI: 1.3-6.8, $p < 0.01$), and large herd size (OR = 3.1, 95% CI: 1.4-6.9, $p < 0.01$). The factors affecting pneumonia and pleuritis seemed to be different.[7]

Lesions consistent with pleuritic are common at time of slaughter in **veal calves**, with one study of calves from 174 farms in France, Belgium, and Italy reporting prevalence of 21% and another of 5825 calves reporting prevalence of 19% in 91 calves examined postmortem.[8,9] Risk factors for increased likelihood of lesions included lower calf weight on arrival at the farm, greater number of calves per pen, presence of slatted or rubber flooring (compared with concrete), and season.[8]

PATHOGENESIS

Contact and movement between the parietal and visceral pleura causes pain as a result of stimulation of pain end organs in the pleura. Respiratory movements are restricted, and the respiration is rapid and shallow. There is production of serofibrinous inflammatory exudate, which collects in the pleural cavities and causes collapse of the ventral parts of the lungs, thus reducing vital capacity and interfering with gaseous exchange. If the accumulation is sufficiently severe, there may be pressure on the atria and a diminished return of blood to the heart. Clinical signs may be restricted to one side of the chest in all species with an imperforate mediastinum. Fluid is resorbed in animals that survive the acute disease, and adhesions develop, restricting movement of the lungs and chest wall, but interference with respiratory exchange is usually minor and disappears gradually as the adhesions stretch with continuous movement.

In all bacterial pleuritis, toxemia is common and usually severe. The toxemia may be severe when large amounts of pus accumulate.

CLINICAL FINDINGS

The clinical findings of pleuritis vary from mild to severe depending on the species and the nature and severity of the inflammation. In peracute to acute stages of pleuropneumonia, there are **fever**, **toxemia**, **tachycardia**, **anorexia**, **depression**, **nasal discharge**, **coughing**, **exercise intolerance**, **breathing distress**, and **flared nostrils**. The nasal discharge depends on the presence or absence of pneumonia. It may be absent or copious, and its nature may vary from mucohemorrhagic to mucopurulent. The odor of the breath can be putrid, which is usually associated with an anaerobic lesion.

Pleural Pain

Pleural pain (pleurodynia) is common and manifested as pawing, stiff forelimb gait, abducted elbows, and reluctance to move or lie down. In the early stages of pleuritis, breathing is rapid and shallow and, markedly abdominal, and movement of the thoracic wall is restricted. The breathing movements may appear guarded, along with a catch at end inspiration. The animal stands with its elbows abducted and is disinclined to move. The application of hand pressure on the thoracic wall and deep digital palpation of intercostal spaces usually causes pain manifested by a grunt, a spasm of the intercostal muscles, or an escape maneuver.

Pleuritic Friction Sounds

Pleuritic friction sounds may be audible over the thoracic wall. They have a continuous to-and-fro character, are dry and abrasive, and do not abate with coughing. They may be difficult to identify if there is a coincident pneumonia accompanied by loud breath sounds and crackles. When the pleuritis involves the pleural surface of the pericardial sac, a friction rub may be heard with each cardiac cycle and be confused with the friction sound of pericarditis. However, there is usually, in addition, a friction sound synchronous with respiratory movements, and the pericardial rub waxes and wanes with expiration and inspiration. Pleural friction rubs are audible only during the initial stages of the disease; they are not audible when fluid accumulates in the pleural space.

Subcutaneous Edema

Subcutaneous edema of the ventral body wall extending from the pectorals to the prepubic area is common in horses with severe pleuritis but is less noticeable in other species. Presumably this edema is attributable to blockage of lymphatics normally drained through the sternal lymph nodes.

Pleural Effusion

In **cattle**, **an inflammatory pleural effusion** is often limited to one side because the pleural sacs do not communicate. Bilateral pleural effusion might indicate either a bilateral pulmonary disease process or a noninflammatory abnormality such as right-sided congestive heart failure or hypoproteinemia.

Dullness on acoustic percussion over the fluid-filled area of the thorax is characteristic of pleuritis in which there is a significant amount of pleural effusion. The dull area has a **horizontal level topline**, called a **fluid line**, which can be demarcated by acoustic percussion. As exudation causes separation of the inflamed pleural surfaces and the pleural effusion accumulates, the pain and friction sounds diminish but do not completely disappear. On auscultation there may still be pleuritic friction sounds, but they are less evident and usually localized to small areas.

In the presence of a pleural effusion, both normal and abnormal lung sounds are diminished in intensity, depending on the amount of the effusion. Dyspnea may still be evident, particularly during inspiration, and a pleuritic ridge develops at the costal arch as a result of elevation of the ribs and the abdominal-type respiration. However, the degree of dyspnea is often subtle, and careful clinical examination and counting of the breathing rate is necessary to detect the changes in breathing.

If the pleurisy is unilateral, movement of the affected side of the thorax is restricted compared with the normal side. In cattle, the pleural effusion is commonly unilateral on the right side, but both sides may be affected. Pain is still evident on percussion on deep palpation of the intercostal spaces, and the animal still stands with its elbows abducted, is disinclined to lie down or move, but is not as apprehensive as in the early stages. Toxemia is often more severe during this stage, the temperature and the heart rate are usually above normal, and the appetite is poor. A cough will be present if there is a concurrent pneumonia, and it is painful, short and shallow. Extension of the inflammation to the pericardium may occur. Death may occur at any time and is attributable to a combination of toxemia and anoxia caused by pressure atelectasis.

Recovery

Animals with pleuritis characteristically recover slowly over a period of several days or even weeks. The toxemia usually resolves first, but abnormalities in the thorax remain for some time because of the presence of adhesions and variable amounts of pleural effusion in the loculi. Rupture of the adhesions during severe exertion may cause fatal hemothorax. Some impairment of respiratory function can be expected to persist, and racing animals do not usually regain complete efficiency. Chronic pleurisy, as occurs in tuberculosis in cattle and in pigs, is usually subclinical, with no acute inflammation or fluid exudation occurring.

Medical Imaging

Radiographic examination may reveal the presence of a fluid line and fluid displacement of the mediastinum and heart to the unaffected side and collapse of the lung. However, in cattle, pleural effusion cannot be located precisely by radiography because only laterolateral radiographs of the thorax can be taken. Ultrasonography is superior for the visualization of small volumes of pleural fluid that cannot be detected by auscultation and acoustic percussion of the thorax.

Ultrasonography

Ultrasonography is more reliable for the detection of pleural fluid in horses and cattle than radiography. Pleural fluid is easily detected as hypoechoic to anechoic fluid between the parietal pleural surface, diaphragm, and lung. Transudative pleural fluid appears homogeneously anechoic to hypoechoic. Exudative fluid is commonly present in horses and cattle with pleuropneumonia and often contains echogenic material. Serosanguineous or hemorrhagic fluid is also more echogenic than transudates. Fibrin appears as filmy and filamentous strands floating in the effusion with loose attachments to the pleural surfaces. Pockets of fluid loculated by fibrin are commonly imaged in horses with fibrinous pleuropneumonia. Adhesions appear as echogenic attachments between the parietal and visceral pleural surfaces; the adhesions restrict independent motion of the surfaces. The presence of small, bright echoes (gas echoes) swirling in pleural or abscess fluid is associated with anaerobic infection of the pleural cavity. Gas echoes are usually most abundant in the dorsal aspects of the pleural cavity. Other lung and pleural abnormalities that may be visualized include compression atelectasis, consolidation, abscesses, and displacement of the lung as pleural effusion accumulates.

Pleuroscopy

Pleuroscopy using a rigid or flexible fiberoptic endoscope allows direct inspection of the pleural cavity. The endoscope is introduced into the pleural cavity in the 10th intercostal space just above the point of the shoulder. The lung will collapse, but pneumothorax is minimized by the use of a purse-string suture placed around the stab incision and blunt dissection of the fascia and muscle layers for insertion of the endoscope. The diaphragm, costosplenic angle, aorta, mediastinal structures, and thoracic wall are clearly visible. By entering the thorax at different locations, the

ventral lung, the pericardium, and more of the diaphragm can be visualized. Lung and pleural abscesses and pleural adhesions may be visible.

Prognosis

The prognosis depends on the severity and extent of the pleuritis and the presence of pneumonia. If the disease is in an advanced stage when first recognized and there is extensive fibrinous inflammation, the response to treatment can be protracted and extensive long-term daily care will be necessary. Also, the common failure to culture the primary causative agent, particularly in horses, makes specific therapy difficult.

CLINICAL PATHOLOGY

Thoracocentesis (Pleurocentesis)

Thoracocentesis to obtain a sample of the fluid for laboratory examination is necessary for a definitive diagnosis. The fluid is examined for its odor, color and viscosity, protein concentration, and presence of blood or tumor cells, and it is cultured for bacteria. It is important to determine whether the fluid is an exudate or a transudate. Pleural fluid from horses affected with anaerobic bacterial pleuropneumonia may be foul smelling. Examination of the pleural fluid usually reveals an increase in leukocytes up to 40,000 to 100,000/µL and protein concentrations of up to 50 g/L (5.0 g/dL). The fluid should be cultured for both aerobic and anaerobic bacteria and *Mycoplasma* spp.

Hematology

In peracute bacterial pleuropneumonia in horses and cattle, leukopenia and neutropenia with toxic neutrophils are common. In acute pleuritis with severe toxemia, hemoconcentration, neutropenia with a left shift and toxic neutrophils are common. In subacute and chronic stages, normal to high leukocyte counts are often present. Hyperfibrinogenemia, decreased albumin–globulin ratio, and anemia are common in chronic pleuropneumonia.

NECROPSY FINDINGS

In early acute pleurisy, there is marked edema, thickening, and hyperemia of the pleura, with engorgement of small vessels and the presence of tags and shreds of fibrin. These can most readily be seen between the lobes of the lung. In the exudative stage, the pleural cavity contains an excessive quantity of turbid fluid containing flakes and clots of fibrin. The pleura is thickened and the central parts of the lung are collapsed and dark red in color. A concurrent pneumonia is usually present, and there may be an associated pericarditis. In the later healing stages, adhesions connect the parietal and visceral pleurae. Type I fibrinous adhesions appear to be associated with pneumonia, whereas type II fibrinous proliferative adhesions are idiopathic.

DIFFERENTIAL DIAGNOSIS

The diagnosis of pleuritis is confirmed by the following:

- The presence of inflammatory fluid in the pleural cavity
- Pleural friction sounds, common in the early stages of pleuritis and loud and abrasive; they sound very close to the surface, do not fluctuate with coughing common in the early stages, and may continue to be detectable throughout the effusion stage.
- The presence of dull areas and a horizontal fluid line on acoustic percussion of the lower aspects of the thorax, characteristic of pleuritis and the presence of pleural fluid.
- Thoracic pain, fever, and toxemia are common.

Pneumonia occurs commonly in conjunction with pleuritis, and differentiation is difficult and often unnecessary. The increased intensity of breath sounds associated with consolidation and the presence of crackles and wheezes are characteristic of pneumonia.

Pulmonary emphysema is characterized by loud crackles, expiratory dyspnea, hyperresonance of the thorax, and lack of toxemia unless associated with bacterial pneumonia.

Hydrothorax and hemothorax are not usually accompanied by fever or toxemia, and pain and pleuritic friction sounds are not present. Aspiration of fluid by needle puncture can be attempted if doubt exists. A pleural effusion consisting of a transudate may occur in cor pulmonale as a result of chronic interstitial pneumonia in cattle.

Pulmonary congestion and edema are manifested by increased vesicular murmur and ventral consolidation without hydrothorax or pleural inflammation.

TREATMENT

The principles of treatment of pleuritis are pain control, elimination of infection, and prevention of complications.

Antimicrobial Therapy

The primary aim of treatment is to control the infection in the pleural cavities using the systemic administration of antimicrobials, which should be selected on the basis of culture and sensitivity of pathogens from the pleural fluid. Before the antimicrobial sensitivity results are available, it is recommended that broad-spectrum antimicrobials be used. Antimicrobial therapy can be required for several weeks.

Drainage and Lavage of Pleural Cavity

Drainage of pleural fluid removes exudate from the pleural cavity and allows the lungs to reexpand. Criteria for drainage include the following:

- An initial poor response to treatment
- Large quantities of fluid causing respiratory distress
- Putrid pleural fluid
- Bacteria in cells of the pleural fluid

Clinical experience suggests that drainage improves the outcome.

Pleural fluid can be drained using intermittent thoracocentesis or indwelling chest tubes. Intermittent drainage is satisfactory in an animal with a small amount of fluid. Small (12-20 French) chest tubes are temporarily inserted at 2- to 3-day intervals to remove the fluid. Aspiration may not be easy in some cases because the drainage tube may become blocked with fibrin, and respiratory movements may result in laceration of the lung. Drainage may be difficult or almost impossible in cases in which adhesion of visceral and parietal pleura are extensive and fluid is loculated.

Indwelling chest tubes may be required unilaterally or bilaterally depending on the patency of mediastinal fenestration and the degree of fluid loculation. A large-bore (24-32 French) chest tube is inserted and secured to prevent it from sliding out. Unidirectional drainage through the tube is facilitated by a Heimlich valve and monitored regularly. Pleural fluid is allowed to drain or drip passively because suction often results in obstruction of the tube with fibrin or peripheral lung tissue. Loculation of fluid may interfere with proper drainage and necessitate replacement of tubes. Complications include subcutaneous cellulitis or pneumothorax.

Pleural lavage may assist in removal of fibrin, inflammatory debris, and necrotic tissue; it can prevent loculation, dilute thick pleural fluid, and facilitate drainage (Fig. 12-11). One chest tube is placed dorsally and one ventrally using ultrasonographic guidance; 5 to 10 L of sterile, warm isotonic 0.9% NaCl solution is infused into each hemithorax by gravity flow. After infusion, the dorsal chest tube is capped, the ventral chest tube is reconnected to a unidirectional valve, and the lavage fluid is allowed to drain.

Thoracotomy has been used successfully for the treatment of pericarditis and pleuritis and lung abscesses in cattle. Claims are made for the use of dexamethasone at 0.1 mg/kg BW IV or IM to reduce the degree of pleural effusion. In acute cases of pleurisy in the horse, analgesics such as phenylbutazone are valuable to relieve pain and anxiety, allowing the horse to eat and drink more normally.

Fibrinolytic Therapy

Pleural adhesions are unavoidable and may become thick and extensive with the formation of loculation, which traps pleural fluid, all of which prevent full recovery. However, some animals will stabilize at a certain level

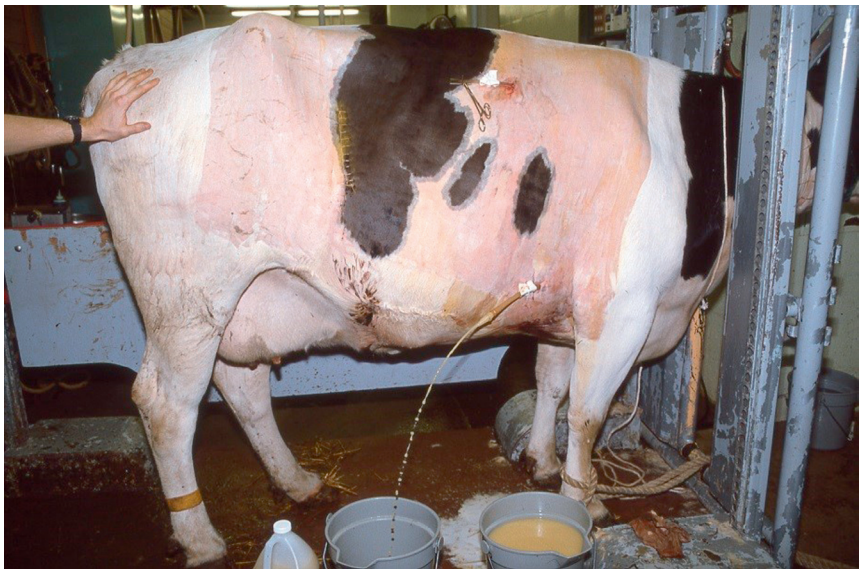

Fig. 12-11 **Pleural lavage of the right pleural cavity of a Holstein Friesian cow with septic pleuritis secondary to a localized abomasal perforation and subsequent development of an abomasal diaphragmatic fistula. Note the dorsal ingress chest tube that is clamped and pleural fluid drainage via the ventral egress chest tube. Ten liters of warmed sterile 0.9% NaCl solution was infused dorsally, and 20 L of pleural fluid was removed via the ventral tube.**

of chronicity, will survive for long periods, and may be useful for light work or as breeding animals. Fibrinolytic agents such as streptokinase have been used in human medicine to promote the thinning of pleural fluid, provide enzymatic debridement of the pleurae, lyse adhesions, and promote drainage of loculi. As reviewed under "Equine Pleuropneumonia" in this chapter, fibrinolytic therapy using recombinant tissue plasminogen activator appears promising in reducing the amount of accumulated fibrin and in hastening recovery of horses with pleuropneumonia.[10-12]

Prevention of the disease is achieved by prevention of exposure to etiologic agents that cause pleuritis, through specific-pathogen-free piggeries, vaccination,[7] and reduction of the effect of noninfectious risk factors.

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PNEUMOTHORAX

Pneumothorax refers to the presence of air (or other gas) in the pleural cavity. Entry of air into the pleural cavity in sufficient quantity causes collapse of the lung and impaired respiratory gas exchange, with consequent respiratory distress.

ETIOLOGY

Pneumothorax is defined as either spontaneous, traumatic, open, closed, or tension. Spontaneous cases occur without any identifiable inciting event. Open pneumothorax describes the situation in which gas enters the pleural space other than from a ruptured or lacerated lung, such as through an open wound in the chest wall. Closed pneumothorax refers to gas accumulation in the pleural space in the absence of an open chest wound. Tension pneumothorax occurs when a wound acts as a one-way valve, with air entering the pleural space during inspiration but being prevented from exiting during expiration by a valve-like action of the wound margins. The result is a rapid worsening of the pneumothorax. Pneumothorax can be unilateral or bilateral. The complete mediastinum of most cattle and some horses means that in many instances pneumothorax is unilateral, provided that the leakage of air into the pleural space occurs on only one side of the chest.

Rupture of the lung is a common cause of pneumothorax and can be either secondary to thoracic trauma, for example, a penetrating wound that injures the lung, or lung disease. Most cases of pneumothorax in cattle are associated with pulmonary disease, notably bronchopneumonia and interstitial pneumonia. Pleuropneumonia is the most common cause of pneumothorax in horses. Pneumothorax in these instances results from "spontaneous" rupture of weakened lung or development of bronchopleural fistulae.

Trauma to thoracic wall can lead to pneumothorax when a wound penetrates the thoracic wall, including the parietal pleura. In cattle, the thoracic wall may be punctured accidentally by farm machinery being used around cattle, as, for example, when bales of hay are being moved among animals. Penetrating wounds of the thoracic wall are common causes in horses that impale themselves on fence posts and other solid objects. A special case of perforating lung injury occurs in newborns in which the rib is fractured during birth and the lung lacerated by the sharp edges of the fractured rib.[1] Bullet and arrow wounds to the chest are not uncommon causes of pneumothorax in regions in which hunting is common.

Pneumothorax also occurs during thoracotomy, thoracoscopy, lung or liver biopsy (in which there is inadvertent damage to the lung), or drainage of pleural or pericardial fluid. Pneumothorax can result from injury or surgery to the upper respiratory tract, presumably because of migration of air around the trachea into the mediastinum and subsequent leakage into the pleural space. Similarly, subcutaneous emphysema, such as commonly occurs with wounds to the axilla, leads to pneumothorax via the mediastinum.[2]

PATHOGENESIS

Entry of air into the pleural cavity results in collapse of the lung. There can be partial or complete collapse of the lung. Collapse of the lung results in alveolar hypoventilation, hypoxemia, hypercapnia, cyanosis, dyspnea, anxiety, and hyperresonance on percussion of the affected thorax. Tension pneumothorax can also lead to a direct decrease in venous return to the heart by compression and collapse of the vena cava.

The degree of lung collapse varies with the amount of air that enters the cavity; small amounts are absorbed promptly, but large amounts compromise tidal volume, minute volume, and gas exchange and can result in asphyxiation.

CLINICAL FINDINGS

There is an acute onset of inspiratory dyspnea, which may terminate fatally within a few minutes if the pneumothorax is bilateral and severe. If the collapse occurs in only one pleural sac, the ribcage on the affected side collapses and shows decreased movement. There is a compensatory increase in movement and bulging of the chest wall on the unaffected side. On auscultation of the thorax, the breath sounds are markedly decreased in intensity and commonly absent. The mediastinum may bulge toward the unaffected side and may cause moderate displacement of the heart and the apex beat,

with accentuation of the heart sounds and the apex beat. The heart sounds on the affected side have a metallic note, and the apex beat may be absent. On percussion of the thorax on the affected side, a hyperresonance is detectable over the dorsal aspects of the thorax.

Affected animals are anxious, tachypneic and in variable degrees of respiratory distress. Because many cases of pneumothorax in cattle and horses are secondary to lung disease, particularly infectious lung disease, there are usually signs of the inciting disease, including fever, toxemia, purulent nasal discharge, and cough. Pneumothorax secondary to chest wall trauma is usually readily apparent, although fractured ribs that lacerate the lung and cause pneumothorax or hemothorax can be easily missed on physical examination, especially in newborns.

Definitive diagnosis is based on demonstration of pneumothorax by radiographic or ultrasonographic examination. Radiography permits the detection of bilateral and unilateral pneumothorax and permits identification of other air leakage syndromes, including pneumomediastinum, pneumoperitoneum, and pneumopericardium. Many cattle with pneumonia and pneumothorax have radiographic evidence of emphysematous bullae. Ultrasonography is also useful in determining the extent of pneumothorax and the presence of consolidated lung and pleural fluid.

Complications of pneumothorax, other than respiratory distress and death, include septic pleuritis secondary to contamination of the pleural space, either secondary to trauma or from ruptured infected lung.

The **prognosis** depends on the underlying disease and its severity. Of 30 cattle with pneumothorax, mostly secondary to pneumonia, 18 survived, 8 were euthanized, and 4 died. Of 40 horses with pneumothorax, 23 survived, 12 were euthanized, and 5 died. The prognosis is better for animals with traumatic pneumothorax or that secondary to surgery than for animals with pneumothorax attributable to pneumonia.

CLINICAL PATHOLOGY

Hematologic and serum biochemical values are indicative of the underlying or concurrent disease—pneumothorax causes no specific changes in these variables. Arterial blood gas analysis reveals hypoxemia and hypercapnia.

NECROPSY FINDINGS

The lung in the affected sac is collapsed. In cases where spontaneous rupture occurs, there is discontinuity of the pleura, usually over an emphysematous bulla. Hemothorax might also be evident.

DIFFERENTIAL DIAGNOSIS

The clinical findings are usually diagnostic. Diaphragmatic hernia may cause similar clinical signs but is relatively rare in farm animals. In cattle, herniation is usually associated with traumatic reticulitis and is not usually manifested by respiratory distress. Large hernias with entry of liver, stomach, and intestines cause respiratory embarrassment, a tympanitic note on percussion, and audible peristaltic sounds on auscultation.

TREATMENT

The treatment depends on the cause of the pneumothorax and the severity of the respiratory distress and hypoxemia. Animals should receive treatment for the underlying disease. Animals with closed pneumothorax that are not in respiratory distress or hypoxemic do not require specific treatment for the pneumothorax, although the animal should be confined and prevented from exercising until the signs of pneumothorax have resolved. An open pneumothorax, as a result of a thoracic wound, should be surgically closed.

Emergency decompression of the pleural cavity using a needle into the pleural cavity, connected to a tubing and submerged into a flask of saline or water, creates a water-seal drainage. Thoracostomy tubes attached to Heimlich thoracic drainage valves are effective in preventing aspiration of air. Continuous suction, using thoracostomy (e.g., 24 French, 40-cm [16-in.] Argyle trocar thoracic catheter) and a standard three-bottle water seal drainage system or commercial equivalent is preferable if there are large continuing air leaks that may be life-threatening. Reinflation of the lung should be gradual because rapid removal of air can result in pulmonary edema.[3] Inflation of the lung can be monitored by repeated ultrasonic examination. The animal should be kept as quiet as possible and permitted no exercise. Horses with wounds to the axilla should be restrained until the wound has closed because this prevents the aspiration of air into the wound.[2] Prophylactic antimicrobial treatment is advisable to avoid the development of septic pleuritis.

Care should be exercised in performing biopsy of the liver or lung, and the former should be done with ultrasound guidance to prevent inadvertent lung damage.[4]

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DIAPHRAGMATIC HERNIA

Diaphragmatic hernia is uncommon in farm animals, in which it can be acquired, usually as a result of trauma, or congenital. Of a series of 44 horses and foals examined because of diaphragmatic hernia, 5 cases were determined to be congenital and 39 acquired.[1]

Congenital diaphragmatic hernias are reported in most large animal species, although details of frequency or risk factors are not available.[2-10] Congenital hernia results from failure of complete formation of the diaphragm during embryogenesis during a process involving the septum transversum, dorsal embryonic mesentery, pleuroperitoneal folds, and body wall mesenchyme. Congenital hernias develop as a result of defects in the diaphragmatic musculature when the septum transversum and pleuroperitoneal folds do not fuse completely or in the dorsal tendinous portion of the diaphragm.[2] Such hernias are characterized by a hernia sac composed of peritoneal and pleural membranes that occurs to one side or the other of midline (usually the right side in horses) in the right ventral (retrosternal hernia) or left dorsal crus.[1,2] The borders of congenital hernias are usually smooth, fibrous, and thickened and have a characteristic histologic appearance. In some cases, the pericardial sac is incomplete and the diaphragm is rudimentary and in the form of a small fold projecting from the chest wall. Affected animals usually survive for a few hours to several weeks, although many can survive for years with the hernia being clinically inapparent.[1] In pigs, a number of animals in each litter can be affected.

Acquired hernias are usually associated with trauma, such as falls, collisions with motor vehicles, foaling (for both mares and foals), or strenuous exercise.[1,10,11] It occurs in cattle, especially in association with traumatic reticuloperitonitis, in which case the hernia is small and causes no respiratory distress, and there may be no abnormal sounds in the thorax.

CLINICAL FINDINGS

Clinical findings in cattle include chronic or recurrent ruminal tympany caused by herniation of reticulum preventing its normal function in eructation. Muffled heart sounds can be detectable on both sides of the thorax.

Clinical signs of diaphragmatic hernia in adult horses are usually referable to herniation with or without incarceration of sections of the gastrointestinal tract. Signs of respiratory compromise are not common. There is sometimes a history of trauma or recent parturition, but this is not invariable, and many cases in adult horses occur without such an event being noticed in the recent past. Clinical signs on examination are those typical of colic of varying severity from nonstrangulation incarceration to acute, strangulating incarceration with severe septic shock and collapse. Rectal examination does not reveal evidence specific for diaphragmatic hernia, with the rare exception of a sense that the

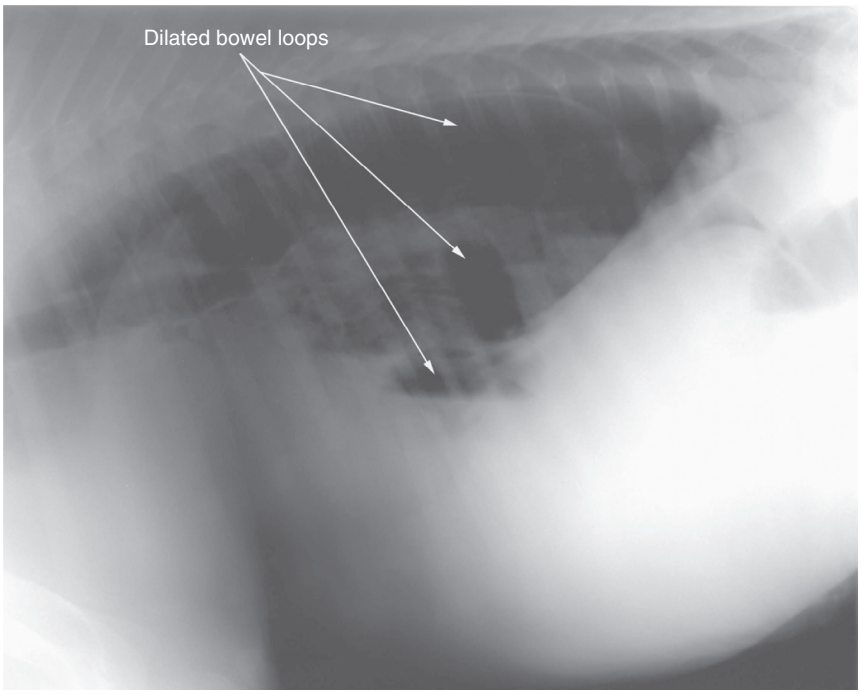

Fig. 12-12 Lateral thoracic radiograph in a foal demonstrating presence of gas-filled intestinal loops in the caudodorsal thorax. (Reproduced with permission.[4])

abdomen is not as full as one would expect. Thoracic auscultation can reveal the presence of borborygmi in the chest fields, but this should be interpreted with caution because such findings can occur in horses with an intact diaphragm. Examination of peritoneal fluid usually does not reveal abnormalities. Radiographic or ultrasonographic examination can provide a definitive diagnosis (Fig. 12-12).

The hernia is usually left dorsal (approximately 2/3 of cases) and right ventral (approximately 1/3 of cases) in horses.[1,4] Congenital retrosternal hernia (Morgagni hernia) is almost exclusively right ventral in horses.[2]

The presence of intestinal sounds in the thorax can be misleading; they are often present in the normal animal, but their presence, accompanied by dyspnea and resonance on percussion, should arouse suspicion. Radiography, ultrasonography, thoracoscopy, and exploratory laparotomy are the most useful diagnostic procedures. Radiography reveals the presence of gas- and fluid-filled intestinal contents in the thorax, apparent in cattle as oval rounded masses over the heart. Ultrasonography demonstrates presence of bowel in the thorax. There can be excessive pleural fluid.

The definitive treatment of acquired or traumatic hernia is surgical replacement of viscera in the abdomen and repair of the defect in the diaphragm. Repair of a diaphragmatic hernia through a standing thoracotomy in a cow has been described. Surgical repair has been performed in calves and foals, and in adult horses.[1-4,10,12,13]

The prognosis is poor to guarded, with short-term survival of affected foals and horses being 7/44 and 8/31.[1,4] Factors related to survival include the presence and extent of incarcerated intestine, size of the rent (smaller associated with increased chance of survival), and location of the rent. Location of the rent is likely related to survival because small, ventrally located rents are more amenable to surgical correction.[1]

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Diseases of the Bovine Respiratory Tract

ENZOOTIC NASAL GRANULOMA OF CATTLE (BOVINE ATOPIC RHINITIS)

Of the three known clinical types of chronic nasal obstruction in cattle, two have been identified etiologically and have clinical or epidemiologic features that distinguish them from enzootic nasal granuloma. One is recorded predominantly in beef cattle and appears to be caused by a fungus, most commonly *Rhinosporidium* spp., *Drechslera* spp., *Nocardia* spp.,[1] or *Pseudoallescheria boydii*.[2] Another is caused by the parasite *Schistosoma nasalis*. The third type, **enzootic nasal granuloma (ENG)**, occurs commonly in southern Australia, less commonly in New Zealand, and is recorded as a sporadic disease in South America and an occasional disease in North America, Britain, and Europe. It is reported as a herd outbreak in Jersey cows in the United Kingdom.[3]

Enzootic nasal granuloma occurs sporadically in some herds but may reach an incidence of 30%. In one area, as much as 75% of herds may have the disease. Animals aged between 6 months and 4 years are most commonly affected, and the chronic disease may or may not be preceded by an attack of acute rhinitis. Most cases commence in the summer and autumn months. It is apparent that nasal granuloma develops as a continuous and progressive response to acute episodes of hypersensitivity to an allergen present in the summer months. This accords with the gradual development of the stertorous respiration and the observations, in biopsies of nasal mucosa, of the presence of mast cells in all seasons, but the regression of eosinophils in the winter months.

An extensive survey of Australian dairying areas showed that 22% of cattle had lesions, and that the prevalence was greater in areas where the average annual rainfall was over 70 cm than where it was less than 70 cm; the prevalence varied between 4% and 48%; Jerseys were more commonly affected than Friesians. In New Zealand, 40% of farms and 36% of culled cattle were affected, whereas only 3.6% of young beef cattle showed lesions.

The disease has been identified as an allergic rhinitis and has been produced experimentally. Specific antigens have not been identified as the cause, but cows with nasal granuloma are much more sensitive to a number of common allergens in the environment than are unaffected cows. Additional possible causes include infestation of the nasal cavities with pasture mites (*Tyrophagus palmarum*). An allied condition has been described in the United States as maduromycosis, but there are nasal granulomas plus multiple granulomatous lesions of the skin of the ears, tail, vulva, and thigh. The granulomas contain many eosinophils and fungal elements identified provisionally as *Helminthosporium* sp.

In enzootic nasal granuloma, acute cases are characterized by a sudden onset of bilateral ocular and nasal discharge and swelling of the nasal mucosa causing difficult, noisy breathing. Affected animals shake their heads and snort and rub their noses in hedges. As a result, they commonly block their nostrils with twigs. This form of the disease is commonest in cattle of the Channel

Island breeds and their crossbreeds. The nasal discharge in these breeds is usually yellow to orange in color.

Established cases of enzootic nasal granuloma have lesions, consisting of granulomatous nodules 1 to 4 mm in diameter and height, in both nostrils. The lesions extend from just inside the nostril posteriorly for 5 to 8 cm. They may be few in number or be packed closely together. Their texture is firm, and the mucosa over them is normal. They have a characteristic histopathology of epithelial metaplasia and hyperplasia, and contain large numbers of eosinophils and mast cells.

The predominant clinical sign is respiratory stertor and dyspnea caused by obstruction to the airflow. The severity of these signs may fluctuate, but in general they progress slowly over several months and then remain static. Although the respiratory distress may be sufficiently severe to cause a loss of condition and marked reduction in milk yield, affected animals do not die. A good proportion of them have to be culled as uneconomic units.

The clinical picture in **mycotic nasal granuloma** is superficially similar with respect to noisy breathing, respiratory distress, and nasal discharge, but there is no seasonal association. Also, the visible and palpable lesions in the anterior part of the nasal cavities are polyps up to 5 cm in diameter that occur singly or in confluent masses. Their cut surfaces are yellow to green, and they are sometimes ulcerated. Histologically, the lesions are eosinophilic granulomas containing fungal spores and sometimes hyphae. Fungi (*Drechslera rostrata*) have been isolated from the lesions.

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TRACHEAL STENOSIS OF FEEDLOT CATTLE

Tracheal stenosis, also known as "honker cattle," occurs in feedlot cattle. The etiology is unknown. It is characterized by extensive edema and hemorrhage of the dorsal wall of the trachea, resulting in coughing (honking), dyspnea, and respiratory stertor. Complete occlusion of the trachea may occur. Affected animals may be found dead without any premonitory signs.

In tracheal stenosis of feedlot cattle, there is marked submucosal hemorrhage dorsal and ventral to the trachealis muscle, resulting in ventral displacement of the mucosa and partial to complete occlusion of the tracheal lumen. Diffuse hemorrhage in the peritracheal connective tissue and surrounding muscles of the neck is common in animals dying of asphyxia. Histologically, there is hyperemia and hyperplastic tracheal mucosa with focal erosions, squamous metaplasia, and loss of cilia. In acute cases, the mucosa is markedly thickened because of hemorrhage and edema. Culture reveals a mixed bacterial flora.

CAUDAL VENA CAVAL THROMBOSIS (POSTERIOR VENA CAVAL THROMBOSIS) AND EMBOLIC PNEUMONIA IN CATTLE

Embolic pneumonia as a sequel to thrombosis of the posterior vena cava is a relatively common disease of cattle in Europe and the United Kingdom. The disease is rare in cattle less than 1 year old, although it can occur at any age. Most affected animals are in feedlots on heavy-grain diets, and there are peaks of incidence at those times of the year when most cattle are on such diets. There is a relationship between the occurrence of this disease and that of hepatic abscessation arising from lactic-acid-induced rumenitis on heavy-grain diets. The disease is reported with deep digital sepsis in cattle.[1] A similar syndrome occurs in horses, although rarely.[2]

The **etiology and pathogenesis** of the disease are based on the development of a thrombus in the posterior vena cava and the subsequent shedding of emboli that lodge in the pulmonary artery, causing embolism, endarteritis, multiple pulmonary abscesses, and chronic suppurative pneumonia. Pulmonary hypertension develops in the pulmonary artery, leading to the development of aneurysms, which can rupture causing massive intrapulmonary or intrabronchial hemorrhage. In most cases the thrombi in the vena cava originate from hepatic abscesses or postdiaphragmatic abscesses. Usually there is an initial phlebitis and the subsequent thrombus extends into the thoracic part of the vessel. When the thrombus occludes the openings of the hepatic veins into the vena cava, there is congestion of the liver and hepatomegaly, ascites, and abdominal distension in some of these cases.

The **most common form** of the disease is characterized by manifestations of respiratory tract disease. Commonly there is a history of the disease for a few weeks or longer, but some animals are "found dead" without prior recorded illness. Affected animals usually have thin to moderate body condition, reduced appetite, reduced rumen motility, and a positive reticular pain response. There is usually fever and an increase in the rate and depth of respiration, coughing, epistaxis and hemoptysis, anemia with pallor, a hemic murmur, and a low packed cell volume. Respirations are painful and a mild expiratory grunt or groan may be audible with each respiration. Subcutaneous emphysema and frothing at the mouth are evident in some. Deep palpation in the intercostal spaces and over the xiphoid sternum might elicit a painful grunt. The lung sounds can be normal in the early stages, but with the development of pulmonary arterial lesions, embolic pneumonia, and collapse of affected lung, widespread abnormal lung sounds are audible on auscultation. There can be ascites. In one series of cases, the presence of anemia, hemoptysis, epistaxis, and widespread abnormal lungs sounds were characteristic features of the disease. There are accompanying nonspecific signs of inappetence, ruminal stasis, and scant feces.

About one-third of affected cattle become progressively worse over a period of 2 to 18 days with moderate to severe dyspnea, and they die of acute or chronic anemia or are euthanized on humane grounds. Almost half of the cases die suddenly as a result of voluminous intrabronchial hemorrhage. It is probably the only common cause in cattle of acute hemorrhage from the respiratory tract that causes the animal to literally drop dead. The remainder have a brief, acute illness of about 24 hours.

Some evidence of hepatic involvement is often present, including enlargement of the liver, ascites, and melena. Chronic cor pulmonale develops in some with attendant signs of congestive heart failure.

Radiography of the thorax of some affected animals has found an increase in lung density and markings. These are irregular, focal or diffuse, and nonspecific. More distinct opacities are present in some and are referable to embolic infarcts and larger pulmonary hemorrhages. Radiographic abnormalities in the lungs are detected in approximately one-third of cows with caudal vena cava thrombosis. **Ultrasonography** can be a useful diagnostic aid in detecting changes in the caudal vena cava. The caudal vena cava in affected cows is round to oval rather than the triangular shape in normal cattle, and the hepatic, splenic, and portal veins can be dilated.[3] The presence of thrombi in the caudal vena cava can be detected (Fig. 12-13).[4]

There is typically anemia and leukocytosis. Neutrophilia with a regenerative left shift and hypergammaglobulinemia as a result of chronic infection are common. Serum gamma-glutamyl transpeptidase activity is high in about one-third of cases.[3]

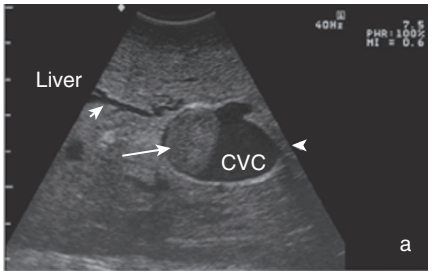

Fig. 12-13 Ultrasonogram of intrahepatic vena cava of an adult cow demonstrating presence of a thrombus (white arrow) and dilated intrahepatic vessels (white arrowhead). (Reproduced with permission.[4])

Fig. 12-14 Cow with cranial vena cava thrombosis causing jugular vein distension and edema of the brisket. (Courtesy of Dr. Christian Gerspach, Vetsuisse Faculty University of Zurich).

The **necropsy findings** include a large, pale thrombus in the posterior vena cava between the liver and the right atrium. Occlusion of the posterior vena cava results in hepatomegaly and ascites. Hepatic abscesses of varying size and number are common and often near the wall of the thrombosed posterior vena cava. Pulmonary thromboembolism with multiple pulmonary abscesses, suppurative pneumonia, and erosion of pulmonary arterial walls with intrapulmonary hemorrhage are also common. The lungs reveal emphysema, edema, and hemorrhage. A variety of bacteria, including streptococci, *E. coli,* staphylococci, and *F. necrophorum,* are found in the abscesses in the liver.

Animals that die suddenly are found lying in a pool of blood, and necropsy reveals large quantities of clotted blood in the bronchi and trachea.[3]

The disease must be differentiated from verminous pneumonia, chronic aspiration pneumonia, pulmonary endarteritis as a result of endocarditis, and chronic atypical interstitial pneumonia. There is no treatment that is likely to have any effect on the disease, and the principal task is to recognize the disease early and slaughter the animal for salvage if possible.

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CRANIAL VENA CAVAL THROMBOSIS

Thrombosis of the cranial vena cava occurs in cows. Cases in young animals are also recorded, and it is suggested that they arise from navel infection. The disease has occurred with reticuloperitonitis in cattle.[1] Clinical signs include cough, tachypnea, muffled heart sounds, exercise intolerance, and excessive pleural fluid. As in caudal vena caval thrombosis, a number of pulmonary abscesses or bronchopneumonia can develop. Pulmonary hypertension is not a feature as it is in the caudal lesion. However, increased jugular vein pressure, dilatation of the jugular vein, and local (brisket) edema can occur (Fig. 12-14). Ultrasound examination can reveal thrombosis of the cranial vena cava extending into the right atrium.

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ACUTE UNDIFFERENTIATED BOVINE RESPIRATORY DISEASE

DEFINITION OF THE PROBLEM

A major problem that large-animal clinicians commonly encounter is a group of cattle that are affected with an acute respiratory disease of uncertain diagnosis.

Acute undifferentiated bovine respiratory disease (BRD) is characterized clinically by dyspnea, coughing, nasal discharge, varying degrees of depression, anorexia, pyrexia ranging from 40 to 41° C (104-105.8°F), evidence of pneumonia on auscultation of the lungs, and a variable response to treatment. Some unexpected deaths may have occurred as the initial indication of the problem. Although in most cases pneumonia is the obvious cause of the disease, determining the etiology is the major diagnostic problem. If lesions typical of any of the common diseases of the respiratory tract of cattle can be recognized clinically, like those of infectious bovine rhinotracheitis (IBR), then on a clinical basis a specific diagnosis can be made. The affected group may be unweaned dairy heifers, weaned beef calves, or yearlings that have recently arrived in a feedlot; cattle that have been in the feedlot for varying periods of time; young growing cattle on summer pasture; mature cows that have recently been placed on a lush pasture; yearling or mature lactating dairy cattle; or a group of veal calves. The morbidity rate can range from 10% to 50% depending on the age of animals affected, the immune status of the animals, the nature of the stressors involved, and the nature of the disease.

Respiratory disease is the most common illness among cattle in feedlots, affecting on average over 16% of cattle placed.[1] In general, bovine respiratory disease accounts for 65% to 79% of the morbidity and 44% to 72% of mortality in feedlot cattle. Despite of intensive research in the field the incidence of respiratory disease in cattle during the first year of life could not be controlled. The proportion of mortality of weaned dairy heifers attributable to respiratory disease increased from 34.8% of all deaths in 1991 to 46.5% in 2007.[2]

The economic impact of respiratory disease on the U.S. beef industry is estimated to exceed $4 billion annually, including treatment cost, disease prevention, and production loss such as decreased growth performance.[3] Cattle with detectable lung lesions at slaughter on average had a reduced daily weight gain of 0.08 kg/d compared with cattle without lung lesions. Protracted effects of respiratory disease in dairy calves on milk production and fertility in later life are more difficult to estimate, but repeated incidences of respiratory disease in a dairy heifer were found to almost double the risk for not completing the first lactation.[4]

The primary goal of the clinician must be to make the most accurate clinical diagnosis as rapidly as possible, based on the clinical and epidemiologic findings that are identifiable on the farm, preferably when examining the animals on the first visit. Giving a prognosis and the formulation of rational and economic treatment that will minimize morbidity and mortality are the next goals. In any group situation, mass medication of each in-contact animal is a major consideration that will increase costs and must be balanced against the economic losses that might occur if all animals are not treated metaphylactically. The clinical management of the outbreak, which includes treatment of the obvious cases and the prevention of new cases if possible, is dependent in part on the diagnosis. However, differentiation between the diseases based on clinical findings can be unreliable, and it is usually necessary to begin antimicrobial therapy that will be effective against the bacterial pathogens most likely to be present. Even after intensive clinical and

laboratory investigation, the specific etiology will often not be determined, and the clinician is left with a diagnosis of **acute undifferentiated respiratory disease of cattle** or **bovine respiratory disease (BRD)**.

The salient clinical and epidemiologic findings of the diseases included in the complex of BRD are summarized in Table 12-7. The common diseases of the respiratory tract of cattle can be broadly divided into those affecting the lower respiratory tract and those affecting the upper respiratory tract. Diseases of the lungs associated with either viruses or bacteria alone or in combination are difficult to distinguish from each other on the basis of clinical findings alone. The presence of toxemia, which causes depression and anorexia in bacterial pneumonias, is a useful guide in categorizing the common diseases when making a differential diagnosis list. Cattle affected with uncomplicated viral diseases of the respiratory tract may show a high fever but are usually not depressed and anorexic because bacterial toxemia is absent.

ETIOLOGY

The major etiologic agents that cause or may be associated with acute UBRD include the following:

Table 12-7 Differential diagnosis of bovine respiratory disease

Disease	Epidemiology	Clinical and laboratory findings	Response to treatment
Pneumonic pasteurellosis (shipping fever)	Common disease in North America. Young cattle recently stressed by weaning or transportation, mixing from many different sources, many animals affected, some found dead, common in feedlots. Epidemics occur 7–10 days after arrival in the lot, but cattle may be sick on arrival or within a few days after arrival.	Acute toxemic bronchopneumonia, moderate dyspnea, fever, increased breath sounds over ventral aspects of lungs, moist crackles, cough, pleuritis.	Good response to treatment in early stages. Failure to respond as a result of advanced lesions, pleuritis, abscesses, inadequate dosage, and incorrect diagnosis.
Pneumonic pasteurellosis (enzootic calf pneumonia)	Common disease in unweaned, housed dairy calves, occasionally in pastured beef calves 2 and 6 months of age.	Acute, subacute, and chronic pneumonia, moderate fever, loud breath sounds ventrally, crackles and wheezes.	Respond favorably to treatment for uncomplicated secondary bacterial bronchopneumonia.
Viral interstitial pneumonia (parinfluenza-3 virus [PI-3V], bovine respiratory syncytial virus [BRSV])	Yearling and adult cattle indoors or outdoors, young cows in closed dairy herd, may occur following addition to herd, high morbidity, low mortality.	Sudden onset of acute pneumonia, moderate dyspnea and toxemia, loud breath sounds and wheezes attributable to bronchiolitis, no moist crackles unless secondary pneumonia. Leukopenia and lymphopenia.	Gradual recovery occurs in 3-5 days. Treat secondary complication with antimicrobials.
Bovine respiratory syncytial virus infection	Young cattle 6–8 months of age, adult dairy cattle, herd outbreaks are characteristic; case-fatality rate varies from 1%–30%. Maternal antibody in calves is not protective.	Inappetence, fever, coughing, dyspnea, and abnormal lung sounds suggestive of interstitial pneumonia. Death common in those with severe respiratory distress. Fourfold or greater seroconversion to BRSV. Immunofluorescence of nasopharyngeal smears and virus isolation. Acute bronchiolitis and alveolitis.	Treat secondary complications with antimicrobials for 3-5 days.
Histophilus somni (formerly *Haemophilus somnus*) pneumonia, pleuritis, and myocardial abscesses	Common in feedlot calves, 6-8 months of age; mean fatal disease onset for pneumonia is 12 days after animal in the lot, and day 22 for myocarditis and pleuritis.	Toxemic suppurative pleuropneumonia, dyspnea, mouth breathing. Persistent fever for several days. Concurrent myocarditis may cause sudden death.	Inadequate response to treatment.
Infectious bovine rhinotracheitis	Common disease. All age groups but mostly young feedlot cattle, outbreaks common, occurrence unpredictable. Most common in unvaccinated herds.	Acute rhinotracheitis with discrete nasal lesions, inspiratory dyspnea, explosive loud coughing, ocular and nasal discharge, high fever for 3–5 days; 1% die of secondary bacterial pneumonia. Virus isolation from nasal swabs. Acute and convalescent serology.	Gradual recovery occurs in 3-5 days in spite of treatment. Treat secondary pneumonia.
Mycoplasma bovis pneumonia	Feedlot cattle with history of respiratory disease. Dairy calves with enzootic pneumonia. Mastitis in lactating dairy cows.	Acute to chronic bronchopneumonia, anorexia, fever, polyarthritis, otitis. Exudative bronchopneumonia, extensive foci of coagulative necrosis. Nasal swabs, transtracheal wash joint fluid, lung tissue, serology (consider presence of maternal antibodies in young calves).	No response to antibiotic therapy.
Atypical interstitial pneumonia (acute pulmonary emphysema and edema, fog fever)	Occurs 4–10 days after adult cattle turned into lush autumn pasture. Outbreaks usual, sudden onset, high case fatality. Incidental occurrence in feedlot cattle toward the end of the finishing period.	Sudden and rapid death, severe loud dyspnea with grunting expiration, loud breath sounds over ventral aspects, crackles, subcutaneous emphysema, severe cases die, laboratory data not helpful, confirm at necropsy.	Most severe cases die; moderate to mild cases recover; treatment difficult to evaluate. Pasture form can be prevented with monensin in the feed for a few days before and after change of pasture.

Table 12-7 Differential diagnosis of bovine respiratory disease—cont'd

Disease	Epidemiology	Clinical and laboratory findings	Response to treatment
Extrinsic allergic alveolitis (bovine farmer's lung)	Not common. Mature cattle housed during winter months and exposed to moldy or dusty feeds. Several animals over period of time.	Chronic coughing, dyspnea, weight loss, reduced milk yield, loud breath sounds, crackles, dull but not toxemic, abnormal nasal discharge.	No response to treatment.
Chronic interstitial pneumonia (diffuse fibrosing alveolitis)	Single animals only. May be chronic form of epidemic acute interstitial pneumonia.	Chronic onset of coughing, dyspnea, weight loss, reduced milk yield, decreased breath sounds, no toxemia, cor pulmonale.	No response to treatment.
Verminous pneumonia (*Dictyocaulus viviparus*)	All ages susceptible, usually young cattle 6–12 months on pasture, wet warm seasons, outbreaks common, enzootic area.	Moderate to severe dyspnea, coughing, fever, loud breath sounds, crackles over **dorsal half of lung**, eosinophilia may occur, larvae in feces 3 weeks after infection.	No response to antimicrobials. Responds to anthelmintics.
Ascaris suis pneumonia	Not common. All ages. On pasture previously occupied by pigs.	Sudden onset, severe dyspnea, rapid deaths, loud breath sounds, crackles over entire lung. Will recover gradually if not too severe.	No specific treatment response.
Allergic rhinitis (summer sniffles)	Mostly late summer, autumn when pasture in flower. Sporadic cases. Mostly Channel Island breeds. Cows may have disease each year.	Sudden onset, dyspnea, inspiratory wheezing, mucopurulent then caseous yellow to orange nasal discharge. Sneeze, rub muzzle in bushes, twigs up nose, bleed.	Into housing, antihistamines, excellent response if early. In cases of long duration, wheezing persists until nasal mucosa sloughs.
Pulmonary abscess	Single animal. History of pneumonia with no response to treatment. Occasionally, several cases in feedlot.	Chronic coughing with epistaxis and hemoptysis, chronic toxemia, mild fever, crackles and wheezes distributed randomly. Neutrophilia.	None.
Calf diphtheria	Young calves, dirty conditions. or on rough dry pasture. Usually only few affected.	Acute toxemia, fever, inspiratory stridor and stertor, necrotic lesions visible in larynx and oral cavity.	Responds to antimicrobials and topical treatment.
Embolic pneumonia attributable to ruptured vena caval abscess	1–8 years of age. History of respiratory disease with hemoptysis and epistaxis and poor response to treatment.	Dullness, polypnea, hyperpnea, thoracic pain, frequent coughing with hemoptysis, epistaxis, temperature variable, anemia. Common, widespread foci of crackles and wheezes with increased breath sounds. May die rapidly from massive hemorrhage. Hepatomegaly and congestive heart failure. Neutrophilia and hypergammaglobulinemia.	No response to treatment. Slaughter for salvage.
Aspiration pneumonia	History is important. Following faulty drenching techniques or regurgitation and aspiration in weak cows (i.e., milk fever).	Acute bronchopneumonia with toxemia 24–48 hours following aspiration. Loud breath sounds ventral half, moist crackles. Marked leukopenia and neutropenia.	May respond to treatment if treated early.
Dusty feed rhinotracheitis	Few days following introduction of finely chopped dry feed. Feed contains high concentrations of "fines,"	Outbreak of coughing, rhinitis with copious serous nasal discharge, conjunctivitis, and ocular discharge. Bright and alert.	Recover in few days following removal of dusty feed.
Enzootic nasal granuloma	In enzootic area up to 30% morbidity in a herd, up to 75% of herd. Coastal regions, autumn is worst, Channel Island breeds most affected. Loss is a result of continuous loss of production. A chronic debilitating disease. All ages, mostly adults.	May be acute "summer sniffles" early. Then chronic dyspnea with stertor, eat indifferently, lose condition, have to be culled. Chronic nasal discharge. Smear nodules on nasal cavity mucosa palpable through nostril,	None.
Contagious bovine pleuropneumonia	Outbreak in susceptible cattle—morbidity up to 100%, mortality up to 50% if cattle stressed, traveling. Aerogenous spread, no mediate contagion. Outbreaks as a result of introduction of cattle often inapparent "carriers" that are detectable by CF test. Incubation period 3–6 weeks.	Acute fibrinous pneumonia, and pleurisy. Dyspnea, fever 40.5°C (104.5°F), deep cough or shallow and, fast, elbows out, grunting respiration. Pain on chest percussion. Pleuritic friction rub early; moist crackles. Course 3 days to 3 weeks,	Not to be treated. Eradication is urgent. Is treated in enzootic areas where eradication is not attempted.

Viruses
- Bovine herpesvirus-1 (BHV-1) causing infectious bovine rhinotracheitis (IBR)
- Bovine respiratory syncytial virus (BRSV)
- Parainfluenza-3 virus (PI-3V)
- Bovine virus diarrhea virus (BVDV)
- Bovine coronavirus (BoCV)
- Bovine adenovirus (BAV)

Bacteria
- *Mannheimia haemolytica*
- *Pasteurella multocida*
- *Histophilus somni* (formerly *Haemophilus somnus*)

Mycoplasma spp.
- *Mycoplasma bovis*
- *Mycoplasma mycoides* causing contagious bovine pleuropneumonia (CBPP)
- *Mycoplasma bovirhinis*
- *Mycoplasma dispar*
- *Ureaplasma diversum*

Uncertain or unknown etiology
- Atypical interstitial pneumonia

Verminous
- *Dictyocaulus viviparus*

Role of Etiologic Agents

The role of the etiologic agents in the cause of BRD is controversial and often uncertain because the major pathogens are ubiquitous in clinically normal animals. The disease is considered to be the result of the effects of stressors causing immunosuppression, which allow colonization of the respiratory tract by opportunistic pathogens. The spectrum of the immune status of the animals is also a major factor. Animals vaccinated well before natural infection may be resistant to clinical disease caused by specific pathogens. Animals that underwent natural infection and developed adequate humoral or cell-mediated immunity may also be immune to clinical disease.

Clearly the **viral–bacterial synergism** is a major factor in the pathophysiology of the BRD complex. It is well recognized that the effect of a virus that usually is nonfatal combined with various bacteria commonly resident in the upper respiratory tract of healthy individuals can cause fatal bacterial pneumonia. Numerous experimental models of synergism between virus and bacteria have been studied. In cattle, the viruses having received the most attention in this context are PI-3 and BHV-1, which in combination with bacteria such as *M. haemolytica* or *P. multocida* can cause clinical disease considerably more severe than experimental infection caused by either viral or bacterial infection alone. Many mechanisms have been suggested behind this viral–bacterial synergism, including impaired neutrophil function or recruitment following initial viral infection, decreased macrophage activity, or altered number and responsiveness of lymphocytes. Most likely several mechanisms are involved in the virus induced suppression of pulmonary antibacterial defense.

Many epidemiologic studies of bovine respiratory disease have attempted to correlate the level of serum antibodies in feedlot calves on arrival at the feedlot and over the first 30 to 50 days of the feeding period with morbidity and mortality as a result of respiratory disease. A low level of antibody to a specific pathogen on arrival followed by significant seroconversion in animals that develop BRD in the first few weeks of the feeding period suggest that the pathogen was an important etiologic factor. Conversely, those animals with a high level of antibody on arrival that do not develop BRD are considered immune. However, some animals with low levels of antibody may remain normal and seroconvert during the early part of the feeding period.

Feedlot cattle commonly seroconvert to the BHV-1, PI-3V, BVDV, and BRSV, and possibly also to *Mycoplasma bovis* and other *Mycoplasma* spp., within the first month after arrival. Seroconversion to these pathogens occurs both in animals that develop respiratory disease and those that remain normal within the same group, but the relative importance of each agent and their causative nature is controversial. Seroconversion to *M. haemolytica* leukotoxin, BRSV, and BVDV were predictive of approximately 70% of all respiratory disease cases in Ontario feedlots. Calves arriving with high serum antibody levels to *Histophilus somni* had less bovine respiratory disease than calves with lower levels.

Many respiratory pathogens that can cause disease are present in diseased and clinically normal individuals alike during outbreaks of BRD. It has been suggested that UBRD in weaned beef calves is not a highly contagious disease and that although respiratory pathogens may be important etiologic factors, the presence of other contributing factors is as important for the development of clinical disease. Stress resulting from weaning, transportation, processing, crowding, or harsh weather is considered to be an important contributor to outbreaks of BRD. Studies exploring the effect of stress resulting from weaning and maternal separation showed that the viral–bacterial synergism was altered when weaning occurred at the time of a primary viral infection with BHV-1 followed by a secondary bacterial infection with *M. haemolytica* resulting in significantly increased mortality rates.[5] Similarly calves weaned immediately before transport to a feedlot developed significantly more UBRD compared with calves adapted to weaning for 45 days.[6] This suggests that identifying and avoiding environmental and management risk factors are crucial to control BRD outbreaks.

The relationships between bacterial and viral antibody titers and undifferentiated fever and mortality in recently weaned beef calves in western Canada were examined. Feedlot calves are commonly exposed to *M. haemolytica, H. somni,* BHV-1, BVDV, and *M. bovis* in the early feeding period. Seroconversion to *M. haemolytica* leukotoxin was associated with a decreased risk of undifferentiated fever. Higher arrival BVDV antibody titer was associated with a decreased risk of undifferentiated fever. Higher arrival *H. somni* antibody titer and increases in *H. somni* antibody titer after arrival were both associated with a decreased risk of undifferentiated fever. The odds of overall mortality (OR 5.09) and histophilosis mortality (OR 11.31) in clinical cases were higher than in the controls. In summary, protective immunity to *M. haemolytica* leukotoxin *H. somni,* BHV-1, BVDV, and *Mycoplasma* spp. may be necessary to reduce the occurrence of undifferentiated fever.

Chronic, antibiotic-resistant pneumonia, sometimes with polyarthritis, occurs in feedlot cattle. *M. bovis,* BVDV, and *H. somni* are commonly found in the tissues at necropsy. This coinfection suggests the possibility of synergism between the BVDV and *M. bovis* in the pneumonia with the arthritis syndrome.

BVDV has been identified as a contributor to respiratory disease in feedlot calves. On arrival in feedlots, 39% of animals were seropositive for BVDV, and those animals treated for UBRD had larger titer increases to the virus than nontreated animals. BVDV-1b strains have been associated with acute pneumonia in commingled calves that were not vaccinated with BVDV vaccines, and in which *M. haemolytica* and *P. multocida* were also present in the pneumonic lesions. Experimental infection of seronegative and immunocompetent calves with BVDV type resulted in primary respiratory disease.

Bovine coronavirus (BoCV) has been implicated as a cause of UBRD based largely on the isolation of the virus from the nasal cavities of cattle with respiratory disease. However, based on seroepidemiology of BoCV titers in feedlot cattle, although higher antibody titers to the virus were associated with a decreased subsequent risk of treatment for UBRD, there was no association between evidence of recent infection (titer increase) and the occurrence of UBRD. Other studies have shown that BoCV infections are not associated with an increased risk of treatment for UBRD. BoCV is widespread in the cattle and can be found in the feces and nasal swabs of recently arrived feedlot cattle and calves with and without clinical signs of BRD. Exposure to BoCV before arrival in the feedlot is common, with 90% of animals being seropositive on arrival. BoCV can be isolated from feedlot cattle in many different locations and most cattle seroconvert to the virus during the first 28

days after arrival in the feedlot. Cattle shedding the virus from the nasal cavity and developing an antibody response to the virus were 1.6 times more likely to require treatment for respiratory disease than cattle that did not shed the virus or develop an immune response. Cattle that shed the virus from the nasal cavity were 2.2 times more likely to have pulmonary lesions at slaughter than cattle that did not shed the virus. In natural outbreaks of shipping fever, more than 80% of affected cattle shed BoCV at the beginning of the epidemic when the *M. haemolytica* infection rate was low.

The role of BoCV in epidemics of shipping fever pneumonia in cattle was examined by the collection of nasal swabs and serum samples before the onset of the epidemic, during the course of the illness, and after death when necropsies were done and samples of lung tissues were examined. Respiratory BoCV was isolated from the nasal secretions before and after transport, from lung tissues of those cattle that died early in the epidemic but not later. *Pasteurella* spp. were isolated from all cattle that had severe pneumonia. All cattle were immunologically naive to both infectious agents at the onset of the epidemic, but those that died after day 7 had rising antibody titers to BoCV and *M. haemolytica*. In contrast, the 18 clinically normal and BoCV-negative cattle had high antibody titers to BoCV from the beginning, and their antibody responses to *M. haemolytica* were delayed.

CLINICAL CASE DEFINITION AND EPIDEMIOLOGY

Clinical Case Definition

The most important part of the clinical and epidemiologic examination is to determine the case definition, which includes the following questions.

What Is the Clinical Disease That Is Present in the Affected Animals?

- Which body system is affected and where in that body system is the lesion?
- Do the animals have pneumonia, rhinitis, laryngitis, tracheitis, bronchitis or combinations of these abnormalities?

The clinician should attempt to make a clinical diagnosis by closely examining several typically affected animals and determine whether the lesions are in the lower or upper respiratory tract. The presence of toxemia, depression, fever, anorexia, and agalactia in lactating dairy cattle indicates a primary or secondary bacterial infection. The presence of loud breath sounds (consolidation) and abnormal lung sounds (crackles and wheezes) indicates the presence of pneumonia. Diseases of the upper respiratory tract are characterized by inspiratory dyspnea, stridor, loud coughing, sneezing, wheezing, and lesions of the nasal mucosa.

Which Animals Are Affected?

Determining which animals are affected includes age of animals affected, a single animal or group of animals, and vaccination history. Recently arrived feedlot cattle mixed from many different origins are susceptible to fibrinous pneumonia associated with *M. haemolytica*.

Where Are the Affected Animals?

Are the infected animals in the feedlot, on pasture, or housed in a barn? If in a barn, with what quality of ventilation?

When Were the Animals Affected?

- How soon after arrival in the feedlot did the animals become affected?
- What stressors may have recently preceded the outbreak?
- What risk factors could have predisposed to this outbreak?
- Have the animals been recently shipped and mixed with animals from another source?

Consideration of the clinical and epidemiologic findings can then be correlated, and hypotheses formulated and tested to determine **why** the disease occurred.

Occurrence

Bovine respiratory disease occurs under many different situations, including all age groups, feedlot animals kept outdoors, housed dairy calves, nursing and recently weaned beef calves, dairy and beef cattle heifers, and adult lactating dairy cows. Epidemics of acute respiratory disease have been described in dairy calves from birth to 6 months of age. Outbreaks of BRSV can occur in dairy cattle heifers and adult dairy cattle.

Pneumonic pasteurellosis is most common in recently arrived feedlot calves (shipping fever) and unweaned dairy calves (enzootic calf pneumonia). In calves 3 to 5 weeks after arrival in the feedlot, *H. somni* pleuropneumonia may be more likely. Atypical interstitial pneumonia (pasture-induced) is the most likely diagnosis when confronted with an outbreak of acute respiratory disease in mature cattle that have been moved from a summer pasture to a lush autumn pasture within the last 4 to 10 days.

Risk Factors

The risk factors that have been identified in outbreaks of respiratory disease in feedlot cattle include the purchase of cattle from auction markets and mixing of cattle from many different sources. An epidemiologic study of fatal fibrinous pneumonia in auction-market-derived feedlot calves in western Canada revealed that peak mortality occurred approximately 16 days after arrival at the feedlot. The risk of fatal fibrinous pneumonia was consistently greater for calves entering the feedlot in November, shortly after the auction sales had peaked, when the feedlot was reaching capacity. Increased mixing at the auction markets was associated with increased fatal disease risk. The distance calves were transported by truck was not associated with fatal disease risk. When the incidence of fatal fibrinous pneumonia was high, the disease clustered within truckloads or pens. Risk factors positively associated with disease clustering included increased mixing of calves from different farms, month of purchase, number of calves passing through the auction markets, and weather conditions at arrival.

Transportation of feedlot calves increases serum concentration of oxidative stress biomarkers, which are related to episodes of bovine respiratory disease. Transportation stress significantly decreases serum total antioxidant capacity and increases malondialdehyde concentrations in steer calves. It is proposed that stressors such as marketing through an auction barn and transportation precipitate oxidative stress, which reduces the antioxidant defense capacity and increases total body lipid peroxidation, resulting in increased susceptibility to BRD. These biomarkers may be useful to measure the oxidative stress of transported cattle. There is some experimental evidence that acidogenic diets and ketoacidosis may affect lymphocyte function, which may affect vaccine efficacy.

The literature on how the adequacy of diets of recently arrived feedlot cattle may affect their health and immunity has been reviewed.[7] Diets for newly arrived stressed beef cattle must be formulated to compensate for decreased feed intake and known nutrient deficiencies.[8]

The literature on the risk factors for bovine respiratory disease in dairy heifers and the effect of the disease on productivity has been reviewed with relevance to commercial dairy farming in the Netherlands. Bovine respiratory disease in dairy heifers increases the risk of mortality directly after the disease episode by up to 6 times, reduces growth during the first 6 months of life by up to 10 kg, and increases the likelihood of dystocia after first calving. Both herd size and other diseases in dairy heifers are clearly associated with the risk of bovine respiratory disease. Season and colostrum feeding are important. The most important risk factors for mild and severe pneumonia in dairy calves aged birth to 3 months were inadequate air circulation and the purchase of cattle.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Individual Animal Diagnosis

Although the clinical diagnosis of BRD can readily be made even by cursory examination in severe or advanced cases, the challenge is to identify newly affected animals in early stages of the disease, which is a critical factor for treatment efficacy. In general,

medical treatment is more effective the earlier in the process it can be initiated, and delaying treatment is considered a major factor contributing to treatment failures. A bayesian estimation of the performance of using clinical observation for diagnosis of BRD yielded an estimated sensitivity and specificity of 0.62 and 0.63 respectively, suggesting that up to 38% of truly diseased animals may go undiagnosed and conversely that up to 37% of animals diagnosed and subsequently treated for BRD are unaffected by respiratory disease.[9] Another study reported that 68% of feedlot cattle with lung lesions at slaughter were never treated for bovine respiratory disease, further suggesting that current methods of diagnosing bovine respiratory disease based on visual appraisal by feedlot pen riders may not always effectively identify sick animals. The limitations of identifying clinically affected animals that need therapy was a major factor in the development of metaphylactic use of antimicrobials.

The subjective clinical findings of distant examination that have been used by animal attendants in commercial feedlots to identify sick animals that need to be closely examined include:

- *Degree of ruminal fill* (1, normal; 2, slightly gaunt; 3, moderately gaunt; 4, excessively gaunt)
- *Attitude* (1, normal; 2, slight lethargy; 3, severe lethargy; 4, nonambulatory)
- *Ocular discharge* (1, none; 2, slight; 3, moderate; 4, abundant)
- *Nasal discharge* (1, none; 2, slight; 3, moderate; 4, abundant)
- *The sounds heard on auscultation of the lungs at three sites along a line extending from the cranioventral to caudodorsal lung fields* (1, normal; 2, slightly harsh; 3, moderately harsh; 4, severely harsh)

Frequently the rectal temperature of animals that appear dull is measured; if temperature is found to be above a predetermined level, they are considered to have BRD if no other clinical findings are detectable that are referable to other organ systems. In most cases, the thorax of these animals is not auscultated for evidence of pneumonia. Although a fever when combined with other clinical signs is a valuable parameter in the process of making a diagnosis, fever of undetermined origin can as well be unassociated with respiratory disease and does not imperatively require treatment. In a feedlot pen of 112 recently arrived bull calves that were fitted with reticuloruminal temperature-sensing boluses, a total of 449 fever episodes were recorded in 110 animals during the first 40 days after arrival. Of these fever episodes, 74% were not associated with any visually apparent clinical signs or respiratory disease, and 75% lasted for less than 48 hours. A negative effect on average daily weight gain of feverish animals with or without clinical signs was recorded with prolonged episodes of fever, with an approximate decrease of 33 g/day for each day of fever.[10]

Because of the difficulty in identifying cattle with early stage respiratory disease in large feedlots, several advanced monitoring techniques have been developed and studied for their suitability to help making a rapid and accurate diagnosis in the field situation. The feeding and watering behavior of healthy and sick animals in a commercial feedlot has been examined using radiofrequency technology to record individual animal behaviors. Eating and drinking behaviors are associated with clinical signs of bovine respiratory disease, but there is no obvious predictive association between signs of bovine respiratory disease in recently arrived weaned beef calves and eating and drinking behavior. Calves that were sick had greater frequency and duration of drinking 4 to 5 days after arrival than calves that were not sick. Sick calves had significantly lower frequency and duration of eating and drinking 11 to 27 days after arrival but had greater frequency of eating 28 to 57 days after arrival than calves that were not sick. Calves at slaughter that had a higher percentage of lung tissue with lesions had lower frequency and duration of eating 11 to 27 days after arrival but had greater frequency and duration of eating 28 to 57 days after arrival. Experimentally, the electronic acquisition of feeding behavior data for feedlot cattle, when analyzed using cumulative sums (CUSUM) procedures, offers the potential for predicting morbidity before conventional visual methods of appraisal. The feeding behavior during the first 30 days when cattle are in a receiving pen may be used to detect animal morbidity approximately 4 days earlier than conventional methods typically employed in commercial feedlots. Overall accuracy, positive predictive value and sensitivity of the CUSUM prediction method were 87%, 91%, and 90%, respectively.

Infrared thermography used to monitor infrared heat loss in calves has been studied as a tool for early detection of animals with BRD, with promising results.[11] Measuring heat loss each time calves accessed waterers allowed to combine thermography data and watering frequency, a procedure that was deemed more sensitive, specific, and cost effective than conventional approaches to monitor animals at risk of BRD.[11]

Temperature-sensing reticuloruminal boluses have been used to monitor calves at risk of developing BRD. This technology allows remote and continuous recording of the reticular temperature of animals fitted with a temperature-sensing bolus and permits the identification of animals with prolonged fever episodes not associated with apparent clinical signs and thereby potentially improves the sensitivity of the detection of animals with BRD in a field setting.[10] Prolonged fever episodes are certainly not always attributable to respiratory disease, but even when not associated with clinical signs, prolonged fever was found to negatively affect average daily weight gain.[10]

Herd-Level Diagnosis

The clinician is limited in most situations to correlating clinical, epidemiologic, and necropsy findings in making a diagnosis. Diagnostic laboratories may not be readily available, and their resources for microbiological and serologic investigations may be much less than is needed for an accurate determination of causes. In case a diagnostic laboratory is not within reach, on-site postmortem examination of fresh carcasses of untreated animals deceased or euthanized in the early stage of the disease can provide valuable information and diagnostic material for further analysis. Coordinating the necropsy with a pathologist who may attend and guide the procedure via teleconference or assess gross findings on digital material and suggest collection of specific specimens is advisable.

A systematic method of data collection from the customized records of large feedlots has been developed and validated for use in the National Animal Health Monitoring System. The current collection of data from large feedlots provides an acceptable level of sensitivity and specificity for the program, but it is important that the veterinarian makes regular clinical observations to validate the data.

The course of the disease, especially when animals have been treated, alters the gross and microscopic appearance of tissues and the microbiological (bacteriologic, virological) and serologic findings so that the animal's status is impossible to determine.

CLINICAL PATHOLOGY

Antemortem Diagnostic Procedures

A number of diagnostic procedures are available that vary in their practicality, their suitability to detect specific pathogens, the rapidity with which test results are available, their economy, the level of stress for the patient, the quality of the material obtained, and the interpretability of the results.

Nasal/Nasopharyngeal Swabs

Nasal and nasopharyngeal swabs are frequently used for antemortem sample collection under field conditions because this procedure is technically less demanding and less invasive than a transtracheal wash or bronchoalveolar lavage. Certainly for upper respiratory tract viral infection such as BHV-1 this method has it merits. In contrast, the significance of bacterial isolates or negative test results must be interpreted cautiously.[12] Isolates of *M. haemolytica* from nasopharyngeal swabs were found to be highly representative for the isolates present in lung tissue in clinically affected animals.[13]

However, because most bacterial pathogens of the lower respiratory tract such as *M. haemolytica* can be part of the normal upper respiratory flora, isolation of one or several of these agents from a nasopharyngeal swab does not necessarily indicate that it is the cause of disease.[14] Although isolation of *M. bovis* from a nasal swab confirms the presence of this pathogen in the herd, the association of the presence of *M. bovis* in the upper respiratory tract with clinical disease or its presence in the lower respiratory tract reported in the literature is more variable. Some authors reported that *M. bovis* is commonly found in the upper respiratory tract in healthy calves; however, this is not confirmed by others.[44-45] On the other hand. cultures form nasopharyngeal swabs were found to yield a positive result in only 33% of animals infected with *M. bovis.*[13]

To collect a nasopharyngeal swab sample, a long, sterile culture swab guarded by an external sheath must be inserted through the nasal cavity of the properly restrained calf. The tip of the swab should be approximately at the height of the medial canthus of the eye before the tip of the swab is extruded beyond the sheath and firmly rotated against the mucosa. The tip of the swab must then be retracted into the sheath before removing the whole swab. Swabs may be submitted for bacterial/mycoplasma culture or virus isolation. Depending on the diagnostic objective, two or three swabs placed into specific transport medium for the analysis requested may be required.[13]

Transtracheal Wash/ Bronchoalveolar Lavage

Transtracheal wash (TTW) and bronchioalveolar lavage (BAL) are antemortem sample collection techniques yielding material suitable for a broader spectrum of diagnostic procedures. Besides of bacterial or fungal cultures, isolation/identification of virus or parasites samples can also be used for cytology.[12] These procedures are technically more demanding and more invasive than swab collection. They require proper restraint, local anesthesia, and aseptic technique. The TTW is performed in the previously clipped and aseptically prepared middle trachea region. A 10-gauge 2-inch needle is advance through a previously placed stab incision through the anesthetized skin between two cartilage rings into to trachea. Sterile polypropylene tubing is then inserted through the needle and advanced to the level approximately 10 cm beyond the thoracic inlet. Twenty to thirty mL of warm sterile saline or lactated Ringer's solution are then infused and rapidly aspirated back. This procedure typically yields between 5 and 10 mL of fluid that can either be submitted in the sealed syringe or transferred into a sterile tube.[15] Transtracheal wash samples can also be collected by endoscopy using the biopsy port.

To perform a BAL, commercially available kits are available that consist of a long tube with an inflatable cuff at the tip. The tube is passed intranasally into the trachea and further into the lungs, where it is lodged into a bronchus. The airway is then sealed by inflating the cuff, and warm lactated Ringer's solution is infused (approximately 30 mL for a calf and up to 180 mL for an adult cow) and immediately aspirated.[12,15]

Serology

Serum samples may be submitted for determination of the levels of specific antibody titers to suspected viral pathogens of the bovine respiratory tract and to *Mycoplasma* spp. Paired acute and convalescent serum samples from both affected and normal animals in the herd are desirable. In a group of animals in a feedlot, or dairy or beef cattle herd, serology for a specific etiologic agent may be followed over a period of time to determine seroconversion and its relationship to occurrence or absence of clinical disease. Although serology is highly sensitive because most respiratory pathogens of cattle induce a strong antibody response, the time delay with which results of paired serum samples become available presents a disadvantage.[16] Moreover, interpretation of serology results in animals with respiratory disease is often complicated by vaccination procedures and the timing of sample collection.[12] Serology is always done in serum and not in plasma.

Serum Biochemistry and Hematology

The suitability of serum concentrations of acute-phase proteins (APPs), such as fibrinogen, serum amyloid A (SAA), or haptoglobin (Hp), as a diagnostic tool to identify calves with BRD has been explored in numerous studies, with variable outcome.[17-21] Although increased concentrations of SAA and Hp in calves with BRD compared with healthy calves suggest that measuring the serum concentrations of these APPs could assist the early detection of calves with respiratory disease, the discriminative ability of Hp by itself for BRD was found to be no better than determination of the rectal temperature.[18] Haptoglobin concentrations vary considerably between calves even in healthy animals and were significantly affected by sex and rectal temperature.[18] The increase in serum Hp was found to occur within 24 hours of experimental challenge with *M. haemolytica* of BHV-1-infected calves but occurred between 4 and 8 days of infection with BRSV or BVD. For SAA, the wide variation between animals and the less pronounced increase in SAA concentration in animals suffering of respiratory disease impair the ability of this parameter to discriminate between healthy calves and animals affected by BRD.[20]

Leukocyte counts are of little value as predictors of respiratory disease because leukocytosis and neutrophilia occur in some animals, but in others there may be a neutropenia or no significant change.[16]

Other Procedures

With ultrasonographic equipment having become more available in routine food animal practice, the use of this imaging technique as potentially suitable ancillary diagnostic tool to diagnose BRD was studied by several authors.[22-24] Thoracic ultrasonography is relatively easy to perform with standard equipment under field conditions but requires a certain degree of expertise and routine of the operator to obtain reproducible results. Results of ultrasonographic examination of pneumonic calves were found to be highly correlated with the results of radiographic and postmortem examination. In contrast, the association between ultrasonographic findings and ancillary tests assessing lung and respiratory functions are less obvious.[25] Although the method was found a useful ancillary diagnostic tool to determine type and degree of pleural and pulmonary lesions, ultrasonography results were not found to be associated with animal health outcomes such as subsequent treatment, chronicity, wastage, or mortality.[26]

More invasive diagnostic procedures such lung biopsies have been studied for their usefulness as ancillary diagnostic tool. Although easy to perform with minimal risk to the animal, this procedure was not found useful for characterizing early respiratory disease in feedlot cattle under field conditions.[27]

Postmortem Samples

Postmortem samples may be collected either during on-site necropsy or at the diagnostic laboratory. Animals selected for postmortem sample collection should have the following characteristics:

- Representative of the typical clinical case of the herd
- In the early phase of the disease
- Untreated

Contacting the diagnostic laboratory before euthanizing the animal is advisable to discuss the diagnostic workup best suited for the specific herd, and thus the required material is advisable.

Bacterial Culture and Antimicrobial Sensitivity

The results of antimicrobial susceptibilities of bacterial pathogens isolated from the lung tissues of cattle with pneumonia over a period of years may provide some indication of trends in antimicrobial sensitivities, but the results are of limited value for making decisions about the selection of antimicrobial in affected animals.

The literature on the principles of antimicrobial susceptibility testing of bacterial pathogens associated with bovine respiratory disease has been reviewed. Two different methods are used. The Kirby–Bauer method

is the traditional in vitro test of bacterial susceptibility or resistance to antimicrobials, which uses a disk containing a standardized concentration of an antimicrobial. Bacteria grow or fail to grow surrounding the disk, and results are interpreted as resistance or susceptibility of the bacteria to certain antimicrobials. The serial-dilution testing uses a broth or agar medium with selected dilutions of antimicrobials in 1:2 dilution steps. Results are expressed as susceptible, intermediate susceptibility, or resistant and also as minimum inhibitory concentrations (MICs), which are considered more reliable. The MIC is defined as "the lowest concentration of an antimicrobial that prevents visible growth of a microorganism in agar or broth dilution susceptibility test."

It is important to adhere to standards set by the National Committee on Clinical Laboratory Standards/Veterinary Antimicrobial Susceptibility Testing Subcommittee (NCCLS/VASTS). Veterinary-specific breakpoints are determined by the NCCLS/VASTS through a consensus process based on reviewing pharmacokinetic, MIC, zone-diameter scattergram and clinical trial data relating to an antimicrobial application. The subcommittee selects MIC breakpoints and zone-interpretative criteria that best fit the definitions of susceptible, intermediate susceptibility, and resistant.

The most veterinary-specific breakpoints for pathogens in bovine respiratory disease have been determined for few antibiotics: ceftiofur crystalline free acid, ceftiofur-hydrochloride, ceftiofur-sodium, danofloxacin, enrofloxacin, florfenicol, spectinomycin-sulfate, tulathromycin, and tilmicosin-phosphate.[28,29] The breakpoints for oxytetracycline and chlortetracycline are adapted from human breakpoints developed for tetracycline.

NECROPSY FINDINGS

Because this clinical syndrome has a multifactorial etiology, necropsy findings will vary with the factors involved in a particular animal or herd, but some form of pneumonia is always present. The most common finding is fibrinous bronchopneumonia with varying degrees of fibrinous pleuritis and pulmonary abscesses (usually bacterial). The pneumonia involves the cranial lung lobes and the ventral portions of the middle and caudal lobes. Affected areas are dark-red and firm (cranioventral consolidation) and may be covered with thick sheets of fibrin on the visceral and parietal pleura. A cut surface of affected lung lobe is dark-red and may be marbled, whereas the bronchi and bronchioles are filled with a purulent exudate. Tracheobronchial and mediastinal lymph nodes are usually enlarged. In subacute or chronic cases, there are areas of coagulative necrosis or encapsulated abscesses within the consolidated lungs, and the fibrin in the pleura is replaced by fibrous tissue resulting in tough adhesions. Microscopically, alveoli are filled with an exudate composed of fibrin mixed with inflammatory cells, mostly neutrophils, macrophages, and fewer lymphocytes. The exudate extends to the lower airways.

With primarily viral infections, the common lesion is an acute, diffuse interstitial pneumonia. The lungs are diffusely red–tan, are enlarged, and do not collapse. All lobes are rubbery, wet, and heavy. Emphysema is present mainly in the diaphragmatic lobes, and there is white froth in the trachea. Microscopically, there will be interstitial pneumonia, necrotizing bronchiolitis, and, in some cases, viral inclusion bodies in epithelial cells or syncytial cells.

Samples for Postmortem Confirmation of Diagnosis

Tissue samples (lung and tracheobronchial lymph nodes) are submitted for histopathology, bacteriology, and virology. However, the length of time usually required to do the diagnostic work and interpret the results means that the procedure is expensive and to an extent inconclusive because the results are available only when the outbreak is over, particularly in feedlot operations.

Interpretation of Results of Clinical Pathology and Necropsy Findings

A large body of information has been generated on the microbiology and, more recently, molecular microbiology of specific pathogens associated with BRD, but only a fraction is applicable clinically. Insufficient effort has been directed toward integrating the information and applying it to the effective control of respiratory disease on the farm. Ideally, investigations of outbreaks of bovine respiratory disease should consist of in-depth examinations of a representative sample of the affected group and normal in-contact animals using a multidisciplinary approach involving clinical, epidemiologic, and laboratory investigation. These procedures, especially those requiring detailed virological and serologic examinations, are expensive, and in the light of the economic status of cattle industries, they are not likely to be lightly borne. But it will only be when such a multidisciplinary approach is brought to bear on bovine respiratory disease that we will improve our position with respect to knowing what actually occurs in outbreaks of the disease.

Of paramount importance is the identification of risk factors, which, if valid, gives the clinician a powerful clinical tool for the clinical management and control of BRD.

TREATMENT

The principles of the clinical management of outbreaks of acute undifferentiated bovine respiratory diseases are as follows:

- The clinician must visit the farm and do the clinical and epidemiologic investigations necessary to solve the problem, to assist the owner or the animal attendants with the clinical management of the disease, and to monitor the problem and the herd until recovery occurs. Simply dispensing antimicrobials to the owner without clinical examination of the animals is inadequate and contradicts the intention of the veterinarian–client relationship. The veterinarian is professionally obliged to provide explicit instructions about medication of affected animals and the drug withdrawal requirements and to keep adequate records of affected animals, treatments given, and the results of laboratory examinations. A final report should be prepared by the veterinarian and sent to the owner.
- Increased surveillance of the group is required to detect affected animals as soon as clinical abnormalities, such as depression, nasal discharge, and dyspnea, are noticeable.
- New cases must be treated as soon as they are detected. Each treated animal should be suitably identified and a record kept of the initial body temperature, the treatment administered and the instructions for follow-up treatment. Treatment failures and disease recurrence a few days after an initial apparent recovery are often attributable to late treatment. Delaying treatment until 48 hours after an experimental aerosol infection of *M. haemolytica* was found to prolong the course of the disease and increase mortality.
- Unless otherwise determined, when toxemia, causing pyrexia and anorexia, is present a primary or secondary bacterial pneumonia should be suspected, in which case antimicrobial therapy is of prime importance. Antimicrobials should be administered parenterally at least initially. Treatment via medicated feed or water bears the risk of underdosage in anorectic animals that are most severely affected. The regular use of a particular antimicrobial in feedlots may increase the level of resistance to *M. haemolytica*. Antimicrobials commonly used for treatment of UBRD are summarized in the treatment box of this section.
- Antiinflammatory therapy should routinely be combined with appropriate antimicrobial therapy in severe cases of BRD that are characterized by dyspnea, pyrexia, and anorexia.

A beneficial response to therapy should be apparent within 12 to 24 hours. The body temperature should decline significantly, and the appearance of the animal and its appetite

should improve. The response to treatment, or lack of it, is valuable information in making a final decision on cause. One of the emerging problems inherent in such broad policies in treatment is public health concern with the amount of antibiotic residue in meat, the spreading of resistance to antimicrobials, and the increased public awareness and concern about indiscriminate antibiotic use in food-producing animals.[30] Pressure is now being applied to use antimicrobials only when necessary, which necessitates a more accurate diagnosis.

A number of antimicrobials are registered for the treatment of BRD, of which some are listed in the treatment box of this section. Some of the antimicrobials with a label for treatment of BRD, such as third- and fourth-generation cephalosporins and fluoroquinolones, are also classified as critically important for human an animal health and should therefore be used restrictively. The World Organization for Animal Health (OIE) issued following recommendations for these classes of antimicrobials:[31]

- Not to be used as preventive treatment applied by feed or water in the absence of clinical signs
- Not to be used as first-line treatment unless justified—when used in a second-line treatment, it should ideally be based on the results of bacteriologic tests.
- Extra-label/off-label use should be limited and reserved for instances where no alternatives are available. Such use should be in agreement with the national legislature in force.

A recent mixed-treatment comparison meta-analysis of antibiotic treatments for BRD attempted a ranking of commonly used antimicrobials based on data published in over 90 publications.[32] Based on the publically available information, the authors estimated tulathromycin followed by enrofloxacin, danofloxacin, and florfenicol as the most effective antimicrobials for the treatment of BRD. Gamithromycin was estimated to be less effective but comparable to tilmicosin at label dose. Oxytetracycline, trimethoprim/sulfur, and ceftiofur injected at the base of the ear all ranked poorly in this analysis. For oxytetracycline, the retreatment risk was estimated to be between 64% and 77% and thus far above the risk calculated for other evaluated antimicrobials.[32]

Antiinflammatory therapy was beneficial in cases of severe BRD that is characterized by marked dyspnea, fever, and feed-intake depression. The best established effects of nonsteroidal antiinflammatory drugs (NSAIDs) in animals suffering of BRD are a more rapid decline of the rectal temperature and faster return to normal feed and water intake. Long-term effects on clinical outcome, disease recurrence, and severity of chronic lung lesions reported in the literature are more variable.[33] The precise mechanism through which NSAIDs act in calves with respiratory disease is not entirely understood, but antiinflammatory properties improving respiratory gas exchange and antipyretic and analgesic properties improving the wellbeing and thereby feed and water consumption are believed to be of prime importance. Numerous studies documented a more pronounced improvement in the initial phase of the disease in calves treated with NSAIDs and antimicrobials compared with animals treated with antimicrobials alone, a finding that is significant from an animal welfare perspective. A recent survey among U.S. feedlots revealed that, on average, NSAIDs were part of the standard initial treatment for respiratory disease in 55.9% of all feedlots, whereas steroids were used in 30.9% of the surveyed U.S. feedlots.[1] Nevertheless, the veterinarian must be aware that the combination of antiinflammatory and antimicrobial therapy complicates the assessment of the antimicrobial effect that should take place no later than 48 hours after first treatment because clinical improvement cannot unequivocally be attributed to the susceptibility of the causative pathogen to the administered antimicrobial drug. Experimental and clinical evaluation of the role of corticosteroid therapy in acute pneumonia in cattle yielded mostly unfavorable results.[34] Steroids are powerful antiinflammatory agents, but their effects on the animal's defensive measures, specifically with repeated use, reduces the value of their use in syndromes of infectious origin unless they have a short duration of action.[35]

TREATMENT AND PROPHYLAXIS

Treatment

Antimicrobial therapy

Tulathromycin (2.5 mg/kg SC as single dose) (R1)

Florfenicol (20 mg/kg q48 IM or 40 mg/kg SC as single dose) (R1)

Tilmicosin (10 mg/kg SC as single dose) (R1)

Gamithromycin (6 mg/kg SC as single dose) (R1)

Enrofloxacin* (2.5-5.0 mg/kg q24 SC/IM for 3 days or 7.5-12.5 mg/kg SC/IM as single dose) (R1)

Danofloxacin* (6 mg/kg q48h SC or 8 mg/kg SC as single dose) (R1)

Ceftiofur* crystalline acid free (6.6 mg/kg SC posterior pinna as single treatment) (R1)

Ceftiofur hydrochloride* (1.1-2.2 mg/kg SC q24 for 3 days) (R1)

Ceftiofur sodium* (1.2-2.2 mg/kg SC/IM q24h for 3 days) (R1)

Cefquinome* (1 mg/kg IM q24 for 3-5 days) (R1)

Oxytetracycline (10 mg/kg IM q24 for 4 days (R2)

Trimethoprim (2.66 mg/kg) + sulfadoxine (13.33 mg/kg) IM q24h for 3 days) (R2)

Antiinflammatory therapy

Flunixin meglumine (2.2 mg/kg IV as single dose) (R2)

Ketoprofen (3 mg/kg IM q24h for 2-3 days) (R2)

Carprofen (1.4 mg/kg IV or SC as single dose) (R2)

Meloxicam (0.5 mg/kg SC/IV as single dose) (R2)

Diclofenac (2.5 mg/kg IM as single dose) (R2)

Tolfenamic acid (2 mg/kg IM/IV q24-48h or 4 mg/kg IM/IV as single dose) (R2)

Prednisolone acetate (0.5 mg/kg IM q24h) (R3)

Dexamethasone (0.01-0.03 mg/kg IM/IV) (R3)

Flumethasone (0.03 mg/kg IM/IV) (R3)

Metaphylaxis

Tulathromycin (2.5 mg/kg SC as single dose) (R1)

Florfenicol (40 mg/kg SC as single dose) (R1)

Tilmicosin (10 mg/kg SC as single dose) (R1)

Gamithromycin (6 mg/kg SC as single dose) (R1)

Oxytetracycline long acting formulation (20 mg/kg IM) (R2)

Enrofloxacin* (7.5-12.5 mg/kg SC as single dose) (R3)

Danofloxacin* (8 mg/kg SC as single dose) (R3)

Ceftiofur* crystalline acid free (6.6 mg/kg SC posterior pinna as single treatment) (R3)

Cefquinome* (1 mg/kg IM q24 for 3-5 days) (R3)

Vaccination

Vaccination against *M. haemolytica* and *P. multocida* (R2)

Vaccination against *H. somni* (R3)

Vaccination against BRSV, PI-3V, BHV-1 (R2)

Vaccination against BVDV (R2)

**These are classified as critically important antimicrobials in human and veterinary medicine. Use as first-line treatment is discouraged.*

CONTROL

When confronted with an outbreak, one of the major decisions to be made is whether or not to recommend metaphylactic antimicrobial mass medication of all in-contact animals in an attempt to treat cases in the preclinical stage.

Mass Medication or Metaphylaxic Antimicrobial Use

Veterinarians frequently recommend metaphylactic mass medication, and field observations claim beneficial results.[8] Treatment of a whole group of animals

is often preferable to selecting individuals for therapy because of the diagnostic challenge of identifying early cases of BRD.[36] Although the metaphylactic use of antimicrobials to control BRD is debatable from the point of view of prudent antimicrobial use, this approach was documented to considerably reduce morbidity and mortality rates in a group, thereby having a significant positive effect on animal health and welfare.[37,38]

The use of tilmicosin at 10 mg/kg BW subcutaneously, florfenicol at 40 mg/kg BW subcutaneously, gamithromycin at 6 mg/kg BW subcutaneously, tulathromycin at 2.5 mg/kg BW, and ceftiofur crystalline-free acid at 6.6 mg/kg BW administered subcutaneously at the base of the ear was found effective in reducing the morbidity rate when given to feedlot calves at high risk of developing respiratory diseases.[39-41] The results obtained with long-acting oxytetracycline at a dose of 20 mg/kg BW or higher reported in the literature are more variable.[37] Although an economical advantage may result from the lower price of oxytetracycline compared with newer antimicrobials, morbidity rates were found to be higher with the use of oxytetracycline compared with tilmicosin.[37]

The mass medication of feed supplies or water of newly arrived feedlot cattle has been investigated as a method of reducing the morbidity and mortality resulting from respiratory disease in a number of studies, but results are equivocal. Although studies suggesting that chlortetracycline and sulfamethazine in feed are effective in reducing morbidity associated with BRD have been published, issues around study design and data analysis questioned the validity of the results.[37] A standard recommendation is to provide 150 mg/kg BW for the first 24 hours and reduce the level to 75 mg/kg BW for the duration of the medication period, which may last 5 to 10 days.

Management of Risk Factors

As a general outline for the control of bovine respiratory disease, the following factors are considered as contributing to disease, and their effects must be minimized with suitable management and disease prevention techniques:

- Young, growing cattle are more susceptible than mature cattle because of a lack of sufficient immunity. The mixing of young cattle of different origins requires increased surveillance to detect evidence of disease. Vaccination of calves at strategic times may be necessary.
- Cattle purchased from various sources and mingled in a feedlot are more likely to develop bovine respiratory disease than cattle that have originated from one source. Some cattle will be highly susceptible and others relatively resistant because of differences in nasal flora and immunologic, genetic, and nutritional backgrounds. A high level of management and constant surveillance are necessary to recognize, isolate, and treat clinical cases early to minimize morbidity and case mortality.
- Rapid fluctuations in environmental temperatures and relative humidity, not only during the fall and winter months but also during warm seasons, will commonly precede outbreaks of respiratory diseases. Every practical and economical management technique must be used to provide as much comfort as possible and to avoid overcrowding.
- Inadequate ventilation is a major predisposing cause of respiratory disease of cattle raised indoors. This is of major importance in dairy herds during the winter months in temperate climates.
- Weaning calves 3 weeks or longer before sale was found to be beneficial for later development and animal health in several studies.[37]
- Weaning of beef calves during inclement weather may exacerbate the stress of weaning and commonly results in an outbreak of respiratory disease.
- Stress associated with the marketing of cattle is a major factor. The movement of cattle through saleyards—where they may be overcrowded; mixed with cattle of many different origins; temporarily deprived of adequate feed and water; handled roughly while being sorted, weighed, tagged, blood sampled, vaccinated, or injected with antibiotics and/or vitamins, and then loaded on to uncomfortable vehicles and transported long distances without adequate rest stops—is stressful. The practice of preconditioning cattle before they enter the feedlot must continue to be examined to determine which aspects are most profitable.

Presale vaccination programs are designed to establish an effective immune response to common respiratory tract pathogens well in advance of any natural exposure that may occur while calves travel through the auction market or after they arrive in the feedlot. These programs usually require calves to be castrated, dehorned, and vaccinated against BHV-1, PI-3V, BRSV, and BVDV. Some programs also require vaccination against *H. somni* and *M. haemolytica.* Presale conditioning programs involve these procedures but also include weaning and nutritional components. Most such conditioning programs require calves to be weaned and adjusted to a roughage and concentrate diet for at least 30 days before sale.

Vaccines

Although vaccines for the control of acute respiratory disease associated with BHV-1, PI-3V, and *Pasteurella* spp. are available and widely used in the field, evidence documenting their efficacy under field conditions is scant.[1,37] According to a large recent survey conducted in the United States, BVD vaccines are used in 96.6 %, BHV-1 vaccines in 93.7%, BRSV vaccines in 89.5%, and PI-3V vaccines in 85.1% of surveyed feedlots to control respiratory disease.[1] The use of vaccines against *Pasteurella* spp. (63.8%) and *Histophilus somni* (69.7%) is less common. Preshipment vaccination of beef calves 3 weeks before weaning with vaccines containing BHV-1, PI-3V, *Pasteurella* spp., and *H. somni* did not reduce the incidence of UBRD compared with those unvaccinated.

Many veterinarians and feedlot owners maintain that vaccination against respiratory disease is an essential component in their disease prevention programs, both to prevent specific disease of the respiratory tract such as clinical infectious bovine rhinotracheitis (IBR) and to reduce losses resulting from respiratory disease in the first few weeks after arrival.

In North America, a large number of bacterial and viral vaccines are available for the control of bovine respiratory disease. There are single-antigen and multiple-antigen vaccines and modified live-virus or inactivated-virus vaccines containing one or more of the following antigens: *M. haemolytica, P. multocida, H. somni,* BHV-1, PI-3V, BRSV, and BVDV. There are many multiple-antigen vaccines containing combinations of the respiratory viruses, BVDV, *H. somni, P. multocida,* and *M. haemolytica.*

Selection of Vaccines

The selection of which vaccine to recommend for the control of BRD in feedlot cattle is currently not possible based on the efficacy information that is available to the veterinarian. The vaccines are used widely, and many anecdotal claims for their effectiveness are made, but there is little scientific evidence that the vaccines are effective and economical in reducing the incidence or the consequences of respiratory disease such as suboptimal weight gain. In most cases the vaccines were approved for sale on the basis of tests for safety in animals, and the potency was measured by a serologic response to the vaccine or experimental challenge in animals under laboratory conditions. Nevertheless, although vaccination is consistently shown to result in antibody production, vaccine-induced titers are not always correlated with protection against disease.[37]

Efficacy of Vaccines

Meaningful field trials to evaluate vaccines for the control of bovine respiratory disease are difficult to achieve. The case definition of what is a "case of respiratory disease" has been very general, such as the presence of anorexia, depression, and a fever. Therefore, when testing a vaccine for the control of pneumonic pasteurellosis, the conclusions reached may be questionable if the cause of the sick animals in either the vaccinated or control group is not known—thus the importance of case definition. In contrast to field trials, the measures used by the manufacturer in the laboratory challenge of the vaccine have been specific. In a field trial, the control group and the vaccinated groups must be comparable. Where more than one vaccine is used to control respiratory disease in vaccinates and controls, it is difficult to evaluate one of the vaccines or the components of a multiple-antigen vaccine unless large numbers of animals are used. Another problem is the difficulty of having the controls and the vaccinates experience approximately the same risk of being affected with respiratory disease.

Field trials for bovine respiratory disease vaccines are often unsatisfactory because of inadequate planning, unsatisfactory experimental design, and lack of monitoring. The following comments on the use of vaccines as an aid in the control of acute undifferentiated respiratory disease in feedlot cattle are based on the current information available.

Pasteurella Vaccines

Because fibrinous pneumonia associated with *M. haemolytica* is the most common lesion associated with bovine respiratory disease in feedlot cattle, much of the emphasis has been on the development of effective vaccines for bovine pneumonic pasteurellosis. Based on the immunologic and microbiological observations of both naturally occurring and experimentally induced pneumonic pasteurellosis, it appears that effective artificial immunization of cattle is possible. High levels of naturally acquired antibody to *M. haemolytica* have been associated with protection against the disease.

Antibodies to *M. haemolytica* leukotoxin and certain bacterial surface components appear to be important for resistance to disease. The basis of a recently introduced pasteurella vaccine is that vaccination of calves with a leukotoxic culture supernatant from pathogenic *M. haemolytica* provided some protection against experimental challenge with the organism.

Vaccination of recently shipped nonpreconditioned calves with the vaccine in Ontario resulted in a slight decrease in morbidity, slight improvement in response rates, and perhaps an important reduction in relapse rates. When the vaccine was combined with an intramuscular modified live BHV-1/PI-3V vaccine, the morbidity rate was increased, the response rate was decreased, and the mortality rate was increased in some groups. It appears that the use of modified live-virus vaccines in recently arrived calves is contraindicated; this is consistent with earlier observations in the Bruce County Project, where fall-placed calves were vaccinated on arrival with a modified live-virus vaccine.

A recent meta-analysis reviewing the evidence behind the recommendation to vaccinate to control BRD in calves identified 18 trials that studied the use of vaccines either against *M. haemolytica* or *M. haemolytica* and *P. multocida*.[42] From these data the authors calculated a risk ratio (RR) of 0.93 (CI 0.89-0.98), indicating a significantly although minimally lower risk of morbidity in vaccinated feedlot cattle compared with controls and thus a potential benefit of using pasteurella vaccines.

Histophilus somni *(Formerly* Haemophilus somnus*) Vaccine*

Few studies have investigated the effectiveness of *H. somni* vaccination of feedlot cattle to control respiratory disease. The antibody response was found to be associated with protection against *H. somni*.[43] When used as part of a preconditioning program, the vaccine tended to have mildly positive or neutral effect on morbidity and mortality related to respiratory disease, whereas the effect appeared to be neutral or even negative when animals were vaccinated on arrival at the feedlot.[43] Observed effects on morbidity and mortality were below the significance level. Little scientific evidence is currently available to support the use of *H. somni* vaccination to control BRD.[42]

Viral Vaccines

Because prior infection of the respiratory tract with either BHV-1, BRSV, or PI-3V may predispose to pneumonic pasteurellosis, the vaccination of beef calves 2 to 3 weeks before weaning and feedlot cattle 2 weeks before shipment to a feedlot has been recommended as part of a preconditioning program. The results are variable, but vaccination of calves at 3 to 6 months of age with an intranasal modified-live BHV-1 and PI-3V vaccine has provided protection against experimental pneumonic pasteurellosis induced by aerosol challenge with BHV-1 followed 4 days later by an aerosol of *M. haemolytica*. It is important to vaccinate the calves at least 2 weeks before they are weaned, stressed, or transported to a feedlot.

A modified-live BHV-1 vaccine given to beef calves before weaning, at weaning, or immediately after arrival in the feedlot was associated with a significant reduction in the treatment rate in one of three groups immunized before weaning and in calves immunized after arrival in the feedlot. There was no significant effect of the vaccine on treatment rate in calves immunized at weaning, in calves immunized after arrival in a bull test station, or in yearlings immunized after arrival in the feedlot. It would appear that the vaccine did provide some protection, but the small reduction may not justify the cost of the vaccination program.

Some feedlot veterinarians recommend that feedlot cattle be vaccinated on arrival with an *M. haemolytica* vaccine, the BHV-1 and PI-3V vaccine, an *H. somni* vaccine, and the BRSV vaccine. The BVDV vaccine is the most commonly used vaccine in feedlots in the United States because BVD virus circulating in feedlots among newly received calves is considered to be an important factor predisposing to BRD.[1] It is expected that control will be achieved if the animals are vaccinated against all the common pathogens that contribute to lesions of bovine respiratory disease. However, there is little, if any, published evidence based on controlled field trials that such blanket recommendations are justifiable.

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PNEUMONIC PASTEURELLOSIS OF CATTLE (SHIPPING FEVER PNEUMONIA)

SYNOPSIS

Etiology *Mannheimia haemolytica* serotype A1 and A6; *Pasteurella multocida* serotype A3.

Epidemiology Young, rapidly growing cattle, especially recently weaned beef calves placed in feedlot (shipping fever) and unweaned dairy calves (enzootic calf pneumonia). Can occur in nursing calves and mature cows. Stressors include transportation, mixing animals from many different sources, weaning, processing procedures, and ineffective ventilation of housed animals

Signs Acute respiratory disease, abnormal lung sounds, fever, toxemia, anorexia, sudden death; respond to treatment with antimicrobials.

Lesions Acute fibrohemorrhagic pneumonia, frequently with pleuritis.

Clinical pathology Culture organism from nasopharyngeal swabs or transtracheal wash/bronchioalveolar lavage fluid.

Diagnostic confirmation Culture organism from lung and histopathology of lung.

Treatment Antimicrobials, NSAIDs.

Control Preconditioning programs. Management strategies to reduce stress. Metaphylactic antimicrobial mass medication on arrival in the feedlot. Viral and bacterial vaccines.

ETIOLOGY

Pneumonic pasteurellosis is an entity within the **bovine respiratory disease (BRD) complex**, characterized clinically by acute bronchopneumonia with toxemia and pathologically by lobar, anterioventrally distributed, exudative pneumonia in which fibrin is usually a prominent part of the exudate and fibrinous pleuritis is common.

M. haemolytica of the order Pasteurellaceae, a gram-negative coccobacillus is considered the most important bacterial pathogen in weaned calves, whereas *Pasteurella multocida* is more commonly isolated from the lower respiratory tract of younger calves with respiratory disease.[1] There are currently 12 recognized serotypes of *M. haemolytica* (A1, A2, A5, A6, A7, A8, A9, A12, A13, A14, A16, and A17), of which serotype A2 and to a lesser extent A4 are commonly isolated from the upper respiratory tract of healthy ruminants and thus are considered normal inhabitants of the nasopharynx and tonsils of ruminants. Serotypes A1 and, more recently, A6 are most commonly associated with respiratory disease in cattle.[2,3]

Pasteurella multocida has 5 capsular serogroups (A, B, D, E, and F) and 16 somatic serotypes (1-16). Whereas *P. multocida* serotypes B and E are associated with hemorrhagic septicemia in cattle and water buffaloes in tropical regions, serotype A3 and, to a lesser extent, D3 are the major pathogens associated with lower respiratory tract infection in cattle.[4] Clearly, the presence of *P. multocida* in the upper respiratory tract does not equal disease because of the ubiquitous nature of this organism, but the proportion of fatal cases of shipping fever attributable to *P. multocida* appears to be increasing, according to the recent literature.[4]

The *Pasteurella* spp. are opportunistic pathogens and the final cause of the pneumonia but rely on predisposing mechanisms allowing the bacteria to enter and colonize the lung and produce the lesions. Viruses or mycoplasmas may act synergistically to allow the bacteria to be pathogenic. Numerous environmental predisposing factors, generally termed as stressors, have been discussed, such as the following:

- Transportation
- Comingling of groups of cattle from different sources
- Confinement of cattle
- Ineffective housing and ventilation
- Extreme temperature changes
- Weaning
- Processing procedures

EPIDEMIOLOGY

Occurrence

Pneumonic pasteurellosis is a common disease of young growing cattle in Europe, the United Kingdom, and North America. The condition has arbitrarily been subdivided into two main categories that are **enzootic calf pneumonia (ECP),** primarily ascribed to *Pasteurella multocida* and **shipping fever** mainly ascribed to *Mannheimia haemolytica*. Whereas ECP is considered a condition predominantly affecting unweaned dairy calves, shipping fever is discussed as disease of older weanling, stocker, or feeder cattle.[1] Nursing beef calves, yearlings, and mature dairy and beef cows may also be affected, but less frequently.

Morbidity and Mortality

Shipping fever is the most common illness among cattle in U.S. feedlots, with an average incidence of 16.2% and a mortality rate of approximately 4%.[5] Considerable regional differences in morbidity rates were reported, with feedlots in the central region of the United States reporting twice the percentage of cattle affected compared with other regions (17.8% vs. 8.8% respectively).[5] The peak incidence of disease occurs within the first 3 weeks after arrival of the calves in the feedlot.

The incidence of respiratory disease in postweaning dairy heifers in the United States was 5.9%, and respiratory disease was determined to be the most common cause of death in weaned and the second most common cause of death in unweaned heifers (after diarrhea) with 46.5% and 22.5% of death losses respectively in the United States.[6] The percentage of death losses attributable to respiratory disease in weaned dairy heifers rose from 34.8% of all deaths in 1991 to 46.5% in 2007.[6]

An observational study of the epidemiology of fatal fibrinous pneumonia in feedlot calves purchased from auction marts in western Canada and placed in a commercial feedlot between September 1 and December 31 over a 4-year period identified some valid information. Peak fibrinous pneumonia occurred approximately 16 days after arrival at the feedlot; the median number of days between arrival and the first treatment of fatal fibrinous pneumonia cases varied from

3 to 8 days. The fatal fibrinous pneumonia rates varied 11-fold (0.25%-2.73%) between years. The fatal fibrinous pneumonia proportionate mortality varied from 10% to 57%. Fully 75% of the calves that died of fibrinous pneumonia already were sick within 2 weeks after arrival. When the incidence of fatal fibrinous pneumonia was high (greater than 2%), the disease clustered, either within certain truckload groups of calves or within certain pens, or within both. Clustering could have been a result of contagious factors, noncontagious factors, or both.

Economic Importance

Pneumonic pasteurellosis is a major cause of economic loss in the feedlot and dairy industry. It is responsible for the largest cause of mortality in feedlots in North America. In addition to the death losses, the costs of treatment (which include the personnel involved in the detection and actual treatment, the drugs used and the vaccines) are considerable. Indirect costs of the disease, which include increased risk of culling or death, losses associated with delayed entry into the milking herd and possibly decreased productivity are difficult to estimate.[7] The average cost of treatments for a single case of respiratory disease in feedlots determined in a recent U.S. survey was $23.40, which was considerably higher than the costs per case of $12.59 reported in similar study from 1999.[5] A study from 1990 estimated the average costs per clinical case with an average $14.71 for unweaned and $1.95 for weaned dairy calves.[8] Long-term costs were estimated at between $15 and $49 for a typical Dutch dairy farm with an overall disease incidence of 60%.[9]

Risk Factors

Animal Risk Factors

The disease occurs most commonly in young growing cattle from 6 months to 2 years of age but all age groups are susceptible. Calves that are nonimmune to *M. haemolytica* are considered to be more susceptible to the disease than calves that have serum neutralizing antibodies to the organism and its leukotoxin (LKT). Calves that have recovered from the experimental disease are resistant to naturally occurring disease. Auction market calves that originate from many different farms and are mixed at the market are at high risk. Calves may develop the disease before weaning if housed in crowded, poorly ventilated barns, when exposed to rapid changes in temperature and humidity, or when subjected to stress.

The disease occurs commonly in outbreaks 7 to 10 days after cattle have arrived in the feedlot following stressful transportation. This forms a major part of the "shipping fever" complex, which is a major hazard in the practice of rearing beef cattle on range country and then transporting them long distances to other centers for growing and finishing. However, the distance that calves were transported by truck from the auction markets to the feedlot was not associated with an increased risk of fatal fibrinous pneumonia, as determined by one study.

Herd outbreaks in dairy herds are not uncommon, especially when recent introductions have been made or cattle are returned to their home farms after summer grazing on community pastures or exhibition at fairs. Outbreaks of peracute pleuropneumonia attributable to *M. haemolytica* have been reported in adult dairy cattle. Many animals were affected of which a high proportion were in the immediate postparturient period. All affected farms had purchased cows and/or heifers within the last 12 months, but there was no history of transportation or movement of affected animals. Mature beef cows are also susceptible to pneumonic pasteurellosis if they are subjected to stressors during the summer months or in the fall of the year, usually associated with moving large groups to or from pasture during inclement weather.

Environmental and Management Risk Factors

A number of environmental risk factors have been discussed in the literature. In addition to prior infection with viral pathogens that are discussed under "Pathogen Risk Factors," other predisposing factors generally summarized as "stressors" include the mixing of cattle from different sources; transportation; sudden and extreme temperature changes; cold coupled with wetness, dust, or acute metabolic disturbances; and feed and/or water deprivation (e.g., during transportation).[4] Mixing of recently weaned beef calves from different sources at auction markets was associated with an increased risk of fatal fibrinous pneumonia in calves moved to feedlots in western Canada, especially in November, shortly after auction sales had peaked and when the feedlot was reaching capacity.

The role of stress as a risk factor in shipping fever pneumonia has been examined experimentally. Experimental transportation and handling to mimic stress, followed by an aerosol of *M. haemolytica,* did not result in significant lesions of pneumonia but did make the animals susceptible to BHV-1. Similarly, stress related to weaning and maternal separation at the time of primary BHV-1 infection increased the innate immune response that correlated significantly with mortality following a secondary bacterial infection.[10]

Confinement in drafty or humid and poorly ventilated barns, exposure to inclement weather, transport, fatigue, and deprivation from feed and water are commonly followed by outbreaks of the disease in cattle. In calves kept at a constant temperature of 16° C (60° F), the bacterial populations in the nasopharynx were at a minimum between 65% and 75% relative humidity and tended to rise at humidities outside that range.

An increase in virulence of the bacteria is often evident after animal passage; at the commencement of an outbreak only those animals that have been subjected to devitalizing influences are affected, but the disease may subsequently spread to other animals in the group. There is little tendency for the disease to become an area problem, with sporadic outbreaks occurring with the appearance of conditions favorable to the development of the disease.

Pathogen Risk Factors

Pasteurella are considered normal inhabitants of the upper- and opportunistic pathogens of the lower respiratory tract of cattle. Although the precise mechanism is not well understood, concurrent viral infection of the upper respiratory tract is considered a key element in the pathogenesis of pneumonic pasteurellosis. Viral infection of the upper respiratory tract causes cellular damage, resulting in microenvironmental changes that facilitate the colonization of the mucosa of the upper respiratory tract with pathogenic serotypes of *M. haemolytica* and/or *P. multocida.*[1] Calves inoculated intranasally with BHV-1 or PI-3V become more susceptible to colonization with *M. haemolytica* even in the presence of antibodies to the organism in the serum and nasal secretion.

The assumption that infection with several different viruses and mycoplasma may predispose to pneumonic pasteurellosis has been a subject of intense research activity and is corroborated by the observation that coinfection of the lower respiratory tract with other pathogens is significantly more common than infection with either *M. haemolytica* or *P. multocida* alone.[4] Seroepidemiologic surveys of cattle in feedlots reveal that BHV-1, PI-3V, BVDV, and BRSV were present, active, and associated with respiratory disease. The presence of antibody indicates current or recent exposure to the virus but does not indicate resistance. Cattle with low titers to BHV-1 or BRSV were at increased risk of subsequent treatment for bovine respiratory disease. Treated cattle also had greater increases to PI-3V and/or BVDV antibodies than control calves. Although there is evidence that BVDV can experimentally affect certain immune mechanisms, there is little direct evidence that the virus is a major predisposing factor in the causation of naturally occurring pneumonic pasteurellosis. Seroepidemiologic surveys indicate that seroconversion to BVDV is related to increased risk of respiratory disease at both individual and group levels. Serologically, there is also evidence of a high prevalence of *M. bovis* and *M. dispar* in feedlot calves. But the relative importance of these pathogens as a cause and effect relationship is controversial. Bovine coronavirus has been associated with some natural outbreaks of shipping

fever in feedlot cattle. Up to 80% of affected animals shed bovine coronavirus from their nasal cavities when the infection rate with *Pasteurella* spp. was low.

The virulence factors of *M. haemolytica* and *P. multocida* include the following:
Surface proteins and carbohydrates

- Adhesion proteins and fimbriae allowing adherence to the mucosal membranes
- Capsular polysaccharides
- Outer membrane proteins (OMPs), some of which are iron regulated and critically important for iron acquisition by the microorganism
- Lipopolysaccharides (also termed *endotoxins,* particularly lipid A) that are responsible for pyrexia and hypotensive shock and contribute to pulmonary lesions
- Lipoproteins (e.g., lipoprotein PlpE)

Toxins and extracellular enzymes

- Leukotoxin (LKT) of *M. haemolytica,* which induces apoptosis or cell necrosis in ruminant leukocytes
- Glycoproteases that can hydrolyze IgG
- Neuramidase

M. haemolytica serotypes A1 and A2 can survive for long periods of time in relatively low-nutrient in vivo fluids. Both strains survived for at least 244 days in ovine and 156 days in bovine tracheobronchial washings, respectively. This may provide an explanation for the long survival of the organism in the nasopharynx of ruminants.

Immune Mechanisms

Calves that have recovered from the experimental disease are resistant to naturally occurring disease. Numerous *M. haemolytica* antigens may stimulate the immune response and resistance to disease. These antigens include many of the previously mentioned virulence factors such as capsular polysaccharides, LKT, and surface antigens, including iron-regulated proteins, a serotype-specific outer membrane protein, and several other antigens that are less well defined. High antibody responses to *M. haemolytica* surface-extract proteins are correlated with resistance to experimental pneumonic pasteurellosis. Resistance to experimental challenge with the organism correlates directly with serum LKT neutralizing titers. Aerosol exposure of calves to *M. haemolytica* results in the development of LKT neutralizing antibodies in pulmonary lavage samples and an accompanying increase in serum neutralizing antibody titer. Because aerosol exposure of calves to viable *M. haemolytica* elicits a protective immune response characterized by enhanced clearance of the organism from the lung and by protection against fibrinous pneumonia, it is possible that the presence of preexisting antibodies to LKT in the lungs provides immunity by protecting phagocytic leukocytes from LKT and by promoting phagocytosis and intracellular killing of the organism.

Passive immunization with antibodies to whole *M. haemolytica* or LKT-containing supernatants provides protection against experimentally induced pneumonic pasteurellosis similar to the protection provided by active immunization with these antigens. In contrast, antibodies to lipopolysaccharide provided little protection against challenge.

Cattle exposed to live organisms produce antibodies to both cell surface antigens and LKT, whereas exposure to the killed vaccine results in the production of antibodies primarily to cell surface antigens.

The experimental lung challenge of calves with formalin-killed *P. multocida* does not provide subsequent protection to challenge with live *P. multocida.*

Method of Transmission

Transmission of pasteurellas probably occurs by the inhalation of infected droplets coughed up or exhaled by infected animals, which may be clinical cases or recovered carriers in which the infection persists in the upper respiratory tract. *M. haemolytica* and *P. multocida* are highly susceptible to environmental influences and it is unlikely that mediate contagion is an important factor in the spread of the disease. When conditions are optimal, particularly when cattle are closely confined in inadequately ventilated barns, when overcrowded in trucks and trains, or held for long periods in holding pens in feedlots, the disease may spread very quickly and affect a high proportion of the herd within 48 hours. In animals at pasture, the rate of spread may be much slower.

PATHOGENESIS

Colonization of Upper and Lower Respiratory Tract

Considerable research has centered on determining how the pasteurellas, which are part of the normal flora of the upper respiratory tract, colonize first the upper- and then the lower respiratory tract. Under normal conditions the bovine lung is practically sterile because of an effective lung clearance mechanism. The current hypothesis is that a combination of a viral infection of the respiratory tract and/or devitalizing influences from transportation, temporary starvation, weaning, rapid fluctuations in ambient temperature, the mixing of cattle from different origins, and the excessive handling of cattle after arrival in a feedlot can collectively promote an increase in the total number and virulence of pasteurellas in the nasopharynx. Enhanced bacterial growth in the upper respiratory tract results in inhalation of aerosol droplets into the trachea and lungs.[2]

In clinically normal cattle, *M. haemolytica* are present in low numbers in the tonsil and nasal passages, and those that are isolated are predominantly serotype A2, which is rarely associated with disease. Exposure of healthy cattle to stressors such as viral infection, change in management practices, and environmental changes leads to an explosive growth and selective colonization by *M. haemolytica* A1 in the upper respiratory tract.

The experimental intranasal exposure of calves to a leukotoxin-deficient *M. haemolytica* elicits an increase in the serum antibody titers against the organism and decreased colonization of the nasopharynx by wild-type *M. haemolytica.* This could allow an immune response to develop before transportation and offer protection from nasopharyngeal colonization and pneumonia by wild-type *M. haemolytica.*

Under normal conditions, alveolar macrophages will effectively clear pasteurellas from the alveoli by phagocytic mechanisms. When the large numbers of organisms enter and colonize the lung, they interact with alveolar macrophages. Neutrophils enter the lung within the first few hours after bacterial inoculation.

Bovine alveolar macrophages release superoxide anion when exposed to *M. haemolytica,* and the response is dependent on the presence of opsonizing antibody and the quantity of organisms presented to the phagocyte. This may have a major role in the pathogenesis of the acute lung injury associated with pneumonic pasteurellosis. It is an important mechanism by which this phagocyte can initiate microbicidal activity and may provide clues to further study of the defense mechanisms of the lung.

Virulence Factors and Cellular and Humoral Reactions

The lung injury caused by the organisms after entry into the lung is dependent on important virulence factors.

Of the previously mentioned virulence factors, the ones that are considered the most relevant for the pathophysiology of pulmonary pasteurellosis of cattle are as follows:

- Fimbriae
- Capsular polysaccharides
- Lipopolysaccharide (LPS or endotoxin)
- Leukotoxin (LKT) for *M. haemolytica*

The interactions of these virulence factors contribute to the pathogenesis of the disease. Fimbriae enhance the colonization of the upper respiratory tract. The capsular polysaccharides of the organism inhibit complement-mediated serum killing and phagocytosis and intracellular killing of the organism. The capsule also enhances neutrophil-directed migration and adhesion of the organism to the alveolar epithelium. The LPS can alter bovine leukocyte functions and is directly toxic to bovine endothelium. It also modifies cardiopulmonary hemodynamics and elevates circulatory prostanoids, serotonin, cAMP, and cGMP. The organism induces morphologic alterations in bovine pulmonary endothelial cells, the effects of

which can be partially inhibited by indomethacin. Tissue factor is involved in intraalveolar fibrin deposition and coagulopathy associated with pneumonic pasteurellosis in cattle.

The migration and activation of neutrophils in inflamed tissue are regulated by a complex network of interactions among cytokines, leukocytes, vascular endothelium, cellular adhesion molecules, and soluble chemotactic factors. The inflammatory cytokines tumor necrosis factor alpha, interleukin (IL)-1 beta, and IL-8 play a central role in the initiation and orchestration of these interactions. IL-8 is the dominant cytokine expressed within the lungs during the acute phase of pneumonic pasteurellosis.

The **lipopolysaccharide (LPS)** of *P. multocida* or *M. haemolytica* is capable of causing direct injury to bovine pulmonary arterial endothelial cells, which may be a contributing pathogenic mechanism. LPS interacts with numerous cell types and humoral mediator systems, resulting in widespread injury to the lung. LPS can readily cross the alveolar wall either from the air or blood and interact with cells and humoral mediators. LPS can be found in the neutrophils in the alveolus, interstitial tissue, and capillary lumen; in intravascular, interstitial, and alveolar macrophages; in endothelial cells; and on alveolar epithelial cell surfaces. The interaction of endotoxin with cells leads to cell activation and death.

Leukotoxin (LKT) is produced by all known serotypes of *M. haemolytica* and is a heat-labile protein exotoxin, a pore-forming cytolysin that affects macrophages, lymphocytes, neutrophils, and platelets of ruminants specifically.[2] The bacterium produces LKT, with maximum production occurring during the log phase of growth, peaking after 6 hours of incubation. Following the inhalation of *M. haemolytica* into the lung, there is an accumulation of neutrophils that, when destroyed by LKT, results in the release of proteolytic enzymes, oxidant products, and basic proteins, which degrade cellular membranes, increasing capillary permeability. This causes fluid accumulation in the interstitium of the alveolar wall, alveolar wall necrosis, and pulmonary edema. LKT also induces histamine release from bovine mast cells.

LKT follows a species-specific dose-dependent activation-inhibition paradox on bovine leukocytes.[2] Exposure to low concentrations of LKT can activate neutrophils and macrophages to stimulate respiratory burst and degranulation, proinflammatory cytokine release, and histamine release from mast cells. At high concentrations, LKT induces apoptosis in bovine leukocytes. Apoptosis is a process of programmed cell death distinguished from necrosis by the orderly shutdown of cell functions. At even higher concentrations, LKT causes transmembrane pore formation, resulting in cell swelling and subsequent cell death.[2]

Supernatants of the organism can also cause rapid cytolysis of platelets. The genes that code for the synthesis and secretion of LKT have been cloned. It is a highly immunogenic protein that is produced by all 15 serotypes of *M. haemolytica.* Cattle with high LKT antibody titers have higher survival rates in natural and experimental cases of pneumonic pasteurellosis than animals with low antibody titers. LKT antigens and bacterial surface component are now used in commercial vaccines to elicit resistance against pneumonic pasteurellosis.

Experimental Pneumonic Pasteurellosis

In an attempt to understand the pathogenesis of bovine pneumonic pasteurellosis, the experimental disease has been reproduced using several different methods; the most commonly used is the sequential aerosol infection of calves with either the PI-3 virus or the BHV-1 virus followed by an aerosol of *M. haemolytica* 3 days or more later. Exposure of calves to aerosols of PI-3V followed by *M. haemolytica* at intervals of 3 to 13 days later results in a purulent bronchopneumonia. The virus interferes with the lung clearance of *M. haemolytica* when an aerosol of the bacteria is given 7 days following the viral infection. There is little interference after only 3 days and a moderate degree at 11 days.

Pneumonic pasteurellosis similar to the naturally occurring disease can be reproduced experimentally by exposing calves sequentially to aerosols of BHV-1 and *M. haemolytica* 4 days apart. The virus infection partly destroys the clearance mechanism of the respiratory tract epithelium and exacerbates the subsequent *M. haemolytica* infection. Both antigens can be detected by immunohistochemical methods in the bronchoalveolar fluid cells.

The viral–bacterial synergism is associated with the release of cytokines, which attract more leukocytes and increase leukocyte expression of CD11a/CD18. In this experimental model, vaccination of the animal against the virus before challenge with the viral–bacterial aerosol sequence is protective. The interaction between the BHV-1 virus and *M. haemolytica* can persist for up to 30 days after infection with the virus. A sequential aerosol infection of BHV-1 and *P. multocida,* or *P. multocida* alone, can also result in pneumonia. Experimental in vitro studies indicate that BHV-1 infection does not have a direct effect on the ability of neutrophils to phagocytize *M. haemolytica;* rather, there is an indirect effect, perhaps through the release of mediators that have an effect on phagocyte function. Large amounts of interferon are produced throughout the course of BHV-1 infection, which reduces chemotaxis and elevates oxidase activity by bovine neutrophils.

The intratracheal injection of *P. multocida* serotype A3 into 8-week-old calves results in clinical and pathophysiologic findings characteristic of bovine pneumonic pasteurellosis and gross pathologic and microscopic changes similar to field cases. Concentrations of the acute-phase proteins haptoglobin, serum amyloid-A, and alpha-1 acid glycoprotein increased, suggesting a role for these proteins as markers of the onset of and progress of the disease.

The intratracheal instillation of live *M. haemolytica* into conscious calves results in acute cardiovascular changes consisting of two systemic hypodynamic and pulmonary vasoconstrictive phases.

Synergism Between Pathogens

Experimentally, synergism may occur between *M. haemolytica* and *M. bovis* in producing pneumonia in gnotobiotic calves but not in conventional calves.

The role of BVDV in outbreaks of pneumonic pasteurellosis is uncertain. In one study the virus did not impair the pulmonary clearance of *M. haemolytica.* In a different study the endobronchial inoculation of calves with the virus and *M. haemolytica* sequentially 5 days apart resulted in a severe fibrinopurulent bronchopneumonia and pleuritis involving up to 75% of the total lung volume. Endobronchial inoculation of the organism only caused a localized noninvasive lesion in the lungs.

In summary, pneumonic pasteurellosis can be reproduced experimentally without a preceding virus infection, and it is likely that the naturally occurring disease can also occur without a preceding viral infection.[4]

CLINICAL FINDINGS

The spectrum of clinical findings depends in part on whether the disease is occurring in groups of young cattle in a large commercial feedlot, in a small farm feedlot, or in individual animals such as lactating dairy cows, in which illness is more easily recognized based on milk production and feed intake. In the feedlot situation, affected animals must be identified primarily by visual observation followed by closer physical examination.

Feedlot

In the feedlot, the disease usually occurs within 10 to 14 days after the animals have been stressed or have arrived in the feedlot. It may occur within 1 day after arrival if the animals have been incubating the disease before arrival. Animals found dead may be the first indication of an outbreak in which many weaned beef calves are obviously affected and some are in the incubation stages of the disease.

When viewed from a distance, affected cattle are depressed, and their respirations are rapid and shallow. There may be a weak protective cough, which becomes more pronounced and frequent if they are urged to

walk. Those that have been ill for a few days will appear gaunt because of anorexia. A mucopurulent nasal discharge, a crusty nose, and an ocular discharge are common. Although affected cattle are anorexic, they may continue to drink maintenance amounts of water.

When the disease has been diagnosed in a group or pen of animals, and new cases are occurring daily, those that are in the earliest stages of the disease are not obviously ill when examined from a distance. If the entire group of animals is put through a chute and examined closely, up to 20%, or even more, of apparently normal animals may have a fever ranging from 40° to 41° C (104°-106° F) and no other obvious clinical abnormalities. Auscultation of the thorax of some of these subclinical cases will reveal rapid, shallow respirations and an increase in the loudness of the breath sounds. These animals respond remarkably well to treatment. If not treated at this stage, they may progress to clinical cases within a few days, or they may recover uneventfully.

When the presence of a fever of 40° C (104.0 F), or higher in animals that are depressed is used to decide whether or not the animal has pneumonia and requires treatment, some animals are treated unnecessarily. In a feedlot pen of 112 recently arrived bull calves that were fitted with reticuloruminal temperature-sensing boluses, a total of 449 fever episodes were recorded in 110 animals during the first 40 days after arrival. Of these fever episodes, 74% were not associated with any visually apparent clinical signs or respiratory disease, and 75% lasted for less than 48 hours. A negative effect on average daily weight gain of feverish animals with our without clinical signs was recorded with prolonged episodes of fever with an approximate decrease of 33 g/day for each day of fever.[11]

Outbreaks of the disease in feedlots may last for 2 to 3 weeks or longer after the first index case, depending on the health status of the cattle when first affected. Outbreaks can be prolonged in feedlots that add groups of newly arrived cattle to an existing pen of cattle every few days to fill the pen to optimum capacity. The disease then occurs in each new group of cattle and may spread to previously resident cattle, perpetuating the disease for several weeks.

The origin of the cattle also influences the severity and length of outbreaks. In well-nourished cattle originating from one ranch and maintained as a single group, the morbidity may be less than 5% and the mortality nil. The outbreak will last only a few days, and the cattle return to normal quickly. In cattle that have originated from a variety of sources and moved through saleyards and then commingled in the feedlot, the disease may persist for several weeks. In these situations, many animals are sick with the disease when they arrive at the feedlot. Some cattle will develop complications, never fully recover, and are culled later.

Early Identification of Affected Animals in Feedlots

Where large numbers of cattle are involved, early identification is crucial to successful therapy. Identification of individual animals with early-stage respiratory disease in a large group presents a major challenge. Methods of diagnosing bovine respiratory disease based on visual appraisal by feedlot pen riders may not always effectively identify sick animals. A bayesian estimation of the performance of using clinical observation for diagnosis of bovine respiratory disease yielded an estimated sensitivity and specificity of 0.62 and 0.63, respectively, suggesting that up to 38% of truly diseased animals may go undiagnosed and conversely that up to 37% of animals diagnosed and subsequently treated for respiratory disease are unaffected by respiratory disease.[12] Improvement of the accuracy of both the diagnosis and the selection of those animals that require treatment will require improvement in the accuracy of the identification of affected animals by visual observation and the use of rapid and reliable clinical examination techniques of individual animals that can identify animals with evidence of pulmonary disease. Close physical examination techniques, such as auscultation of the lungs, are not routinely used in feedlots because of the time required to examine individual animals and the perceived inaccuracy of the examination in making a clinical diagnosis.

More recently, novel approaches to monitor calves at increased risk of developing respiratory disease, such as the use of temperature-sensing reticuloruminal boluses or infrared thermography to monitor heat loss in calves, have been studied, with promising results.[11,13] There is certainly a need to further improve our clinical diagnostic techniques and to develop new ones that can be applied to making a rapid and accurate diagnosis beside the animal in the field situation.

Close Physical Examination

The typical case of pneumonic pasteurellosis reveals a fever of 40 to 41° C (104-106° F), bilateral mucopurulent nasal discharge, gaunt abdomen with rumen atony, coughing, varying degrees of polypnea and dyspnea, and evidence of bronchopneumonia. In the early stages there are loud breath sounds audible over the anterior and ventral parts of the lungs. As the disease progresses, these breath sounds become louder and extend over a greater area; crackles become audible, followed by wheezes in a few days, especially in chronic cases. Pleuritic friction rubs may be audible, although their absence does not preclude the presence of extensive adherent pleuritis. In severe cases or those of several days' duration, the dyspnea is marked, commonly with an expiratory grunt, although the respiratory rate may not be elevated.

The course of the disease is only 2 to 4 days. If treated early, affected cattle recover in 24 to 48 hours, but severe cases and those that have been ill for a few days before being treated may die or become chronic despite prolonged therapy. Some cattle recover spontaneously without treatment.

A mild diarrhea may be present in some cases but is usually of no consequence. On an affected farm, calves may be affected with pneumonia, and young calves may die of septicemia without having shown previous signs of illness.

CLINICAL PATHOLOGY

Bacterial Culture

Nasopharyngeal swabs taken from clinical cases before treatment often yield a pure culture of pasteurellas, but *M. haemolytica* serotype A1 is the most common isolate obtained from weaned cattle with acute pneumonic pasteurellosis. The same serotype can usually be isolated from the upper respiratory tract of apparently healthy in-contact calves. *P. multocida* is the predominant pathogen cultured from swabs or bronchioalveolar lavage fluid in unweaned calves with pulmonary pasteurellosis. In recent years the number of clinical cases of bovine pneumonic pasteurellosis in older calves attributed to *P. multocida* in the absence of other Pasteurellaceae has increased considerably.[4]

The antimicrobial sensitivity of the pasteurellas isolated can be determined, but interpretation of the results is often difficult because it is not known whether the isolates from nasopharyngeal swabs represent those causing the lesions. Significant differences may exist between the antimicrobial sensitivities of isolates from nasopharyngeal swabs and those from the lung tissues. Thus it is not yet possible to recommend routine culturing and antimicrobial sensitivity determination of pasteurellas from nasal cavity or nasopharyngeal mucus from cattle with acute shipping fever pneumonia. In healthy calves monitored from the farm to the feedlot, there was no relationship between the nasal flora and pulmonary lesions.

Serum Biochemistry and Hematology

The suitability of serum concentrations of acute-phase proteins (APPs) such as fibrinogen, serum amyloid A (SAA) or haptoglobin (Hp) as a diagnostic tool to identify calves with BRD has been explored in numerous studies with variable outcome.[14-19] Although increased concentrations of SAA and Hp in calves with BRD compared with healthy calves suggest that measuring the serum concentrations of these APPs could assist the early detection of calves with respiratory disease, the discriminative ability of Hp by itself for BRD was found to be no better than

determination of the rectal temperature.[15] The concentration of Hp varies considerably between calves even in healthy animals and is significantly affected by sex and rectal temperature.[15] The increase in serum Hp was found to occur within 24 hours of experimental challenge with *M. haemolytica* of BHV-1 infected calves but occurred between 4 and 8 days of infection with BRSV or BVDV. For SAA the wide variation between animals and the less pronounced increase in SAA concentration in animals suffering of BRD impairs the ability of this parameter to discriminate between healthy calves and animals affected by BRD.[17]

Leukocyte and differential cell counts are of little value as predictors of respiratory disease because leukocytosis and neutrophilia occur in some animals, but in others there may be a neutropenia or no significant change.

NECROPSY FINDINGS

There is marked pulmonary consolidation, usually involving at least the anteroventral third of the lungs. The stage of pneumonia varies within the affected tissue, commencing with congestion and edema and passing through various stages of airway consolidation with serofibrinous exudation in the interlobular spaces. A catarrhal bronchitis and bronchiolitis, and a fibrinous pleuritis are usually present and may be accompanied by a fibrinous pericarditis. The lung is firm and the cut surface usually reveals an irregular, variegated pattern of red, white, and gray tissue as a result of hemorrhage, necrosis, and consolidation. Coagulation necrosis of pneumonic lungs is the most characteristic lesion in pneumonic pasteurellosis. In chronic cases there are residual lesions of bronchopneumonia with overlying pleural adhesions. Occasionally, sequestra of necrotic lung tissue are found. *P. multocida* causes a fibrinopurulent bronchopneumonia without the multifocal coagulation necrosis that is characteristic of the fibrinous lobar pneumonia associated with *M. haemolytica.*

The sequential gross and microscopic lesions of experimental bovine pneumonic pasteurellosis have been described and may provide guidelines for aging the lesions in naturally occurring cases. On days 2 to 3 after infection the lesion is characterized by soft grayish-purple consolidation; on day 6 the affected areas are firm and nodular; on days 9 to 10 the nodular lesions are more prominent and fibrous tissue encapsulates the lesions and becomes obvious. The initial microscopic changes consist of flooding of the alveoli with edema, fibrin, and hemorrhage. Large numbers of neutrophils and macrophages move into the alveoli by day 2. The classical lesion is visible by day 4 and consists of necrotic tissue surrounded by a dark zone of inflammatory cells. The elongate, "oat-cell" profile of some of these leukocytes is a useful marker in culture-negative cases. In nonfatal cases a walling-off reaction by fibrous tissue isolates the necrotic tissue. Determination of the age of the lesions by gross and/or microscopic examination may assist in correlating the occurrence of the disease with specific health management procedures in the herd. In feedlot cattle, determining the age of bacterial pneumonia can help to assess whether or not the pneumonia was present in the animal on arrival or if treatment failure resulted from late detection or from inadequate drug therapy. The degree of necrosis and fibrosis are the main lesions used to age pneumonia.

In general, *M. haemolytica* causes a fibrinous pleuropneumonia with extensive thrombosis of interstitial lymph vessels and limited evidence of bronchitis and bronchiolitis. In contrast, bronchopneumonia attributable to *P. multocida* is associated with a suppurative bronchitis, minor thrombosis of interstitial lymph vessels, and considerably less exudation of fibrin.

The organism is easily cultured from acute, untreated cases, but other species of bacteria, including anaerobes, are often found in more chronic cases. More sophisticated tests such as PCR and immunoperoxidase techniques are available for the detection of *M. haemolytica* but are seldom required in diagnostic cases.

Samples for Confirmation of Diagnosis

- Bacteriology—lung, bronchial lymph node (CULT)
- Histology—formalin-fixed lung (LM)

DIFFERENTIAL DIAGNOSIS

The differential clinical diagnosis of pneumonic pasteurellosis is summarized in Table 12-6.

As a general guideline the common pneumonias of cattle may be divided into bronchial, interstitial, and hematogenous.

- The **bronchial pneumonias** include pneumonic pasteurellosis and other less common bacterial pneumonias characterized by toxemia and shallow respiration and a good response to early treatment.
- The **interstitial pneumonias** include the viral and parasitic pneumonias and acute interstitial pneumonias characterized by marked respiratory distress and a slow or no response to treatment. In viral pneumonias the animals may die acutely in a few days or recover over a period of several days.
- The **hematogenous pneumonias** are associated with vena caval thrombosis and pulmonary aneurysm and are characterized by acute respiratory distress, hemoptysis, and no response to treatment.

Pneumonic pasteurellosis of cattle is an acute, toxemic bronchopneumonia with a high fever and a good response to treatment in the early stages. Depression and anorexia are common. The disease is most common in young beef and dairy calves that have been recently stressed following weaning or mixed in auction markets and shipped to feedlots. The disease can also occur in mature cattle as a primary or secondary pneumonia.

In **viral interstitial pneumonia** of calves, young and adult cattle there is characteristic dyspnea, a moderate fever, only a mild toxemia, and loud breath sounds over the ventral aspects of the lungs followed by crackles and wheezes in a few days, and recovery may take several days. Pneumonia attributable to BRSV may be mild with uneventful recovery or severe with dyspnea and subcutaneous emphysema and a high case-fatality rate.

Lungworm pneumonia occurs most commonly in young pastured cattle and is characterized by dyspnea, coughing, only mild toxemia, and a moderate or normal temperature; the course may last several days. Usually many cattle are affected. Crackles and wheezes are usually audible over the dorsal aspects of the lungs, and the response to treatment is usually favorable if treatment is initiated early when signs are first noticed.

Less common causes of acute pneumonia in calves and young cattle include infection with *Klebsiella pneumoniae, Streptococcus* spp., and *Fusobacterium necrophorum,* all of which are characterized by a bronchopneumonia indistinguishable clinically from pneumonic pasteurellosis.

Acute/atypical interstitial pneumonia (fog fever) usually occurs in outbreaks in pastured cattle that have been moved from dry to lush pasture (or just a different species of pasture or on to a recently harvested cereal grain field); the onset is sudden, and some cattle may be found dead, whereas others are in severe respiratory distress with an expiratory grunt.

Infectious bovine rhinotracheitis (IBR) is characterized by rhinitis, usually with discrete lesions in the nares, tracheitis, loud coughing, high fever, and no toxemia unless secondary bacterial pneumonia is present. Recovery usually occurs gradually over 4 to 7 days.

Contagious bovine pleuropneumonia resembles pneumonic pasteurellosis but occurs in plague form; there is severe, painful, toxemic pleuropneumonia, and the case-fatality rate is high.

TREATMENT

Antimicrobial Therapy

The recommendations for the treatment of bovine pneumonic pasteurellosis are based on clinical experience and the results of clinical field trials. Approximately 85% to 90% of affected cattle recover within 24 hours if treated in a timely manner.

Choice of Antimicrobial

Choice of antimicrobial will depend on the cost, availability, expected efficacy based on previous experience with the antimicrobial in a particular area, ease of administration, frequency of administration required, concentrations of the antimicrobial that can be achieved in the lung tissues of affected animals, and length of the withdrawal period required before slaughter or withholding of milk in case of lactating dairy cattle. According to a recent survey among feedlots in the United States, the most commonly used antimicrobials used for the first-line treatment of BRD were tulathromycin (66.3%), followed by fluoroquinolones (43.1%), florfenicol, or third-generation cephalosporins (both 34.8%) and tetracyclines (28.1%).[5] Commonly used antimicrobials registered for the treatment of BRD and their dosages are listed in the treatment table of this section.

Several novel antimicrobials, such as the macrolides tilmicosin, tulathromycin, or gamithromycin, have successfully been used for treatment and control of bovine respiratory pasteurellosis. Other commonly used antimicrobials with proven efficacy include florfenicol, an analog of thiamphenicol, and the chinolones enrofloxacin difloxacin and danofloxacin. Ceftiofur preparations have been evaluated and also found to be effective for the treatment of bovine respiratory pasteurellosis. Ceftiofur crystalline-free acid sterile suspension (CCFA-SS), a long-acting ceftiofur administered subcutaneously in the middle third of the posterior aspect of the ear, is effective, safe, and practical for the treatment of experimental pneumonic pasteurellosis and the control and treatment of bovine respiratory disease in feedlot cattle.

Some of the antimicrobials with a label for treatment of BRD, such as third- and fourth-generation cephalosporins and fluoroquinolones, are also classified as critically important for human an animal health and should therefore be used restrictively. The World Organization for Animal Health (OIE) issued following recommendations for these classes of antimicrobials:[20]

- Not to be used as preventive treatment applied by feed or water in the absence of clinical signs
- Not to be used as first-line treatment unless justified. When used in a second-line treatment, it should ideally be based on the results of bacteriologic tests
- Extra-label/off-label use should be limited and reserved for instances where no alternatives are available. Such use should be in agreement with the national legislature in force.

Antimicrobial Sensitivity

The antimicrobial sensitivity of *M. haemolytica* varies, depending on the geographic origin of the animals and the previous use of the drug in the herd or the feedlot. Most isolates of *M. haemolytica* have some degree of multiple antimicrobial resistance, associated with continued use.

Surveys of antimicrobial sensitivity trends for pathogens isolated from cattle with respiratory disease from all over the world based on MIC indicate that, overall, resistance to ampicillin, tetracycline, erythromycin, and sulfamethazine is frequently encountered among isolates of *M. haemolytica* and *P. multocida*.[3] The widespread resistance to erythromycin may account for the wide variation in sensitivity to tilmicosin because of cross-resistance. Ampicillin- and tetracycline-resistant *Pasteurella* isolates from dairy cattle (dairy herds and calf ranches) with pneumonia were spatially clustered within certain geographic areas in California. The percentage of *M. haemolytica* isolates resistant to ampicillin was 21.3%; to *P. multocida,* 12.3%. The percentage of *M. haemolytica* isolates resistant to tetracycline was 37%; to *P. multocida,* 52.5%. This reinforces the need to establish regional estimates of percentages of bacterial isolates which are susceptible to commonly used antimicrobials.

It was noticed that *M. haemolytica* serotype A6, the prevalence of which increased significantly over the last decade, exhibited significantly higher antimicrobial resistance rates than serotytpes A1 and A2.[21]

Tetracycline resistance (*tet*) genes have been found in isolates of *P. multocida, M. haemolytica, M. glucosida,* and *M. varigena* from cases of respiratory diseases in cattle and pigs in Germany. Tetracycline resistance in *P. multocida* and *M. haemolytica* is mediated by at least three different *tet* genes, most of which are located on the chromosomes. A new *tet* (H)-carrying plasmid has been identified, and *tet* (B) has been detected in *P. multocida* and *tet* (G) in *M. haemolytica*. More recently, a plasmid carrying chloramphenicol–florfenicol resistance gene *floR* was identified in *M. haemolytica* isolates from cattle.[22]

Medication of Feed and Water Supplies

There is much interest in mass medication of the drinking water or feed supply or both. The rationale is that the medication of the feed or water would successfully abort an outbreak by treating those animals incubating the disease, provide convalescent therapy to those that have already been treated individually, and deal with mild cases before they become acutely ill and need individual treatment. However, there are problems. The amount of water that cattle drink is directly proportional to feed consumption. If they are inappetent or anorexic, water consumption will decline to only maintenance requirements, and therapeutic levels of drug will not be achieved if the concentration in the water is provided at a level for normal consumption. The other major problem is the provision of a uniform concentration of drug in the water supply, either through automatic water proportioners in the waterline or placing the drug directly into water tanks. Both can be unreliable. The mass medication of feed supplies or water of newly arrived feedlot cattle has been investigated as a method of reducing the morbidity and mortality as a result of respiratory disease in a number of studies, but results are equivocal. Although studies suggesting that chlortetracycline and sulfamethazine in feed are effective in reducing morbidity associated with BRD in calves have been published, issues around study design and data analysis questioned the validity of the results.[23]

Antiinflammatory Agents

Antiinflammatory therapy was found to be beneficial in numerous studies in cases of severe respiratory disease that is characterized by marked dyspnea, fever, and feed intake depression. The best established effects of nonsteroidal antiinflammatory drugs (NSAIDs) in animals suffering of respiratory disease are a more rapid decline of the rectal temperature and faster return to normal feed and water intake. Long-term effects on clinical outcome, disease recurrence, and severity of chronic lung lesions reported in the literature are more variable.[24] The precise mechanism through which NSAIDs act in calves with respiratory disease is not entirely understood, but antiinflammatory properties improving respiratory gas exchange and antipyretic and analgesic properties improving the well-being and thereby feed and water consumption are believed to be of prime importance.[25] Numerous studies documented a more pronounced improvement in the initial phase of the disease in calves treated with NSAIDs and antimicrobials compared with animals treated with antimicrobials alone, a finding that is significant from an animal welfare perspective. Notwithstanding the veterinarian must be aware that this combination treatment complicates the assessment of the antimicrobial effect that should take place 48 hours after first treatment, as clinical improvement cannot unequivocally be attributed to the susceptibility of the causative pathogen to the administered antimicrobial drug. Experimental and clinical evaluation of the role of corticosteroid therapy in acute pneumonia in cattle yielded mostly unfavorable results.[26] Steroids are powerful antiinflammatory agents, but their effects on the animal's defensive measures, specifically with repeated use, reduces the value of their use in syndromes of infectious origin unless they have a short duration of action.[27]

A recent survey among U.S. feedlots revealed that on average NSAIDs were part of the standard initial treatment for respiratory disease in 55.9% of all feedlots, whereas steroids were used in 30.9% of the surveyed U.S. feedlots.[5]

Failure to Respond

The causes of failure to respond to therapy include the following:

- Delayed initiation of treatment resulting in complications such as pulmonary abscess, bronchiectasis, and pleuritis
- Presence of viral or interstitial pneumonia or some other pneumonia that is not responsive to antimicrobials
- Inadequate dose of antimicrobials, inadequate treatment duration
- Antimicrobial resistance of the bacteria

TREATMENT AND CONTROL

Treatment

Antimicrobial therapy

Tulathromycin (2.5 mg/kg SC as single dose) (R-1)

Florfenicol (20 mg/kg q48 IM or 40 mg/kg SC as single dose) (R-1)

Tilmicosin (10 mg/kg SC as single dose) (R-1)

Gamithromycin (6 mg/kg SC as single dose) (R-1)

Enrofloxacin* (2.5–5.0 mg/kg q24 SC for 3 days or 7.5–12.5 mg/kg SC as single dose) (R-1)

Danofloxacin* (6 mg/kg q48h SC or 8 mg/kg SC as single dose) (R-1)

Ceftiofur* crystalline acid free (6.6 mg/kg SC posterior pinna as single treatment) (R-1)

Ceftiofur hydrochloride* (1.1–2.2 mg/kg SC q24 for 3 days) (R-1)

Ceftiofur sodium* (1.2–2.2 mg/kg SC/IM q24h for 3 days) (R-1)

Cefquinome* (1 mg/kg IM q24 for 3–5 days) (R-1)

Oxytetracycline (10 mg/kg IM q24 for 4 days (R-2)

Trimethoprim (2.66 mg/kg) + sulfadoxine (13.33 mg/kg) IM q24h for 3 days (R-2)

Antiinflammatory therapy

Flunixin meglumine (2.2 mg/kg IV as single dose) (R-2)

Ketoprofen (3 mg/kg IM q24h for 2–3 days) (R-2)

Carprofen (1.4 mg/kg IV or SC as single dose) (R-2)

Meloxicam (0.5 mg/kg SC/IV as single dose) (R-2)

Diclofenac (2.5 mg/kg IM as single dose) (R-2)

Tolfenamic acid (2 mg/kg IM/IV q24–48h or 4 mg/kg IM/IV as single dose) (R-2)

Prednisolone acetate (0.5 mg/kg IM q24h) (R-3)

Dexamethasone (0.01–0.03 mg/kg IM/IV) (R-3)

Flumethasone (0.03 mg/kg IM/IV) (R-3)

Metaphylaxis

Tulathromycin (2.5 mg/kg SC as single dose) (R-1)

Florfenicol (40 mg/kg SC as single dose) (R-1)

Tilmicosin (10 mg/kg SC as single dose) (R-1)

Gamithromycin (6 mg/kg SC as single dose) (R-1)

Oxytetracycline long-acting formulation (20 mg/kg IM) (R-2)

Enrofloxacin* (7.5–12.5 mg/kg SC as single dose) (R-3)

Danofloxacin* (8 mg/kg SC as single dose) (R-3)

Ceftiofur* crystalline acid free (6.6 mg/kg SC posterior pinna as single treatment) (R-3)

Cefquinome* (1 mg/kg IM q24 for 3–5 days) (R-3)

Vaccination

Vaccination against *M. haemolytica* and *P. multocida* (R-2)

Vaccination against *H. somni* (R-3)

Vaccination against BRSV, PI-3V, BHV-1 (R-2)

Vaccination against BVDV (R-2)

**These are classified as critically important antimicrobials in human and veterinary medicine. Use as first-line treatment is discouraged.*[20]

CONTROL

Satisfactory economical control of the disease depends on the successful integration of management and perhaps the use of vaccines and antimicrobials metaphylactically. It is unrealistic to depend on a vaccine, an antimicrobial, or a single management technique to control the disease. Successful control begins with the adoption of effective management techniques, the judicious use of efficacious vaccines, and care in handling and transportation of cattle.

Management Strategies

Preconditioning Programs

Because of the common occurrence of the disease at the time of shipment from the ranch to the feedlot, much attention has been given to reducing the incidence of disease at this time. This led to the development in North America of the concept of preconditioning. The objective of preconditioning was to prepare the weaned calf for the feedlot environment by vaccinating it for all the commonly anticipated diseases before weaning and distributing all stressful procedures such as castration, dehorning, branding, and deworming over a period of time rather than concentrating these at weaning time. Weaning at least 2 weeks before shipment is considered as one of the most desirable preconditioning practices. This was to result in a weaned calf that could be moved into a feedlot in which the feed bunks and water bowls would not be strange but familiar and the calf would adjust quickly. The most common vaccinations were for BHV-1, PI-3V, BVDV, BRSV, and clostridial disease. In some situations, calves were also vaccinated for *H. somni* and against pneumonic pasteurellosis.

Weaning Procedures

Beef calves should be weaned at least 2 to 3 weeks before shipping and well in advance of anticipated inclement weather. A common successful practice is to begin feeding hay and providing water to calves at least 2 weeks before weaning in the same corral or paddock into which they will subsequently be weaned. Following such a weaning program, the calves require only a minimum of adjustment; the only adjustment necessary should be to the loss of their dams. Recently weaned calves should be observed at least twice daily for evidence of respiratory disease and treated promptly if necessary. They should not be transported long distances until they appear to be healthy and are eating liberal quantities of hay and drinking water normally. During transportation liberal quantities of bedding are necessary, and cattle should not be without feed and water for more than 24 to 30 hours. For long trips, calves should be rested for 8 to 12 hours and fed water and hay at intervals of 24 hours. This will minimize the considerable loss of body weight as a result of shrinkage and the effects of temporary starvation.

Creep Feeding

The use of creep feed for calves for several weeks before weaning has been successful but may not always be economical. A high-energy ration containing cereal grains, a protein supplement, and the necessary vitamins and minerals is provided for the calves in a creep arrangement to which the dams do not have access. At weaning time the dams are removed from the calves, and the stress on the calves is minimal. This program has been very successful for purebred herds, where it may be economical, but in commercial herds, it is only economical when the market value of the calves warrants it.

Conditioning Programs

In the absence of preconditioning programs, conditioning programs have become the usual procedure for preparing beef calves or yearlings for the feedlot. This begins with movement of the animals from their farm source to the feedlot. The ideal situation would be to avoid public saleyards and move the cattle directly from the ranch to the feedlot. This avoids the stress of handling, overcrowding, temporary starvation, exposure to aerosol infection from other cattle, and the unnecessary delays associated with buying and selling cattle. However, large intensified feedlots are unable to buy cattle directly from the herd of origin according to their needs at a particular time and thus inevitably purchase large groups of cattle of different backgrounds. This has necessitated the development of **conditioning procedures** or processing procedures in which,

after arrival, the cattle are individually identified; injected with a mixture of vitamins A, D, and E; treated with a residual insecticide; perhaps given an anthelmintic; injected with a long-acting antimicrobial; and vaccinated for clostridial and respiratory diseases. The issue of whether the cattle should be processed immediately after arrival or after a rest period of 2 to 3 weeks remains unresolved because there are few data to support one time over the other. However, the feedlot industry feels that processing immediately after arrival is most economical.

Feeding Newly Arrived Cattle

The feeding and nutritional status of newly arrived cattle is important, but there are few scientific data to formulate a sound economical feeding program that will promote rapid recovery from shipping stress. Good results can be achieved when stressed calves are fed a receiving ration consisting of 50% to 75% concentrate with good-quality hay in a total mixed ration for the 2 to 3 weeks until the cattle have become adapted to their new environment.

Vaccines

General Comments

The use of vaccines against respiratory viruses and to a lesser extent against bacterial pathogens to control shipping fever and enzootic calf pneumonia is widespread. According to a large recent survey conducted in the United States, BVD vaccines are used in 96.6%, BHV-1 vaccines in 93.7%, BRSV vaccines in 89.5%, and PI-3 vaccines in 85.1% of surveyed feedlots to control BRD.[5] The use of vaccines against pasteurellas (63.8%) and *Histophilus somni* (69.7%) is less common. Notwithstanding the evidence of efficacy for this practice is equivocal at best. A review of the literature in 1997 on the efficacy of the vaccines available for the control of bovine respiratory disease concluded that there were few documented data to support the use of vaccines against respiratory disease under feedlot conditions. Since that time progress has been made in understanding immunity to pneumonic pasteurellosis, and some vaccines with varying degrees of efficacy have been developed. Various commercial vaccines differ in the rapidity and intensity of serum antibody responses to *M. haemolytica* whole cells and leukotoxin. Although the vaccines have been evaluated by experimental challenge of vaccinated animals with specific pathogens in a laboratory environment, there is little scientific evidence available that the vaccines are protective against bovine respiratory pasteurellosis as it occurs in the real-world situation.[23] Respiratory vaccines have consistently resulted in antibody production against the specific antigen, but obtained titers did frequently not protect against respiratory disease. Failures of vaccination can certainly not exclusively be attributed to the vaccines themselves. Factors such as timing of vaccination or animal stress hampering the immune response to vaccination are likely to contribute to the poor vaccine performance.[23] Optimal vaccine response can only be expected in a fully immunocompetent animal, takes 2 to 3 weeks to develop, and may require multiple doses of vaccine to elicit protective immunity.[28]

Pasteurella Vaccines

Based on the immunologic and microbiological observations of both the naturally occurring and experimental disease, it appears that immunization of cattle is possible. Calves that recover from experimentally induced pneumonic pasteurellosis possess increased resistance to subsequent experimental challenge. Cattle that have recovered from the natural disease are resistant to the disease. The **challenge** in the development of an efficacious vaccine against pneumonic pasteurellosis has been to **determine the most effective protective antigens** of the organism. Several different *Pasteurella* vaccines have been developed based on the virulence factors, including leukotoxin, lipopolysaccharides, capsular polysaccharides, and outer membrane proteins. Each of the vaccines produced may provide some protection against experimental and naturally occurring disease, but none provides a high degree of protection.

Modified live and killed vaccines are currently available. Live streptomycin-dependent *P. multocida* vaccines have been associated with improved health and weight-gain performance compared with unvaccinated calves in some studies. Killed pasteurella vaccines did not show significant effects on morbidity, mortality, or extent of pulmonary damage and even were reported to result in increased morbidity in some cases.[29]

Leukotoxin Extract Vaccine

High leukotoxin-neutralizing antibody titers induced by natural infections have been associated with reduced susceptibility to pneumonic pasteurellosis. Vaccination of calves with a leukotoxic culture supernatant from pathogenic *M. haemolytica* provides some protection against experimental challenge with the organism.

The efficacy of the leukotoxin extract vaccine has been evaluated in clinical field trials against naturally occurring bovine respiratory disease in weaned beef calves 6 to 8 months of age entering feedlots in Ontario and Alberta. In an initial field trial in Alberta, auction-market-derived calves were given two doses of the vaccine within 1 to 5 days of arrival. Mortality from all causes was significantly lower in vaccinated calves (4.2% vs. 2.1%), and mortality as a result of fibrinous pneumonia was lower (2.2% vs. 1.1%). In a trial in Ontario feedlots, recently shipped nonpreconditioned calves were vaccinated within 24 hours after arrival. The vaccine resulted in a slight decrease in morbidity, slight improvement in treatment response rates, and a reduction in relapse rates. When the vaccine was combined with a modified live-virus vaccine containing the BHV-1 and PI-3 viruses, the mortality rate increased. However, the number of calves in each group was insufficient to adequately evaluate the differences. In another trial in Alberta the vaccine did not result in a change in morbidity or weight gain. Total mortality rates were increased significantly, and mortality rates from respiratory disease tended to be increased in ranch calves vaccinated at the ranch. In summary, there were no major benefits from vaccination.

A single vaccination of a *M. haemolytica* bacterin-toxoid given to calves on arrival in the feedlot reduced overall crude mortality, but there were no differences between vaccinates and nonvaccinates in bovine respiratory disease-specific mortality, morbidity, and/or average daily gain.

Passive Immunity to *Mannheimia haemolytica*

Vaccination of pregnant dairy cows at 6 and 3 weeks before parturition with a leukotoxin extract vaccine induced leukotoxin-neutralizing serum antibody titers in the cows, increased titers in colostrum, and increased passive leukotoxin colostral antibody titers in the calves. Vaccination was also associated with increased indirect agglutinating serum antibody titers in the cows. The protective effect of the antibodies against naturally occurring disease in the calves was not determined.

Vaccination of beef cows with a combined genetically attenuated leukotoxin *M. haemolytica* vaccine and an *H. somni* vaccine once at 4 weeks prepartum increases passive antibody titers to both organisms in their calves. Double vaccination of the calves with preexisting maternal antibodies at 1 and 2 months of age will increase antibody titers to both organisms until 6 months of age. Vaccination of beef calves with low levels of preexisting antibody at 3 and 4 months of age will increase antibody titers to *H. somni* until 6 months of age and to *M. haemolytica* until 5 months of age. Thus prepartum vaccination may be an effective measure for the control of pneumonia in calves under 2 months of age, and vaccination of the calves at 3 and 4 months of age may provide additional protection until the calves are 6 months of age.

Evaluation of Efficacy of *Mannheimia haemolytica* Vaccines

Meta-analysis of the published literature on the efficacy of the various vaccines against pneumonic pasteurellosis of cattle indicates that culture supernatants and/or potassium-thiocyanate-extracted outer-membrane protein vaccines perform as well

as live vaccines. Live vaccines are considered to be the best in terms of protective immunity induced against pneumonic pasteurellosis because they replicate at the site of injection and produce the important immunogens that stimulate a protective immune response. However, live vaccines are associated with side effects such as fever, local abscessation, and lameness.

Commercial vaccines have been evaluated by measuring antibodies in 4- to 6-week-old calves vaccinated against leukotoxin, capsular polysaccharide, whole-cell antigens, and iron-regulated outer-membrane proteins. A bacterin-toxoid, a leukotoxin culture supernatant, a modified live *M. haemolytica* and *P. multocida* vaccine, and an outer-membrane extract of the organism were evaluated. All vaccines induced antibodies to the antigens, but there were wide variations between the vaccines: some vaccines demonstrated little if any antibody to leukotoxin or outer-membrane proteins. The highest leukotoxin antibody titer did not reach its peak until 14 days after the booster dose of vaccine, which suggests that a second dose of vaccine is necessary for protection.

The efficacy of three commercial vaccines was evaluated against experimental pneumonic pasteurellosis. Protective immunity was evaluated by assessment of clinical scores and lung lesions after endobronchial challenge with virulent *M. haemolytica.* There was significant correlation between lung and serum antibody levels against leukotoxin, capsular polysaccharide, and outer-membrane proteins. The vaccines did not provide optimal protection, but the bacterin-toxoid vaccine was superior to the others. The culture supernatant containing leukotoxin, lipopolysaccharide, and capsular polysaccharide provided the best protection against experimental disease compared with a sodium salicylate extract containing outer-membrane proteins, lipopolysaccharide, and capsular polysaccharide, and a combination of these two. Leukotoxin is an important virulence factor in the disease, and its use in vaccines provides significant protection. Muramyl dipeptide analogs may increase the humoral and protective response of calves to capsular polysaccharide.

Adverse Vaccine Reactions

Some adverse reactions are associated with live vaccines. Systemic infection attributable to *M. haemolytica* occurred 2 to 18 days following vaccination with an avirulent live culture of *M. haemolytica.* Lesions included injection site inflammation, purulent meningitis, and polyarthritis. Abscess formation at injection sites after vaccination with modified live *M. haemolytica* vaccines is also possible. The purified capsular polysaccharide of *M. haemolytica* used in combination with other antigens did not provide protection but rather caused a high incidence of anaphylaxis.

Histophilus somni Vaccines

Few studies have investigated the effectiveness of *H. somni* vaccination of feedlot cattle to control respiratory disease. The antibody response was found to be associated with protection against *H. somni.*[1] When used as part of a preconditioning program the vaccine tended to have mildly positive or neutral effect on morbidity and mortality related to respiratory disease, whereas the effect appeared to be neutral or even negative when animals were vaccinated on arrival at the feedlot.[1] Observed effects on morbidity and mortality were below the significance level. Little scientific evidence is currently available to support the use of *H. somni* vaccination to control BRD.[29]

Viral Vaccines

Because prior infection of the respiratory tract with either BHV-1, BRSV, or PI-3V may predispose to pneumonic pasteurellosis, the vaccination of beef calves 2 to 3 weeks before weaning and feedlot cattle 2 weeks before shipment to a feedlot has been recommended as part of a preconditioning program. A modified live-virus BRSV vaccine given to beef calves before weaning, at weaning, or immediately after arrival in the feedlot was associated with a significant reduction in the treatment rate in one of three groups immunized before weaning and in calves immunized after arrival in the feedlot. There was no significant effect of the vaccine on treatment rate in calves immunized at weaning, in calves immunized after arrival in a bull test station, or in yearlings immunized after arrival in the feedlot. It would appear that the vaccine did provide some protection, but the small reduction may not justify the cost of the vaccination program. Vaccination of calves at 3 to 6 months of age with an intranasal modified live BHV-1, BRSV, and PI-3V vaccine provides protection against experimental pneumonic pasteurellosis induced by aerosol challenge with BHV-1 followed 4 days later by an aerosol of *M. haemolytica.* Using this principle of control, it would be necessary to vaccinate the calves at least 2 weeks before they are weaned, stressed, or transported to a feedlot. Vaccination on arrival with modified live-virus vaccines, although commonly done, may be associated with increased mortality.

Antimicrobial Metaphylaxis

The early onset of pneumonic pasteurellosis in cattle within a few days after arrival in combination with the limitations of identifying clinically affected animals that need therapy was a major factor in the development of metaphylactic use of antimicrobials. Although the metaphylactic use of antimicrobials to control BRD is debatable from the point of view of prudent antimicrobial use, this approach was documented to considerably reduce morbidity and mortality rates in a group, thereby having a significant positive effect on animal health and welfare. The administration of antimicrobials to high-risk calves immediately after arrival is particularly effective under commercial feedlot conditions. Meta-analysis of the literature on mass medication for bovine respiratory disease indicates that metaphylactic parenteral mass medication of calves with long-acting antimicrobials such as oxytetracycline, florfenicol, tilmicosin, or other novel macrolide antimicrobials on arrival at the feedlot will reduce bovine respiratory disease morbidity rates.

The use of tilmicosin at 10 mg/kg BW subcutaneously, florfenicol at 40 mg/kg BW subcutaneously, gamithromycin at 6 mg/kg BW subcutaneously, tulathromycin at 2.5 mg/kg BW and ceftiofur crystalline-free acid at 6.6 mg/kg BW administered subcutaneously at the base of the ear were found effective in reducing the morbidity rate when given to feedlot calves at high risk of developing respiratory diseases.[30-32] The results obtained with long acting oxytetracycline at a dose of 20 mg/kg BW or higher reported in the literature are more variable.[23] Although an economical advantage may result from the lower price of oxytetracycline compared with newer antimicrobials, morbidity rates were found to be higher with metaphylactic treatment with oxytetracycline compared with tilmicosin.[23]

Mass Medication of Feed and Water Supplies

The mass medication of feed supplies or water of newly arrived feedlot cattle has been investigated as a method of reducing the morbidity and mortality resulting from respiratory disease in a number of studies, but results are equivocal. Although studies suggesting that chlortetracycline and sulfamethazine in feed are effective in reducing morbidity associated with BRD in calves have been published issues around study design and data analysis questioned the validity of the results.[23] A standard recommendation is to provide 150 mg/kg BW for the first 24 hours and reduce the level to 75 mg/kg BW for the duration of the medication period, which may last 5 to 10 days.

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DISEASES OF THE RESPIRATORY TRACT ASSOCIATED WITH *MYCOPLASMA* SPP.

Several mycoplasmas have been isolated from pneumonic and nonpneumonic lungs of cattle, sheep, and goats (Table 12-6), but attempts to reproduce respiratory tract disease with the isolates have resulted in inconclusive findings.

Sheep

In sheep, *M. ovipneumoniae* is the most important species of mycoplasma affecting the respiratory tract. The pathogen is the cause of **atypical** or **ovine nonprogressive pneumonia**, also known as summer pneumonia in New Zealand and Australia.[1] *M. ovipneumoniae* is believed to cause disease by predisposing the lower respiratory tract to invasion by other organisms, such as *Parainfluenza-3 virus* or *Mannheimia haemolytica*. *M. ovipneumoniae,* although in low numbers, is frequently isolated from the upper respiratory tract of healthy sheep, which may act as source of infection to young lambs. A high prevalence of *M. ovipneumoniae* in association with *M. haemolytica* in lung tissue of lambs has been reported from Turkey and more recently from Italy.[2] Lambs get infected during the first days of life but usually do not show clinical signs before 5 or 10 weeks of age. Clinical disease is commonly triggered by stress, inclement weather, or a secondary bacterial infection.[3]

The clinical presentation can vary from mildly affected dull lambs with increased respiratory rate, coughing, increased rectal temperature, anorexia, and poor growth to severely affected animals with acute fibrinous pneumonia, lung consolidation, pleuritis, and pulmonary abscesses formation. Most commonly the disease results in chronic, persistent, and irregular cough, with mucopurulent nasal discharge.[3] Treatment with fluoroquinolones, tetracyclines, or macrolid antibiotics often results in a rapid improvement that is followed by a relapse once the treatment is discontinued.

The diagnosis is complicated by the common occurrence of *M. ovipneumonia* in the field. Whereas isolation of the pathogen from the upper respiratory tract is of little diagnostic value, the presence of it in the lower respiratory tract, particularly when associated with lung lesions is highly suggestive of an etiologic role of this pathogen. Paired serum samples obtained 2 to 3 weeks apart from acutely affected animals to determine rising antibody titers give a good indication of an active infectious process and are of more diagnostic value than the simple determination of an antibody titer at one time.

In the United States, *M. ovipneumonia* has recently been associated with several outbreaks of epizootic pneumonia, a devastating respiratory tract disease of yet unknown etiology in sheep.[4] Although a number of other pathogens, such as *Mannheimia haemolytica* and *Pasteurella multocida,* have consistently been isolated from sick animals along *M. ovipneumoniae;* this latter pathogen was the only agent with a significantly higher prevalence in animals from outbreaks (95%) than unaffected control animals (0%).[4]

Other *Mycoplasma* spp. occasionally associated with respiratory disease in sheep include *M arginini, M. agalactiae, M. putrefaciens, M. mycoides* subsp. *capri* (formerly *M. mycoides* subsp. *mycoides* LC), *M. capricoloum* subsp. *capricolum,* and *M. bovis*. When found in clinically affected animals, these *Mycoplasma* spp. are frequently isolated in combination with other pathogens, obscuring their role in ovine respiratory disease. Only *M. capricoloum* subsp. *capricolum* is considered an important pathogen and the major cause of pneumonia in sheep in North Africa.

Goats

Mycoplasma infections in goats most commonly involve the *Mycoplasma mycoides* cluster organisms that include *M. mycoides* subsp. *capri, M. capricolum* subsp. *capricoloum,* and *M capricolum* subsp. *capripneumoniae,* the etiologic agent of the **contagious caprine pleuropneumonia** (see also "Contagious Caprine Pleuropneumonia").[5] Although contagious caprine pleuropneumonia is characterized by clinical disease specifically affecting the respiratory tract, the other members of the *M. mycoides* cluster have also been associated with polyarthritis.[6]

Contagious agalactia is another economically important disease of sheep and goats caused by mycoplasmal infection and occasionally associated with respiratory disease (see also "Contagious Agalactia"). *Mycoplasma* spp. incriminated as etiologic agents of contagious agalactia are *Mycoplasma agalactia,* the classical etiologic agent of the disease, *Mycoplasma putrefaciens,* and the two species of the *mycoides* cluster, *Mycoplasma mycoides* subsp. *capri* and *M. capricolum* subsp. *capricolum.*

Other *Mycoplasma* spp. associated with respiratory disease in goats include *M. arginini, M. bovis,* and *M. ovipneumoniae.*[7]

The most common syndrome in goats associated with mycoplasma is a chronic interstitial pneumonia with cough, unthriftiness proceeding to extreme emaciation, chronic nonpainful bony enlargement of joints, and chronic indurative mastitis. The pneumonia in some cases progresses to the point where cor pulmonale develops, with a subsequent appearance of the signs of congestive heart failure.

Cattle

M. bovis is a major cause of calf pneumonia (see also "*Mycoplasma bovis* Pneumonia"). In

England and Wales, serologic screening between 2000 and 2009 revealed a herd seroprevalence of *M. bovis* of over 30%, and *M. bovis* was isolated on average in 40% of pneumonic lungs submitted during that time period.[8] In Israel, *M. bovis* was isolated from 26% to 65% of samples from pneumonic calves submitted between 2004 and 2008.[9] *M. bovis* was also the strain isolated from more than half of the 1000 samples from calves with bronchopneumonia that were submitted to different diagnostic laboratories in France between 2003 and 2008.[8] In a series of cases of chronic, antibiotic-resistant pneumonia, sometimes with concurrent polyarthritis, in feedlot cattle in western Canada, *M. bovis* was present in the lung tissues of more than 90% of cases, and bovine viral diarrhea virus (BVDV) was present in 60% of the cases suggesting a possible synergism between *M. bovis* and the BVDV. Outbreaks of pneumonia and arthritis in beef calves associated with infection caused by *M. bovis* and *Mycoplasma californicum* have been described in a mixed dairy cattle and beef cattle herd kept under extremely poor housing and hygienic conditions. The prevalence of infection of *M. bovis* in Danish cattle appeared to increase over a period of several years.

A large increase in the detection of *M. alkalescens* since 2003 has been reported from Great Britain, where this species accounted for 26% of all isolated from bovine lungs in 2009. A similar trend has been observed in France and Israel, but the significance of this development is not clear.[8]

M. arginini, M. dispar, Ureaplasma diversum, M. bovirhinis, M. canis, M. canadense, and *M. bovigenitalium* have also been isolated from the lungs of pneumonic cattle, but it is uncertain if they are primary causes of disease.[8] During a 3-year period in Belgium, in calves with respiratory disease, the prevalence of *M. bovis* was 31.5%, *M. dispar* 45.5%, *M. canis* 10.7%, and *Ureaplasma diversum* 14.8%, and in half the cases they occurred in association with *Pasteurella* and/or *Mannheimia* species. In a survey of pneumonic bovine lungs submitted to a diagnostic laboratory in Denmark, 83% were found infected with mycoplasmas. The predominant mycoplasmas were Ureaplasma spp. (72%), *M. dispar,* (48%), and *M. bovis* (24%). Multiple species mycoplasma infections were predominant.

M. dispar is capable of producing pneumonia without clinical signs in gnotobiotic calves, and in conjunction with *Ureaplasma* spp. it has been found commonly in "cuffing" pneumonia of calves. It could, therefore, be a precursor to other infections causing enzootic pneumonia in calves or with pasteurellae producing fibrinous pneumonia of calves.

Horses

Mycoplasma spp. appear to be opportunistic pathogens in equine respiratory tract infection. *M. felis* has been associated with outbreaks of lower respiratory tract disease and pleuritis of horses. In a retrospective study reviewing the occurrence of different pathogens isolated from specific anatomic sites of horses revealed that *Mycoplasma* spp. were isolated from 6 out of nearly 200 horses with respiratory disease and in each case were part of chronic, mixed infection.[10]

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CONTAGIOUS BOVINE PLEUROPNEUMONIA (LUNG SICKNESS, CBPP)

SYNOPSIS

Etiology *Mycoplasma mycoides* subsp. *mycoides* (small colony) (*MmmSC*).

Epidemiology A major plague of cattle, endemic in Africa but considered as eradicated in most other parts of the world. Reportable disease to the World Organization for Animal Health (OIE, list A). Insidious nature of disease allows it to spread undetected for months.

Signs Fever, agalactia, anorexia, depression, coughing, thoracic pain, back arched, dyspnea, expiratory grunting, pleuritic friction rubs, dull areas of lung, edema of throat and dewlap.

Clinical pathology Complement fixation test (CFT), competitive enzyme-linked immunosorbent assay (C-ELISA). Nucleic acid recognition of causative organism with polymerase chain reaction (PCR).

Lesions Remarkable pleuritis, marked consolidation and marbling of lung, pleural adhesions.

Diagnostic confirmation Isolation of organism from tissue (lung) or pleural or synovial fluid.

Treatment Not recommended because of usually poor treatment response and the risk of developing carrier animals.

Control Identification and slaughter of sick animals. Vaccination followed by test and slaughter. Antimicrobial therapy of exposed animals may reduce disease transmission but is prohibited in many countries. Establish disease-free areas. Control movement of cattle in control areas.

ETIOLOGY

Mycoplasma mycoides subsp. *mycoides* small colony (SC) (*MmmSC*) is the cause of the contagious bovine pleuropneumonia (CBPP). This pleomorphic organism belongs to the *mycoides* cluster, which consists of six closely related mycoplasma species, that are subdivided into two groups, *capricoloum* and *mycoides* (Table 12-8). Only two of the six species cause disease in cattle, *MmmSC,* which is the cause of CBPP, and *Mycoplasma bovine group 7* (Bg7), which may cause arthritis and bovine mastitis. The four others, two subspecies within the *Mycoplasma mycoides* group and two subspecies within the *Mycoplasma capricolum* group, are responsible for goat respiratory and other diseases.

Although *MmmSC* has been isolated from buffalos, sheep, and goats, which may thus function as hosts, the pathogen only causes disease in ruminants of the *Bos* genus (i.e., bovine and zebu cattle). The disease is not communicable to other species.[1] *MmmSC* is very similar culturally and antigenically to the causative organism of caprine contagious pleuropneumonia, but the two can be differentiated culturally and biochemically.

EPIDEMIOLOGY

CBPP is considered one of the economically most important cattle plagues affecting Africa, and with relaxation of import controls and increase in international trade, it presents a constant risk for disease-free countries. The World Organization for Animal Health (OIE) has categorized CBPP as so-called "List A" disease. List A currently lists a total of 15 diseases notifiable to the OIE that are considered "transmissible diseases that have the potential for very serious and rapid spread, irrespective of national borders, that are of serious socioeconomic or public health consequence and that are of major importance in the international trade of animals and animal products."[2] The Pan African Programme for the Control of Epizootics (PACE), which is implemented in 32 African countries, identified CBPP as the second most common transboundary disease in Africa after rinderpest.[3]

Occurrence and Prevalence of Infection

Under natural conditions, CBPP occurs in cattle of the species *Bos.* Although buffaloes can be infected by artificial means, only one single case has been reported in an American buffalo and none in African buffaloes or other wild ruminants. Wild and small ruminants are currently not considered to play a relevant role in the epidemiology of CBPP.[1,3]

Currently CBPP affects around 30 countries in sub-Saharan Africa, of which several are endemically infected. Although the condition was eradicated from many countries during the nineteenth and twentieth centuries through stringent movement control and culling of infected cattle, it has reemerged

Table 12-8 Contagious pleuropneumonia-1: Members of the *Mycoplasma mycoides* cluster

Name	Main disease	Main (and other) hosts
M. mycoides subsp. *mycoides* SC variant	Contagious bovine pleuropneumonia	Cattle (goats, sheep, buffalo)
M. mycoides subsp. *mycoides* LC variant	Caprine pneumonia, contagious agalactiae	Goats (sheep, cattle)
Mycoides subsp. *capri*	Caprine pneumonia	Goats (sheep) but rare
M. capricolum subsp. *capricolum*	Caprine pneumonia, contagious agalactiae	Goats (sheep, cattle)
M. capricolum subsp. *capripneumoniae*	Contagious caprine pleuropneumonia	Goats (sheep)
M. bovine group 7 (Bg7)	Arthritis, also mastitis, calf pneumonia	Cattle

in Africa during the second half of the past century, and several outbreaks have been recorded in southern Europe until the end of last century. Reasons behind the failure to contain CBPP in Africa include the following:[4,5]

- **Changes of ecological/ environmental factors,** such as availability of water, droughts, and floods, leading to increased movement of livestock over long distances
- **Civil strife and political and economic difficulties** complicating the strict movement control of apparently infected cattle
- **Lack or insufficient resources allocated to CBPP control**, particularly limited funding for vaccination, livestock movement control, and mass education of farmers and veterinarians
- **Limited potency and field performance of CBPP vaccines**
- **Reduced disease surveillance** (e.g., serologic screening and abattoir studies)
- **Distraction by control and prevention of other economically important diseases** (e.g., rinderpest or avian influenza)

Historically CBPP spread throughout the European continent during the eighteenth century through uncontrolled movement of livestock caused by transhumance and wars. During the nineteenth and twentieth centuries the disease was introduced into the United States, Asia, Australia, and Japan. CBPP was first introduced into **Africa** through an infected Holstein Friesian bull imported from the Netherlands to South Africa in 1854.[4] From there the disease spread to other South African countries, causing the death of over 100,000 cattle.[6] By 1939 the disease was eradicated in most South African countries but not in war-torn Angola and Namibia, where it still remains a threat for neighboring disease-free countries.[6]

CBPP was reported from 27 African countries between 1995 and 2002, of which 13 were in West Africa, two in Central Africa, six in East Africa and the remainder in South Africa.[3] In East Africa CBPP is endemic in Burundi, Rwanda, large parts of Tanzania, southern Sudan, Ethiopia, and Somalia.[3,7,8] Guinea, Mali, Niger, and Mauritania are endemically infected countries in West Africa and Angola and Zambia in the South African region.[3] Numerous epidemic outbreaks of the disease, spreading from endemic neighboring regions through livestock movement have been reported, including Burundi and Zambia in 1997, Tanzania in 1996 and 1999, and Botswana in 1995 and 1998.[3,9]

The infection prevalence varies considerably in endemic regions of the world. Whereas a seroprevalence of 8% of the cattle population has been reported in Sudan, prevalence rates in the range of 25% have been given for Ethiopia, Chad, Guinea, and Tanzania. For Burkina Faso and Uganda, prevalence rates below 5% have been reported.[3]

In **Asia**, CBPP has been reported from Assam in India, Bangladesh, and Myanmar. Sporadic outbreaks have been recognized in the Middle East, probably derived from importation of cattle from Africa. The latest Asian outbreaks reported to the OIE occurred in Mongolia, India, and Pakistan in 1973, 1990, and 1997 respectively.[10] In China CBPP first occurred at the beginning of the twentieth century through livestock shipments from Australia.[10] In the early 1950s China implemented its first control measures, including a vaccination program, after having suffered severe economic damage from the plague. The country declared itself free of CBPP in 1996 and is currently officially recognized as CBPP-free by the OIE.[11]

The disease was introduced into **Australia** in 1858 by dairy cattle imported from England into the colony of Victoria. It spread rapidly throughout Australia by cattle being driven to take up new pastoral lands everywhere, aided by the bullock teams, which provided the only form of transportation of goods and supplies in those days. In 1958 a national eradication campaign was commenced. Australia was declared free from the disease 1973.

The disease was introduced into the **United States** in 1842 through cattle imports from England. CBPP became so widespread over the following 40 years that the federal government initiated the first intensive campaign to control an animal disease in 1887. By implementing strict quarantine and culling diseased animals the disease could be eradicated by 1892. CBPP was never recorded to occur in **South America**.

In **Europe,** after having been eradicated at the end of the nineteenth century, the disease reemerged in Portugal in 1951 and in Spain in 1957.[4] In the 1980s, after an apparent absence of 13 years, CBPP was diagnosed on the borders of France and Spain, from where the disease apparently spread to Portugal, where another outbreak was recorded in 1983. Although the disease could be rapidly controlled in France and Spain, recurrent outbreaks occurred in Portugal until the end of last century. Italy, which had been free of the disease since 1899, saw an outbreak of CBPP between 1990 and 1993 that could never be traced back to its source. Within these 3 years the disease spread over an area 59 km^2, affecting nearly 100 cattle herds and requiring the slaughter of over 24 000 cattle. The disease was eradicated in 1993. The last reported outbreak in Europe occurred in northern Portugal in 1999. As of 2004, Europe is free from CBPP and the European Union rules prevent the importation of live animals from affected areas. A recent phylogenetic analysis indicated all *MmmSC* strains isolated in Europe since 1980 were derived from a common ancestor, suggesting that a single strain may have spread, largely unnoticed, in southern Europe between 1980 and 1993.[12]

Source of Infection

The focus of infection is often provided by recovered "carrier" animals in which a pulmonary sequestrum preserves a potential source of organisms for periods as long as 3 years. For many years, it was thought that conditions of stress attributable to starvation, exhaustion, or intercurrent disease can cause the sequestrum to break down and convert the animal into an active case. Experimental evidence throws some doubt on this explanation, but droplet infection is usually associated with a donor lesion in the lungs. Renal lesions are not uncommon and large numbers of viable *MmmSC* are passed in the urine of infected animals, and inhalation of urine droplets may be a route of infection. The organism has been **isolated from the semen** and preputial washings of two young bulls that were the result of frozen embryos implanted into Portuguese cows and were

being considered for entry into a breeding center.

Methods of Transmission

Transmission occurs from direct and repeated contacts between sick and healthy animals. The principal route of infection is by the inhalation of infective droplets from active or carrier cases of the disease. Mediate infection by contamination of inanimate objects is unlikely under natural conditions, but it has been effected experimentally. Infected hay remained infective for up to 144 hours. Other inanimate objects such as placenta and urine can also remain infective for long periods. It has been suggested that urine may be a mode of transmission, especially in European countries with temperate climates, where cattle are reared intensively in restricted geographic areas and many herds share the same watercourse. Spread of the pathogen may also occur by discharges from local tail lesions resulting from vaccination with virulent culture.

A separation of 6 m between animals is usually considered to be sufficient, but transmission over 200 m is suspected to occur.[3] Cattle may be infected for periods of up to 8 months before the disease becomes apparent, underscoring the importance of sufficiently long quarantine period before a herd can be declared to be free of the disease.

CBPP is usually transmitted through movements of live animals; trade in animal products is not thought to be a significant risk.

Risk Factors

Animal Risk Factors

CBPP occurs only in cattle; a rare natural case has been observed in one buffalo but has not been detected in other wildlife. In sheep and goats, the injection of culture causes a local cellulitis without pulmonary involvement. There is no difference in the susceptibility of *Bos taurus* and *Bos indicus* cattle and both races respond equally to vaccination.

Immune Mechanisms

The exact nature of the immunity conferred by vaccination or by naturally occurring disease is not entirely understood, although it can be transferred by the administration of serum from an immune animal. The lack of a cell wall and endotoxins may enable mycoplasmas to colonize the animal without inducing an immune response, and the predilection for the mucosal membranes may also limit the humoral response. For these reasons it is suggested that the organism is a poor immunogen, which may account for the frequent lack of good circulating antibody responses in experimentally infected and vaccinated cattle. There is a poor relationship between antibody titer and the severity of lesions; animals with high antibody titers may have no visible lesions, and those with severe lesions may have low or undetectable titers.

Management Risk Factors

The occurrence and incidence of CBPP is heavily influenced by management systems, disease control policies and regulations of a country, knowledge of the disease by farmers and veterinarians, and livestock field officers. The diagnostic capability of veterinary laboratories, disease-surveillance and monitoring systems, adequacy of vaccination programs, government budgets allocated to control programs, the effectiveness of education programs, and the desire of cattle owners and traders to control the disease are critically important management factors that influence the effectiveness of control of the disease in a country.

Pathogen Risk Factors

M. mycoides subsp. *mycoides* is sensitive to all environmental influences, including disinfectants, heat, and drying, and does not ordinarily survive outside the animal body for more than a few hours. A low incidence can be anticipated in arid regions because of the rapid destruction of the organism in exhaled droplets.

The organism can be grouped into two major, epidemiologically distinct, clusters. One cluster contains strains isolated from different European countries since 1980, and a second cluster contains African and Australian strains collected over the last 50 years.

The current European strains lack a substantial segment of genetic information, which may have occurred by a deletion event. The strains found in reemerging outbreaks of CBPP, which occurred after the eradication of the epidemic in Europe in the middle of the twentieth century, represent a phylogenetically newer cluster that has been derived from a strain of the older cluster of *MmmSC* that is still endemic on the African continent. The genome of *MmmSC* type strain PG1^T has been sequenced to map all genes and to facilitate further studies regarding the cell function of the organism.

A variety of potential virulence factors have been identified, including genes encoding putative **variable surface proteins**, enzymes, and transport proteins responsible for the production of hydrogen peroxide and the capsule that is thought to have toxic effects on the animal. **Galactan** is also associated with pathogenicity of the organism, but its mode of action is uncertain. Galactan can cause necrosis and a connective tissue response in cattle similar to the sequestra in chronically infected animals.

The phylogeny of the *Mycoplasma mycoides* cluster according to sequencing of putative membrane protein genes has been examined. Molecular epidemiology of CBPP by multilocus sequence analysis of *MmmSC* strains found a clear distinction between European, south-western African, and sub-Saharan strains. This indicates that the CBPP outbreaks that occurred in Europe were not a result of introduction from Africa and confirms true reemergence. Strains of *MmmSC* isolated from recent outbreaks of CBPP in Africa have been compared with vaccine strains and older isolates. A Botswanan field isolate differed from all other strains of *MmmSC* tested by a variety of criteria. The new isolate may possess a set of protective antigens different from those of other strains of *MmmSC,* including vaccine strains. Such findings have implications for the control of CBPP in Africa.

The last strains isolated from an epidemic are usually of lower virulence than the first strains. Strains are most virulent when first isolated and lose their virulence after subculture.

Economic Importance

CBPP is considered as one of the two economically most important diseases of cattle (beside rinderpest) in Africa. Direct losses are from mortality, reduced milk yield, vaccination costs, and disease surveillance. The indirect costs associated with the chronic nature of the disease are more difficult to assess and include decreased weight gain or loss of body weight, impaired working ability, reduced fertility, losses resulting from quarantine, and losses related to restrictions of trade and cattle movement.

Annual losses attributed to mortality, decreased beef and milk production, and loss in draught power occurring in 12 endemically infected sub-Saharan African countries were estimated to be €30.1 million.[3]

Costs associated of managing and eradicating the disease after the CBPP outbreak of 1995 in Botswana were estimated at $98 million.[13] The eradication campaign after the most recent European outbreak of CBPP in Portugal at the end last century, which required the slaughter of over 85,000 cattle was estimated to cost more than €200 million.[14]

PATHOGENESIS

Even after more than 100 years since CBPP was discovered, the pathogenesis is not well understood. The disease is an acute lobar pneumonia and pleurisy. The organism invades the lungs of cattle and causes a mycoplasmemia; this results in localization in numerous other sites including the kidneys, joints and brain, resulting in high morbidity and mortality. An essential part of the pathogenesis of the disease is thrombosis in the pulmonary vessels, probably before the development of pneumonic lesions. The mechanism of development of the thrombosis is not understood, but there is no general increase in blood coagulability and no generalized tendency to spontaneous thrombosis.

The production of hydrogen peroxide and other active oxygen species is widely believed to play an important role in mycoplasma pathogenicity, and it has been demonstrated to result in lysis of erythrocytes, the peroxidation of lipids in *M. mycoides* infected fibroblasts, and inhibition of ciliary movement in tracheal organ cultures infected with *M. mycoides* and *M. ovipneumoniae*. European *MmmSC* strains appear to be distinguished from other *M. mycoides* strains by their lack of glycerol phosphate oxidase activity and ability to oxidize glycerol.

Death results from anoxia and presumably from toxemia. Under natural conditions a proportion of animals in a group do not become infected, either because of natural immunity or because they are not exposed to a sufficiently large infective dose. These animals may show a transient positive reaction to the complement fixation test (CFT). Approximately 50% of the animals that do become infected go through a mild form of the disease and are often recognized as clinical cases.

CLINICAL FINDINGS

There is considerable variation in the severity of clinical disease from hyperacute to acute to chronic and subacute forms. With acute presentation, high disease incidence of nearly 90% and fatality rates of 50% and higher are observed. Acute disease is the common presentation in outbreaks occurring in previously unaffected regions.[15] Mild or even subacute forms with low mortality rates are common presentation in zones where the disease in endemic. Approximately 25% of the infected cattle have been estimated to remain as recovered carriers with or without clinical signs.

Acute Form

After an incubation period of 3 to 6 weeks (in occasional instances up to 6 months), there is a sudden onset of high fever (40°C [105°F]), a drop in milk yield, anorexia, and cessation of rumination. There is severe depression, and the animals stand apart or lag behind a traveling group. Coughing, at first only on exercise, and thoracic pain are evident; affected animals are disinclined to move, standing with the elbows out, the back arched, and the head extended. Respirations are shallow, rapid, and accompanied by expiratory grunting. Pain is evidenced on percussion of the chest. Auscultation reveals pleuritic friction sounds in the early stages of acute inflammation, and dullness, fluid sounds, and moist gurgling crackles in the later stages of effusion. Dullness of areas of the lung may be detectable on percussion. Edematous swellings of the throat and dewlap may occur, and swelling of the large movable joints may be present. In calves, valvular endocarditis and myocarditis may occur. In fatal cases death occurs after a variable course of disease from several days to 3 weeks. In the **peracute form**, affected cattle may die within 1 week after the onset of respiratory distress.

Chronic and Subacute Forms

Recovered animals may be clinically normal but in some an inactive **sequestrum** forms **in the lung**, with a necrotic center of sufficient size to produce a toxemia causing unthriftiness, a chronic cough, and mild respiratory distress on exercise. These sequestra may break down when the animal is exposed to environmental stress and may cause an acute attack of the disease. During the Italian outbreak of 1990, less than 5% of cattle in an infected herd had evidence of clinical disease. This was possibly a result of the use of antimicrobials and antiinflammatory agents, which may have masked clinical signs and facilitated the formation of chronic lesions. In Africa, up to one-third of acute cases that recover become potential carriers.

CLINICAL PATHOLOGY

Culture and Nucleic Acid Recognition

Culture of the organism is the reference method for detection of the pathogen. However, mycoplasmas are labile, making it necessary to use a special transport medium protecting this microorganism and preventing proliferation of other bacteria. Long-distance transport of samples, particularly when unrefrigerated drastically affects the viability of the bacteria rendering them unfit for culture.[16] Frequently, attempts to isolate *MmmSC* fail because the organism is labile, is present in too little quantities, and is so demanding in its growth requirements. Negative results should therefore always be regarded as inconclusive.[1]

In case of successful culture final identification of mycoplasmas is usually made by means of a biochemical test such growth inhibition, the fluorescent antibody test (FAT) or the immunofluorescence tests (IMF). Specific nucleic acid recognition using the **polymerase chain reaction (PCR)** has become common practice over the last two decades.

Although most PCR protocols rely on previous culture, preenrichment, or extraction of mycoplasma, PCR is also used without prior culture, directly using samples taken from nasal swabs, bronchioalveolar lavage or transtracheal wash fluid, pleural fluid, blood, urine, or pulmonary tissue. The PCR can identify the organism in bacterial isolates or clinical material within 2 days of extraction and is sensitive and highly specific.[1] An inconvenience of the PCR results from its high sensitivity, which makes it susceptible to false-positive results caused by contamination.

More recently **isothermal loop-mediated amplification (LAMP)** of DNA sequences specific for *MmmSC* has been developed. The LAMP assay detects *MmmSC* DNA directly form crude samples of pulmonary or pleural fluid and serum or plasma within 1 hour using a simple dilution protocol.[16]

Immunologic Tests

A number of immunologic test to identify the causative agent or its antigen in tissue, biological fluids, or cultures are available. Such tests include the **indirect fluorescent antibody test (IFA)** and the **fluorescent antibody test (FAT)**, which both use hyperimmune rabbit serum against *MmmSC* and labeled antibovine IgG. The **growth inhibition test (GIT)** is based on direct inhibition of growth of *MmmSC* by a specific hyperimmune serum. Although this is a simple test to perform cross-reactions within the *mycoides cluster* are common.[1] The **antigen immunodiffusion test (AGID)** has also been used to detect specific antigens present on the surface of *MmmSC*. The AGID is considered to lack sensitivity, and little is known about its specificity.[1] Because all these tests depend on the presence of a minimum number of organisms, only positive results should be considered conclusive.

Serologic Tests

Serologic tests that identify an immune reaction of an individual animal to infection with *MmmSC* include the complement fixation test (CFT) and the competitive enzyme-linked immunosorbent assay (C-ELISA). Both are prescribed tests for international trade according the OIE. This group of diagnostic tests has important limitations because of the nature of the pathogenesis of the disease with its long incubation period and the relatively rapid decline of the antibody titer.

The **complement fixation test (CFT)** is rapid to perform and easy to interpret. With a sensitivity in the range of 70% to 80% and specificity of 98% it is best suited to diagnose clinically affected animals with acute lesions but less suitable to identify either animals in early stage of the disease, chronically infected or carrier animals with low antibody titers.[1,17] The therapeutic use of antimicrobials further increases the risk of a false-negative test results. Vaccinated animals give a positive reaction for about 6 weeks, although this period may be much longer if severe vaccination reactions occur. Because of the limited sensitivity the CFT is considered unreliable on an individual animal level, but it is deemed to be highly effective in detecting infected herds when testing the entire population. The test is widely used in to determine freedom of disease on a herd level.[1] Because false positive results caused by serologic cross-reactions with other species of the *mycoides cluster* can occur, it is advisable to confirm a positive test result by postmortem and bacteriologic examination.

The **C-ELISA** has a similar or even greater specificity than the CFT.[1,17] The sensitivity of the C-ELISA was found to be superior to the CFT particularly to detect animals in the chronic stage of the disease, whereas the CFT appears to outperform the C-ELISA in the detection of animals in the acute phase of the disease.[17,18] An indirect ELISA based on a **recombinant protein, LppQ-NX (LppQ ELISA**), has been developed and provides good sensitivity and specificity for the diagnosis of CBPP and is robust under harsh climatic conditions. The CFT, competitive ELISA, and LppQ ELISA, all used for detection of antibodies to *MmmSC,* were compared with postmortem inspection for the diagnosis of CBPP in naturally infected cattle in an endemic area in Zambia between 2007 and 2008.[17] The percentage of positive sample was 67.5% for post postmortem examination, 59.0% for the C-ELISA, 52.6% for the CFT, and 44.4% for the LppQ ELISA. Of the three serologic tests the CFT identified the largest number of animals in the acute phase of the disease, whereas the C-ELISA was the most sensitive test to detect animals in advanced stages of the disease. The LppQ ELISA had a very poor sensitivity (10.8%) to identify animals in the early stage of the disease, whereas in the chronic stage it had a sensitivity ranging above the CFT but below the C-ELISA.

The **immunoblotting test (IB)** is based on an immunoenzymatic reaction with higher sensitivity and specificity than the CFT. The IB is recommended as a confirmatory test on positive samples previously analyzed with another test because IB is not suitable for mass screening and may be difficult to standardize.[1]

No single serologic test is capable of detecting all CBPP affected animals in the field. These tests are most useful for diagnosis at the herd level. In the absence of a "gold standard" test for the serologic diagnosis of CBPP, some uncertainties remain unresolved. Suspicious CBPP cases identified by positive serology must be confirmed by further investigations that demonstrate the presence of antigen in the respiratory tissues of animals.

NECROPSY FINDINGS

Lesions are confined to the thoracic cavity and lungs, and the lesions are usually unilateral. The pleural cavity may contain large quantities of clear, yellowish-brown fluid with pieces of fibrin. This fluid is ideal for culture of the organism. Caseous fibrinous deposits are present on the parietal and visceral surfaces of the pleura. The interlobular septae are prominently distended with amber-colored fluid surrounding distended lymphatics. This fluid distinctly outlines the lobules, which vary in color, with red, gray, or yellow hepatization. Consolidation of the lungs with a typically marbled appearance is characteristic. In chronic or advanced cases, a **sequestrum** of necrotic lung varying size from 1 to 10 cm in diameter is surrounded by a fibrous capsule. If these sequestrae rupture and are drained by a bronchus, they can be a source of aerosol infection to cattle. Such a mechanism may contribute to epidemics in closed herds. In affected calves, exudative peritonitis, arthritis, bursitis, and fibrinous arthritis of carpal and tarsal joints may be present.

Histologically, in the early stages the typical lesion consists of bronchiolar necrosis and edema, progressing to exudative serofibrinous bronchiolitis with extension to the alveoli and adjacent lymphatics. This process extends to the tracheobronchial lymph nodes and pleural lymphatics. The mediastinal, sternal, aortic, and intercostal lymph nodes are enlarged, edematous, and hemorrhagic. Lymphatics become thrombosed and fibrosed. The pulmonary lobules become consolidated with alveolar edema, fibrin, and inflammatory cells. Coagulation necrosis is common, and the organism can be demonstrated in these lobules by immunohistochemistry.

Perivascular organization foci, or "organizing centers," in the interlobular septa are considered typical of CBPP. They consist of a center occupied by a blood vessel with proliferation of connective and inflammatory cells surrounded by a peripheral zone of necrotic cells. Type I foci contain more proliferative cells in the central zone, which is larger than the peripheral zone. In Type II foci, the proliferative cells are scarce, and the peripheral zone is relatively larger. Immunoreactive antigen is visible in the central zone inside blood vessels. Immunocytochemical tests can be used to detect the organism in tissue sections and provide valuable confirmatory diagnosis after slaughter. Stained antigen is visible in the smaller bronchioles and alveoli and within the interlobular septa of the lung. Immunofluorescent staining of impression smears of lungs may be more sensitive and rapid than culture.

Renal lesions are frequently detectable in CBPP in field and experimental cases. In the acute phase of the disease, multiple renal infarcts are common. In subacute and chronic cases, the infarcts progress to form large areas of fibrosis accompanied by tubular dystrophic calcification, tubular atrophy, and lymphocyte interstitial infiltrates. Immunohistochemically, the *MmmSC* antigen is present in several renal structures.

Samples for Confirmation of Diagnosis

- **Histology**—formalin-fixed lung (LM, IHC)
- **Mycoplasmology**—effusion fluid in serum tube, lung, bronchial lymph node (MCULT, FAT, PCR, C-ELISA)

DIFFERENTIAL DIAGNOSIS

A diagnosis based on a history of contact with infected animals, clinical findings, a complement fixation test, necropsy findings, and cultural examination is necessary.

Diseases that must be differentiated from CBPP include the following:

Rinderpest Erosive stomatitis, dysentery, and erosions throughout the alimentary tract

Foot and mouth disease Salivation, lameness, fever, and vesicular stomatitis

Hemorrhagic septicemia Acute disease with death in 6 to 72 hours. Edema of the neck and brisket, lung lesions similar to CBPP. Culture of *Pasteurella* spp.

Theileriosis (East Coast fever) Coughing, nasal and ocular discharge, diarrhea, enlargement of peripheral lymph nodes, ulceration of abomasum. No lung lesions.

Ephemeral fever Ocular discharge, drooling saliva, lameness, enlarged joints, self-limiting disease of short duration; most affected cattle recover quickly; fluctuating fever; secondary pneumonia may occur.

Pulmonary abscesses Large abscesses containing foul-smelling purulent material; may have total destruction of lung.

Tuberculosis Tubercular nodules may resemble CBPP sequestra, but they are degenerative cheese-like lesions, often calcified.

Farcy Abscesses of lungs containing foul-smelling material and enlarged local lymph nodes.

Actinobacillosis Generalized lesions of lung and other adjacent tissues.

Echinococcal (hydatid cysts) Pulmonary cysts with a double wall and containing clear fluid, often calcified when old.

TREATMENT

Official conventional wisdom in the past held that treatment of clinical cases of CBPP with antimicrobials is counterproductive to contain the disease because it gives rise to persistent infection and may produce symptomless carrier animals.[5] Accordingly, the use of antimicrobials is legally banned in many endemically affected countries. Nevertheless, the use of antimicrobials in affected regions is widespread, mainly because, with limited availability of vaccines, it is considered the only available and effective treatment and control measure.[5,19] In recent years the popularity of antibiotic treatment and the perception of positive results led to some research activity suggesting that antimicrobial use may be of value primarily to control disease transmission.[5]

Despite the perception of veterinarians and farmers that antibiotics can alleviate the clinical course of the disease, enabling some

improvement in condition, field studies suggest that antimicrobial therapy had little to no effect on severity of signs, course of the disease, and mortality rate in clinically affected animals.[20,21] Treatment failures in clinically affected animals may be attributable to inadequate dosage or duration of treatment and to the chronic nature of the condition. Treatment success of antimicrobial therapy to treat mycoplasma infection greatly depends on a timely initiation of the treatment, but clinically affected animals in endemic areas frequently are in an advanced or even chronic stage of disease and thus are unlikely to show strong treatment response.[21] Because of the generally poor treatment response and because these animals present a source of infection for herdmates, clinically affected animals should rather be culled than treated.

In contrast the treatment of in-contact animals appeared to considerably reduce disease transmission, which resulted in a marked decrease in the disease occurrence, morbidity, and mortality rates in affected herds.[21] An increasing body of evidence suggests that use of antimicrobials, primarily as a part of a disease control program, should be reconsidered.[5,15,19]

The major classes of antimicrobials that are effective against mycoplamsas are tetracyclines, macrolides, florfenicol, and fluoroquinolones. A number of in vitro and in vivo studies have been published in recent years with results supporting the use of fluoroquinolones, several different macrolides, and tetracyclines to reduce the shedding of MmmSC.[21-24] Beta-lactam antibiotics and sulfonamides are inherently ineffective against the *Mycoplasmas* that do not have a cell wall and do not synthesize folic acid.

TREATMENT AND CONTROL

Treatment*

Tulathromycin (2.5 mg/kg SC as single dose) (R-3)

Florfenicol (20 mg/kg q48 IM) (R-3)

Tilmicosin (10 mg/kg SC as single dose) (R-3)

Gamithromycin (6 mg/kg SC as single dose (R-3)

Danofloxacin (2.5 mg/kg q24h SC) (R-3)

Oxytetracycline (10 mg/kg IM q24 for at least 4 days (R-3)

Control*

Danofloxacin (2.5 mg/kg q24h SC over three consecutive days) (R-2)[21]

Tulathromycin (2.5 mg/kg SC as single dose)

Tilmicosin (10 mg/kg SC as single dose)

Gamithromycin (6 mg/kg SC as single dose)

Florfenicol (20 mg/kg q48 IM)

Oxytetracycline long-acting formulation (20 mg/kg IM)

Vaccination

Vaccination with T1/44 or T1SR MLV vaccines (R-1)

**The use of antimicrobials is legally prohibited in many countries affected by CBPP.*

CONTROL

There are four essential tools in CBPP control and eradication: **livestock movement control**, **stamping out, vaccination,** and **treatment**.[3]

The possible strategies used for control in affected countries or regions are as follows:

- **Slaughter of all sick and in-contact cattle.** This requires full cooperation of cattle owners and an adequate and timely compensation system. This strategy is impractical in developing countries with a pastoral economy.
- **Slaughter of all sick cattle and vaccination of in-contact cattle.** This strategy is used frequently and usually perpetuates the disease.
- **Vaccination of healthy cattle with slaughter of sick cattle in an epidemic and revaccination of cattle at risk.** This method depends on the ability of the authorities to detect epidemics rapidly, most effectively, by abattoir surveillance and to maintain vaccination for at least 3 years. Vaccination in endemic areas must be done annually, whereas newly infected areas require repeat vaccinations aimed at eradication of the disease.

Although the combination of movement control, quarantine, and culling of infected animals, when strictly enforced, can successfully eradicate CBPP, as is documented by numerous examples of the past centuries, this approach requires a financial and logistic effort that is beyond the means of many endemically infected countries in Africa. In these countries social and civil disturbances interfere with effective disease control. Farmers fleeing civil unrest may move their cattle to endemic areas and then return with them when the threat is over, making strict livestock movement control logistically and politically unenforceable. Culling of infected and in-contact animals requires a system of financial reimbursement for farmers that is far beyond the financial resources of many affected countries.

In view of the epidemiologic situation of CBPP and the socioeconomic structure of many African countries, control of the condition is largely based on vaccination in Africa, whereas treatment of affected animals—although widely practiced—is legally prohibited in many countries.[3] Although there are examples of successful eradication of CBPP on the continent, efforts to contain the disease keep failing in many sub-Saharan countries, which lead to a reemergence of the disease in the region over the past decades and regular recurrent outbreaks in countries where the disease has previously been eradicated.

Removal of Sources of Infection

To control or eradicate CBPP, enforcing suitable surveillance strategies is imperative. Surveillance needs to cover the susceptible species (cattle and possibly buffaloes) and should comprise **clinical surveillance**, **serologic surveillance,** and **pathologic surveillance**.[1] Because of the limited sensitivity of available tools randomized surveillance is discouraged, and the interpretation of **surveillance results should be interpreted on a herd level rather than an individual animal level.**[1] All infected and suspicious reactors and clinical cases should be destroyed or transported under close control to abattoirs. Where this cannot be done without a chance of spread to animals along the route, destruction on the farm is necessary. Animals that eventually go to abattoirs should be kept under quarantine until slaughter, irrespective of their status.

Clinical Surveillance

Clinical surveillance aims at identifying clinical signs consistent with CBPP in a herd by closely examining susceptible individual animals. It requires good knowledge of the possible clinical presentation of the condition particularly in endemic regions, where affected animals may only show mild and subtle signs. Although the diagnosis of CBPP cannot be confirmed solely on the basis of physical examination, clinical surveillance can greatly contribute to the level of confidence in the overall surveillance strategy when a large number of animals in a susceptible herd are regularly examined. Animals suspected to be infected must be followed up, either by serologic or pathologic surveillance.

Pathologic Surveillance

Systematic pathologic surveillance conducted on slaughterhouse material, thus including large numbers of clinically unapparent animals is considered the most effective approach to screen for the presence of the disease within a herd and, more important, within a region or a country.[1] Appropriate training of the personnel conducting pathologic surveillance to identify characteristic and suspicious lesions is essential. Suspected cases must be followed up to confirm the presence of the specific pathogen in the tissue.

Serologic Surveillance

Because of the limited sensitivity of the available serologic tests, serology is unsuitable as

a stand-alone screening procedure but may be used in the framework of epidemiologic investigations. Serologic surveillance results should primarily be interpreted at a herd level, and positive results should be confirmed by clinical or pathologic examination and identification of the specific pathogen. Because animals in the incubation phase and early stages of the disease may test negative, it is necessary to have two negative tests 2 months apart. After vaccination a positive reaction occurs; this usually disappears within 2 months but may persist for 5 months.

Vaccination

Vaccination against CBPP has been used in countries where rigorous cattle movement control, quarantine, and stamping out cannot be implemented, as is the case in many African countries that cannot afford the prohibitive costs of culling entire infected herds. Although CBPP vaccination is doomed to fail when used as stand-alone strategy to control CPBB, a systematic vaccination strategy in combination with intense surveillance and removal of infected animals can contribute to the containment of the disease and reduction of the infection prevalence to the point where vaccination can be discontinued and remaining infected animals can be culled to achieve complete eradication of the disease.[3,25] Extensive vaccination in Australia reduced the incidence of the disease to an extremely low level, and complete eradication of the disease was achieved shortly afterward. Vaccine application is usually controlled by local legislation.

All effective CBPP vaccines have been based upon live versions of the disease-causing *MmmSC,* either attenuated or not. Currently the only vaccines in use are live vaccine derived from the T1 strain (**T1/44 and T1SR**) of live attenuated *Mycoplasma mycoides* subsp. *mycoides* SC and attenuated through repeated passage in embryonated eggs before production in artificial growth media.[26] Unfortunately these vaccines are characterized by poor and variable efficacy, with only between 30% and 60% of vaccinated animals being protected. In some situations, the T1/44 vaccine induces a good immunity, especially when herds are revaccinated annually, in which case the level of protection exceeds 85%. Induced immunity is short lived, particularly for the T1SR strain, requiring revaccination at least on a yearly basis. Either systemic or local **adverse reactions are common,** particularly for the T1/44 strain. Within 2 to 4 weeks following injection, an invading edema develops known as the "Willems" reaction. The incidence of these reactions varies from area to another. The reversion to virulence of the T1/44 vaccine has also been observed when it was serially passaged by endobronchial intubation resulting in the development of lesions of CBPP in animals that were infectious to in-contact animals. This suggests that animals given the currently used vaccines (T1/44 and T1SR) subcutaneously could be reservoirs for *MmmSC* and infect other animals in areas previously free of CBPP.

The value of calfhood vaccination is limited because arthritis, myocarditis, and valvular endocarditis occur 3 to 4 weeks after vaccination of calves less than 2 months old. Vaccination of calves after this age is recommended because it avoids the occasional deaths which occur after vaccination of adults.

Historically, pleural exudate from natural cases (natural lymph) was used in an attempt to immunize cattle at risk. Vaccination was carried out by injection into the tough connective tissue at the tip of the tail with a high-pressure syringe. "Natural lymph vaccination" caused severe reactions with sloughing of the tail and extensive cellulitis of the hindquarters, necessitating destruction or causing death of the animal in many instances. Draining infected injection sites may have contributed to the spreading of this disease and others.

Inactivated CBPP vaccines have been field tested but results have been inconclusive. Immunostimulating complex (ISCOM) protein subunit vaccines have been developed, and early results are encouraging. The capsular polysaccharide (CPS) of *MmmSC* is an important surface antigen and pathogenicity factor previously known as a galactan. The immune response in mice of capsular polysaccharide conjugate vaccines against CBPP indicates that protection against *MmmSC* mycoplasmemia in mice is cell-mediated rather than humoral immunity.

Antimicrobial Use

As mentioned previously the use of antimicrobials for treatment and control of CBPP is banned in many countries affected by the disease because it is considered to be counterproductive for the control and eradication of the disease because it may result in subclinically infected animals carrying and possibly shedding the pathogen for prolonged period of time. Despite the ban, the use of antimicrobials is widespread mainly because vaccine coverage vaccine efficacy is limited.[5,15] Recent field trials and epidemiologic studies provided evidence corroborating the empirical observation that the use of antimicrobials in infected herds is able to contain disease transmission.[5,15,21] Using a homogeneous model based on publically available data, one study concluded that the potential impact of reducing the infectious period by the use of antimicrobials on disease persistence and mortality was in the same range as the impact of currently available vaccines.[25] In single isolated herds of 500 head of cattle, a 50% reduction in length of the infectious period caused the fade-out of the disease in essentially 100% of herds, a 60% decline in the number of cases, and a 73% decline in mortality.[25] Accordingly a significant decline in mortality, seropositivity, frequency of morphologic lesions, and severity of clinical scores was observed in herds where all in-contact animals received a treatment with danofloxacin at a dose of 2.5 mg/kg on 3 consecutive days compared with untreated herds.[21] This mounting body of evidence warrants the reconsideration of the use of antimicrobials in an CBPP eradication or control program.

Disease Control on an Area Basis

The prevention of entry of infected animals into a free area is a difficult task. Only the following classes of cattle should be permitted to enter:

1. Cattle that have neither been in an infected area nor in contact with infected animals for at least 6 months. This may be relaxed to permit entry of cattle going to immediate slaughter after a clinical examination and a period of 1 month in a free area.
2. Cattle that have given negative reactions to the CFT or C-ELISA on two occasions within the preceding 2 months and have not been in contact with infected animals during this period. Less rigid measures than these will permit introduction of the disease.

When the disease is already present in an area, two methods of control are possible: vaccination and eradication by test and slaughter of reactors. The method chosen will depend largely on the economy of the cattle industry in the affected area. A vaccination program may be the first step to reduce the incidence of the disease to the point where eradication becomes possible.

In areas where farms are large, fencing is poor, and the collection of every animal cannot be guaranteed, eradication of the disease by test and slaughter is impractical. Vaccination can be practiced whenever the cattle are brought together. Animals moving out of or into infected areas and groups of cattle that contain active cases must be vaccinated. Moving cattle that develop the disease should be halted, clinical cases slaughtered, and the remainder vaccinated. Results are usually good provided the vaccination is carried out carefully, but some further cases as a result of prevaccination infection are to be expected.

When outbreaks occur in small areas where herds can be adequately controlled, complete eradication should be attempted by periodic testing and the destruction of reactors, and in-contact animals should be vaccinated. To avoid unnecessary contact between cattle, retesting is delayed until 5 to 6 months after the first test when vaccination reactions have usually subsided. Under most circumstances all nonreactors should be

vaccinated. This practice is particularly applicable in feeder cattle that will be slaughtered subsequently and when extensive outbreaks occur in closely settled areas where the chances of spread are great. Simple test and slaughter in these latter circumstances will be too slow to control the rate of spread. In either case the herd should not be released from quarantine until two tests at an interval of more than 2 months are completely negative.

World Organization for Animal Health (OIE)—CBPP Status for Countries or Regions

CBPP is a so-called "List A" disease, making it a notifiable disease to the OIE.[2] To qualify as a CBPP-free country or zone according to the rules of the OIE the country must fulfill the following requirements:

- Have a record of regular and prompt animal disease reporting
- Submission of a declaration to the OIE on a yearly basis stating the following:
  - There has been no outbreak of CBPP during the past 24 months.
  - No evidence of CBPP infection has been found during the past 24 months.
  - No vaccination has been carried out during the past 24 months.

This declaration has to be supported by evidence documenting that a surveillance program is in operation and that regulatory measures for the prevention and control of CBPP have been implemented.[1] In case a country loses the status as CBPP-free because of an outbreak, one of the following waiting periods applies:

- 12 months after the last case, where a stamping-out policy combined with serologic surveillance and strict movement control is enforced.
- 12 months after slaughter of the last vaccinated animal, where vaccination was used.

As of May 2015 the following countries are recognized by the OIE as CBPP-free: Argentina, Australia, Botswana, Canada, China, France, India, Portugal, Singapore, Switzerland, and the United States.[2]

In Europe, legislation exists to prevent the spread of CBPP. Any outbreak in a previously CBPP-free country must be reported to the European Commission within 24 hours of confirmation of the disease; the Commission will then inform other member states. Unaffected regions may export only to other member states if cattle come from herds in which all animals over 12 months of age have been serologically negative in the previous 12 months. All animals for export must have been serologically tested negative 30 days before being loaded. Cattle from restricted areas must not be exported to other member states until all herds in the area have passed three clear herd tests on all animals over 12 months of age at intervals greater than 3 weeks apart.

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MYCOPLASMA BOVIS PNEUMONIA, POLYARTHRITIS, MASTITIS, AND RELATED DISEASES OF CATTLE

SYNOPSIS

Etiology *M. bovis*

Epidemiology Occurs in dairy and beef cattle of all ages. Pneumonia, otitis, and polyarthritis primarily seen in feedlot cattle and dairy calves; mycoplasmal mastitis in dairy cows.

Clinical findings Unresponsive pneumonia, polyarthritis, otitis media/interna, mastitis in dairy herds.

Diagnostic confirmation Culture or detection of antigen or bacterial DNA from respiratory secretions, joint fluid, milk.

Treatment Antimicrobial therapy, often with poor treatment outcome.

Control Biosecurity and biocontainment procedures. Prevent entry of infected animals into herds. Purchase animals free of mycoplasma. Pasteurization of milk of cows with mycoplasma mastitis before feeding to calves. Metaphylactic antimicrobial therapy might be justified in herds with high morbidity and mortality rates. Vaccines have been unsuccessful.

ETIOLOGY

Mycoplasma spp. belong to the class of Mollicutes, a group of bacteria enveloped by a complex plasma membrane but lacking a cell wall. They are characterized by their small size, their tiny genome, and their intimate association with host cells that is essential for their survival. Mycoplasmas typically inhabit mucous membranes, including those of the respiratory tract, urogenital tract, the mammary gland, or the conjunctivae.[1] *M. bovis* is a major cause of disease of cattle causing pneumonia, otitis media, arthritis, tenosynovitis, keratoconjunctivitis, mastitis, meningitis, and reproductive disorders, including abortion.[2] It is the etiologic agent of the so-called **chronic pneumonia and polyarthritis syndrome (CPPS)** of feedlot cattle that has been recognized in the United States and Canada.

Mycoplasma bovis is highly adapted to cattle but has occasionally been isolated from small ruminants, buffaloes, and, in rare instances, even from humans with bronchopneumonia.

EPIDEMIOLOGY

Occurrence

M. bovis that was first isolated in 1961 from cow with mastitis in the Unites States, has spread to many countries of the world via animal movements and is now recognized as a worldwide pathogen of intensively farmed cattle.[1] Of 1600 isolates of *Mycoplasma* species recovered from ruminant animals in

Britain over a 10-year period, *M. bovis* was the most common species, mostly from pneumonic calves, but occasionally also from cattle with mastitis and arthritis. A serologic survey of pneumonic cattle found *M. bovis* antibody–positive samples in 18%.

Mycoplasma bovis that is a common although not ubiquitous inhabitant of the upper respiratory tract of cattle is considered a major cause of **respiratory disease** affecting beef and dairy calves alike. In England and Wales, serologic screening between 2000 and 2009 revealed a herd seroprevalence of *M. bovis* of over 30% and *M. bovis* was isolated on average in 40% of pneumonic lungs submitted during that time period.[2] In Israel, *M. bovis* was isolated from 26% to 65% of samples from pneumonic calves submitted between 2004 and 2008.[3] *M. bovis* was also the strain isolated from more than half of the 1000 samples from calves with bronchopneumonia that were submitted to different diagnostic laboratories in France between 2003 and 2008.[2] In a recent Italian study, 37% of lung tissue samples from pneumonic dairy calves less than 1 month old submitted to a diagnostic laboratory tested positive on PCR for *Mycoplasma* spp. Of the positive samples, 31% were identified as *M. bovis*.[4]

The **chronic pneumonia and polyarthritis syndrome** (CPPS) of feedlot cattle has been reported in Canada and the United States. It occurs commonly in young feedlot cattle usually affecting many animals a few weeks after arrival and mingling in the lot. In Canada, the disease has been seen commonly in young cattle (6-8 months of age) following shipment from western rangelands to eastern feedlots, which suggests that long transportation and mixing of cattle of different origins may be important epidemiologic characteristics. The morbidity ranges from 20% to 85% and the case-mortality rate from 3% to 50%. Calves affected with arthritis commonly have necropsy evidence of mycoplasmal pneumonia, and it is proposed that the pneumonia precedes the development of the arthritis. In feedlot calves the prevalence of *M. bovis* infection was found to be below 7% at the time animals enter the feedlot but increases dramatically within to first weeks in the feedlot to values between 40% and 100% in most studies.[5] In a group of feedlot cattle, from Alberta, Canada, with chronic unresponsive pneumonia and polyarthritis, *M. bovis* was the most common pathogen isolated, having been detected in 82% of cases, including 71% in lungs and 45% in joints. In a series of cases of chronic, antibiotic-resistant pneumonia, sometimes with concurrent polyarthritis, in feedlot cattle in western Canada, *M. bovis* was present in the lung tissues of more than 90% of cases, and the BVDV was present in 60% of the cases, suggesting a possible synergism between *M. bovis* and the BVDV. Outbreaks of pneumonia and arthritis in beef calves associated with infection attributable to *Mycoplasma bovis* and *Mycoplasma californicum* have been described in a mixed dairy cattle and beef cattle herd kept under extremely poor housing and hygienic conditions.

Mycoplasma arthritis of cattle has been reported in a number of countries including Canada, the United States, several European countries and the United Kingdom. Commonly, arthritis occurs in association with respiratory disease or otitis media in calves, or mastitis in adult cattle, and it is proposed that the pneumonia precedes the development of the arthritis. Calves suckling cows with experimental mastitis attributable to this organism may develop mycoplasmal arthritis, and a high incidence is recorded in calves in dairy herds where mycoplasmal mastitis was occurring. In Ireland, infection has occurred in housed adult dairy cattle, without any evidence of pneumonia, producing severe polyarthritis with a clinical incidence in 12 farms that varied from 2% to 66%.

Otitis media/interna has been described in preweaned Holstein dairy calves in dairy herds that have expanded in size. Affected calves were 2 to 5 weeks of age, morbidity was 3% to 10%, and case-fatality rates estimated at 50%. In a retrospective study in calves submitted for necropsy in California, affected calves were 2 weeks to 4 months of age, 92% were from dairy herds, and most cases occurred during late winter and spring. *M. bovis, M. bovirhinis,* and *M. alkalescens* were isolated from the ears of affected calves. Outbreaks of suppurative otitis media and pneumonia associated with *M. bovis* have been described in calves on a dairy farm in the United Kingdom with a disease incidence of 20%.[6] Outbreaks in beef cattle farms in Japan with morbidity and mortality rates of 8% to 40% and 30% to 100%, respectively have also been reported.

Mycoplasma bovis has been recognized as pathogen of the bovine **mammary gland** (see also "Mastitis Caused by *Mycoplasma* spp.") that is widespread within the dairy cattle population in the United States. A national survey conducted in 2002 in the United States determined a prevalence of mycoplasma culture positive bulk tank milk samples of 7.9%, of which 86% were identified as *M. bovis*.[7] In contrast the herd prevalence of *M. bovis* in Canadian dairy herds was estimated to be 1.7%, 1.5% in Belgium, and 0.56% in Japan.[8,9] *M. bovis* was not detected in recent survey in dairy herds in New Zealand.[10]

Economic Importance

M. bovis has been associated with respiratory disease in calves and mastitis in dairy cows, that both have a considerable prevalence at least in some parts of the world. Costs related to infection with mycoplasma include expenses for treatment and diagnosis, death and culling losses and implementation of control measures. The chronicity and poor treatment response of most diseases associated with *M. bovis* contribute considerably to these expenses. The costs to the U.S. beef industry were estimated with approximately $32 million per year as result of decreased weight gain and lost carcass value. Mycoplasma mastitis was estimated to cost the U.S. dairy industry approximately $108 million per year.[11]

Risk Factors

Pathogen Risk Factors

The virulence factors of *M. bovis* and mechanisms of pathogenicity are not well understood, but the organism's ability to vary the expression of **variable surface proteins** (VSPs), a family of lipoproteins on the bacterial surface with high frequency is currently being investigated. The organism has 13 VSP genes involved in antigenic variation that alter the antigenic character of its surface components and may act to enhance colonization and/or adherence to host cells or to evade the host's immune defense systems.[5] These VSP proteins and some unrelated proteins such as pMB67 and P48 are the primary antigenic targets of the host's antibody response, which, however, does not appear to be protective.

M. bovis can induce apoptosis of lymphocytes, and a C-terminal fragment of VSP-L is able to impair the lymphocyte proliferative response to mitogens. The bacterium can furthermore adhere to neutrophils and block the oxidative burst in these cells.[5] Several strains of *M. bovis* produce **biofilm**, protecting the organism from heat and desiccation and possibly playing a role in evading the host's immune response and in resistance to antimicrobial therapy in vivo.[5] *M. bovis* produces **hydrogen peroxide** in quantities that vary between strains. Peroxide production can result in oxidative injury to host tissues. The organism is also able to penetrate through lung epithelial junctions and cause systemic infections. There is some evidence of variability of *M. bovis* strains to cause arthritis.

Animal Risk Factors

The immune status of the individual animal is important in determining the susceptibility to respiratory disease, particularly in young ruminants. An association between failure of transfer of passive immunity and the risk and severity of respiratory disease in young calves is well established. It is however not clear whether maternal antibodies against *M. bovis* have a protective effect in calves. An association between *M. bovis* specific serum antibody titers in the first weeks of life and the risk of developing respiratory tract disease could thus far not be established.[1]

Age appears to influence the susceptibility at least to some forms of *M. bovis* infection. Otitis media is most commonly observed in calves 2 to 6 weeks of age and is uncommon in older calves. Age-related susceptibility is also observed in other species.[1]

Although a genetic effect on the susceptibility to mycoplasma infection in cattle has not been confirmed, the genetic background is considered an important determinant of resistance to mycoplasmal respiratory disease in nonruminants.[1]

Environmental Risk Factors

With direct animal contact being the main route of infection, introducing infected animals into a herd with no or low *M. bovis* infection prevalence presents a major risk for disease transmission. Similarly, high infection prevalence within the herd or mingling calves from different origins presents a high risk of infection for young calves. Feeding colostrum or waste milk of cows with clinical or subclinical *M. bovis* mastitis was shown to result in colonization of the upper respiratory tract with this pathogen and was associated with increased occurrence rates of mycoplasmal otitis.[11]

Specific and nonspecific immune response that is a critical determinant of the susceptibility to respiratory tract infection can be compromised by a number of environmental factors, such heat or cold stress, overcrowding, poor ventilation, transportation, inadequate nutrition, or stress related to processing procedures.

Concomitant infection of the respiratory tract with other viral or bacterial pathogens may compromise the nonspecific immune response. *M. bovis* infection may predispose to superinfection of the respiratory tract with other bacterial pathogens, and previous infection of the respiratory tract with other pathogens may facilitate to progression of *M. bovis* into the lower respiratory tract. Experimental infection studies have confirmed a synergistic effect of M. bovis with other common pathogens of the respiratory tract, such as *M. haemolytica* or *P. multocida*.[1]

Environmental temperatures have been found to affect the degree of nasal shedding and the incidence rate of clinical disease. Sudden drops of the environmental temperature were associated with increased rates of nasal shedding of *M. bovis* and higher occurrence rates of respiratory disease caused by M. bovis.[1]

Methods of Transmission

Direct transmission of *M. bovis* from infected to uninfected animals is considered the primary route of disease transmission, an assumption that is corroborated by epidemiologic evidence indicating that the seroprevalence in feedlot cattle increases dramatically in the first weeks in the feedlot. Clinically normal cattle in infected herds harbor *M. bovis* in the upper respiratory tract with no apparent adverse effect and may shed the organism through the nasal discharge for months to years.[5] *M. bovis* might be transmitted with respiratory secretions, via aerosols, direct nose-to-nose contact, or fomites. Although mycoplasmas in general do not easily survive in the environment, *M. bovis* can survive for prolonged periods outside the host, particularly in cool and humid conditions. Nonetheless the role of fomites and environmental contamination deserves further investigation but, with exception of the transmission of mycoplasma mastitis, is currently considered to be of limited epidemiologic relevance.

Oral ingestion of *M. bovis* contaminated milk or colostrum results in colonization of the oral cavity and upper respiratory tract with this pathogen and increased occurrence of otitis media.[12] Feeding colostrum or milk of cows shedding *M. bovis* through the mammary gland must therefore be considered as an effective route of disease transmission.

Intrauterine infection of calves appears to occur infrequently. Transmission of mycoplasma mastitis is considered to primarily occur in the milking parlor through contaminated milk.

PATHOGENESIS

As with many mycoplasmas, *M. bovis* is both immune reactive and immunosuppressive. Upon incubation with *M. bovis,* alveolar macrophages are activated and produce TNF-alpha and nitric oxide, two powerful initiators of immune activity. *M. bovis* is also immunosuppressive by inhibiting neutrophil degranulation and oxidative bursts and proliferation of lymphocytes by mitogens. *M. bovis* also induces bovine lymphocyte apoptosis through the production of a protein that is different from other mycoplasmas both pathogenic and nonpathogenic. The protein is an immuno-inhibitory peptide that can suppress Concanavalin A (ConA)–induced proliferation of bovine lymphocytes. This represents a unique immunosuppressive peptide produced by the *M. bovis.*

Despite its deleterious effects on lymphocytes, infected cattle are able to generate measurable humoral and cellular immune responses against *M. bovis.* Serologic analysis indicates that *M. bovis* stimulates increased production of antigen-specific IgG1, whereas very little IgG2 is produced.

There is a systemic phase of *M. bovis* infection, including a potential interaction of the pathogen with endothelial cells. It is one of the most invasive bovine mycoplasmas capable of invading through lung epithelial junctions and causing systemic infections such as arthritis and mastitis following pneumonia. Localized lung vasculitis and the presence of thrombi within subsynovial vessels has been observed, both suggestive of interaction of *M. bovis* with epithelial cells.

Arthritis is normally regarded as a sequel to pneumonia or mastitis and infection in the respiratory tract or in the mammary gland is believed to lead to bacteremia and localization in joints. However, arthritis can suddenly occur in regions or countries where mycoplasmal pneumonia has been recognized for many years, suggesting that a new strain with different virulence or tropism has been introduced. The intraarticular injection of *M. bovis* into calves causes severe fibrinosuppurative synovitis and tenosynovitis, erosion of cartilage, and its replacement by polypoid granulation tissue. Erosion of the cartilage is accompanied by chronic osteomyelitis and formation of pannus tissue. Histologically, there is extensive ulceration of synovial membranes of leukocytic infiltration of the subsynovium, congestion, hyperemia, and thrombosis of the subsynovial vessels. Intratracheal inoculation of the organism results in pneumonia and severe lameness, which suggests that *M. bovis* is involved in pneumonia–arthritis syndrome.

In **otitis media/interna** of calves there is facial nerve paralysis because of proximity of CN VII to the tympanic cavity. Although hematogenous spreading of the pathogen to the middle and inner ear is considered a possible route of infection of this organ, an ascending infection from the oral cavity through the Eustachian tubes to the middle ear, resulting in clinical otitis media could be experimentally induced by feeding milk replacer contaminated with *M. bovis*.[1,12] Varying degrees of peripheral vestibulocochlear dysfunction occur because of the involvement of the vestibulocochlear receptors and nerve. The spontaneous regurgitation and dysphagia may be associated with lesions involving the glossopharyngeal nerve (CN IX) with or without the vagus nerve (CN X). These nerves may be affected by the inflammation associated with meningitis because both CN IX and CN X travel through the jugular foramen.

CLINICAL FINDINGS

Pneumonia and polyarthritis associated with *M. bovis* may occur alone or together in cattle of all ages, including dairy and beef calves in their original herds, in growing dairy and beef cattle heifers, and in mature dairy and beef cows.

Chronic Pneumonia and Polyarthritis Syndrome

The disease is most common in feedlot calves within a few weeks after arrival in the feedlot. The morbidity rate may be up to 25%. Affected calves commonly have had a history of respiratory disease with poor to no treatment response to antimicrobial therapy. Auscultation of the lungs reveals areas of loud bronchial tones, crackles and wheezes, and areas of muffled lung sounds indicating consolidation and occlusion of the bronchi with exudate. Depression, inactivity, inappetence, coughing, nasal discharge, fever, and progressive weight loss are common.

Arthritis

Although mycoplasmal arthritis is most commonly seen concurrent with

pneumonia, cattle of any age can be affected. Cases tend to occur sporadically, but outbreaks have been reported in calves and adult cows and as part of CPPS in feedlot cattle.[11] There is stiffness of gait, acute, non-weight-bearing lameness, inappetence, moderate fever, and progressive loss of weight. Swelling of the large movable limb joints and distension of tendon sheaths, associated with fibrinous synovitis and synovial fluid effusions, are characteristic. Both forelimbs and hindlimbs can be affected, and involvement of the carpal joints, the fetlocks and the proximal and distal interphalangeal joints commonly can be clinically detected. In calves, pneumonia is a common finding in the affected group. Some affected cattle spend considerable time in recumbency, lose weight, and develop decubitus ulcers, and they must be destroyed. Mildly affected cases recover spontaneously over a period of several weeks, but severe cases become progressively worse, may develop discharging sinuses over affected joints, and must be culled.

Otitis Media/Interna

Otitis media/interna occurs in young beef and dairy calves as enzootic disease or as outbreaks. Feedlot calves are sporadically affected.[11] Clinical findings depend on the extent of the inflammation, which can involve only the middle ear or middle and inner ear. Varying degrees of depression, coughing, nasal discharge, inappetence, and fever are common in affected groups of calves. Head shaking, scratching, or rubbing of the ear are signs of ear pain. Otitis externa that is characterized by purulent exudate in the external ear may occur as result of a ruptured tympanic membrane. A unilateral head tilt and paralysis of the lip, eyelid, and ear muscles on the same side are common. When the eye on the affected side is threatened, the eyeball may retract, but there is no palpebral fissure closure. An intermittent loss of balance on the affected side may be apparent when the animal attempts to walk. Bilateral peripheral CN VII and VIII deficits (bilateral ear, lip, and eyelid paresis; bilaterally absent menace and palpebral reflexes; normal gait; balance loss to either side) are suggestive of bilateral otitis media/interna. Dysphagia, spontaneous regurgitation of milk and difficulty in sucking from a bottle or prehending feed may occur. Partially chewed feed may accumulate in the oral cavity, along with difficult prehension and mastication. Bilateral vestibular disease (balance loss to either side) may occur. Endoscopy of the pharynx may reveal collapse of the nasopharynx, dorsal displacement of the soft palate, and a widely dilated, hypomotile esophagus. Opisthotonus and nystagmus are common, and ataxia, recumbency, and death in several days may occur. The mortality rate is about 50%.

CLINICAL PATHOLOGY

Clinical and pathologic signs are not characteristic for *M. bovis* infection, so laboratory diagnosis is necessary for identification. The organism can be detected by culture, identification of specific bacterial DNA by polymerase chain reaction (PCR), or identification of specific bacterial antigen, for example, with a sandwich-ELISA or immunohistochemistry.

Culture

Culture methods for the detection of *M. bovis* are typically used on lung tissue, nasal swabs, bronchioalveolar lavage (BAL), or transtracheal wash (TTW) fluid and synovial fluid. Culture methods have the advantage that they can isolate multiple mycoplasma species at the same time and may reveal novel or unexpected species. Nonetheless culture methods require complex growth media, special equipment, and technical skills. They are time consuming, laborious, difficult, and expensive.[5] The sensitivity of mycoplasma culture in clinical material is rather low for several reasons. Infected animals may shed the pathogen intermittently and the distribution within affected tissue is uneven. Poor handling of samples and long shipping times will affect the viability of this labile microorganism. Cultures may also fail in cases sampled animals were previously treated with antimicrobials or samples are contaminated with other pathogens, as is commonly the case with samples collected from the respiratory tract. Although mycoplasma colonies can be identified by their characteristic morphology, they cannot readily be differentiated from each other, making speciation by immunologic methods or PCR necessary.[1]

DNA Probe and Polymerase Chain Reaction

Molecular tests to detect bacterial DNA of *M. bovis* have been developed in recent decades and have been widely adopted for clinical diagnostic *M. bovis* infection. The main advantages of these methods are lower costs per sample, a considerably shortened turnaround time, and the compatibility with molecular testing for other pathogens. Because this method does not require the presence of living organisms, it is suitable to be used on previously stored samples.[1]

Immunohistochemistry

Immunohistochemical (IHC) techniques can be used to detect the antigen of *M. bovis* in the tissues of cattle. IHC can be performed using formalin-fixed and paraffin-embedded tissue. Histology and immunohistochemistry can be used to analyze the lesions and distribution of the *M. bovis* antigen in the lungs of cattle with pneumonia and can be performed retrospectively.

Enzyme-Linked Immunosorbent Assay

A monoclonal antibody-based sandwich ELISA (sELISA) for the detection of *M. bovis* in clinical material has been developed in Europe. The sensitivity of this ELISA is similar to that of conventional culture but can be improved when samples are incubated for a short period before antigen capture.[1]

Serology

Because *M. bovis* infection induces a robust humoral response, several methods of detecting antibodies against *M. bovis* have been developed. Available tests that can be used on serum and other body fluids such as milk or synovia include the passive or indirect hemagglutination (HA) test, the indirect ELISA, and the film inhibition test. A variety of ELISA test kits are now commercially available.

Antibodies against *M. bovis* are detectable with the ELISA as early as 6 to 10 days post experimental inoculation. Notwithstanding correlation between antibody titers and clinical disease was found to be poor, and seroconversion in feedlot cattle is observed in healthy and sick calves alike, suggesting that paired serum samples may not be a good predictor of *M. bovis* respiratory disease.[5] Furthermore, serum antibody titers can remain elevated for months to years after an infection, meaning that a high titer may not necessarily be consistent with ongoing or recent infection.[1] Maternal antibodies may result in high antibody titers in young calves, but with a half-life of 12 to 16 days normally wane within the first months of life.[1]

Serology is currently considered to be of limited diagnostic value on an individual animal level but is useful on a herd level to screen a group of animals.

Sample Collection and Handling

The choice of the specimen submitted for mycoplasma diagnostics and handling of the sample can have a great impact on the final test result and its validity. A number of antemortem diagnostic procedures are available for the diagnosis of respiratory disease that vary in their practicality, their suitability to detect a specific pathogen, the rapidity with which test results are available, their economy, the level of stress for the patient, the quality of the material obtained, and the interpretability of the results. Nasal, nasopharyngeal or conjunctival swabs, bronchioalveolar lavage (BAL), and transtracheal washes (TTW) are the most commonly used procedures to detect *M. bovis* in living animals. Nasal/nasopharyngeal swabs are frequently used under field conditions because this procedure is technically less demanding and less invasive than a TTW or BAL. Although presence of *M. bovis* on swab material is useful to confirm the presence of

the pathogen in a herd, the diagnostic value on an individual animal level is limited because of the high occurrence of upper respiratory tract infection with *M. bovis* in clinically healthy cattle of infected herds. Comparison of paired culture results from nasopharyngeal swabs and BAL samples in cattle with respiratory disease indicate that the correlation between the presence of *M. bovis* in the upper respiratory tract and its presence in the lower respiratory tract or clinical disease is poor.[1] In contrast, excellent agreement between paired BAL fluid samples and corresponding tissue cultures obtained during necropsy have been reported, suggesting that samples collected from the lower respiratory tract such as BAL or TTW fluid are better suited to make a diagnosis on an individual animal.[1]

When using swabs, wooden cotton swabs should be avoided because they can inhibit growth of mycoplasma.[11] The tip of the swab should be inserted approximately to the height of the medial canthus of the eye and must be firmly rotated against the mucosa to harvest many cells to which mycoplasmas are adhered. Swabs should then be stored in aerobic bacterial or mycoplasma transport media. Samples submitted for culture must either be kept refrigerated when shipping time does not exceed 24 hours or otherwise frozen. Because prolonged frozen storage significantly decreases the isolation of *M. bovis,* storage time should not exceed 7 to 10 days.[1]

Tissue samples can be formalin-fixed when used for histology and IHC or must be placed on ice and transported to the diagnostic laboratory immediately for culture.[11]

NECROPSY FINDINGS

At necropsy, the characteristic lung lesion of *M. bovis* is a **caseonecrotic bronchopneumonia** comprising raised, white, sharply demarcated, friable foci of caseous necrosis within consolidated areas mostly in the cranioventral lung lobes.[13-15] The necrotic foci often range from 1 to 10 mm in size but can coalesce and grow up to 5 cm in diameter. Larger foci are frequently surrounded by pale firm connective tissue. Between 10% and 50% of total lung surface may be consolidated. When the lung is squeezed, the necrotic material falls out as a single or multiple pieces, and sequestra may be seen.[14]

Microscopically, the specific lesions are areas of coagulative to caseous necrosis originating mostly from bronchioles and bronchi. Well-developed foci of necrosis contain an eosinophilic coagulum at the center surrounded by accumulations of mostly degenerate neutrophils, macrophages and an outer zone of plasma cells, lymphocytes, and degenerate bronchiolar/bronchial epithelial cells. Adjacent lung tissue shows typical suppurative bronchopneumonia and atelectasis. Within the necrotic lesions, and especially in the inflammatory cells at the margin, *M. bovis* antigen or DNA can be detected by immunohistochemistry or molecular techniques respectively.

Fibrinous polysynovitis is remarkable at necropsy. One or more joints are swollen (as detected clinically). Acute lesions consist of a serofibrinous exudate within joint cavities and tendon sheaths, and the synovium is reddened and hyperplastic. Later, the exudate becomes purulent or fibrinopurulent, and there may be foci of necrosis as described in the lung. Microscopically, large numbers of lymphocytes and plasma cells are found within the hypertrophic synovial villi. Immunohistochemical and molecular techniques are used for specific diagnosis in the tissues of feedlot cattle.

Involvement of the ears by *M. bovis* can lead to **otitis media.** A fibrinous, purulent or caseating exudate is present in one or both middle ears with or without concurrent involvement of the lungs and joints.

Samples for Confirmation of Diagnosis

- **Histology**—lung, synovial membrane (LM, IHC)
- **Mycoplasmology**—lung, culture swab from joint cavity and affected middle ear (MCULT)
- **PCR**—lung, synovial membrane or exudate from middle ear

DIFFERENTIAL DIAGNOSIS

A diagnosis of infection by *M. bovis* should be considered when pneumonia and arthritis, synovitis, and possibly otitis occur at about the same time. The disease must be differentiated from other causes of joint swelling and lameness in feedlot cattle. With *M. bovis* infection there are usually several animals affected in a short period of time, which serves to distinguish it from other sporadic causes of arthritis.

Differential clinical diagnosis for respiratory disease:

- **Pneumonic pasteurellosis of cattle** is an acute, toxemic bronchopneumonia with a high fever and a good response to treatment in the early stages. Depression and anorexia are common. The disease is most common in young beef and dairy calves that have been recently stressed following weaning or mixed in auction markets and shipped to feedlots. The disease can also occur in mature cattle as a primary or secondary pneumonia.
- In **viral interstitial pneumonia** of calves, young and adult cattle there is characteristic dyspnea, a moderate fever, only a mild toxemia, and loud breath sounds over the ventral aspects of the lungs followed by crackles and wheezes in a few days, and recovery may take several days. Pneumonia attributable to BRSV may be mild with uneventful recovery or severe with dyspnea and subcutaneous emphysema and a high case-fatality rate.
- **Lungworm pneumonia** occurs most commonly in young pastured cattle and is characterized by dyspnea, coughing, only mild toxemia, and a moderate or normal temperature; the course may last several days. Usually many cattle are affected. Crackles and wheezes are usually audible over the dorsal aspects of the lungs, and the response to treatment is usually favorable if treatment is initiated early when signs are first noticed.
- **Atypical interstitial pneumonia (fog fever) and atypical interstitial pneumonia of feedlot cattle.** The former usually occurs in adult pastured cattle that have been moved from dry to lush pasture (or just a different species of pasture or on to a recently harvested cereal grain field); the latter is incidentally observed in feedlot cattle most commonly in the finishing period; the onset is sudden and some cattle may be found dead, whereas others are in severe respiratory distress with an expiratory grunt. This condition is usually not associated with toxemia.
- **Infectious bovine rhinotracheitis (IBR)** is characterized by rhinitis, usually with discrete lesions in the nares, tracheitis, loud coughing, high fever, and no toxemia unless secondary bacterial pneumonia is present. Recovery usually occurs gradually over 4 to 7 days.
- **Contagious bovine pleuropneumonia** (CBPP) resembles pneumonic pasteurellosis but occurs in plague form; there is severe, painful, toxemic pleuropneumonia, and the case-fatality rate is high.

Differential clinical diagnosis for arthritis:

- **Traumatic aseptic arthritis** may be associated with history of trauma and sudden onset of lameness without signs of systemic disease. Synovial fluid collected by arthrocentesis is colorless or yellow to red-tinged (as a result of hemorrhage). Synovia is clear with moderate cellularity.[16] Culture of synovia is negative.
- **Traumatic septic arthritis** with bacterial contamination of joint cavity commonly involves a single joint with worsening lameness. A skin lesion over the affected joint might be present. Synovial fluid is cloudy, with decreased viscosity, high cellularity, fibrin content, and a tendency to clot.
- **Septic arthritis as a result of hematogenous infection** is a common complication of omphalitis or septicemia (e.g., diarrhea) in calves. Frequently involves several joints and is associated with clinical omphalitis. Common pathogens are *Trueperella* (formerly *Arcanobacterium*) *pyogene, E. coli, Salmonella* spp., *Streptococcus* spp.,

Staphylococcus spp. *Histophilus somni,* and others.
- For a definitive diagnosis, joint fluid must be placed immediately into laboratory media specially prepared for *Mycoplasma* spp. The failure to isolate the mycoplasma from the fluid of joints that have been affected for more than 14 days does not preclude a diagnosis of mycoplasma arthritis because the organism may have been eliminated from the joint.
- Other pathogens isolated from the ear of animals with otitis include mites, nematodes, *Mycoplasma* spp., and variety of bacteria.[17]

Differential clinical diagnosis for otitis in cattle:
- **Bovine parasitic otitis** caused by *Rhabditis bovis. Diagnosis is made by identifying the nematode in the secretion of the external ear*
- **Otitis externa caused by mites** such as *Raillietia auris.*
- **Otitis media/interna as a complication of respiratory tract disease** caused by pathogens such as *M. haemolytica, P. multocida, H. somni, Streptococcus* spp., and *Staphylococcus* spp.

TREATMENT

In general, treatment response to antimicrobial therapy is fair at best for respiratory tract disease and is particularly disappointing for mycoplasmal arthritis. Limited drug distribution into infected caseous lung tissue where *M. bovis* is present in largest numbers and the fibrin deposition and biofilm production that characterizes some *M. bovis* strains all contribute to resistance to antimicrobials in vivo that may contrast in vitro susceptibility.[2] Mycoplasmas have a theoretical susceptibility to antimicrobials that disrupt protein or DNA synthesis, such as tetracyclines, macrolides, florfenicol, and fluoroqinolones. In contrast they are inherently resistant to all β-lactam antibiotics because they lack a cell wall and to sulfonamides because they do not produce folic acid.

Although a large number of studies document the efficacy of different antimicrobials for treatment of cattle experimentally infected with *M. bovis,* little information is available about treatment efficacy under field conditions. Several antimicrobials, including tylosin, oxytetracycline, lincomycin, spectinomycine, and oleandomycin, have been used in the past to treat naturally occurring *M. bovis* infection and were reported to result in clinical improvement. However, because of the common resistance of *M. bovis* against most of these antimicrobials that has been documented in recent years, these substances can no longer be considered appropriate choices.[1,2]

The published results of in vitro antimicrobial testing of isolates of *M. bovis* recovered from various locations are highly variable. The antimicrobial susceptibility of *M. bovis* strains, cultured from cases of pneumonia, arthritis, and mastitis of cattle, measured in vitro indicate that enrofloxacin, florfenicol, and spectinomycin all exhibited good to excellent activity.[18] The in vitro susceptibilities of Belgian field isolates of *M. bovis* to 10 antimicrobials found that tiamulin was the most active against the organism. The fluoroquinolones, danofloxacin, enrofloxacin, and marbofloxacin were effective against strains of *M. bovis*, whereas gentamicin was ineffective. In a series of British isolates of *M. bovis,* most isolates were susceptible to danofloxacin but less susceptible to florfenicol. An industry sponsored study found tulathromycin and florfenicol at label dose to be effective for the treatment of respiratory disease in cattle caused among others by *M. bovis* under field conditions.[19] Gamithromycin at label dose was found to be effective in treating naturally occurring clinical pneumonia caused by *M. bovis.*[20]

In calves with a high incidence of respiratory disease associated with *M. bovis* and *Pasteurella* spp. the use of valnemulin in the milk of the calves for 4 days resulted in improved weight gains and fewer cases of mycoplasmal infection and required fewer treatments with antibiotics than those in the placebo treated group.

Little evidence to support recommendations for an **appropriate treatment duration** with antimicrobials is available in the literature. Common wisdom holds that early treatment and prolonged therapy are the two most important factors contributing to the success of treatment of mycoplasmal infection.[2] Given that *M. bovis* infection often becomes chronic, prolonged use of antimicrobials at least until clinical signs resolves appears warranted.[11] This implies that in many cases antimicrobials would have to be administered in an extra-label manner.

In addition to antimicrobial therapy short-term use of antiinflammatory drugs for management of pain and respiratory distress is certainly indicated in severe cases.

In case of early stage **septic arthritis** involving a single or few joints joint irrigation may be attempted. The objective of this procedure is to drain infected synovial fluid thereby reducing the number of bacteria and removing fibrin, debris, and other harmful products of inflammation. Joint lavage can be performed either by "tidal flush" through the same needle inserted into the infected joint or, preferably, by "through-and-through" lavage in which fluid is injected and drained through different 14-gauge needles that are placed into the joint as far apart as possible. Depending on the size of the joint, a minimum of 250 mL and up to several liters are needed for effective joint lavage.[21-22] Warmed polyionic solutions (e.g., lactated Ringer's or isotonic saline solution) are commonly flushed through the joint applying pressure until draining fluid becomes clear. Adding polyvidone-iodine to the irrigation solution to obtain a 0.01% to 0.1% solution has been recommended for its bactericidal effect.[23] Aseptic technique is required to prevent secondary bacterial infection. Because treatment efficacy is greatly impaired by fibrin accumulation in the joint, joint irrigation should mainly be considered in early stages of the disease. Arthroscopic joint debridement has been used to treat septic arthritis in cattle in other species, with good outcome. This approach allows to effectively remove fibrin and debride to synovial membrane but requires adequate surgical equipment and skills.[24] Arthrotomy is rarely done because it is a major surgery with long after care and often unrewarding outcome.

In cases of **otitis media** with ruptured tympanic membrane irrigation of the middle ear has been recommended. Irrigation solutions used include various apparently empirical dilutions of povidone iodine, hydrogen peroxide or chlorhexidine.[17] Perforating the still intact tympanic membrane (myringothomy) with a sharp object has been proposed to allow drainage and irrigation of the middle ear. The advantages and risks of this procedure do not appear to have been properly evaluated.

TREATMENT AND CONTROL

Treatment

Antimicrobial therapy

Tulathromycin (2.5 mg/kg SC as single dose)

Florfenicol (20 mg/kg q48 IM)

Tilmicosin (10 mg/kg SC as single dose)

Gamithromycin (6 mg/kg SC as single dose)

Enrofloxacin (2.5–5.0 mg/kg q24 SC)

Danofloxacin (6 mg/kg q48h SC)

Oxytetracycline (10 mg/kg IM q24)

β–lactam antibiotics (R-4)

Erythromycin (R-4)

Sulfonamides (R-4)

Antiinflammatory therapy

Flunixin meglumine (2.2 mg/kg IV as single dose) (R-2)

Ketoprofen (3 mg/kg IM q24h for 2–3 days) (R-2)

Carprofen (1.4 mg/kg SC/IV as single dose) (R-2)

Meloxicam (0.5 mg/kg SC/IV as single dose) (R-2)

Diclofenac (2.5 mg/kg IM as single dose) (R-2)

Tolfenamic acid (2 mg/kg IM/IV q24–48h or 4 mg/kg IM/IV as single dose) (R-2)

Metaphylaxis

Tulathromycin (2.5 mg/kg SC as single dose)

Florfenicol (40 mg/kg SC as single dose)

Continued

Tilmicosin (10 mg/kg SC as single dose)

Gamithromycin (6 mg/kg SC as single dose)

Oxytetracycline long-acting formulation (20 mg/kg IM as a single dose)

Enrofloxacin* (7.5–12.5 mg/kg SC as single dose)

Danofloxacin* (8 mg/kg SC as single dose)

Vaccination

Vaccination against *M. bovis* (R-3)

**These are classified as critically important antimicrobials in human and veterinary medicine. Use as first-line treatment is discouraged.*[25]

CONTROL

Biosecurity

Effective control consists in maintaining a closed herd or screen and quarantine newly purchased animals. Aggressive surveillance and culling of infected animals is advisable for herds with low infection prevalence. In high-prevalence herds and operations where maintaining closed herds or enforcing a quarantine is impractical, as is the case in feedlots, the focus of the control measures must be on limiting stress, controlling concomitant and potentially debilitating diseases such as bovine viral diarrhea (BVD) and segregating clinically affected animals from new arrivals.

Bioecurity and biocontainment procedures should be implemented to prevent the introduction of infection into the herd and to minimize the spread of infection in the herd.

Biosecurity measures applicable to **dairy herds** include the following:[11]

- Bulk-tank cultures from the herd of origin of newly purchased dry cows or heifers
  - Recur to bulk-tank culture history of the herd of origin or if unavailable.
  - Obtain cultures from at least 3 bulk tank samples collected at least 3 to 4 days apart.
- Individual milk samples of newly purchased lactating cows should be submitted for detection of mycoplasma (culture, PCR, C-ELISA) before introducing these animals into the herd. Be aware of low sensitivity of a single milk sample to detect subclinical infection.
- Test for *M. bovis* antibodies in milk or serum to identify infected animals.
- Screen calf health records from herd of origin of newly purchased animals for history of clinical signs consistent with *M. bovis* infection (e.g., polyarthritis or otitis) when available.

Procedures that have been recommended to control ***M. bovis* transmission in calves** include the following:[1]

- Avoid exposure of calves to milk/colostrum contaminated with *M. bovis.*
  - Avoid feeding milk/colostrum from infected cows.
  - Pasteurize milk/colostrum of cows of unknown status in infected herds before feeding to calves.
  - Feed milk replacer.
- Reduce airborne exposure to *M. bovis.*
  - Segregate calves with suspected or confirmed clinical *M. bovis* infection.
  - Prevent overcrowding.
  - Provide adequate ventilation.
  - Promptly treat calves with respiratory disease.
- Prevent fomite transmission.
  - Sanitize nipples, bottles, tube feeders, buckets, and so forth.
  - Wear gloves when feeding newborn calves and assisting sick calves to nurse, and change gloves between animals.
- Consider all-in, all-out procedure and sanitize pens between calves or separate younger from older calves to prevent disease transmission.
- Consider metaphylactic use of antimicrobials in situations with high morbidity/mortality rates.
- Use nonspecific measures to stimulate calf health.
  - Ensure adequate transfer of passive immunity.
  - Provide adequate nutrition.
  - Minimize stress.
  - Control other potentially debilitating pathogens (e.g., other pathogens of the respiratory tract or BVDV.

In dairy herds, pasteurization of mycoplasma mastitis milk at 65°C (149 F) for 1 hour can kill mycoplasmas and reduce the incidence of respiratory disease in calves. A temperature of 65°C (149 F) killed *M. bovis* and *M. californicum* after 2 minutes of exposure, whereas *M. canadense* remained viable for up to 10 minutes. Exposure to 70°C (158 F) inactivated *M. bovis* and *M. californicum* after 1 minute, but *M. canadense* samples were positive for up to 3 minutes.

Metaphylactic Use of Antimicrobials

Although the prophylactic/metaphylactic use of antimicrobials is undesirable from a standpoint of prudent use of antimicrobials, it is well established that treatment of mycoplasmal infection is most effective when initiated early in the course of disease.[11] Metaphylactic use of antimicrobials in animals at high risk of developing respiratory disease of undetermined etiology has clearly been demonstrated to reduce the incidence and severity of disease in feedlot cattle.[26-28] For the treatment of other mycoplasmal infection in cattle such as contagious bovine pleuropneumonia (CBPP), the metaphylactic treatment of in-contact animals was reported to significantly reduce disease transmission, severity scores in affected animals, and mortality rates within herds.[29] This suggests that metaphylactic treatment of mycoplasmal infection might be more successful than initiating treatment after disease is clinically apparent. Given limited evidence, available metaphylactic antimicrobial therapy of animals at high risk of developing *M. bovis* infection is probably justified in herds with high morbidity and mortality rates.[11]

Vaccines

Some vaccines have been developed, but they have not been sufficiently efficacious or have yielded poor results. A quadrivalent inactivated vaccine containing BRSV, PI-3 virus, and *M. dispar* and *M. bovis* provided some protection against naturally occurring outbreaks of bovine respiratory disease. A vaccine containing formalin-inactivated strains of *M. bovis* and *Mannheimia haemolytica* from affected herds reduced losses from pneumonia and the cost of treatment in newly arrived feedlot calves.

A single dose of vaccine for *M. bovis* pneumonia, inactivated with saponin, provided protection against experimental challenge of calves 3 to 4 weeks of age with a virulent isolate of *M. bovis.* The vaccine also reduced the spread of *M. bovis* to internal organs. Attempts to vaccinate against *M. bovis* arthritis have been unsuccessful. Experimental vaccines against mycoplasma vaccines have been unsuccessful and may even exacerbate the mastitis.

Currently there are several *M. bovis* bacterin vaccines that have been licensed in the United States for the control of *M. bovis* pneumonia in calves, and several U.S. companies are permitted to produce custom autogenous bacterin vaccines. No registered *M. bovis* vaccine is currently available in Europe. There is currently no convincing evidence documenting the efficacy of vaccines to control *M. bovis* infection under field conditions.[11]

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ENZOOTIC PNEUMONIA OF CALVES

SYNOPSIS

Etiology Bovine respiratory syncytial virus (BRSV), bovine coronavirus (BoCV), Parainfluenza-3 virus (PI-3); less frequently, bovine herpesvirus-1 (BHV-1; infectious bovine rhinotracheitis, IBR) and other viruses; *Mycoplasma bovis, Mycoplasma* spp.; secondary opportunistic bacterial infection with *Pasteurella multocida,* rarely with *Mannheimia haemolytica.*

Epidemiology Housed dairy calves under 3-5 months; high morbidity low mortality; veal calves; beef calves crowded in calving grounds; poor colostral immunoglobulin status; inadequately ventilated calf barns; excessive infection pressure on newborn calf because of close proximity to adult cattle in barns; economically important.

Signs Mild to severe dyspnea, fever, loud breath sounds over cranioventral lungs (consolidation), coughing, high morbidity; low case-fatality rate, secondary bacterial pneumonia with toxemia.

Clinical pathology Isolate pathogens from nasal swabs, transtracheal aspirates. Serology for seroconversion to viruses.

Differential diagnosis:
- Pneumonic pasteurellosis caused by *Mannheimia haemolytica*
- *Histophilus somni* pleuropneumonia
- Aspiration pneumonia
- Dyspnea of enzootic muscular dystrophy
- Chronic cases may resemble congenital cardiac defects

Treatment Antimicrobials to prevent and treat secondary bacterial pneumonia.

Control Ensure adequate colostral intake. Good housing and ventilation. Prevent crowding in calf barns. Raise dairy calves separate from adult cattle or in calf hutches.

ETIOLOGY

The term *enzootic pneumonia* should be considered more as a description than a diagnosis, similar to the term *shipping fever*. **Enzootic pneumonia** refers to clinical respiratory disease in dairy and veal calves raised in confinement under 6 months of age, whereas **shipping fever** refers to clinical respiratory disease in recently weaned beef calves shortly after transportation.

The cause of enzootic pneumonia is multifactorial, associated with various species of viruses, mycoplasma, bacteria, and environmental and host risk factors contributing to the pathogenesis, severity and nature of the pneumonia. Respiratory pathogens such as bovine respiratory syncytial virus (BRSV), bovine coronavirus (BoCV), and mycoplasma act as primary pathogens, and bacterial infection with *Pasteurella multocida* acts as a common secondary opportunistic complication. *Mannheimia hemolytica* is isolated from calves with enzootic pneumonia at a much lower rate than *P. multocida*.[1,2]

Bovine Respiratory Syncytial Virus

Bovine respiratory syncytial virus (BRSV) causes pneumonia in both dairy and beef cattle of all ages, but primarily in dairy calves under 6 months of age. BRSV isolates belong to an antigenic grouping different from that of Human Respiratory Syncytial Virus (HRSV), and distinct antigenic subgroups of BRSV exist.

Bovine Coronavirus

Bovine coronavirus (BoCV) is one of the more newly identified viral respiratory pathogens of cattle, being first described in 1993. As a consequence, the clinical significance of BoCV in bovine respiratory disease, and enzootic pneumonia in particular, is still being determined.

The current evidence indicates that BoCV plays a primary and important role in enzootic pneumonia. BoCV was the most commonly identified viral pathogen identified in nasal swabs from calves with respiratory disease in Ireland, being present in 23% of calves.[3] BoCV was identified throughout the year, but at a much lower rate in summer.[3] BoCV was the only virus detected in approximately 75% of respiratory disease outbreaks in two to 3-month calves in Italy.[4]

Parainfluenza-3 Paramyxovirus

The evidence for viruses as primary etiologic agents is based on virus isolation, serologic evidence of active infection, lesions of viral pneumonia, and experimental infection. The parainfluenza-3 (PI-3) virus has been isolated most commonly from affected calves and inoculation of the virus into colostrum-deprived calves results in a pneumonia that resembles the naturally occurring disease.

Mycoplasma bovis and *Mycoplasma* spp.

Mycoplasma bovis is a major cause of calf pneumonia, and in conjunction with BRSV and BoCV, is considered one of the three most important and common etiologic agents of calf pneumonia. In addition, *Mycoplasma dispar, M. bovirhinis, and Ureaplasma diversum,* are frequently isolated from the lungs of pneumonic calves. *Acholeplasma laidlawii* and *M. arginini* are also found but of dubious significance.

Mixed Viral and Other Pathogen Infections

A survey of viral infections of the respiratory tract of calves over a 3-year period revealed that BRSV, the PI-3 virus and bovine virus diarrhea virus (BVDV) were significantly associated with respiratory disease. Seroepidemiologic and clinical surveys of calves raised as herd replacements in dairy herds commonly reveals evidence of BRSV and PI-3 virus infections associated with respiratory disease. The rhinoviruses, adenoviruses, reoviruses, and enteroviruses were also isolated but in much lower frequency, and were considered not to be important. *Chlamydia* spp. has been associated with respiratory disease in calves and usually as part of a mixed infection with viruses and bacteria. A recent metagenomics study identified the presence of bovine adenovirus, bovine rhinitis A virus, and bovine influenza D virus either alone or in combination in 62% of calves with respiratory disease.[5]

Bacteria

P. multocida is frequently isolated from the lungs of calves with enzootic pneumonia. Because it is a commensal of the upper respiratory tract, *P. multocida* is **typically considered as an opportunistic pathogen** in calves with enzootic pneumonia.[6] *M. haemolytica* and *P. multocida* may both be recovered from the lungs of calves with pneumonia and may act synergistically with the *Mycoplasma* spp. to cause a more severe and fatal pneumonia; however, *M. haemolytica* is not commonly isolated from calves with enzootic pneumonia.[1,2] Several other bacterial species may also be recovered from pneumonic lungs, including *Histophilus somni* (formerly *Hemophilus somnus*), *Trueperella* (formerly *Arcanobacterium* or *Actinomyces or Corynebacterium*) *pyogenes, Fusobacterium* spp., *Streptococcus* spp., and *Staphylococci* spp., particularly from chronically infected animals.

Bibersteinia trahalosi (formerly *P. haemolytica* Biotype T) has been infrequently isolated from young dairy calves and adult cows with respiratory disease. It is currently uncertain whether *B. trahalosi* acts as a primary respiratory pathogen in bovine respiratory disease or plays a secondary and opportunistic role. Calves challenge exposed with a virulent isolate of *B. trahalosi* and previously vaccinated with a modified live leukotoxin positive *M. haemolytica* vaccine had less severe clinical signs of respiratory disease, lower mortality, and reduced lung lesions scores than unvaccinated calves.[7] In contrast, intratracheal inoculation of 2- to 3-month-old dairy calves with leukotoxin positive or negative strains of *B. trahalosi* failed to induce clinical signs of respiratory disease.[8] Interestingly, *B. trahalosi* inhibits the growth of *M. haemolytica* via a proximity-dependent mechanism,[9] leading to speculation that most of the pathology in field cases attributed to *B. trahalosi* pneumonia was actually attributable to *M. haemolytica* infection that has been overgrown. Additional studies are needed to clarify the role of *B. trahalosi* in bovine respiratory disease.

EPIDEMIOLOGY

Occurrence

Dairy Calves

Enzootic pneumonia occurs most commonly in housed dairy calves from 2 weeks to 5 months of age being raised as herd replacements. Pneumonia can be responsible for up to 30% of all deaths of calves in dairy herds from birth to 16 weeks of age, second to enteritis, which can account for 44% of all deaths. Some farms report many cases of pneumonia, whereas others have none, emphasizing the role that management and environment play in the incidence of enzootic pneumonia.

Pneumonia can be the single largest cause of death in veal calf farms. The calves are purchased at about 10 days of age, assembled into large groups of 25 to 50 per group and fed a milk substitute diet for about 16 weeks and then sent to slaughter. The peak incidence of disease occurs about 5 weeks after arrival in the calf house during which time PI-3 and BRSV are recovered most often.

Beef Calves

Enzootic pneumonia occurs in nursing beef calves and can account for significant reductions in weaning weight and a significant cause of economic loss as a result of disease in the neonatal period. In cow–calf herds in northwestern Quebec, one of the major causes of a low percentage of weaned calf-crop was the occurrence of diarrhea and pneumonia in calves under 2 weeks of age. Pneumonia can also occur after beef calves have been housed.

Morbidity and Case Fatality

Morbidity rate and case-fatality rates vary depending on the quality of housing and management provided, and the type and amount of viruses and bacteria that predominate in the environment at any one time. The morbidity rate may reach 100%, and the case-fatality rate is usually less than 5%.

On veal calf farms, pneumonia can be the largest single cause of death, with mortality rates up to 3.7% and culling rates at 5.1%. Peak death and cull losses occur during the 7th and 8th week of production.

In Ontario Holstein dairy herds, 15% of calves were treated for pneumonia before the age of weaning. Treatment rates for pneumonia increased slightly until about the 6th week of life and then declined until weaning. Calves that had pneumonia during the first 3 months of life had an increased risk of mortality before they reached calving age. In Holstein herds in New York, the crude incidence rate for respiratory disease within 90 days of birth was 7.4%. In those same herds, dullness of calves and unspecified diagnosis within 90 days of birth increased the hazard rate of death after 90 days of age 4.3-fold above that for heifers without dullness within 90 days of birth. These data indicate pneumonia in dairy calves in the first 3 months of age can have an adverse effect on long-term survival and subsequent growth rate.

Methods of Transmission

Aerosol infection and **direct contact** are the methods of transmission and both are accentuated in crowded, inadequately ventilated conditions. Newborn calves raised in individual pens may become infected within 5 to 15 days after an experimentally infected calf is placed in the calf house.

Risk Factors

Because most of the pathogens described under etiology can be found in the respiratory tract of normal calves, it has been generally accepted that environmental risk factors, such as ambient temperature, relative humidity, air quality, and population density, are necessary to precipitate the disease. In addition, several animal risk factors make calves susceptible to the pathogens in their environment. There are also pathogen risk factors that determine the disease outcome.

Animal Risk Factors

The onset of calf pneumonia occurs between 2 and 4 weeks of age when the concentration of serum IgG_1, IgG_2 and IgA in the nasal secretions are lowest. When the concentrations of serum IgG_2 begin to increase at about 2 to 4 months of age, the incidence of new cases of pneumonia begins to decline. The spectrum of colostral antibodies present in home-raised calves will depend on the spectrum of infection in the adult cows. In herds infected with BRSV, newborn calves acquire colostral antibodies to BRSV, which declines to undetectable levels in an average of about 100 days with a range of 30 to 200 days.

Most calves that recover from clinical enzootic pneumonia are resistant to further attacks of the disease associated with the same infectious agents. Herd immunity to one or more viruses develops, and severe outbreaks of disease usually occur following the introduction of animals that may be carriers of infectious agents to which the resident animals are nonimmune. In commercial veal calf units where market-purchased calves are being introduced on a regular basis, there is commonly a succession of minor epidemics of enzootic pneumonia. The incidence is highest in the recently introduced calves and the disease will occur in a small percentage of resident calves.

In a study of range beef calves from birth to 45 days of age, respiratory disease accounted for a total mortality of 1% and was associated with twins, which may result in a less viable calf at birth that may be neglected and abandoned. The risk of respiratory disease was also higher for male calves. The recent advancement of calving dates of beef cattle herds in the cold areas of North America from April–June to January–March results in crowded conditions in calving yards, which creates the environmental conditions similar to those of housed dairy calves. This has increased the incidence risk for enzootic pneumonia in beef calves.

Environmental and Management Risk Factors

Environmental risk factors, such as inadequate housing and ventilation are major contributors to the disease process. These include calving area, calf housing, spatial separation between calves, mixing calves of different age groups, and seasonal effects. Dairy herds that do not house calves in groups before weaning, or that house calves in groups of seven or fewer calves per group, are less likely to be affected with high mortality rate as a result of respiratory disease. The

calving area and environment can affect calf health through stress and the degree of exposure to infectious agents. Inadequate ventilation, improper climate control, and poorly constructed facilities can induce stress in calves. Crowding results in close contact and promotes spread of infection, and also results in excess moisture that, in the presence of inadequate ventilation (movement of air) and supplemental heat, causes a high relative humidity and chilling of calves. Many calf barns are old, adapted barns that are occupied for several months without depopulation and disinfection. Monitoring 48 dairy herds over 1 year in the National Animal Health Monitoring System revealed that mortality was lower in herds that used calf hutches compared with those that did not. In commercial veal units, the longer the disinfection and vacancy break, up to 6 to 7 days, the lower the incidence of disease in new calf crops entering the unit. Ventilation is commonly inadequate where dairy calves are raised because of poor design of the building.

Rapid changes in weather, particularly during the winter months, are often followed by outbreaks of acute pneumonia because of inadequate ventilation. A common practice during cold weather is to close the air inlets and turn off the ventilating fans in an attempt to maintain the inside temperature at a comfortable level. This results in increased relative humidity, condensation of moisture on walls and on the calves, leading to wet conditions, and the reduced ventilation results in an increase in the concentration of droplet infection. Attempts to correlate meteorologic data with the daily morbidity rate have not yet provided evidence for the hypothesis that climatic factors have an influence on incidence. This may be because of the difficulties associated with accurately monitoring meteorologic data, and the lack of a direct relationship between the environment outside a calf barn and the microclimate of the calf inside the barn. The disease appears to be most common during the winter months when calves are housed continuously and when ventilation is commonly inadequate.

Humid weather results in a marked increase in the percentage of bacterial colony-forming particles of less than 4 to 7μm in size. This provides the beginnings of a sound physical framework for the explanation of this and other, as yet empirical, relationships between the microenvironment in calf barns and the etiology and epidemiology of calf pneumonia.

The management risk factors that can influence the incidence rate and mortality of calves with pneumonia include the following:

- Colostrum feeding practices
- General feeding practices
- Quality of perinatal care provided by the personnel
- Age at weaning
- Use of prophylactic antimicrobials
- Health management of the dams.

The feeding of a coccidiostat to preweaned calves may be associated with an increase in the risk of enzootic pneumonia because herds with a history of disease would be more likely to feed a coccidiostat.

Factors associated with mortality to 21 days of life in dairy heifers in the United States include:

- First colostrum-feeding method, timing and volume
- Time of separation from dam
- Calving ease
- Twin birth.

Inadequate transfer of passive immunity has consistently been identified as a major risk factor for enzootic pneumonia.[10] Optimizing the feeding of colostrum, as summarized in Chapter 20, is an important method for decreasing morbidity and mortality associated with enzootic pneumonia. Up to 31% of mortality is associated with ineffective colostrum feeding. The longer the calf is left with the dam after birth, the greater the mortality, presumably as a result of greater exposure of the calf to pathogens harbored by the dam. Difficult calving also may interfere with the optimum ingestion of colostrum and absorption of immunoglobulins.

A path model of individual-calf risk factors for calfhood morbidity and mortality in New York Holstein herds indicated that management appeared to affect, directly and indirectly, the risk of respiratory disease within 90 days of birth. Being born in loose housing is strongly related to development of clinical signs of calf diarrhea within 14 days of birth, which in turn increases the risk of respiratory disease within 90 days of birth.

Calves reared as herd replacements may be born inside and raised indoors until they are about 6 months of age and then turned out to pasture for the summer. In the case of veal calf-rearing units, the calves are kept and fed indoors under intensive conditions from a few days of age until they reach 150 kg body weight (BW) at 12 weeks of age. In the barley-beef units, the calves are fed indoors on an intensive basis from weaning until they reach market weight at 10 to 12 months of age. In all of these situations, young, growing calves are raised together in confined conditions that promote the spread of respiratory disease associated with several viruses, *Mycoplasma* spp., and *Pasteurella* spp.

Based on serologic surveys, most calves raised in close confinement will have become infected by several viruses, including the BRSV, PI-3 virus, adenoviruses, BHV-1, and bovine viral diarrhea virus. If natural exposure to these viruses, *Mycoplasma* spp., and bacteria is so widespread and inevitable, it raises serious questions about the rationale for vaccination. In most cases the effects of the viruses and *Mycoplasma* spp. are minimal. The stress factors associated with inadequate ventilation, high relative humidity, chilling, and secondary bacterial complications are responsible for the onset of clinical disease.

Pathogen Risk Factors

The infectious agents are ubiquitous in the respiratory secretions of the animals and in their environment, and more numerous in crowded poorly ventilated conditions. The spectrum of infectious agents that are present and acting in a calf population and the severity of clinical disease will vary between farms, between countries, and from season to season. It has been assumed that older calves and mature animals in a herd are the source of infection for the young calves. This assumes major importance in control measures that are commonly designed to rear calves separate from older animals.

Bovine Respiratory Syncytial Virus

Infection with BRSV may be subclinical, mildly clinical, or highly fatal. Raising calves in close proximity to older cattle may result in constant exposure to infectious agents to which the mature animals are immune. The disease may be endemic on particular farms in which almost every calf experiences clinical disease. Herd epidemics may occur following the introduction of a different virus, such as BRSV, or following a breakdown in the ventilation system. The disease occurs specifically in nursing beef calves from 1 to 4 months of age while on pasture. Veal calves that are seronegative for BRSV on arrival to the veal unit were twice as likely to develop respiratory disease within the first 3 weeks compared with seropositive calves.[10]

Bovine Coronavirus

Information regarding pathogen risk factors is just starting to be identified. Veal calves that are seronegative for BoCV on arrival to the veal unit are 1.7 times as likely to develop respiratory disease within the first 3 weeks as are seropositive calves.[10]

Parainfluenza-3 Virus

This is commonly subclinical in a group of calves, and clinical disease may not occur until other pathogens are present or when adverse environmental conditions precipitate clinical disease. Following natural infection of young calves, the PI-3 virus may persist for several weeks. However, the presence of PI-3 infection may predispose to respiratory disease by interfering with normal pulmonary clearance mechanisms and allowing secondary invasion by bacteria or mycoplasmas. PI-3 decreases the phagocytic ability of alveolar macrophages, enhances the production of arachidonic acid signally cascade, a proinflammatory response, and enhances contraction of respiratory smooth muscle, resulting in bronchoconstriction.[11]

The number and types of ***Mycoplasma* spp**. that colonize the nose and trachea of calves are influenced by the age of calves and

not by the environmental temperature or relative humidity. *Mycoplasma* spp. start to colonize the upper respiratory tract of calves within days after birth, and the peak isolation rate from their nasal cavities occurs at about 2 to 6 weeks of age, and from the trachea at 6 to 8 weeks of age. Over 92% of calves collected from farms and reared in a controlled environment can harbor *Mycoplasma* spp. in their noses when they are 2 weeks of age. The rate of recovery falls gradually thereafter.

Mycoplasma dispar colonizes the respiratory tract of experimentally infected young calves for several months and can be isolated from nasal swabs and transtracheal samples throughout the period of colonization. *M. dispar* and *P. multocida* have been cultured from transtracheal aspirates of dairy calves with pneumonia under 3 months of age. In calves aged 1 to 5 months in calf-rearing farms that purchase calves from dairy farms, the prevalence and level of colonization of the respiratory tracts with *Mycoplasma* spp. can be more than 90% over a 2-year period. *M. dispar, M. bovirhinis,* and *Acholeplasma laidlawii* have all been isolated from such calves. A high degree of colonization with *M. dispar* among 1- to 2-month-old calves on these rearing farms indicates the ability of the pathogen to spread among the calves and colonize the respiratory tract. *M. dispar* is able to spread very rapidly among groups of calves, and airborne transmission is considered to be an important mode of transmission in addition to direct contact. The infection rate in the calves at the farms of origin is small.

Pasteurella multocida is a normal component of the upper respiratory tract of calves but is frequently isolated from the lungs of calves with enzootic pneumonia. It appears that isolation of *P. multocida* from pneumonic lung reflects the overwhelming of the calf immune system rather than a primary pathogenic response. Different strains of *P. multocida* exist in the upper respiratory tract of calves, particularly if calves come from different sources, and *P. multocida* infection can amplify the severity of lower respiratory tract disease in the presence of impaired defense mechanisms.[12] *P. multocida* is more frequently isolated from the nasal passages of dairy calves less than 10 weeks of age than similarly aged beef calves;[13] this is thought to reflect differences in housing and management and is consistent with the predominance of enzootic pneumonia cases in dairy calves.

Mixed Flora

Although a mixed flora of viruses, mycoplasma, and bacteria can be isolated from the respiratory tract of calves with pneumonia, and the unpassaged respiratory material can cause disease similar to the naturally occurring disease, the inoculation of pure cultures of *M. bovis, M. dispar,* and *Ureaplasma* spp., or pure cultures of BRSV, BoCV, or PI-3, into calves does not produce the severe clinical disease seen in the field. The failure of pure cultures of a pathogen to produce a severe pneumonia may be for one of three reasons:

- Combinations of organisms are required for disease
- Laboratory passage of the pathogens, necessary for purification causes their attenuation
- Material in the respiratory secretion other than the pathogens identified is required for disease, which may include agents that were not detected by routine culture techniques.

Economic Importance

The economic losses associated with enzootic pneumonia may be considerable. One estimate reports that the disease accounts for 50% of all calf mortality and a reduction of 7% in live weight gain. In commercial veal units, the presence of enzootic pneumonia may be associated with a prolonged time in the unit because of reduced daily weight gain.

The economic loss attributable to calfhood morbidity and mortality is well recognized by the dairy industry. However, the long-term effects of morbidity from diseases such as enzootic pneumonia on health and performance may constitute an even greater economic loss to the herd. Calfhood diseases occurring in the first 3 months of life may have serious long-term consequences. Heifer calves that are treated for pneumonia during the first 3 months of life are 2.5 times more likely to die after 90 days of age than heifers that are not treated for pneumonia. Heifer calves without respiratory disease are twice as likely to calve, and calved for the first time 6 months earlier, compared with calves with respiratory illness as calves. Some studies have found no significant independent association with calfhood disease status with first lactation milk production. However, the population selected did not include all heifers affected as calves; a heifer could have a suboptimal rate of growth or unthrifty appearance and would be removed from the herd before milk production was measured.

PATHOGENESIS

Viruses

The respiratory viruses can cause a viral interstitial pneumonia affecting the cranial lobes of the lung that may be subclinical, mildly clinical, or severe and highly fatal. The pathogenesis of BRSV and BHV-1 pneumonia is described elsewhere in this chapter. The pathogenesis of experimental respiratory bovine coronavirus infection has not been well described.

Parainfluenza-3

Subclinical viral pneumonia associated with the PI-3 virus uncomplicated by secondary bacterial invasion is usually of minor importance. In subclinical PI-3 infection in calves, seroconversion will occur, and at necropsy there are microscopic lesions consisting of bronchiolitis, bronchial and bronchiolar epithelial hyperplasia, alveolar epithelialization, and giant-cell syncytial formation. In the mild form there are slight clinical signs such as coughing and polypnea. In the severe form of viral pneumonia, such as in respiratory syncytial viral infection, there is severe dyspnea, with mouth breathing and an expiratory grunt, but a marked absence of toxemia compared with a bacterial pneumonia. Death can occur without secondary bacterial bronchopneumonia. Atelectasis and consolidation of the anterior lobes of the lungs are characteristic and account for the loud bronchial tones audible on auscultation over the anterior ventral aspect of thorax.

The experimental intranasal inoculation of the PI-3 virus into colostrum-deprived calves results in a pneumonia that is grossly and histologically similar to the naturally occurring disease. Within 2 to 4 days following infection there is bronchiolitis and bronchitis and cellular exudate in the bronchiolar lumina. These lesions become more severe and are accompanied by alveolar cell thickening and hyperplasia. Beginning at about 14 days following infection, there is healing of the bronchiolar and alveolar lesions. The bronchiolar exudate becomes organized by fibroblasts, and mononuclear cells predominate in the alveolar exudate. Bronchiolitis obliterans is widespread, but reepithelialization of damaged bronchiolar mucosa and alveoli occur.

Experimentally, the PI-3 virus can affect alveolar macrophages, which may impair the lung clearance mechanisms and allow *M. haemolytica* to produce a secondary bacterial bronchopneumonia. However, aerosols of PI-3 followed by *M. haemolytica* 7 days later do not necessarily result in significant pulmonary disease.

After the primary viral pneumonia is established, bacterial invasion may occur and the resulting pneumonia will vary with the species of bacteria that are present. Secondary bacterial pneumonias usually respond to treatment, although relapses are common if the viral pneumonia is extensive. Viruses are capable of reducing the resistance of mucous membranes, allowing bacteria such as pasteurellae to invade tissues. They are also capable of destroying the cilia on the bronchial mucosa that act as an escalator and help to keep the lower respiratory tract free of potential pathogens. In animals where there is an uncomplicated viral pneumonia with very extensive lesions, there may be minimal clinical signs and almost complete resolution.

Mycoplasma

The pathogenesis of *M. bovis* pneumonia is described elsewhere in this chapter.

The endobronchial or intratracheal inoculation of gnotobiotic calves with *Mycoplasma* spp. does not usually result in significant clinical disease. However, 2 or 3 weeks following inoculation, there is microscopic evidence of pneumonia. The lesions produced by experimental inoculation of calves with *M. bovis, M. dispar,* or *Ureaplasma* spp. are characterized by **peribronchiolar and perivascular "cuffing**," catarrhal bronchiolitis, and atelectasis. Intranasal inoculation of *Ureaplasma diversum* into SPF calves results in thick cuffs of round cells surrounding the bronchi, bronchioli, and blood vessels and a lobular catarrhal pneumonia. However, clinical signs of pneumonia are not observed. Inoculation of *M. canis* results in only a slight pathologic change that disappears 9 days after infection. *M. dispar* produces an alveolitis without cuffing lesions. It is thought that the *Mycoplasma* spp. are synergistic with each other, viruses, and bacteria in producing the lesions of subclinical and clinical enzootic pneumonia.

Bacteria

The pathogenesis of *P. multocida* and *M. haemolytica* pneumonia is described elsewhere in this chapter, and the pathogenesis of *Histophilus somni* pneumonia is described in Chapter 22.

CLINICAL FINDINGS

Regardless of the identity of the causative pathogen, the clinical findings in almost all enzootic pneumonias of calves are similar. In the **experimental viral pneumonia**, a febrile reaction occurs on about day 5 and is followed by the appearance of rhinitis, pneumonia, and mild diarrhea. The fever is only moderate (40–40.5°C [104–105°F]). A harsh, hacking cough, easily stimulated by pinching the trachea, is characteristic.

Clinical scoring systems have been developed to assist in the field diagnosis of respiratory disease in calves with enzootic pneumonia.[14,15] Factors considered of potential clinical utility, such as rectal temperature, presence and nature of nasal and ocular discharges, presence and nature of a cough, respiratory rate, and degree of depression, are assigned a whole integer score and the individual scores added to provide a summative score. Ultrasonographic examination of the cranioventral lung fields may provide helpful additional information.[16] Current scoring systems for dairy calves[14,15] have not been well validated,[16] and the use of a summative clinical score is statistically illegal, even when weights are assigned to measured factors. Despite these limitations, appropriately designed and validated clinical scoring systems show promise as providing a practical method for implementing effective treatments earlier to calves with respiratory disease.

In **naturally occurring cases**, the clinical findings are similar, although the fever is usually higher. This may be attributable to bacterial invasion in the early stages. The nasal discharge is only moderate in amount and is mucopurulent. On auscultation of the thorax the major abnormalities can be detected over the ventral aspects of the apical and cardiac lobes. The breath sounds are loud and harsh and represent breath sounds transmitted through consolidated lung. The intensity of the heart sounds is increased because of shrinkage of lung tissue in the cardiac area. The usual course ranges from 4 to 7 days. Some peracute cases of uncomplicated viral pneumonia die within 1 day after the onset of signs. Infections with the PI-3 virus generally cause mild respiratory disease characterized by coughing, nasal discharge, slight fever, and recovery in a few days.

M. bovis pneumonia in young calves is characterized by the sudden onset of severe dyspnea, fever, and rapid deterioration in spite of therapy.

In **BRSV pneumonia** there may be a sudden onset of acute pneumonia in 80% to 90% of a group of calves. The clinical findings are characteristic of a severe viral pneumonia. Affected calves are usually mentally alert, and there is only a mild fever. There is polypnea and dyspnea, which in a few days become worse, with mouth breathing and an expiratory grunt. Loud breath sounds, indicating consolidation, are audible over the anterior lobes of the lung. Squeaky, wheezing sounds as a result of the bronchiolitis are also commonly audible over the periphery of the consolidated areas. Loud, crackling sounds as a result of interstitial emphysema may also be audible over the dorsal aspects of the lungs. Death may occur in 2 to 4 days in spite of intensive therapy.

When secondary bacterial bronchopneumonia occurs, the fever, dyspnea, and toxemia are usually more severe. When secondary infection with *Pasteurella* spp. occurs, the temperature rises to 41° to 41.5°C (106-107°F), the area of lung affected is much increased, and loud harsh breath sounds as a result of edema are followed by crackles and a pleuritic friction rub. These cases usually respond rapidly to adequate treatment. When *Trueperella pyogenes* is the secondary invader, consolidation is marked, and there is a profound toxemia and loud breath sounds. In cases where *Fusobacterium necrophorum* is present, the clinical findings are similar, and pulmonary abscesses are likely to develop. The calf has lost a substantial amount of weight and stands with its neck stretched out in an attempt to decrease upper airway resistance (Fig. 12-15). Necrotic lesions are often present in the mouth and pharynx in these cases, and the pulmonary infection probably originates from here. With both of these latter infections there may be some response to antibiotic treatment, but there is a predisposition to relapse soon after treatment is terminated. Coughing, dyspnea, anorexia, and emaciation continue, and the animal eventually has to be destroyed.

CLINICAL PATHOLOGY

The etiologic cause of a case of bovine respiratory disease cannot be differentiated in most cases based on clinical examination in conjunction with consideration of history and signalment.[17] As a result, it is sometimes helpful to submit appropriately collected and transported samples to a laboratory for analysis. Detailed methods for primary respiratory pathogens are described elsewhere in the appropriate section of this book.

Acute-phase reactants, such as serum haptoglobin and haptoglobin-matrix metalloproteinase 9 (Hp-MMP 9), are biomarkers

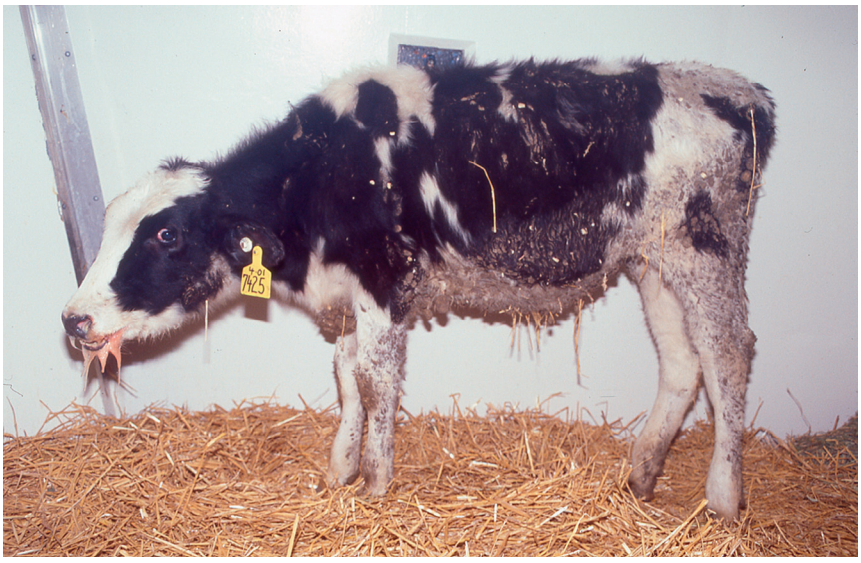

Fig. 12-15 **Holstein Friesian heifer with enzootic pneumonia. Notice the poor body condition, anxious look, conjunctivitis, nasal discharge, open-mouth breathing with blood-tinged foamy saliva, and extended neck in an attempt to facilitate breathing.**

of inflammation and may therefore indicate the presence or absence of active lung pathology in calves at risk of developing respiratory disease.[18] However, the clinical utility of serum biomarkers needs to be compared with tests that can be conducted rapidly and calf-side, such as rectal temperature, presence and nature of nasal and ocular discharges, or ultrasonographic examination of the cranioventral lung fields,[14,15,16] before they are widely adopted.

The oxygen tension (Po_2) in arterial (preferably) or venous blood is decreased in calves with respiratory disease, primarily as a result of ventilation-perfusion mismatch.[19,20] The partial pressure of oxygen in arterial blood (Pao_2) is negatively associated with the extent of lung lesions in calves with experimentally induced BRSV infection, with a 0.6% to 0.8% increase in the proportion of affected lung for every 1 mm Hg decrease in Pao_2 from the reference value.[20] As such, arterial Po_2 provides an excellent method for quantifying the proportion of diseased lung and monitoring the response to treatment.

Isolation of Pathogens

Nasopharyngeal swabs, transtracheal aspirates, and lung lavage samples may be taken for isolation of viruses, mycoplasmas, and bacteria, and the methods have been described in detail.[21,22] Special laboratory media are required to isolate *Mycoplasma* spp. Determination of drug sensitivity to the bacteria may be valuable, particularly when a number of calves are involved in an outbreak. The isolation of BRSV from natural infections is difficult because of the labile nature of the virus. The immunofluorescent antibody test for antigen detection is one of the most rapid, reliable, and sensitive tests for BRSV from tracheal aspirates, nasal swabs, and lung samples.

After experimental infection with PI-3, the median time to shedding is 1 day, the median time to peak shedding is 4 days, and the median time until shedding ceased is 10 days.[23]

Serology

Serologic tests have been more extensively used for confirmation of suspected BRSV infections. The standard serologic test is a virus-neutralization test using microtiter plates. Others include a modified indirect fluorescent antibody test, indirect hemagglutination, and an ELISA test, the latter of which is considered to be sensitive and specific and has the advantage of giving test results within several hours, whereas the virus-neutralization test requires 5 to 6 days for completion. The complement fixation test is less specific and less sensitive than the ELISA test.

NECROPSY FINDINGS

In uncomplicated viral pneumonia, irrespective of the specific cause, there are areas of atelectasis and emphysema in the apical and cardiac lobes, with little macroscopic involvement of the diaphragmatic lobes. In the later stages, a dark red consolidation featuring a hobnail appearance of the pleural surface affects most of the ventral portions of the apical and cardiac lobes. The lesions are always bilateral. Histologically, there is a bronchointerstitial pneumonia. Acute inflammation of the nasal mucosa, particularly on the turbinate and ethmoid bones, is usually accompanied by a marked, mucopurulent exudation. In PI-3 infection, intracytoplasmic inclusion bodies are widespread in the lungs; after experimental infection, they are present on day 5, but they have disappeared by day 7 after infection.

In respiratory syncytial viral pneumonia there is severe interstitial pneumonia and interstitial emphysema. Histopathologically, there is severe bronchiolitis, alveolitis with multinucleated syncytia (which often contain eosinophilic intracytoplasmic inclusion bodies), and alveolar epithelial cell hyperplasia.

When bacterial or mycoplasmal invasion has occurred, the lesions vary with the agent present. Extensive hepatization with mottled red and gray lobules and considerable interlobular aggregations of serofibrinous fluid, often accompanied by a fibrinous pleuritis, is characteristic of *P. multocida* infection. Extensive consolidation and suppuration occur with *T. pyogenes* and *F. necrophorum* infections. In the latter case there may be necrotic lesions in the mouth and upper respiratory tract.

Confirmation of this diagnosis at necropsy is somewhat awkward because the population of pathogens responsible may change between the time of disease onset and the death of the calf. In severe outbreaks it may be necessary to euthanize animals early in the course of the disease or to perform serologic surveys for respiratory pathogens among surviving herdmates.

Samples for Confirmation of Diagnosis

- **Histology**—lung (several sections), trachea, turbinate (LM, IHC)
- **Virology**—lung (several sections), trachea (FAT, ISO)
- **Mycoplasmology**—lung (MCULT, FAT)
- **Bacteriology**—lung (CULT)

DIFFERENTIAL DIAGNOSIS

Clinically, the diagnosis of pneumonia is usually readily obvious, but the causative agents are usually not determined. Young calves raised indoors and affected with a cough, nasal discharge, and pneumonia are usually affected with enzootic pneumonia associated with the agents described under etiology. The common diseases of the respiratory tract of young calves that may resemble enzootic pneumonia include the following:

- Bacterial pneumonia caused by *M. haemolytica* or *H. somni* in young calves is characterized by severe toxemia, fever, dyspnea, grunting, and a poor response to therapy.
- *M. bovis* pneumonia is characterized by sudden onset of dyspnea, fever, depression, and poor response to therapy in a group of calves.
- Calf diphtheria usually affects a single calf and is characterized by inspiratory dyspnea, stridor, toxemia, fever, and obvious lesions of the larynx.
- Lungworm pneumonia occurs in young calves at pasture, and marked dyspnea, coughing, and a few deaths are characteristic. A fever is common in lungworm pneumonia, and there are loud breath sounds over the ventral aspects of the lungs, and loud and moist crackles over the dorsal aspects.
- Acute myocardial dystrophy in young calves, following turnout on pasture, is characterized by sudden onset of weakness, polypnea and dyspnea as a result of pulmonary edema and lesions of the diaphragm, tachycardia and arrhythmia, and skeletal muscular weakness.
- Aspiration pneumonia occurs occasionally in calves that have been force-fed colostrum or milk. There is a sudden onset of marked dyspnea, anxiety, and distress, and death may occur within a few minutes. However, some calves survive, and there is marked dyspnea with abdominal breathing and loud breath sounds and crackles over the dorsal and ventral aspects of both lungs. Some calves will recover completely in a few days.
- BRSV interstitial pneumonia in weaned beef calves must be differentiated from pneumonic pasteurellosis. In BRSV pneumonia there is a sudden onset of marked dyspnea; fever; anxiety, but not toxemia; mouth breathing in advanced cases; loud breath sounds and wheezes over both lung fields, especially over the ventral aspects; and subcutaneous emphysema. Several animals are usually involved. Affected animals fail to respond to treatment with antimicrobials, and the case-fatality rate is usually over 75%. There may be a history of mild respiratory disease in the affected group about 10 days previously. In pasteurellosis, depression, toxemia, fever, loud breath sounds over the ventral aspects of the lungs, and a favorable response to treatment are characteristic.
- Chronic enzootic pneumonia is characterized by bronchiectasis and pulmonary abscessation, causing unthriftiness and a poor response to therapy.

TREATMENT

Antimicrobial Therapy

Uncomplicated enzootic pneumonia associated with mycoplasma or viruses is unlikely to respond to treatment, but antimicrobial therapy daily for 3 days is indicated because of the high probability of secondary bacterial pneumonia. Any of the antimicrobials used commonly for the treatment of acute undifferentiated bovine respiratory disease (shipping fever) are effective, with a preference for antimicrobials that are effective against *Mycoplasma* spp. and *P. multocida, H. somni,* and *M. haemolytica* These are described in detail in the section on pasteurellosis earlier in the chapter.

Early treatment is necessary to avoid the development of incurable secondary complications, such as pulmonary abscesses, pleuritis, bronchiectasis, and suppurative pneumonia. In commercial veal calf units, the case-fatality rate can be kept to a low level by early and adequate treatment. In some cases it may be sufficient to treat animals once only, but a proportion of cases are likely to relapse after an initial response. Such cases require repeated daily therapy for 3 to 5 days. If the number of relapses in an area or on a farm is excessive, all cases should receive multiple treatments.

Adjunctive Therapy

Bronchodilators and NSAIDs as adjunctive therapy for enzootic pneumonia in calves are used, but their efficacy is questionable.

Correction of Adverse Environmental Conditions

The clinical management of an outbreak of enzootic pneumonia in calves must include correction of adverse environmental conditions that may have precipitated the disease.

CONTROL

Environmental and Management Practices

Control of the disease in housed calves is dependent on effective animal and environmental management. Overcrowding, drafty or inadequately ventilated housing, exposure to inclement weather, and sudden changes in environmental temperatures are major risk factors. Recently purchased calves should be isolated for several weeks before being introduced to the group.

Ideal Environmental Conditions

Control is especially difficult and expensive in countries where the calves are housed for several months during the winter months in northern climates. The most comfortable ambient temperature for young calves ranges from 13° to 21°C (55–70°F) with a relative humidity of 70%. To achieve these environmental conditions requires a suitable insulation material in the walls and ceilings, ample bedding to absorb moisture from feces and urine, and adequate movement of air to remove aerosol particles that may be infectious. This requires an adequate air inlet and outlet system, adequate capacity fans, and supplemental heat during very cold periods. The installation of recirculating air filter units can lead to a substantial reduction in the concentration of airborne bacteria to which calves are exposed. Field studies in veal calf units indicate that mean aerial bacteria concentration in filtered barns can be reduced by 45%, the number of calves requiring treatment reduced by 19%, the number of repeat courses of treatment and the total antibiotic usage reduced by 29% and 35%, respectively. At slaughter, the average area of lung consolidation in calves from filtered barns can be reduced by 35%. In general, air filtration can result in a reduction in both the incidence and severity of clinical and subclinical pneumonia in calves and in improved weight gain.

In spite of ideal hygiene and management it may not be possible to prevent the development of new cases if the infection already exists in a herd, or if cattle from other herds are moved into the herd. At present, it is feasible only to be vigilant and treat new cases urgently and vigorously because a strict hygiene program may not be feasible in the average commercial herd. If management is inadequate and the general resistance of the animals is low, losses resulting from calf pneumonia with significant bacterial or mycoplasmal invasion can be sufficient to make calf-rearing unprofitable.

Calf Barns or Hutches

Where economics permit, the ideal situation is to construct a calf barn completely removed from the main adult cow barn to minimize the spread of infection from adults that may be symptomless carriers. After the colostral feeding period, calves are removed from the calving barn and placed in individual pens in the calf barn. The raising of young calves outdoors in calf "hutches" or "igloos" is highly satisfactory and economical, even in countries where the outside temperatures go well below freezing. With adequate bedding, protection from the prevailing winds and adequate nutrition, calves will grow satisfactorily. Dairy herds that have had difficulty controlling enzootic pneumonia of calves have found this system to be an excellent alternative to the construction of a standalone, well-ventilated calf house. Nutritional deficiencies, usually of energy and protein, are common in young calves and often accentuate the severity of the pneumonia. Young calves should receive a balanced calf starter grain ration supplemented with essential vitamins and minerals and good-quality hay beginning by at least 3 weeks of age.

Vaccines and Immunization

There is **insufficient information** available from field trials to make firm recommendations for the use of vaccines for the control of enzootic pneumonia in calves. It is difficult to evaluate the results of vaccination trials because investigators use so many combinations of vaccines and different vaccination schedules, and there are many different management variables and differences in methods of evaluation. In addition, many vaccination trials are not randomized controlled trials. Recommendations regarding vaccination protocols for BRSV, BHV-1, *M. haemolytica, P. multocida,* and *M. bovis* are presented elsewhere in this chapter; recommendations for a BVDV vaccination protocol are presented in Chapter 9, and recommendations for an *H. somni* vaccination protocol are presented in Chapter 22.

Any successful vaccine would have to be multivalent and would have to be effective when given before 2 months of age or earlier in the "**window of susceptibility**" to coincide with the decline in passive (maternal) immunity, the increase in active immunity, and the occurrence of enzootic pneumonia in calves (Fig. 12-16).

There is good field evidence that the colostral immunologic status of the calf has a significant effect on the susceptibility of the calf to pneumonia. There is a clear association between low levels of IgG_1, IgG_2 and IgA of calves at 2 to 3 weeks of age, and subsequent susceptibility to pneumonia at 2 to 3 months of age. Calves with signs of pneumonia had low levels of IgG_1 compared with nonpneumonic calves that had relatively higher levels. In addition, calves with high levels of serum immunoglobulin do not respond normally to vaccine and any vaccine for enzootic pneumonia would have to be administered during this relatively refractory period. However, for veal calves, which are purchased at a few days of age and with low levels of immunoglobulin, this may not be a problem. A 2015 meta-analysis concluded that in natural exposure trials, 2-week-old to 4-month-old dairy calves vaccinated with commercially available vaccines against BHV-1, BVDV, BRSV, and PI-3 for protection against respiratory disease had **no reduction in morbidity or mortality** compared with age-matched unvaccinated controls.[24]

Vaccines currently available for BoCV are only licensed to control neonatal diarrhea in calves. Their efficacy in controlling respiratory disease is unknown but is likely to be poor. This is because respiratory isolates belong to subclades 2a, 2b, and 2c, whereas enteric isolates and a vaccine strain for control of enteric disease in calves belong to the antigenically different clade 1.[25]

The intranasal inoculation of calves with virulent or a modified strain of PI-3 virus stimulates the development of both serum antibody and nasal secretion antibody. The nasal secretion antibody is dose dependent. Challenge exposure of these calves provides protection against clinical disease. These factors should be considered in the development and administration of PI-3 viral

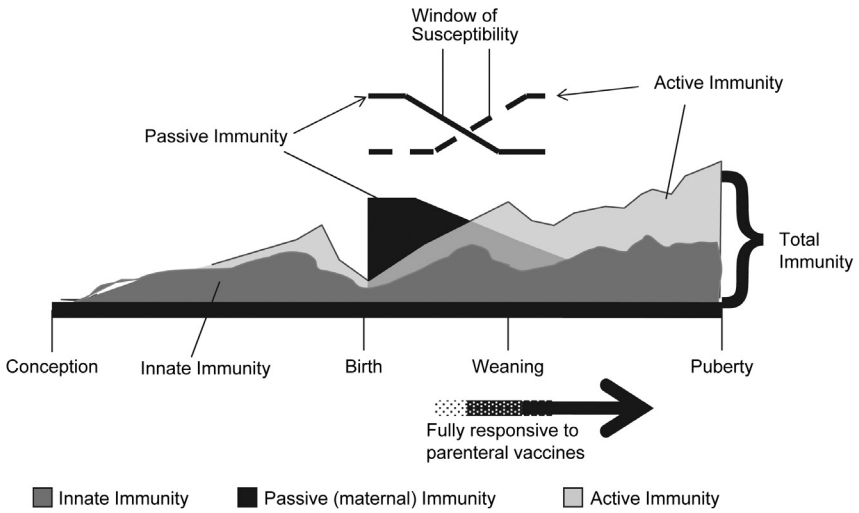

Fig. 12-16 Development of the immune response in the calf from conception to puberty. The window of susceptible is the optimal time for vaccination against enzootic pneumonia and represents the time during which passive (maternal) immunity is waning and active immunity is increasing. (Reproduced, with permission, from Chase CCL, Hurley DJ, Reber AJ. Neonatal immune development in the calf and its impact on vaccine response. *Vet Clin Food Anim* 2008; 24:87-104.)

vaccines if the objective is to establish an optimal concentration of antibody in the nasal secretion. The parenteral administration of two sequential doses, 2 weeks apart, of an inactivated PI-3 virus vaccine with adjuvant will induce high levels of serum antibody and prevent virus excretion in nasopharyngeal secretions after challenge. Successful immunization of calves against PI-3 infection may be useful for protection against pneumonic pasteurellosis if PI-3 precedes the bacterial infection. This is presented in greater detail in the section on pneumonic pasteurellosis. A single dose of an experimental vaccine for *M. bovis* pneumonia, inactivated with saponin, given subcutaneously to 3- to 4-week-old calves followed by experimental challenge 3 weeks later with a virulent strain of *M. bovis* provided protection against clinical pneumonia. Unvaccinated calves developed clinical signs of disease as a result of lung lesions. The vaccine also reduced the spread of *M. bovis* to internal organs. Calves tested 6 months after immunization had high levels of humoral immunity. The successful use of saponin in vaccines has been demonstrated for other mycoplasma infections. such as contagious caprine pleuropneumonia (CCPP) and contagious agalactia. The saponin may preserve the major antigens seen in untreated whole cells.

TREATMENT AND CONTROL

Treatment

Antimicrobial treatment for animals to address concurrent bacterial and *Mycoplasma bovis* pneumonia (see treatment recommendations for *Pasteurella multocida* and *Mannheimia hemolytica* in this chapter, with a preference for antimicrobials that are also effective against mycoplasma organisms) (R-1)

Anti-inflammatory treatment (see treatment recommendations for *Pasteurella multocida* and *Mannheimia hemolytica* in this chapter)

Non-steroidal anti-inflammatory agents (R-2)

Corticosteroids (R-3)

Control

Optimize transfer of passive immunity via colostrum. (R-1)

House in a well ventilated area, preferably in individual calf hutches before weaning, (R-1)

Vaccination of dairy calves less than 6 months of age with killed BRSV vaccine. (R-1)

Vaccination of dairy calves less than 6 months of age with combined modified live or inactivated vaccine against BHV-1, BVDV, BRSV, and PI-3. (R-3)

Vaccination of dairy calves less than 6 months of age with BoCV vaccine. (R-3)

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BOVINE RESPIRATORY SYNCYTIAL VIRUS

SYNOPSIS

Etiology Bovine respiratory syncytial virus (BRSV); subtypes A, B, AB, and untyped.

Epidemiology Prevalence of infection high; disease most common in calves under 6 months of age but adult cattle also affected; recurrent infections and disease in herds common; persistent infection in few seropositive cows. Immunity following natural infection or vaccination short-lived. Antibodies following natural exposure are different than those following experimental infection or vaccination. Maternal antibody does not prevent infection, but high levels decrease severity of clinical disease.

Signs Mild, moderate, or severe dyspnea; fever, agalactia, coughing, wheezes of lungs; most animals recover; small percentage develop severe fatal viral interstitial or bacterial pneumonia. Outbreaks occur in cattle under 6 months of age and also in adult cattle.

Clinical pathology Difficult to isolate or detect virus in tissues. Immunohistochemical tests of nasopharyngeal swabs and lung tissue. Serology.

Differential diagnosis Bacterial pneumonia. Other viral interstitial pneumonias. Infectious bovine rhinotracheitis. Lungworm pneumonia.

Treatment Nothing specific.

Control Minimize stressors. Control by natural exposure and treat secondary bacterial pneumonia. Modified live-virus and inactivated virus vaccines available but efficacy uncertain because lack of field trials.

ETIOLOGY

Bovine respiratory syncytial virus (BRSV) is a cause of a viral pneumonia primarily in calves under 6 months of age and also in yearlings and adult cattle. BRSV is a pneumovirus in the family Paramyxoviridae. Antigenic and genetic subtypes have been identified. Using monoclonal antibodies against a glycoprotein (G protein on the surface of the virus), four subgroups have been identified: A, B, AB, and untyped. There are six genetic subgroups based on G and five subgroups based on F (a fusion protein on the surface of the virus that causes fusion of cell membranes with resultant formation of syncytia) or N (nucleoprotein).[1] The evolution of BRSV into subtypes may have been driven, in part, by selection pressure applied by vaccination.

BRSV is genetically and antigenically related to human respiratory syncytial virus (HRSV). Other pneumoviruses include ovine respiratory syncytial virus (ORSV) and caprine respiratory syncytial virus (CRSV), with BRSV being most closely related to CRSV. BRSV provides a good model for HRSV, and consequently many studies related to identifying virulence factors and understanding the immunopathogenicity of BRSV infection have been conducted.

EPIDEMIOLOGY

BRSV was first reported in Europe in 1970 and was subsequently reported in the United States in 1974. Distribution is currently worldwide. A case could be made that BRSV is the most important viral cause of respiratory disease in calves less than 6 months of age and in adult dairy cattle.[2] BRSV is a rare cause of respiratory disease in adult beef cows.[3]

Occurrence

Prevalence of Infection

The virus is ubiquitous in the cattle population and new infections occur most commonly in autumn and winter annually and may result in severe respiratory disease. In longitudinal studies in dairy herds, 90% of primary infections occur in calves and heifers; very few occur in cattle over 2 years of age, and all cattle in the herds are seropositive when they are over 3 years of age. Recurrent infections occurring annually at the same time, and in cows of all ages, without new introductions into the herd, are characteristic of BRSV infections in a herd. The virus appears to circulate during summer at very low levels or not at all, with almost all isolations coming from winter and spring.[4] Persistent BRSV infection in some of the cows in a herd might be a means for the virus to survive during the summer, but a steady state of reinfection of seropositive cows throughout the year at a low level might also maintain a reservoir of infection. Monthly data on the prevalence of BRSV antibodies in dairy herds suggest that persistent infection in seropositive cows is more likely than population persistence.

When the prevalence of infection in the cattle population is high, the incidence of clinical disease is much lower.[2] It can be assumed that most mature cattle have been exposed to the virus, but unexposed cattle are susceptible to developing clinical disease after infection. Surveys in the United States, England, Denmark, Sweden, and France found seropositive rates in herds ranging from 50% to 80%. Cattle entering feedlots may seroconvert to the virus, which may be associated with an increased risk to subsequent treatment for respiratory disease. A high percentage of young beef bulls aged about 6 months and entering performance test stations may seroconvert to BRSV and adenovirus, both of which may be associated with clinical respiratory disease.

Occurrence of Clinical Disease

In general, clinical infection is most common in calves under 6 months of age and some BRSV infections are undoubtedly associated with enzootic pneumonia of housed dairy calves. In **dairy herds**, recent introductions of young cattle purchased from public saleyards may introduce the infection to home-farm cattle that have had no previous exposure to the viruses or those in which their immunity to a previous infection with the virus has declined. Thus adult dairy cows may be affected with a highly fatal pneumonia attributable to the virus. A high prevalence of infection exists in Swedish cattle, and annual outbreaks of disease have occurred in adult cattle, with pregnant or recently calved cows being most severely affected. Outbreaks have occurred in beef cattle on pasture. The disease occurs in **nursing beef calves** 1 to 8 months of age on pasture with their dams without any history of previous stress. A common occurrence is in **weaned beef calves** 6 to 8 months of age within 2 to 3 weeks following weaning and commingling in confinement. Yearling cattle in feedlots are also susceptible.

In North America, herd epidemics of clinical disease usually occur during the **fall** and **winter months.** Nursing beef calves may be affected with clinical disease during the summer months. Some outbreaks have occurred in nursing beef calves between 1 and 2 months of age while they are still in nursery pastures or in the calving areas.

A spontaneous outbreak of respiratory disease in goats attributable to BRSV has been described, and sheep can be infected with the virus.

Morbidity and Case Fatality

The morbidity rate in herd epidemics of clinical disease can vary from 30% to 50% or higher. The case-fatality rate is usually low, 3% to 5%, but may be higher.

Methods of Transmission

The mode of transmission has not been defined, but aerosol infection and direct contact are probable. Infection spreads rapidly among susceptible cattle.

Risk Factors

Animal Risk Factors

Naturally occurring BRSV infection affects both dairy and beef cattle, and those under 6 to 10 months of age are most susceptible to clinical disease. Nursing beef calves with colostral BRSV antibody are not protected from infection, but the incidence and severity of clinical disease is inversely related to the level of maternal antibodies in calves younger than 3 months. The highest percentage of reinfections occurs most commonly in cows during their first lactation. Older animals may have a more effective immunity because of previous exposure.

Seroepidemiologic surveys in **feedlot cattle** found that seroconversion to the virus may occur in up to 70% of animals within 1 month after arrival. Animals with low titers to the virus on arrival are at increased risk of subsequent treatment for respiratory disease, which suggests that the virus may be a factor in bovine respiratory disease. In some situations in feedlot cattle, high BRSV serum antibody levels on arrival were related to a lower risk of respiratory disease.

Environmental Risk Factors

The highest incidence of clinical disease occurs in autumn and winter months. Outbreaks have been associated with changes in weather, especially declining ambient temperatures and atmospheric pressure.

Pathogen Risk Factors

BRSV has a narrow host range, affecting primarily cattle. Important antigenic differences between BRSV isolates have been described. Subgroups A and AB are associated with severe respiratory disease and circulate in the Dutch cattle population. In natural outbreaks of infection in closed dairy cattle herds and veal calf units in Denmark, using DNA sequence data, identical viruses were isolated within a herd during outbreaks, but viruses from recurrent infections varied by up to 11% even in closed herds. It is possible that a quasispecies variant swarm of BRSV persisted in some of the calves in each herd and that a new and different highly fit virus type (master and consensus sequence) became dominant and spread from a single animal in connection with each outbreak.

Antigenic subtypes may have relevance both in explaining differences in virulence between subtypes and in the development of new vaccines for the control of clinical disease. The production and characterization of monoclonal antibodies to a vaccine strain of BRSV has been described. The respiratory syncytial virus of goats and sheep, caprine respiratory syncytial virus, and ovine respiratory syncytial virus are antigenically related, but not identical, to BRSV.

The BRSV may act synergistically with a concurrent experimental challenge of the virus and 3-methylindole to produce more severe pulmonary disease similar to BRSV pneumonia seen in feedlot cattle, than either agent alone. But vaccination of cattle with BRSV vaccines does not protect the potential synergism between the 3-MI and BRSV infection.

Whether or not the virus persists in individual animals in spite of the presence of maternal or naturally acquired antibodies has been a major question. Serologic findings indicate persistence of the virus, but the virus could not be detected in lung lavage fluid or nasal swabs. Experimentally, the virus persisted in tracheobronchial and mediastinal lymph nodes for up to 71 days after infection. In vitro, the virus was still able to replicate in bovine B-lymphocyte cell lines 6 months after infection. This may explain the absence of the virus between epidemics, recurrent infections in the same individuals and inapparent reinfection of adults.

Immune Mechanisms

After a natural BRSV infection, the protection is short-lived and multiple reinfections are common. In endemic areas, the absence of BRSV-associated disease in adult cattle is possibly as a result of repeated infections. This places a constraint on vaccine development because one or two vaccinations would have to induce immunity that only repeated natural infections can provide. BRSV infections can occur in the presence of high to moderate levels of maternal antibodies. Maternal antibodies, which are directed against the F, G, and N proteins of BRSV, are commonly present in calves but do not protect against infection. However, the incidence and severity are inversely related to the level of specific maternal antibody, and natural infection does not prevent reinfection but appears to offer good protection against clinical disease after infection. Primary BRSV infections in calves less than 1 month of age are less severe than those in calves 2 to 4 months of age, probably as a result of decreased proinflammatory TNF-α production in calves less than 1 month of age.[5]

The BRSV colostral antibody of dairy calves varies dependent on season of the year when the calves are born. Dairy calves born during the winter months in the Netherlands have lower BRSV colostral antibody titers than those born during the summer months. Whether this may be attributable to the seasonal periodicity of BRSV circulation or to other factors influencing antibody development or colostrum intake is uncertain. Calves born in the summer have higher antibody titers at 14 to 19 weeks of age, most likely attributable to BRSV exposure. Calves born during the season of infection may be primed with BRSV field virus during the period of maternally derived immunity and may be better protected against disease by cellular immunity during the next season of infection.

IgM and IgA are the predominant antibody isotypes in the respiratory tract after BRSV infection, with IgA especially prominent after reinfection. Both serum antibody responses and local antibody responses are suppressed by maternal antibodies. After natural BRSV infection of cattle, antibodies are predominantly directed against the epitope A, whereas after experimental infection, or vaccination with an inactivated vaccine, antibodies against epitope B and nonneutralizing epitope C are markedly increased compared with the same epitopes in naturally infected cattle.

The subgroups of the virus are based on antigenic differences of the G protein, and BRSV infection protects against reinfection by homologous strains of the virus. It is also known that a complete BRSV can partially protect against a BRSV infection with a strain that contains an antigenic dissimilar G protein. Therefore incorporation of representative viruses of different BRSV subgroups in vaccines for cattle does not seem necessary to achieve cross-protection. Vaccination of calves with a formalin-inactivated BRSV vaccine followed by challenge exposure to virulent virus increased the severity of clinical disease and lesions compared with calves nonvaccinated and challenged. Vaccination did not induce neutralizing antibodies, but IgG antibodies were detected with ELISA. Immunization with formalin-inactivated BRSV vaccine mainly primes a Th2-like inflammatory response characterized by a significant eosinophilic influx in the bronchia alveolar lung field and lung tissues and high levels of immunoglobulin E serum antibodies.

PATHOGENESIS

BRSV causes rhinitis, tracheitis, bronchitis, bronchiolitis, and mild interstitial pneumonia. In naturally occurring cases, the main lesions are bronchitis and bronchiolitis in the cranioventral portions of the lungs combined with widespread emphysema and edema throughout the lungs. BRSV infection causes airway obstruction and hyperactivity that may persist for up to 30 days following viral exposure. In naturally occurring cases, the cranioventral lung fields are particularly poorly ventilated, and there is arterial hypoxemia associated with mismatching of ventilation and perfusion. Radiographic and radionuclide lung perfusion imaging reveals the presence of bullous emphysema and areas of marked atelectasis.

The pathogenesis of acute fatal pneumonia as a result of BRSV is not clear. The characteristic lesions are exudative or necrotizing bronchiolitis, atelectasis, interstitial edema, and emphysema. The acute fatal disease is commonly preceded by a mild respiratory disease several days previously, which suggests that hypersensitivity may be a pathogenic mechanism causing lung injury. The second stage may follow initial improvement or recovery from the first stage and is associated with the onset of extreme respiratory distress. The virus-specific IgE antibody may play a role in the pathogenesis of the severe disease as part of a hypersensitivity reaction. The IgM and IgA antibodies are not involved in a hypersensitivity reaction. In experimentally induced infection in calves, there is considerable injury to bronchiolar epithelium including hypertrophy, hyperplasia, and formation of syncytia, which facilitates movement of virus between cells.[1] In the alveoli, BRSV infection results in necrosis of type I pneumocytes; the response of type II pneumocytes includes hypertrophy, hyperplasia, and syncytial formation. It is suggested that an immune-mediated mechanism may be responsible for the widespread lesions over the entire lung.

The severe, highly fatal form of the disease, also known as the "malignant" form, or **paroxystic respiratory distress syndrome** (PRDS), is associated with extensive pulmonary mast cell degranulation. In a series of naturally occurring paroxystic respiratory disease in calves, paired serum samples were taken 3 weeks apart and lungs examined at necropsy. The serum concentration of tryptase was used as a marker of mast cell degranulation. Tryptase is a preformed serine protease stored in mast cell granules and causes significant changes in the respiratory tract smooth muscle tone and vascular permeability. The substances released by the mast cells are at least partially responsible for the pulmonary edema, in particular by means of venoconstriction and the increase in the vascular permeability induced by the histamine. The edema and bronchoconstriction caused by the mast cell leukotrienes impede bronchiolar flow, which causes ventilatory asynchronism. The mechanical constraints caused by the asynchronism are aggravated because the bovine lungs consist of a number of compartments, which prevents any collateral ventilation and any dissipation of interlobular pressure gradients. The breaking point is reached when the level of the mechanical constraints exceeds the level of tissue resistance, causing interstitial emphysema.

Calves that die of the BRSV-associated PRDS have a uniform pattern of gross

lesions. The trachea and bronchi are filled with a white-to-pink froth, and the lungs are heavy and voluminous and fail to collapse. The most characteristic lesions were the dramatic lung distension by edema, alveolar hyperinflation, and severe interstitial and subpleural emphysema, often with large dissecting bullae on the dorsal edge of the diaphragmatic lobes.

Microscopically, the most characteristic lesions are bronchitis, bronchiolitis, alveolar edema, mononuclear cell infiltration, hyaline membrane deposition, and scattered hyperplasia of type-2 pneumocytes. There is a clear gradient in the severity of inflammatory changes in the airway along a craniocaudal axis, with lesions being more frequent and severe in the cranial parts except for hyperinflation and emphysema. Extensive mast cell degranulation occurs in the diaphragmatic lobes, where neither the virus nor the epithelial syncytia nor the bronchiolitis typically observed in cranioventral zones are found.

Experimental Reproduction of BRSV Pneumonia

Experimental reproduction of the disease has been difficult; in most cases, infection results in only mild clinical disease with limited lesions.

Severe respiratory tract disease and lesions can be reproduced experimentally in conventionally reared calves, and the virus can be recovered from tissues. Severe disease similar to the naturally occurring disease can be induced with a single aerosol of a low-passage clinical isolate of the virus. Moderate to severe BRSV-induced pneumonia can be reproduced in colostrum-fed calves, and nasal shedding of the virus and demonstration of the antigen in the lungs at necropsy provides evidence that the virus causes the disease.

In neonatal calves with experimental acute infection with BRSV, there is increased pulmonary resistance and decreased compliance, which explains the severe dyspnea observed in some calves. There is no evidence that transplacental infection occurs. Experimental infection of young lambs with BRSV can result in severe pathologic changes with only mild clinical disease.

In experimentally infected calves, the virus can be detected in the bronchiolar epithelial cells and in alveolar cells, including bronchial ciliated and mucous cells, and bronchiolar ciliated and nonciliated epithelial (Clara) cells. Syncytia are often observed in the bronchiolar walls and in the alveoli, and such syncytia were always replicating the virus. However, syncytial cell formation is not unique to infection with BRSV because it may also occur in other viral infections of the lung. Ultrastructural studies of experimental BRSV pneumonia reveal that BRSV replicates primarily in the superficial layer of respiratory ciliated epithelium and to a lesser extent in type II pneumocytes. BRSV infection of ciliated cells in the airway can result in the loss of cilia and ciliated cells, which may interfere with lung clearance mechanisms and predispose to bacterial pneumonia. The severity of clinical signs following experimental BRSV infection in calves is positively associated with the magnitude of decreased clearance of an inhaled protein marker, indicating that BRSV-induced ciliated epithelial damage affects the effectiveness of the mucociliary escalator.[6]

Experimental BRSV infection in calves induces an acute-phase-protein response. Strong and reproducible acute-phase proteins haptoglobin and serum amyloid A will peak at 7 to 8 days after inoculation of the virus. The proinflammatory cytokine, tumor necrosis factor (TNF-α), can be detected in the bronchoalveolar lung lavage fluids, and high levels appear on the days when severe lung lesions and clinical signs are obvious. It may be involved in mechanisms leading to increased permeability of endothelium.

CLINICAL FINDINGS

The clinical findings vary considerably from herd to herd and from year to year. In dairy cattle, disease occurs most commonly in young calves under 6 to 10 months of age, although outbreaks of severe disease in mature dairy cattle also occur. Clinical signs of infection in older cattle, particularly those with previous exposure to the virus, are less severe. In large dairy herds, episodes of infection are usually mild and often unnoticed, despite cattle having a fever, slight inappetence, and a corresponding decrease in milk production that lasts 3 to 5 days. Primary infections in lactating dairy cattle may cause a considerable decrease in daily milk production. However, reinfections are not associated with an important loss of milk production.

A **sudden outbreak of acute respiratory disease** in a group of animals is a characteristic of a primary BRSV infection. The disease is more severe in animals with no previous exposure to the virus. A dry, nonproductive cough, severe dyspnea and polypnea, and bilateral nasal discharge are characteristic. A fever of 40° to 42°C (104-108°F) is common and milk production in lactating cows declines markedly. Feed consumption in the affected group declines for a few days. The fever usually persists for 3 to 5 days in spite of therapy with antimicrobials. Toxemia is not a feature unless there is secondary bacterial pneumonia. On auscultation of the lungs there are loud breath sounds over the ventral aspects, indicating consolidation, and wheezes that indicate bronchiolitis. These are the findings of a viral interstitial pneumonia. Most animals recover within 5 to 7 days. Approximately 1% to 2% of affected animals will develop a fatal viral pneumonia characterized by severe dyspnea with abdominal breathing and an expiratory grunt, the head stretched out horizontally, mouth breathing with foamy salivation, marked anxiety, persistent fever, and death within 2 to 5 days after onset. Feed and water consumption are decreased because of severe dyspnea, which results in a gaunt abdomen and dehydration. Affected animals are reluctant to move or lie down. The loud breath sounds audible over the ventral two-thirds of both lung fields indicate that extensive consolidation is becoming pronounced. Subcutaneous emphysema over the withers may also occur. Occasionally, some animals that are not being observed closely will die with peracute pneumonia within a few days and represent the index case of an outbreak.

In outbreaks of BRSV infection in **young dairy cattle** under 12 to 16 months of age, the first clinical abnormalities usually noticed are coughing and a mild nasal discharge in 50% to 75% of the animals. Inappetence with a fever of 40°C (104°F) or higher lasts for about 3 days followed by recovery in most cases. Coughing, nasal discharge, and conjunctivitis may persist for several days or a few weeks in 10% to 30% of the animals with no long-lasting complications. Abdominal breathing, and loud and abnormal lung sounds may occur in approximately 50% of the animals, but these commonly resolve within 10 days.

In an outbreak of BRSV in **recently weaned beef calves** 6 to 8 months of age, nasal and lacrimal discharge, polypnea and dyspnea, fever of 40° to 42°C (104-108°F), decreased feed intake, coughing, and lethargy are common. In a small percentage of affected animals, within a few days the dyspnea becomes marked, with mouth breathing and the production of frothy saliva created by the labored respirations. Subcutaneous emphysema over the withers as a result of severe interstitial emphysema also occurs. Loud breath sounds, wheezing, and crackling sounds are audible over the ventral aspects of the lungs. Death may occur within a few days after the onset of the dyspnea. Secondary bacterial bronchopneumonia may occur but is uncommon.

CLINICAL PATHOLOGY

It is difficult to obtain a definitive etiologic diagnosis of BRSV infection because the virus is highly labile in tissue samples, and virus detection in specimens is poor because of inadequate laboratory techniques. The virus replicates slowly, classical virus isolation is laborious, and several blind passages are often necessary before any cytopathic effect can be seen. Nasopharyngeal swabs for virus isolation and paired serum samples are necessary to make a definitive etiologic diagnosis. Successful laboratory diagnosis of BRSV is generally based on one of the following four criteria:

- Virus isolation
- Detection of BRSV antigen in suspected tissues

- Indications of BRSV seroconversion
- Histopathology

The high prevalence of antibody titers to the virus and the need for skilled personnel to process and interpret the diagnostic tests have hindered development of a routine diagnostic test. Successful isolation of the virus from typical clinical cases of disease is often unsuccessful and can take 11 to 21 days because of the late appearance of any noticeable cytopathic effect. Because of these difficulties, isolation of the virus is not commonly recommended as a routine diagnostic approach.

Virus Isolation or Detection

After experimental infection, the median time to shedding is 3 days, the median time to peak shedding is 5 days, and the median time until shedding ceased is 9 days.[7]

The ideal sample for **isolation** of the virus is a transtracheal aspirate in the very early stages of the disease. The sample also provides cells for **immunofluorescent antibody** (IFA) **staining**. Nasopharyngeal swabs are also useful, but sampling technique must ensure good contact with the most caudal part of the pharyngeal cavity, and the samples must be placed in viral transport medium and shipped on cold packs and not frozen.

The **PCR assay** is rapid and sensitive and is the preferred method for the analysis of clinical specimens because of advantages in speed, sensitivity, sample lability, and cost.[4] The presence of the virus can be determined by using PCR on moistened cotton nasal swabs taken in the acute phase of a suspected outbreak.[4] The virus can be detected and quantified in cell cultures using real-time quantitative RT-PCR and quantitative competitive RT-PCR assays. A rapid patient-side immunomigration assay designed to detect HRSV can accurately detect BRSV in field studies, thereby providing a rapid calf-side diagnostic test.[8]

The fluorescent antibody test for virus **detection** is one of the most rapid, reliable, and sensitive tests for the diagnosis of BRSV infection. For tracheal aspirates, an aliquot of the sample is centrifuged onto a microscopic slide to obtain a cell preparation for the IFA test. The virus can be detected in tissues with monoclonal or polyclonal antibodies and avidin–biotin complex immunohistochemistry. This is typically done on formalin-fixed, paraffin-embedded tissues.

Serology

The standard serologic test for specific BRSV antibodies is the **virus-neutralization** (VN) **test**, usually done with microtiter plates. Paired acute and convalescent samples from both affected and normal animals in the herd are desirable. The majority of dairy calves do not have detectable maternal antibodies directed against BRSV after 5 months of age;[9] consequently, a positive titer in cattle aged 6 months or more indicates exposure to infection within the population. The **indirect ELISA** is a rapid and reliable test for detecting antibodies to BRSV in milk, bulk-tank milk, and serum. A **microneutralization ELISA** has been developed that correlates well with other assays and is useful in assessing antibody responses to the virus both in naturally occurring disease and in vaccination studies.

Arterial Blood Gas Analysis and Blood L-Lactate Concentration

The partial pressure of oxygen in arterial blood (Pao_2) is negatively associated with the extent of lung lesions in calves with experimentally induced BRSV infection, with a 0.6% to 0.8% increase in the proportion of affected lung for every 1 mm Hg decrease in Pao_2 from the reference value.[10] As a consequence, measurement of Pao_2 provides clinical useful information regarding the proportion of damaged lung and the response to treatment. In contrast, arterial blood L-lactate concentration was not useful in predicting the proportion of damaged lung and usually remained within the reference interval, even in the presence of severe arterial hypoxemia.

NECROPSY FINDINGS

Affected lungs are voluminous and heavy, and they fail to collapse when the thoracic cavity is opened. The cranioventral portions of the lung are consolidated and usually dark red or plum-colored. The interlobular septa are edematous, and mucoid exudate can often be expressed from small bronchi. Severe interstitial emphysema and edema are prominent over the dorsal and caudal lobes. Subpleural emphysema is often obvious in the cranial and caudal lobes. The caudodorsal lung regions may be "meaty" in consistency. The caudal lobes are often markedly distended because of interstitial emphysema, and large bullae are common. The interlobular septa of the caudal lobes are usually distended because of emphysema and edema. Subcutaneous emphysema over the withers, thorax, and neck are common. Secondary bacterial bronchopneumonia with pleuritis may occur.

Histologically, there is bronchiolitis and bronchitis. Large multinucleated syncytia are present, projecting from the bronchiolar walls or lying free in the lumen. Hyperplasia is necrosis of the bronchiolar epithelium are common. Exudates consisting of neutrophils, macrophages, desquamated epithelial cells, and syncytia are present in the bronchiolar lumina. Small airways are often occluded with exudate. Alveolar changes include cellular infiltration and thickening of alveolar septae with multinucleate giant-cell syncytia in the alveoli. Epithelial syncytia containing eosinophilic intracytoplasmic inclusion bodies are often present on alveolar walls. The presence of epithelial syncytia is a useful feature, but the numbers and prominence of these structures can vary considerably. Other viruses can also induce these syncytia. In the caudodorsal lung regions, there is severe emphysema, often with rupture of alveolar walls and alveolar edema, sometimes with hyaline membrane formation and swelling of alveolar epithelial cells.

In experimental BRSV pneumonia, the findings include bronchitis, bronchiolitis, proliferative and necrotizing bronchiolitis, interstitial pneumonia with areas of atelectasis and alveolar edema, epithelial syncytium formation on bronchiolar and alveolar walls, and pneumocyte hyperplasia. Viral antigen can be demonstrated by immunoperoxidase or immunofluorescent staining of bronchiolar and alveolar epithelium.

Isolation of the BRSV from natural field cases has always been difficult because of the long duration required for the appearance of characteristic cytopathic effects. Fluorescent microscopy can be used for detection of the antigen in the cranioventral lung areas, but PCR is a more sensitive technique. It is advisable to collect and **sample several areas of lung** because viral antigen/nucleic acid will be most abundant in areas of acute infection. The virus can also be demonstrated in formalin-fixed paraffin-embedded bovine lung tissue using immunohistochemical techniques.

Samples for Confirmation of Diagnosis

- **Histology**—fixed lung (several sites) (LM, IHC)
- **Virology**—chilled lung (several sites) (FAT, PCR); nasal swab (ELISA, PCR)

DIFFERENTIAL DIAGNOSIS

The differential diagnosis includes those infectious diseases of the respiratory tract of young cattle that commonly affect groups of animals in a short period of time.

It is not usually possible to make a definitive etiologic diagnosis based on the clinical findings. However, the combination of the epidemiologic and clinical findings is usually suggestive of an acute viral respiratory disease. It is not usually possible to be more specific than making a clinical diagnosis of acute undifferentiated respiratory disease.

- Acute respiratory disease attributable to BRSV infection in weaned beef calves is characterized by marked dyspnea, anorexia, mouth breathing, fever, subcutaneous emphysema, loud breath sounds, and death in a small percentage of animals in a few days or less. In some cases there may be a history of respiratory disease in the affected group several days previously.
- Infectious bovine rhinotracheitis (IBR) is characterized by outbreak of coughing,

profuse nasal discharge, fever, inappetence, and the presence of typical nasal lesions; pneumonia is not common. Recovery occurs in several days
- Pneumonic pasteurellosis is characterized by anorexia, toxemia, fever, abnormal lung sounds, coughing, nasal discharge, and response to treatment with antimicrobials. fibrinous pneumonia at necropsy is typical.
- Lungworm pneumonia occurs most commonly in groups of young cattle on summer pasture and is characterized by coughing, nasal discharge, tachypnea, abdominal breathing, fever and inappetence, and increased breath sounds with crackles. A necropsy diagnosis is usually necessary.
- BRSV infection in mature dairy cattle may be mild and is characterized by a slight drop in milk production, fever for a few days, inappetence, and recovery in a few days. Adult cattle lacking immunity may develop severe fatal pneumonia, which must be distinguished from bacterial pneumonia, infectious bovine rhinotracheitis, and other causes of interstitial pneumonia.

TREATMENT

Antimicrobial Therapy

Broad-spectrum antimicrobials given daily for 3 to 5 days for secondary bacterial pneumonia are commonly administered but may not be necessary. Recovery usually occurs gradually over a period of 3 to 5 days. Severely affected animals will become worse in spite of therapy.

Corticosteroids and Nonsteroidal Antiinflammatory Agents

Corticosteroids and NSAIDs are used on the basis that widespread dissemination of the virus into the caudodorsal lung field is proinflammatory and results in extensive emphysema and severe respiratory distress. There is no evidence that such treatment is efficacious. Currently, there are no effective postinfection treatments for HRSV infection in humans, other than supportive care.[11]

CONTROL

The ubiquitous nature of the virus, the persistency of infection in herds, the movement of cattle between herds, the expansion of herds, the replacement practices used in herds, and recurrent infections make control difficult. However, in BRSV-seronegative herds, effective biosecurity measures, including maintaining a closed herd, preventing nose-to-nose contact with cattle on adjacent farms, quarantining and testing new additions to the herd, and providing boots for visitors, may be effective in preventing infection from entering the herd.[9] A rational approach to control would be management of the herd to minimize stressors such as inadequate ventilation. Herd replacements brought into the herd should be quarantined from the rest of the herd for 2 to 3 weeks before mixing with the remainder of the herd.

Vaccines and Immunization

An effective vaccine must be able to stimulate an effective immune response in the presence of maternally derived antibody resulting from colostrum ingestion. This is because the majority of adult cattle are seropositive, and clinical disease appears to be most common in calves aged 2 to 4 months. Currently, several types of immune response, influenced by vaccination protocol and vaccine composition, appear to provide protection against BRSV, and optimal protection in the face of maternally derived antibodies may require both live and inactivated vaccines.[12] However, immune responses to parenterally administered modified live-virus (MLV) vaccines appears to be substantially inhibited in neonatal calves. As a result, the parenteral administration of a MLV BRSV vaccine is not expected to engender a protective immune response to BRSV infection.[13]

Several MLV and inactivated virus vaccines are available for the control of respiratory disease resulting from BRSV infection, but appropriately controlled randomized clinical trials evaluating the efficacy of the vaccine under naturally occurring conditions against BRSV infection or clinical disease are lacking. Protection induced by BRSV vaccines is short lived and usually less than 4 months.

A MLV vaccine administered by the intranasal route appears to provide the best vaccine candidate, based on our current understanding of the pathogenesis of BRSV infection. Such a vaccine was first made commercially available in 2007. However, administration of a MLV vaccine runs the risk of reversion to virulence and spread. The risk of reversion is reduced if gene deletion is employed.[12] In addition, maternal antibodies appear to inhibit priming of protective immune responses when intranasal BRSV vaccines are administered.[13,14] Cattle vaccinated with MLV BRSV vaccines generally develop high concentrations of virus neutralizing antibodies (VN) and F (fusion) inhibiting antibodies, compared with low to moderate concentrations of total BRSV-specific IgG. A 2015 meta-analysis concluded that in experimentally induced exposure trials, beef or dairy calves vaccinated with modified live BRSV vaccines had no reduction in morbidity or mortality compared with unvaccinated controls. This meta-analysis included calves vaccinated by the intranasal route.[15] Cattle receiving **inactivated virus vaccines** develop lowered concentrations of VN antibodies and higher concentrations of virus-specific (nonneutralizing) IgG than cattle administered an MLV vaccine. The clinical significance of this difference has not been determined. Inactivated BRSV vaccines have been successful when tested by experimental challenge of vaccinated calves, which contrasts to the enhanced disease that may occur in children vaccinated with a formalin-inactivated alum adjuvanted HRSV vaccine. A 2015 meta-analysis concluded that in experimentally induced exposure trials, dairy calves less than 6 months of age vaccinated with inactivated BRSV vaccines had no change in morbidity but **decreased mortality** compared with unvaccinated controls.[15]

None of the 81 commercial BRSV vaccines available in 2014 enable the **differentiation of infected from vaccinated animals** (**DIVA**).[16] Subunit BRSV vaccines provide an attractive alternative to MLV BRSV vaccines in that there is no potential for reversion to virulence, and vaccinated animals can be distinguished from naturally infected animals based on serologic testing.[12,16] The use of BRSV-**immunostimulating complexes** (**ISCOMs**) has been evaluated in calves with BRSV-specific maternal antibodies. The vaccine overcame the suppressive effect of colostral antibodies and induced a strong clinical and virological protection against a BRSV challenge. Clinical protection was associated with a marked reduction in virus replication in the upper and lower respiratory tract and rapid antibody and T-helper-cell responses, which may be attributable to the effects of the adjuvant in antigen presentation.[17]

TREATMENT AND CONTROL

Treatment

Antimicrobial treatment for animals with a fever to address concurrent bacterial pneumonia (see treatment recommendations for *Mannheimia hemolytica* in this chapter) (R-1)

Antiinflammatory agents

Nonsteroidal antiinflammatory agents (R-2)

Corticosteroids (R-3)

Control

Vaccination of dairy calves less than 6 months of age with inactivated BRSV vaccine. (R-1)

Vaccination of beef or dairy calves with modified live BRSV vaccine. (R-3)

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INFECTIOUS BOVINE RHINOTRACHEITIS (RED NOSE), BOVINE HERPESVIRUS-1 INFECTION

SYNOPSIS

Etiology Bovine herpesvirus-1 subtypes: BHV-1.1 (respiratory); BHV-1.2a and 1.2b (genital); BHV-1.3 (renamed BHV-5; encephalitic).

Epidemiology Worldwide occurrence in cattle; high prevalence of infection; low incidence of disease; transmitted directly; latent infection characteristic; economic losses as a result of deaths and abortions, latent infection in breeding animals cause international trade problems and entry into artificial insemination units.

Signs Rhinitis with typical nasal lesions, tracheitis, fever, conjunctivitis, coughing, nasal discharge, and recovery in few days; severe systemic disease in newborn calves, abortion outbreaks.

Clinical pathology Isolation or detection of virus with tissue culture or polymerase chain reaction (PCR); serology with serum-neutralizing titer, enzyme-linked immunosorbent assay (ELISA). Bulk-tank milk antibodies.

Lesions Rhinotracheitis, bronchopneumonia, nonsuppurative encephalitis, alimentary tract necrosis in calves with systemic disease, aborted fetuses autolyzed.

Differential diagnosis All diseases associated with bovine respiratory tract disease: pneumonic pasteurella, viral interstitial pneumonia, *Haemophilus pleuropneumoniae,* allergic rhinitis.

Treatment Antimicrobials for secondary bacterial infections.

Control Vaccination of young breeding herd replacements using modified live virus or inactivated virus vaccines. Subunit and marker vaccines are preferred to conventional vaccines. Some countries have eradicated infection by identifying and eliminating seropositive animals.

ETIOLOGY

The bovine herpesvirus-1 (BHV-1), or the infectious bovine rhinotracheitis (IBR) virus, is an alpha-herpesvirus and the cause of the respiratory disease, abortion, conjunctivitis, and other clinical forms of the disease complex. Genetic analyses of various clinical isolates have found at least four distinct **BHV-1 subtypes**: a **respiratory subtype, two genital subtypes,** and an **encephalitic subtype** designated as BHV-1.1, BHV-1.2a, BHV-1.2b, and BHV-1.3, respectively. BHV-1.3 as a neuropathic subtype has been renamed as three genotypes, BHV-5a, BHV-5b, and BHV-5non-a/non-b.[1] Antigenic differences between isolates of the virus may account for some of the diverse epidemiologic and pathologic patterns of behavior of this herpesvirus, although development of rhinotracheitis or vulvovaginitis/balanoposthitis depends more on the route of infection than on the subtype of the virus.

Four ruminant alpha-herpesviruses are related to BHV-1 and have the potential for cross-infection of cattle in Europe: bovine herpesvirus-5, caprine herpesvirus-1 (CpHV-1), cervine herpesvirus-1 (CvHV-1), and cervine herspesvirus-2 (CvHV-2). Buffalo herpesvirus-1 and elk herpesvirus are also closely related to BHV-1. BHV-5 is the cause of fatal meningoencephalitis in calves. CpHV-1 causes enteritis and generalized infection in neonatal kids. Most CpHV-1 infections in adults are subclinical, the virus can cause vulvovaginitis, balanoposthitis, or abortion. CvHV-1 can cause ocular disease in red deer and is widespread in free-living and farmed red deer. CvHV-2 has been isolated from reindeer in Finland, and serologic evidence of infection with a virus similar to BHV-1 has been reported in caribou in Canada. Although these viruses differ considerably in their virulence, they are closely related both genetically and antigenically, and they can establish latent infections similar to that of BHV-1. An immunofluorescence assay using monoclonal antibodies can discriminate between these related herpesviruses. Bovine herpesvirus-4 has been associated with mastitis in cattle.

EPIDEMIOLOGY

Prevalence of Infection and Occurrence of Disease

Reproductive disease as a result of BHV-1 was first reported in Germany in 1841 as the cause of infectious pustular vulvovaginitis (IPV) and infectious pustular balanoposthitis (IPB). A more virulent disease form attributable to BHV-1.1 (infectious bovine rhinotracheitis) emerged in Colorado feedlots in the United States in 1950, and this subtype has been widely disseminated, most likely as a result of the export of live cattle. It is believed that this subtype developed because of an adaption to multiply in respiratory epithelium associated with large susceptible populations congregated on one feedlot. The virus is now distributed worldwide, but it has been eradicated from Austria, Denmark, Finland, parts of Germany, Sweden, parts of Italy, Switzerland, and Norway.[2] Control programs are running in several other countries. The respiratory form of clinical disease is most common in feedlot cattle and cattle on dairy and beef farms without a routine vaccination program.

Wildlife

Bovine herpesvirus infections exist in wild ruminants. Infections may be endemic in white-tailed deer in certain parts of Canada, and it is suggested a mild form of the disease occurs in these animals. Mule deer are susceptible to infection, the disease has occurred naturally in a goat, and antibodies to the virus have been found in pronghorn antelope in western Canada and in Tanzania in game animals and cattle. According on serologic surveys, the virus is widespread in African wildlife, particularly the buffalo, which may be a reservoir of infection among the wildlife population. The virus has been recovered from the wildebeest in Africa, which suggests further that wildlife may serve as reservoirs. Antibodies to the alpha-herpesviruses were found in reindeer, roe deer, and in red deer in Norway. In Saskatchewan, Canada, 52% of Woodland caribou were seropositive for BHV-1.

Morbidity and Case Fatality

The uncomplicated form of the respiratory disease in cattle is not highly fatal, with most losses being mainly attributable to secondary bacterial bronchopneumonia. The morbidity and case-fatality rates in dairy cattle are about 8% and 3%, respectively, whereas in feedlot cattle the morbidity rate is usually 20% to 30% in unvaccinated cattle and may rarely reach 100%. The case-fatality rate in feedlot cattle is invariably associated with secondary bacterial tracheitis and bronchopneumonia and may reach 10%, but is usually no more than 1%. Morbidity and mortality are **higher in feedlot cattle** than in dairy herds because of the frequent introduction of susceptible animals into an enzootic situation. The case-fatality rate in the systemic form of the infection in newborn calves is almost 100%.

Methods of Transmission

The main sources of infection are the nasal exudate and coughed-up droplets, genital secretions, semen, and fetal fluids and tissues. Aerosol infection is the method of spread of the respiratory disease. Experimentally,

BHV-1 can be shed from calves into the environment and transported by air over a distance of at least 3.9 m to sentinel calves housed in a separate building. The virus is stable for at least 1 month at 4° C (39 F), 50 days at 22° C (72 F), 10 days at 37° C (98.6 F), and 21 minutes at 56° C (132 F), and can survive 30 days in feed. Venereal transmission is the method of spread of the genital diseases. The virus may survive for up to 1 year in semen frozen at −196° C (−321° F).

Introduction of animals into a group often precedes an outbreak of the disease. However, it can arise simultaneously in a number of dairy farms in an area and spread from these farms to adjacent farms until the entire area is affected. The same pattern of occurrence simultaneously in a number of foci is seen in feedlots, and from these foci infections it spreads to other pens in the lot. An outbreak usually reaches its peak in week 2 or 3 and ends by week to 6.

Risk Factors

Animal Risk Factors

Age and Breed Susceptibility

All ages and breeds of cattle are susceptible, but the disease occurs most commonly in animals over 6 months of age, probably because of their greater exposure. There is no seasonal variation in incidence, except possibly a higher occurrence in feedlot cattle in the fall and winter months when large numbers of susceptible animals are assembled. The disease complexes associated with the virus occur most commonly in animals that lack acquired immunity from previous natural infection or vaccination. **An unvaccinated herd of breeding cattle or a group of feedlot animals is highly susceptible to epidemics of respiratory disease and abortion.** Newborn calves are highly susceptible to the systemic form of infection if the level of specific antibody to the virus in the colostrum is inadequate or if there is failure of transfer of passive immunity.

The analysis of the relationship between interferon genotype and severity of clinical disease in cattle experimentally inoculated with BHV-1 revealed that certain alleles of the interferon were significantly associated with the more severe clinical phenotype. A second allele at another locus was associated with the milder disease genotype. Thus selective breeding programs aimed at altering the frequency of these alleles in cattle populations may potentially improve animal health and lessen the economic impact of BHV-1 infections. This potential control method has not been pursued with the introduction of national vaccination and eradication campaigns.

Environmental and Management Risk Factors

Several management factors have been associated with BHV-1 infection in a herd. Infected herds purchase cattle and participate in cattle shows more often than negative farms. The positive farms have more visitors and are situated closer to other cattle farms. The failure to vaccinate regularly and keep reliable records of vaccination dates is commonly associated with inadequate disease control. In countries with BHV-1 eradication programs, the loss of certification is commonly associated with yearly number of cattle purchased, farm density within a 1-km radius, and cattle density within a 1-km radius.

Pathogen Risk Factors

The **IBR-like viruses** are now designated **BHV-1.1**, and the **IPV-like viruses** are designated **BHV-1.2**, with the latter subtype being further divided into two groups given the letter designations a and b. **Subtype 1.2a isolates cause abortion**; 1.2b isolates are not abortifacient. **Subtype 1.3 (now renamed BHV-5)**, is the encephalitic strain and consists of three subtypes as identified earlier. Currently available vaccines, which are made with 1.1 subtype vaccines, cannot be given to pregnant cattle because they are abortifacient. The currently available MLV BHV-1 vaccines can cause infertility in cattle infected 14 days after breeding.

The virulence of the virus or its host tissue specificity changes as a result of unknown factors. The BHV-1 genome is not stable during host animal passage, and variations can occur in the restriction endonuclease patterns of the viruses within individual animals during both acute infections and after viral reactivation or after viral reactivation followed by superinfection with a different subtype of BHV-1 than was used for the primary inoculation. The virus of IBR is similar to the virus causing IPV in cows and IPB of bulls. It has been suggested that the IPV was transmitted to North America from Europe in infected cattle, but continued to cause lesions in only the genital tract until its introduction into dense populations of cattle in feedlots encouraged rapid passage through many hosts and thus encouraged adaptation to the respiratory tract. Only rarely do the respiratory and genital forms of the disease occur together. However, by routine methodology it is difficult, and usually impossible, to distinguish between isolates obtained from the reproductive tract and the respiratory mucosa. Likewise, with the exception of temperature-sensitive mutants, vaccine strains cannot be distinguished from field isolates.

The virulence of several strains of one genotype can vary widely. The outcome of BHV-1 infection can vary from subclinical to a systemic infection in neonatal calves that is often highly fatal. Vaccine strains of BHV-1.1 have been associated with outbreaks of meningoencephalitis in feedlot cattle within 7 to 10 days after routine vaccination intranasally with a vaccine intended for the intramuscular route. Newborn calves under 3 days of age are susceptible to the highly fatal systemic form of IBR if vaccinated intramuscularly with a modified live-virus BHV-1 and PI-3 vaccine. An outbreak of a subclinical form of IBR has been described in a dairy herd of high health status and managed under high standards of biosecurity, and known to be serologically negative for the virus for the previous 15 years. Although 70% of the cows had seroconverted to the virus, no clinical signs were observed, with the exception of an ocular discharge in a few cows, and their performance and productivity were unaffected. The causative virus was isolated after reactivation with corticosteroids and had the DNA profile of a BHV-1 strain normally associated with severe respiratory disease.

The glycoprotein E (gE) gene is a virulence factor of BHV-1 is important in the development of **gE-negative marker vaccines** used in eradication programs in Europe. These marker vaccines, either inactivated or live attenuated, are deleted in the gene coding for the nonessential glycoprotein E (gE) of BHV-1 to allow serologic differentiation between vaccinated and infected cattle.

Immune Mechanisms

Immunity to the virus is complex and consists of relationships between local and systemic antibody and cell-mediated immunity. Following natural infection or vaccination with the modified live-virus (MLV) vaccines, both cell-mediated and humoral components of the immune system are activated. The level of humoral immunity has been used as an indicator of previous infection and an indirect measure of resistance to clinical disease. However, the level of serum neutralizing (SN) antibody is not a reliable indicator of resistance to clinical respiratory disease. Animals with low levels of antibody may be immune because of cell-mediated immunity. The level of cell-mediated immunity can be evaluated using the delayed-type hypersensitivity test. Experimentally, the virus-neutralizing (VN) titers are lower in calves inoculated with both the IBR and parainfluenza-3 (PI-3) viruses than in calves infected with a single virus. This suggests that mixed viral infections may result in greater immunosuppression, although infectious virus synthesis may be suppressed by interference.

Following intranasal infection or the use of a MLV IBR virus vaccine intranasally, local secretory antibody and interferon are produced. The interferon appears in 3 days and persists for 10 days. The presence of the interferon does not protect calves against experimental challenge 3 days after vaccination. However, the presence of even low levels of antibody in the serum or nasal secretion, which appears by day 7 following vaccination, provides varying degrees of resistance to clinical disease for 9 months.

Colostral Immunity

Calves acquire colostral antibodies from dams with humoral antibody. The duration of the colostral immunity varies from 1 to 6 months of age depending on the initial level acquired by the calf. Maternal antibody in the calf may interfere with the successful vaccination of calves before 6 months of age.

Economic Importance

BHV-1 infection can cause major economic consequences in a dairy or beef cattle breeding herd or in a beef feedlot. Losses are incurred because of epidemics of abortion, infertility as a result of IPV and IPB in bulls, loss of production and deaths from the respiratory form of the disease in all ages of cattle, deaths from the highly fatal systemic form of the disease in newborn calves, and the cost of treatment when secondary bacterial infections of the respiratory tract occur.

PATHOGENESIS

The virus causes disease through several different pathways including a primary infection restricted to the respiratory tract, eyes, and reproductive tract. Systemic spread to many organs by viremia occurs and neuronal spread. In addition, the virus can establish latency in neuronal or lymphoid cells. Upon reactivation, the viruses reestablish the lytic cycle of replication. The innate immune response is primarily activated in animals infected with IBR though Toll-like receptors 2 and 4, and the development of an effective cytotoxic T-cell response is critical for the elimination of cells infected with virus.[3]

Respiratory Disease

The BHV-1 virus infects the nasal cavities and upper respiratory tract, resulting in rhinitis, laryngitis, and tracheitis. The pharyngeal tonsil is readily infected by the virus and may be an important lymphoid tissue for early antiviral responses. There is extensive loss of cilia in the trachea, leaving the tracheal epithelium covered by microvilli. Intratracheal administration of the virus results in almost complete denudation of tracheal columnar cells, which presumably has an adverse effect on the defense mechanisms of the respiratory tract. Spread from the nasal cavities to the ocular tissues probably occurs by way of the lacrimal ducts and causes conjunctivitis with edema and swelling of the conjunctiva, multifocal plaque formation on the conjunctivae, peripheral corneal edema, and deep vascularization. The virus can also enhance the prevalence and severity of IBR in calves. In neonatal calves, potentially fatal infection, associated with the continued presence of viral antigen and active inflammation, contrasts with repair and clearance of viral antigen in weanling calves. Experimentally, the endobronchial inoculation of calves with the BHV-1 causes an interstitial pneumonia. The viral antigen can be detected in the desquamated cells and macrophages of bronchoalveolar fluid.

Encephalitis

The mechanism by which the brain is infected is presumed to be spread of the virus from the nasal mucosa via the trigeminal peripheral nerve to the trigeminal ganglion, resulting in a nonsuppurative encephalitis. However, a viremia has been suspected. Severe encephalitis can be produced experimentally in colostrum-deprived calves with neurovirulent type BHV-1.3. Experimental infection with BHV-1.1 produces respiratory disease and a mild encephalitis. Intranasal inoculation of young calves and adult cows with BHV-1 can result in nonfatal trigeminal ganglionitis and encephalitis, which may be an important mechanism for latent infection.

Abortion

Systemic invasion by the virus is followed by localization of the virus in several different tissues. The virus may be transported by peripheral leukocytes to the placenta and transferred to the fetus to cause abortion. The fetus is highly susceptible to the virus, which causes a peracute infection that is usually fatal. Infection in the last trimester of gestation may result in mummification, abortion, stillbirth, or weak calves with the usual lesions of IBR and the lesions of the stomachs and intestines that have been produced by experimental administration of the virulent virus to newborn calves.

The systemic form of the infection in newborn calves is characterized by severe inflammation and necrosis of the respiratory and alimentary tracts, including the pharynx, esophagus, lungs, larynx, lymph nodes, and liver, and nephritis and encephalitis. There is severe laryngeal edema and respiratory distress that results in difficulty in swallowing and aspiration pneumonia. A severe, highly fatal syndrome characterized by diffuse erosion and ulceration of the upper alimentary tract, including the oral cavity, has occurred in beef feedlot cattle.

Latency

The **BHV-1 virus can become latent** following a primary infection with a field isolate or vaccination with an attenuated strain. The virus may remain latent indefinitely, and recrudescence, reactivation, and shedding of the virus can occur following the use of large doses of corticosteroids that mimic the effects of stress. Transportation of cattle with latent infection can reactivate the virus, resulting in reexcretion of the virus and a rise in neutralizing antibodies. Attenuated vaccine strains can remain in a latent stage, and vaccination does not provide protection against the establishment of latent infection with a wild strain. Vaccination also does not inhibit reexcretion of a wild strain that was in the latent form at the time of vaccination. The vaccine virus and the field isolates can be excreted after live-virus vaccination and subsequent field isolate challenge. Colostral antibodies in calves do not prevent initial virus replication, and latency can persist after the decline in colostral immunity and the calves are seronegative.

The location of latency of the virus in the body varies; the virus remains localized near the site of its first multiplication and during recrudescence will be reexcreted by the tissue primarily infected. The BHV-1 can be isolated from the **trigeminal ganglion** of clinically normal cattle during the latent period, and trigeminal ganglionitis can be observed during recrudescence. Latent infection with virulent BHV-1 virus may occur in the trigeminal ganglion of calves previously vaccinated with the MLV vaccine. The virulent virus may spread along the trigeminal peripheral nerve despite the presence of humoral antibodies in vaccinated calves. Recrudescence of the virus from the trigeminal ganglion and spread along the peripheral nerves by intraaxonal flow to the nasal mucosa can occur in calves treated with corticosteroids and, presumably, occurs following stress. The virus has been isolated from the trigeminal ganglia of 10% of clinically normal cattle at slaughter, 40% of which had SN antibody to the virus.

The practical aspect of latency is that all cattle from endemic herds must be considered as potential sources of BHV-1 virus and capable of spreading infection to previously unexposed animals. Some latent carriers do not possess detectable antibodies. The only method of identification is by treatment with dexamethasone to initiate recrudescence and detection of the virus from nasal secretions, or the PCR examination of the trigeminal ganglion at necropsy.

A combined serologic and clinical surveillance of 20 dairy herds over three consecutive years revealed wide variations in the circulation of the virus. In some herds there was no identification of active infection, whereas in others one or two cycles of infection occurred in calves and yearlings, often without any clinical evidence of disease. Reactivation and shedding of the virus can occur in known carrier bulls at the time of mating, which may explain the higher incidence of titers in bulls than cows in some beef herds. Breeding bulls in an artificial insemination center that were vaccinated with a MLV vaccine were shedding the vaccine virus in the semen, and the virus could be recovered from preputial washings 2 to 3 months after the last immunization. However, the frequency of recurrent infections and the amount of virus excreted are reduced after vaccination.

The presence of passively acquired antibodies in calves does not prevent virus replication and establishment of latent infection. It is also possible to experimentally produce BHV-1 seronegative passively immunized

calves that do not have antibody response after infection but develop a cell-mediated immune response after infection detected by a specific interferon gamma assay. The failure to easily detect such animals presents an epidemiologic threat for the control of BHV-1 infections. Marker glycoprotein E–negative vaccines can also establish latency not only in naïve but also in passively immunized neonatal calves after a single intranasal inoculation. This indicates that gE-negative vaccines, when used in calves with passive antibodies, can result in seronegative vaccine virus carriers.

The experimental intrapreputial infection of young bulls with BHV-1.2 caused acute balanoposthitis, latent infection, and detection of viral DNA in regional neural (sacral nerve ganglia, pelvic sympathetic plexus) and nonneural tissues (lymph nodes) 50 days after experimental reactivation. Following experimental infection in calves the BHV-5 also can result in latent infection of surviving animals.

Parturition may also be a stimulus for reactivation and shedding of a thermosensitive vaccine strain of the virus in vaccinated animals. Reactivation and shedding of the virus has also been observed in cattle that recovered from the respiratory form of the disease and 5 months later were experimentally infected with *Dictyocaulus viviparus.* The placenta may harbor the virus in a latent stage for up to 90 days without transmitting the virus to the fetus. Recrudescence may be differentiated from primary infection and reexposure by the intranasal route based on the distribution of antiviral antibody activity among serum IgM, IgG_1, and IgG_2 isotypes.

Predisposition to Pneumonia

The role of the virus in affecting the lung clearance mechanism of cattle in the pathogenesis of pneumonic pasteurellosis has been reviewed and is presented in the section on shipping fever pneumonia in cattle. Experimental aerosol exposure of calves with the BHV-1 virus impairs the function of alveolar macrophages, which allows *Mannheimia haemolytica* to persist and proliferate in the lung and produce the characteristic lesion. In vitro studies indicate that the BHV-1 virus can interfere with the function of effector cells, such as macrophages, neutrophils, and lymphocytes. Aerosol exposure of calves to BHV-1 can affect the composition of alveolar phospholipids, which can alter the function of lung surfactant and compromise pulmonary defense mechanisms. The BHV-1 can cause alteration in the glycoconjugate composition of bovine nasal epithelial surfaces, which may promote *M. haemolytica* proliferation in the early stages of pneumonic pasteurellosis. The virus also causes varying degrees of obstructive lung disease, resulting in increased resistance to breathing, retention of carbon dioxide, and increased resting lung volume. Excessive airway constriction and impairment of bronchial relaxation occurs, which may compromise lung defense mechanisms and allow development of secondary bacterial pneumonia. A severe fatal BHV-1 pneumonia can occur.

Experimentally, active BHV-1 infection triggers cytokine expression on bronchial epithelial cells that facilitates recruitment of neutrophils.[4] BHV-1 infection also affects bovine peripheral blood neutrophils, enhances the binding of *M. haemolytica* leukotoxin to bronchoalveolar leukocytes, and increases their killing. The virus increases the number of bronchoalveolar leukocytes, resulting in many more leukotoxin-responsive cells being present in the lung. The net effect is that BHV-1 infection amplifies the detrimental effect of *M. haemolytica* in the lung.[4]

Reproductive Failure

The **intrauterine inoculation of the BHV-1 into cattle results in an acute necrotizing endometritis** in the uterine body and caudal portions of the uterine horns but minimal lesions in the anterior parts of the horns. Experimental inoculation of the virus into heifers on the day after estrus and insemination can result in lesions of the ovaries consisting of focal necrosis and cellular infiltration. Commercially available vaccinal strains of the BHV-1 virus can produce similar lesions. The ovarian lesions have marked effects on luteal function, and plasma progesterone values in the first estrus after inoculation are markedly lower than those in subsequent normal cycles. Whether the BHV-1 virus causes reproductive failure as a result of necrosis of the corpus luteum or embryonic infection remains to be determined. Recently hatched bovine embryos can be infected with any of several strains of BHV-1 and such infection in vitro is embryocidal. Experimentally induced infection during early pregnancy (7-28 days) will cause oophoritis and, in some cases, embryonic mortality. The effects of the virus on the genital tract and on reproductive performance in cattle have been reviewed.

Bovine Mastitis

BHV-1 and BHV-4 have been associated with mastitis in cattle. Both viruses, including the foot-and-mouth disease virus, and the PI-3 virus have been isolated from milk. BHV-4 has been isolated from cows with clinical mastitis that also developed antibodies against the virus at the time of the mastitis, and no bacteria were isolated from the milk samples. Bovine umbilical cord endothelial cells were used to culture the virus. Experimental inoculation of the ductus papillaris of the teat has resulted in replication of the virus and subclinical mastitis after BHV-4 infection. Simultaneous intramammary and intranasal inoculation of lactating cows with BHV-4 did not induce clinical but subclinical mastitis. It is unlikely that BHV-4 is a major mastitis pathogen.

CLINICAL FINDINGS

Rhinitis (Red Nose), Tracheitis, and Conjunctivitis

After experimental infection there is an incubation period of 3 to 7 days, but in infected feedlots the disease occurs 10 to 20 days after the introduction of susceptible cattle.

There is considerable variation in the severity of clinical signs following natural infection, dependent on the strain of the virus, the age susceptibility, and environmental factors. In North America, where the disease is endemic, the clinical disease is usually mild in dairy cattle and in range beef cattle. A severe form of the disease can occur in feedlots where crowding and commingling from several sources occur. A severe form of upper respiratory tract disease and encephalitis has been reported in neonatal beef calves. Clinical disease is most common after 6 months of age in colostrum-fed calves as a result of waning colostral IgG (passive immunity).

There is sudden onset of anorexia, loud coughing, fever (up to 42° C [108° F]), severe hyperemia of the nasal mucosa with numerous clusters of grayish foci of necrosis on the mucous membranes of the nasal septum visible just inside the external nares, a serous discharge from the eyes and nose, increased salivation, and sometimes a slight hyperexcitability. A marked fall in milk yield may be the earliest indication in dairy cattle. The respirations are increased in rate and are shallow, but only an increase in the loudness of breath sounds is audible on auscultation of the lungs unless secondary pneumonia is present. A severe primary viral, or secondary bacterial, tracheitis may cause inspiratory dyspnea with abnormal tracheal breath sounds transmitted to the lungs. Respiratory distress is evident on exercise. A short, explosive cough is characteristic of some outbreaks but not in others. Sudden death within 24 hours after first signs appear can result from extensive obstructive bronchiolitis.

In dairy cattle, many animals in a herd become affected within a few days. The disease is usually mild, characterized by inappetence, coughing, profuse bilateral serous nasal discharge, excessive salivation, nasal lesions, moderate fever, moderate drop in milk production, and recovery in a few days. Several animals may have the conjunctival form of the disease with obvious conjunctivitis and profuse ocular discharge. The affected animals as a group do not return to full production for 10 to 14 days. The outbreak of respiratory disease will be followed by abortions in several days up to 90 days after the index case occurred.

In feedlot cattle the illness is often more prolonged, the febrile period is longer, the

nasal discharge becomes more profuse and purulent, and the convalescent period is longer. Some deaths may occur in the acute febrile period, but most fatalities are attributable to a secondary bronchopneumonia and occur after a prolonged illness of up to 4 months in which severe dyspnea, complete anorexia, and final recumbency are obvious signs. Some recovered animals may have a persistent snoring respiration and a grossly thickened, roughened nasal mucosa accompanied by nasal discharge.

Ocular Form of IBR

Conjunctivitis is a common finding in typical "red nose," but outbreaks of conjunctivitis may occur as the major clinical finding. One or both eyes may be affected, which is easily misdiagnosed as infectious keratoconjunctivitis (pinkeye) associated with *Moraxella bovis*. However, the IBR lesions are confined to the conjunctiva and there are no lesions of the cornea except diffuse edema. The conjunctiva is reddened and edematous, and there is a profuse, primarily serous, ocular discharge. The cornea is initially unaffected, but occasionally may be damaged as a result of secondary bacterial infection. Calves less than 6 months of age may develop encephalitis, which is marked by incoordination, excitement alternating with depression, and a high mortality rate. Salivation, bellowing, convulsions, and blindness are also recorded.

Systemic Disease in Newborn Calves

In newborn calves under 10 days of age, the systemic form of the disease is severe and highly fatal. Sudden anorexia, fever, excessive salivation, and rhinitis, often accompanied by unilateral or bilateral conjunctivitis, are common. The oral mucous membranes are usually hyperemic, erosions of the soft palate covered by tenacious mucus are common, and an acute pharyngitis covered by tenacious mucopurulent exudate is characteristic. The larynx is usually edematous, and respiratory distress is common. Bronchopneumonia is common, and loud breath sounds, crackles, and wheezes associated with consolidation are present. Outbreaks of the disease commonly occur in highly susceptible herds where the herd immunity has declined, the dams are not vaccinated, and there is minimal, if any, specific colostral immunity. Diarrhea and dehydration, referred to as the alimentary form of BHV-1 infection, occur in some affected calves. The cause of the diarrhea is uncertain, but it may be related to the ruminal lesions.

Abortion

Abortion is a common sequela and occurs some weeks after the clinical illness or parenteral vaccination of nonimmune pregnant cows with the MLV vaccine of bovine tissue culture origin. Abortion may occur up to 90 days following vaccination if the virus becomes latent in the placenta and infects the fetus much later than usual. This raises the possibility that vaccination even with safe vaccines may appear to be the cause of abortion if natural infection preceded vaccination. It is most common in cows that are 6 to 8 months pregnant. Retention of the placenta often follows, but residual infertility is unimportant. However, endometritis, poor conception, and short estrus can occur after insemination with infected semen. The infectious bovine rhinotracheitis virus has been isolated from semen 12 months after storage.

Infectious pustular vulvovaginitis is characterized by frequent urination, elevation of the tail, and a mild vaginal discharge. The vulva is swollen, and small papules, then erosions and ulcers, are present on the mucosal surface. Mucosal ulcers may coalesce, and sloughing of brown necrotic tissue may occur. Recovery usually occurs in 10 to 14 days unless there are complications.

Balanoposthitis is characterized by similar lesions of the glans penis and preputial mucosa.

CLINICAL PATHOLOGY

Virus Isolation or Detection

After experimental infection, the median time to shedding is 2 days, the median time to peak shedding is 4 days, and the median time until shedding ceases is 14 days.[5] Isolation of the virus from nasal swabs using tissue culture combined with a fourfold rise in antibody titers between acute and convalescent phase sera are desirable for a positive diagnosis of the disease. When using nasal swabs, cotton and polyester swabs are recommended rather than calcium alginate swabs, which are viricidal within 2 hours. The virus can be detected in nasal swabs by the use of an ELISA, direct and indirect immunofluorescence techniques, immunoperoxidase, and by electron microscopic examination that may reveal herpes-like viral particles. The sensitivity of the direct immunofluorescence techniques is comparable to the cell culture technique. The ELISA is highly sensitive. A combination of the indirect immunofluorescence test and virus isolation from both ocular and nasal swabs of several animals will increase the recovery rate.

The PCR assay is as sensitive as virus isolation and is a practical alternative for the rapid detection of the virus. The results are available in 1 day, compared with virus isolation, which requires 7 days. The PCR assay can be used for detection of virus in semen and is considered equivalent to that of standard virus isolation and dot blot hybridization. The PCR assay with Southern blot hybridization is considered to be highly sensitive and can detect the virus in semen before they develop any detectable antibody. The PCR assay can also detect 5 times as many positive semen samples as the virus isolation on egg yolk–extended semen. PCR is considered the diagnostic test of choice for routine diagnosis of BHV-1 in aborted fetuses.[6]

Using restriction endonuclease analysis of viral DNA, it is now possible to distinguish field isolates of the virus from vaccine strains, which may be useful in the investigation of vaccine-induced epidemics of the disease.

Serology

Several serologic tests are available for the detection of antibody and a rise in titer between the acute and convalescent phases of the infection.

The primary immune response to BHV-1 experimental inoculation of cattle is characterized by the formation of IgM and IgG antibodies, primarily IgG_1, by postinoculation day 7. Secondary immune responses are characterized primarily by the formation of IgG_2 antibody. A secondary immune response resulting from abortion induced by intraamniotic virus inoculation is characterized by a substantial increase in IgM antibody. A secondary BHV-1 exposure by the intranasal route does not result in secondary IgM antibody formation.

The VN test has been widely used and is the standard by which other techniques have been evaluated. The ELISA is a specific, sensitive, and practical test for detection of BHV-1 antibodies and has advantages over the SN test. The IgM–ELISA test is useful for the diagnosis of recent infection with BHV-1 in calves.

The detection of latent BHV-1 infection in cattle is important in control programs and in international trade activities. Therefore tests to detect specific antibodies in serum must be highly sensitive to detect low levels of BHV-1-specific antibodies. This emphasizes the need for international standardization of tests to detect BHV-1-specific antibodies in cattle. In endemically infected herds, BHV-1 transmission is not continuous but is sufficient to produce detectable antibodies. A serologic test negative carrier status (latent infection) occurs when there is no reexposure to stimulate humoral immunity. In other words, it remains very challenging to identify latently infected cattle when they are housed in a population with minimal BHV-1 transmission.[7]

An immunofluorescence assay using monoclonal antibodies can discriminate between the four ruminant alpha-herpesviruses related to the BHV-1. They include the bovine herpesvirus-5, caprine herpesvirus-1 (CpHV-1), cervine herpesvirus-1 (CvHV-1), and cervine herspesvirus-2 (CvHV-2). Buffalo herpesvirus-1 and elk herpesvirus are also closely related to BHV-1.

Bulk-tank milk testing for BHV-1 antibodies may be useful in eradication and monitoring programs because it offers the possibility of rapid and inexpensive screening. The correlation between the bulk milk test and the within-herd prevalence of

seropositive animals can be as high as 0.86. If BHV-1 is detected in the bulk milk, there is a high probability that more than one animal in a herd is infected and that the infection has spread. The BHV-1 blocking ELISA is in use on bulk milk samples as part of the Danish surveillance system for BHV-1 infection in dairy herds. The test can detect seropositive herds, with prevalence proportions as low as 1 seropositive cow out of 70 cows.

Specific antibody against BHV-1 may be detectable in fetal fluids and increases the rate of diagnosis of abortion.

NECROPSY FINDINGS

In **adult cattle**, gross lesions are restricted to the muzzle, nasal cavities, pharynx, larynx, and trachea, and they terminate in the large bronchi. There may be pulmonary emphysema or secondary bronchopneumonia, but for the most part the lungs are normal. In the upper respiratory tract there are variable degrees of inflammation, but the lesions are essentially the same in all anatomic regions. In mild cases there is swelling and congestion of the mucosae. Petechiae may be present, and there is a moderate amount of catarrhal exudate. In severe cases the exudate is profuse and fibrinopurulent. When the exudate is removed, the mucosa is intact except for small numbers of necrotic foci in the nasal mucosa, but there may be diffuse denudation of epithelium in the upper part of the trachea. Lymph nodes in the throat and neck region are usually swollen and edematous. Histologically, there is acute, catarrhal inflammation of the mucosa. Inclusion bodies are rarely seen in natural cases but do occur transiently in the nuclei of respiratory epithelial cells in experimentally infected animals. Secondary bacterial invasion will cause a more severe necrotizing change, which is usually followed by the development of bronchopneumonia. The virus is usually isolated from affected tissues using cell culture techniques. It can also be demonstrated in paraffin-embedded tissues by utilizing immunohistochemical techniques.

In the systemic form in **neonatal calves** a severe epithelial necrosis has been observed in the esophagus and rumen, with the adherent necrotic epithelium having the pultaceous quality of milk curd. The laryngeal mucosa is congested and edematous, with multiple focal lesions in the mucosa. Bronchopneumonia is common, with a thick white exudate coating the tracheal lumen and extending into the bronchi. Histologically, there is necrosis of the pharynx, larynx, associated lymph nodes, esophagus, and liver. Inclusion bodies are evident in many surviving epithelial cells. Systemic infection in neonatal calves may be more common than is currently realized; 2.1% of 2980 calves examined at necropsy between 1 and 30 days of age had lesions consistent with BHV-1 infection, although it was not clear whether BHV-1 was the primary cause of death in all cases.[8]

The **encephalitic form** lacks gross lesions but is characterized microscopically by nonsuppurative inflammation, neuronal degeneration and gliosis, located particularly in the cerebral cortex and the internal capsule. Inclusion bodies are sometimes present. Both immunoperoxidase and PCR tests are capable of detecting BHV-5 antigen in formalin-fixed brain tissues affected with bovine herpesvirus encephalitis.

Aborted fetuses show moderately severe autolysis and focal necrotizing hepatitis. Microscopically, foci of necrosis rimmed by very few leukocytes are visible in the liver and many other organs. Occasionally, intranuclear inclusion bodies can be seen. Viral antigen can be demonstrated in sections of the lung, liver, spleen, kidney, adrenal gland, placenta, and in mummified fetuses using the avidin–biotin complex system. Using this system, the viral antigen can be found in fetal tissues from which the virus could not be isolated in cell culture.

Samples for Confirmation of Diagnosis

- **Histology**—formalin-fixed samples: *abortion/neonate:* lung, liver, trachea, kidney, adrenal gland, rumen, esophagus, pharynx; *respiratory form:* nasal turbinate, trachea, pharynx, lung; *encephalitic form:* half of midsagittally sectioned brain (LM, IHC)
- **Virology**—*abortion/neonate:* lung, liver, kidney, rumen; *respiratory form:* lung, trachea, nasal swab; *encephalitic form:* half of midsagittally sectioned brain (FAT, ISO, PCR)

DIFFERENTIAL DIAGNOSIS

Infectious bovine rhinotracheitis is characterized by acute rhinotracheitis, coughing, profuse nasal discharge, nasal septum lesions, bilateral conjunctivitis, anorexia, fever, and gradual recovery in a few days. Secondary bacterial tracheitis and pneumonia can occur. It must be differentiated from the following:

- Pneumonic pasteurellosis is characterized by marked toxemia and depression, coughing, anorexia, gauntness, fever, abnormal lung sounds, and good response to antimicrobials.
- Bovine virus diarrhea is characterized by depression, anorexia, salivation, oral erosions and ulcers, persistent diarrhea, dehydration, and death in a few days.
- Malignant head catarrh is characterized by remarkable mental dejection, prominent lesions of nares, severe erosive lesions in oral cavity, interstitial keratitis, enlarged peripheral lymph nodes, high persistent fever, hematuria, terminal encephalitis, and death in several days.
- Calf diphtheria occurs usually in a single animal and there is depression, fever, inability to suck or eat, inspiratory dyspnea and stridor, fetid oral and laryngeal lesions, and severe toxemia.
- Viral pneumonia of calves occurs in a group of calves and is characterized by mild depression, inappetence, fever, coughing, dyspnea, abnormal lung sounds, no nasal lesions, and recovery in a few days.
- Allergic rhinitis occurs in cattle on pasture in summer months and is characterized by sneezing and wheezing with inspiratory dyspnea, mouth breathing, normal temperature, and profuse thickened nasal discharge that is caseous and greenish-orange in color.
- Systemic form of IBR in newborn calves must be differentiated from acute pneumonia, septicemia, and toxemias.

TREATMENT

Antimicrobial Therapy

Broad-spectrum antimicrobials are indicated if secondary bacterial tracheitis and pneumonia are present. Affected cattle should be identified, isolated, and monitored frequently for evidence of secondary bacterial disease accompanied by anorexia and toxemia and treated accordingly. The tracheitis is particularly difficult to treat; antimicrobials daily for several days are necessary.

CONTROL

The diseases associated with the virus may occur unpredictably at any time, and even closed herds with no introductions may remain free of the disease for several years and suddenly experience an outbreak. The current strategies for control are **natural exposure, biosecurity, vaccination**, or **eradication** of the virus from a herd or even the cattle population of a country.

Natural Exposure or Vaccination

Natural Exposure

Cattle that have recovered from a natural infection with the virus are immune to further clinical disease. However, to depend on natural exposure of the herd is risky because not all animals will become infected and become immune. Abortion storms occur in herds that are not vaccinated and depend on natural exposure. Vaccination is therefore recommended in areas where the prevalence of infection is high and eradication is not feasible because of the extensive nature of the cattle population and movement of animals from one area to another. The virus is sensitive to many disinfectants including 1% quaternary ammonium bases, 1% phenolic derivatives, and 10% Lugol's iodine.

Biosecurity

Biosecurity is any practice or system that prevents the spread of infectious agents from infected animals to susceptible animals or that prevents the introduction of infected animals into a herd, region or country in which the infection has not yet occurred. Biosecurity is an integral part of any successful livestock enterprise and reduces the risks and consequences of introducing an infectious disease. The components of biosecurity include management and placement programs, farm layout, decontamination, pest control, and immunization. All of these factors directly affect productivity and profitability.

The introduction of new infections into herds can be prevented or minimized by purchasing animals directly from herds known to be free of a particular disease. The adoption of this principle requires awareness of the possibility of purchasing unknown infected animals and testing animals for the infection before entry into the herd. It may also require keeping the introduced animal in quarantine for several weeks after arrival before it is mixed with the other animals.

Veterinarians need to work with their clients to develop a specific disease control and biosecurity protocol for each farm. The benefits of a rigidly enforced biosecurity program need to be stressed. Veterinarians can assist producers in developing methods to handle livestock and to purchase replacement stock by designing protocols that concentrate on general and specific aspects, such as design and construction of isolation rates.

Closed Herd

A closed farming system to prevent the introduction of infectious diseases into dairy farms is technically possible and is economical. A closed dairy farming system could prevent the introduction of BHV-1 and can be a good starting point for eradication of infectious diseases from the herd.

In the cattle industry, animals are moved freely from their farms of origin to veterinary clinics, cattle shows and sales, auction markets, 4H club events, and community grazing pastures. Cattle are commonly returned to their farms of origin after being at shows and sales, veterinary clinics and other events where animals from other farms are mixed. Animals may commingle with those from adjacent herds (broken fences or cattle breaching fences from one pasture to another). Breeding bulls may be leased from their farm or origin, used on another farm, and then returned to the farm of origin. The mixing of animals that occurs in all of these circumstances provides opportunities for the transmission of important infectious agents.

Vaccination

With currently available diagnostic tests, it is not possible to identify animals that have a latent BHV-1 infection. The next best strategy is to use a well-planned vaccination program.

Rationale for Vaccination

Vaccination protects animals from severe clinical signs of infection and assists in control and eradication programs. The rationale for vaccination is based on the following:

- The virus is ubiquitous and the occurrence of the disease unpredictable.
- Economic losses from abortion, neonatal disease, and respiratory disease can be high.
- Colostral immunity in calves wanes by 4 to 6 months of age.
- *The vaccine will prevent abortions caused by the virus and provide protection against respiratory disease if given at least 10 days before natural exposure.*

Several attenuated live and inactivated BHV-1 vaccines are currently available, with attenuated vaccines being administered intranasally or intramuscularly. The vaccine strains have usually undergone multiple passages in cell culture to induce attenuation.

Modified live-virus (MLV) **vaccines** offer three advantages over inactivated vaccines:

- Induction of a rapid immune response
- Relatively long duration of immunity
- The induction of local immunity

Protection from infection and disease has been observed within 40 to 96 hours following intranasal or IM vaccination with MLV vaccines. This rapid development may be attributable to interferon induced locally, but intranasal vaccination also induces secretory IgA antibody and cell-mediated immunity. Vaccination trials have found that the traditional MLV vaccines are safe and effective in preventing clinical disease and are more effective than inactivated vaccines.

Both the intranasal and intramuscular stimulate the production of humoral antibody. The **intranasal vaccine** stimulates the production of local interferon and local antibody in the nasal mucosae, is safe for use in pregnant cows, and is highly effective for the prevention of abortion caused by the virus. The **intramuscular vaccine** of bovine tissue culture origin is abortigenic, especially in nonimmune cows. The intranasal vaccine provides protection against respiratory disease induced by experimental challenge 72 hours after vaccination. In general, the intranasal vaccine provides effective protection against the respiratory form of the disease but occasionally disease occurs in vaccinated animals. The intranasal vaccines do not cause a significant systemic reaction and have been used in the face of an outbreak where all in-contact animals are vaccinated in an attempt to reduce the number of new cases. **A major requirement of the intranasal vaccine is that the vaccine virus must multiply on the nasal mucous membranes.** If the vaccine is not administered into the nasal cavities carefully, or if the animal is difficult to handle or snorts out the vaccine, vaccination will not occur. The careful administration of a temperature-sensitive vaccine in 2 mL of diluent into one nostril is as effective as a two-nostril vaccination method using a total of 5 mL of diluent. Serum antibody titers were similar for beef cattle vaccinated in high (>32° C, >90 F) or moderate (21° C, 69 F) ambient temperatures with an intranasal vaccine.[9] The preexistence of some local antibody from natural exposure or coinfection with a virulent strain of the virus may also restrict the multiplication of the vaccine virus, especially the temperature-sensitive mutants.

Temperature-Sensitive BHV-1 Modified Live Vaccine. An intranasal BHV-1 vaccine containing an MLV strain whose growth is restricted to the upper respiratory tract has been developed in Europe. The vaccine strain is chemically treated to produce a temperature-sensitive characteristic, so that it cannot replicate at the body temperature of the animal. Prebreeding vaccination of replacement heifers with the vaccine provides fetal protection. The vaccine is efficacious and safe for use in pregnant cattle. Intranasal vaccination stimulates both systemic and local cell-mediated immunity and antibody.

Disadvantages of Modified Live Vaccines. The extensive use of MLV vaccines has reduced the incidence of clinical disease but there are some potential disadvantages. MLV vaccines must be stored and handled properly to avoid loss of potency. The parenteral MLV vaccine is potentially abortigenic and cannot be used on nonimmune pregnant cattle. The virus in MLV vaccines can also become latent following vaccination. Fatal, generalized BHV-1 infection has been associated with vaccination of beef calves under 3 days of age with MLV containing BHV-1 and PI-3. An outbreak of meningoencephalitis occurred in purchased Holstein Friesian male calves vaccinated intranasally at 1 and 3 weeks of age with a commercial MLV vaccine containing BHV-1, bovine virus diarrhea virus (BVDV), PI-3, bovine adenovirus infection type-7 and bovine respiratory syncytial virus (BRSV). Parenteral vaccination was recommended as the proper vaccination protocol. The isolated virus was classified as BHV-1.1.

Shedding of Virus by Vaccinated Animals. There is some concern that MLV-vaccinated calves may shed the vaccine virus, which could then spread to pregnant cattle, resulting in abortion. In calves vaccinated with the intranasal vaccines, the virus replicates in the respiratory tract and is shed for 7 to 14 days. In nonimmune calves, replicating virus can

be detected 9 hours after vaccination, with peak shedding occurring at 4 days. However, the intranasal vaccination of feeder calves at 7 months of age does not result in transmission of the vaccine virus to nonvaccinated animals comingled with vaccinated calves. Calves vaccinated with a live temperature-sensitive mutant of BHV-1 vaccine were protected against clinical illness from experimental challenge, but excreted the virus 2 months later following treatment with corticosteroids. This emphasizes the general principle that the use of a MLV vaccine implies a continuing commitment to vaccination that may reduce the incidence of disease but is unlikely to eradicate the infection.

Inactivated Vaccines. Inactivated virus vaccines were developed because of some of the disadvantages of MLV vaccines. The vaccines contain high levels of inactivated virus or portions of the virus particle (glycoproteins) supplemented with an adjuvant to stimulate an adequate immune response. Inactivated vaccines are given intramuscularly or subcutaneously. They do not cause abortion, immunosuppression, or latency, although they do not prevent the establishment of latency by field strains. They do not cause shedding and are safe for use in and around pregnant animals. They are also relatively stable in storage.

Inactivated vaccines, however, may not be as efficacious as MLV vaccines because of the potential for destruction of some of the protective antigens during the inactivation process. They require two doses of the vaccine and protection is not observed until 7 to 10 days following the second dose of the vaccine, which is usually given 10 to 14 days after the primary vaccination.

A major disadvantage of both the MLV and inactivated vaccines is that neither allows for differentiation between vaccinated and naturally infected animals. These factors render conventional vaccines ineffective for a concurrent vaccination and eradication strategy and inappropriate for use in breeding bulls for export market or artificial insemination units that demand BHV-1-free animals. These limitations, along with major advances in molecular biology and protein purification techniques, have encouraged the development of genetically engineered attenuated vaccines and nucleic acid–free subunit vaccines.

Subunit Vaccines. A subunit vaccine contains only one or more of the antigens of the pathogen necessary to evoke protective immunity, and lacks the components that might cause unwanted side effects. The major surface glycoproteins of the BHV-1 are the antigens responsible for stimulating protective immunity. To produce a subunit vaccine containing only surface glycoproteins, the proteins are isolated from the virus of virus-infected cells, or the peptides can be synthesized. The major glycoproteins of BHV-1 originally designated gI, gIII, and gIV are now named gB, gC, and gD, and they induce high levels of antibody in cattle that are fully protected from experimental disease. The level of immunity based on serum antibody titers and protection against experimental challenge is much greater with the individual glycoproteins than are those immunized with commercially available inactivated vaccines.

BHV-1 subunit vaccines provide a number of advantages:

- They do not contain live virus and therefore cannot be shed to other animals, cause abortion, or establish latent infections.
- They prevent infection and disease.
- They are not immunosuppressive.
- Serologic assays, based on one or more antigens not present in the vaccine, provide a potential to differentiate vaccinates from naturally infected animals.

Prevention of infection by the use of a BHV-1 subunit vaccine combined with the use of a diagnostic test to identify infected cattle offers the potential for vaccination of breeding bulls for artificial insemination units and export and for eradication of the virus.

The potential disadvantages of subunit vaccines include the following:

- Because of the amount of glycoprotein needed, two immunizations may be necessary for protection.
- Subunit vaccines will have to be compatible with the commonly available multivalent vaccines.
- The efficiency of subunit vaccines is highly dependent on the use of an effective adjuvant.

Marker vaccines or **DIVA** (**differentiation of infected from vaccinated animals**) attenuated or inactivated vaccines are based on deletion mutants of one or more viral proteins, which allows the distinction between vaccinated and infected animals based on respective antibody responses. This vaccine approach was very successful in eradication programs for pseudorabies. A marker vaccine must be accompanied by a diagnostic test, which enables distinction of infected from vaccinated animals. These tests detect antibodies against a **glycoprotein that is lacking in the vaccine.** The desirable characteristics of the companion diagnostic test include the following:

- Antibodies are detectable in 2 to 3 weeks after infection, both in vaccinated and unvaccinated cattle.
- Antibodies must persist for at least 2 years, preferably lifelong.
- A low level of virus replication gives rise to detectable antibody formation.
- Cattle repeatedly given the matching marker vaccine remain negative in the test.
- The test should be suitable to detect antibodies in milk.
- The test has high sensitivity and specificity in comparison with conventional antibody tests.

Mutants of BHV-1 have been developed by deleting one or more of the nonessential glycoproteins. Marker vaccines offer the advantage of evaluating the effect of vaccination on the circulation of the field virus under naturally occurring conditions. Using a gE-deleted BHV-1 strain, both a killed virus and MLV marker vaccine have been developed. These vaccines induce all the relevant immune responses against BHV-1-specific immune reactions, including antibodies against gE. Both vaccines have the capacity to reduce, and even to stop, the spread of BHV-1. A serologic test that detects gE-specific antibodies in serum and milk is also available. These vaccines have been tested according to the current European requirements for the development of bovine vaccines. The live vaccine is safe in pregnant cattle and is considered safe for all kind of breeding cattle, including bulls. The live-virus marker vaccine is also efficacious in the presence of maternal antibody, and vaccination of very young calves, irrespective of their BHV-1 status, can be recommended. An inactivated BHV-1 gE-negative vaccine resulted in only a slight decrease of about 1.4 liters per cow in milk production after a double vaccination. One concern with this use of modified live BHV-1 gE-negative vaccines is the potential for recombination of vaccine-virus and field-virus strains resulting in the emergence of virulent BHV-1 virus that is gE-negative on serologic testing. This potential can be mitigated by development of double mutant vaccine strains, such as a gE and thymidine kinase mutant Bo-HV-1 strain.[10]

Combination or Multivalent Vaccines. The vaccines available for the control of diseases associated with BHV-1 infection are mostly multivalent antigen vaccines containing other respiratory pathogens such as PI-3, BRSV, and BVDV. Some also contain the antigens for the control of leptospirosis and campylobacteriosis. Vaccines containing only BHV-1 are not in common use. A Canadian field trial to compare the serologic responses in calves to eight commercial vaccines against BHV-1, PI-3, BRSV, and BVDV found some differences. Antibody responses to BHV-1 were higher in calves vaccinated with MLV vaccines than in those vaccinated with the inactivated vaccines. There were no differences in seroconversion rates and titers to BHV-1 between intranasal and MLV IM vaccines following a single vaccination. However, after double vaccination with MLV BHV-1 vaccines, both seroconversion rates and changes in titers to the virus were higher

in calves vaccinated IM than in those vaccinated intranasally. Whether or not these differences in antibody titers reflect differences in vaccine efficacy against naturally occurring disease in the field situation is unknown.

The vaccination of calves with multivalent vaccines containing MLV or MLV and inactivated BHV-1 is associated with virus-specific interferon gamma production and protection from clinical disease as a result of challenge 5 days after a single vaccination.

Immunization and Latency. Immunization with vaccines, as with natural infection, does not prevent subsequent infection and the possibility of latency.

Vaccination Programs in Herds

Beef Breeding Herds. Beef calves should be vaccinated 2 to 3 weeks before weaning as part of a preweaning preconditioning program. Calves vaccinated with the parenteral MLV BHV-1 vaccine before colostral BHV-1 antibody titers reach low levels do not develop an immediate, active serologic response, as indicated by serologic titers, but are sensitized to the virus. Revaccination at a later date, when maternal antibodies have decreased to undetectable levels, results in a marked serologic response. **Heifer and bull replacements are vaccinated at least 2 weeks before breeding.** When outbreaks of the respiratory disease occur in unvaccinated beef herds, all cattle in the herd may be vaccinated with the intranasal vaccine. Whether or not beef herds should be vaccinated annually following the initial vaccination is uncertain. There are field reports of outbreaks of abortion as a result of the virus in beef cattle that were vaccinated 3 years previously, which suggests that revaccination of breeding females every 2 years may be indicated. Because both natural infection and vaccination results in latent infection, it may be that the persistence of the virus, combined with natural exposure, may result in persistence of antibody. The duration of protective immunity following vaccination is uncertain, but usually lasts 1 year. Antibodies last for at least 5.5 years in heifers following experimental infection and complete isolation during that time.

The MLV BHV-1 vaccine given intranasally or parenterally can enhance the prevalence of infectious bovine keratoconjunctivitis in beef calves vaccinated between 4 and 10 months of age, when the risk for the ocular disease is highest. The explanation for the pathogenic mechanism is uncertain.

Feedlot Cattle. Feedlot cattle should be vaccinated at least 10 days before being placed in the lot, especially one in which the disease may be enzootic. If this is not done, a high incidence of the respiratory form of the disease may occur in recent arrivals. If vaccination before arrival is not possible, the next best procedure is to vaccinate the cattle on arrival and place them in an isolation starting pen for 7 to 10 days, during which time immunity will develop. A 2015 meta-analysis concluded that in natural exposure trials, beef calves vaccinated with commercially available vaccines against BHV-1, BVDV, BRSV, and PI-3 had slightly less than half the risk of developing clinical signs of pneumonia and approximately 1/5th the risk of dying from respiratory disease. Moreover, vaccination with modified live or inactivated IBR vaccine decreased the risk of developing clinical signs of respiratory disease by 39% to 46%, respectively, in beef and dairy calves to experimental challenge compared with unvaccinated controls.[11] Collectively, this is strong supportive evidence that vaccination against IBR is effective in beef calves in North America.

Dairy Cattle. The necessity of vaccinating dairy cattle will depend on the prevalence of the disease in the area and in the herd and the movement of cattle in and out of the herd. A closed herd may remain free of BHV-1 infection indefinitely and vaccination may not be indicated. But to avoid unpredictable abortion storms as a result of the virus in dairy herds, **heifer replacements should be vaccinated for the disease 2 to 3 weeks before breeding.** Vaccination of a large dairy herd with a persistent BHV-1 infection has been successful in controlling the respiratory form of the disease. The intranasal vaccine has been used extensively in newborn calves in problem herds, but its efficacy at such an age is unknown. **The parenteral vaccination of beef calves under 3 days of age with an MLV BHV-1 and PI-3 vaccine caused high mortality.** If the systemic form of the disease poses a threat to a potential calf crop, the pregnant cows could be vaccinated with the intranasal vaccine in late pregnancy; this will increase the level of colostral antibody available to the newborn calf and will provide newborn calves with protection against the highly fatal systemic form of the disease. A 2015 meta-analysis concluded that in natural exposure trials, dairy calves vaccinated with commercially available vaccines against BHV-1, BVDV, BRSV, and PI-3 had similar risk of developing clinical signs of pneumonia and dying from respiratory disease than unvaccinated controls.[11] The markedly different effect of vaccination in dairy calves to that seen in beef cattle may be because respiratory disease occurs most frequently before 6 months of age in dairy calves. However, as indicated previously, vaccination with modified live or inactivated IBR vaccine decreased the risk of developing clinical signs of respiratory disease to experimental challenge by 39% to 46%, in beef and dairy calves, respectively, compared with unvaccinated controls.[11] Collectively, there is moderate supportive evidence that vaccination against IBR is effective in dairy calves in North America.

Bulls intended for use in artificial insemination centers present a special problem of disease control because the virus in semen can have severe consequences on reproductive performance. Bulls that are seropositive to the virus must be considered as carriers and potential shedders of the virus, and should not be allowed entry to these centers. Not all bulls that are seronegative can necessarily be considered free of the virus, and regular attempts at the isolation of the virus must be made from preputial washing and semen. Bulls that become infected while at the centers should be kept isolated, culled, and replaced with clean bulls. Bulls from herds that routinely vaccinate against BHV-1 should not be vaccinated with conventional vaccines if destined for an artificial insemination center. Cattle destined for export should not be vaccinated in case importing countries prohibit the introduction of seropositive cattle. This will not guarantee that such animals will not become positive from natural infection. The use of marker vaccines has some potential in breeding bulls intended for artificial insemination units and for export.

Eradication

Eradication of the BHV-1 virus from a single herd or the cattle population in a country can be considered as an alternative to vaccination, particularly when the initial prevalence of infection based on serology is low.[2] Serologically positive animals are removed or culled, and only seronegative animals introduced into the herd. Control is focused on segregation and elimination of seropositive animals and reduction of animal movement to prevent spread. This approach is not feasible in countries with extensive cattle populations and where management practices result in movement of cattle from one region to another.

Eradication Using Marker Vaccines. Some countries are beginning an immunization program with the marker vaccines, which will protect the cattle against disease but still allow differentiation between vaccinated animals and those that have been naturally infected and are potential carriers of the latent virus. These infected animals could be eliminated over a period of time. Successful eradication depends not only on the efficacy of the vaccine but also on the quality of the tests. False-positive test results can lead to unnecessary culling of cattle, an increase of costs, and reduced cooperation of farmers in the eradication program. As an example, a compulsory eradication program for BHV-1 began in The Netherlands in 1998. The program required that farms either vaccinate all cattle twice yearly or be approved for a certified BHV-1-free or specific-pathogen-free (SPF) status. To become a certified BHV-1 free herd, cattle have to be sampled individually and all seropositive animals

culled as soon as their status is known. The BHV-1-free herd status is monitored by monthly bulk milk samples. The spread of BHV-1 between herds can be prevented using a surveillance system of sampling herds annually, both individual milk samples and blood samples.

Herds with BHV-2 infected (seropositive) animals are required to vaccinate with a glycoprotein E (gE)-negative BHV-1 vaccine. The vaccine may be either an inactivated or live vaccine both based on a spontaneous BHV-1 mutant without the complete gE gene. These so-called marker vaccines or DIVA vaccines allow the identification of cattle infected with the wild-type BHV-1 within a vaccinated population using a gE-ELISA or a commercially available gE-blocking ELISA that both specifically detect gE antibodies. The eradication program is based on the presumption that all BHV-1 wild-type strains express gE and induce antibodies that can be measured with a gE-blocking ELISA.

Loss of Certification. The probability of and risk factors for the introduction of BHV-1 into SPF Dutch dairy farms has been examined. A total of 95 SPF dairy farms were monitored for 2 years, during which time 14 introductions of infectious diseases occurred on 13 of the 95 farms, for a total incidence rate per herd-year at risk of 0.09. Outbreaks were usually associated with allowing cattle to return to their farm, cattle grazed more often at other farms, and protective clothing less often provided to the veterinarian. For a successful eradication program, farms should remain BHV-1 free, which can be achieved by a more-closed farming system. A more-closed farming system is one that rules out the possibility of direct contact with other cattle from other farms. Also, the farmer requests that professional visitors such as veterinarians and artificial insemination (AI) technicians to wear protective farm clothing when handling cattle. Protective farm clothing includes coveralls or overcoats and boots that can be worn over "off-farm" clothing and that the farmer provides to the visitors before handling cattle. A sanitary barrier is a covered area outside the barn in which visitors put on protective farm clothing over their off-farm clothes. A sanitary barrier has a "dirty" side, where visitors leave their off-farm boots, and a "clean" side, where visitors wear protective clothing and can enter the barn. All of these measures would be economical.

TREATMENT AND CONTROL

Treatment

Antimicrobial treatment for animals with a fever to address concurrent bacterial pneumonia (see treatment recommendations in this chapter for *Mannheimia hemolytica*) (R-1)

Control

Vaccination of beef calves ≥ 6 months of age against BHV-1 (preferably with modified live glycoprotein E–negative vaccine) or with modified live or killed vaccine against BHV-1, BVDV, BRSV, and PI-3 (R-1)

Vaccination of dairy calves less than 6 months of age against BHV-1 (preferably with modified live glycoprotein E–negative vaccine) (R-2)

Vaccination of dairy calves less than 6 months of age with modified live or killed vaccine against BHV-1, BVDV, BRSV, and PI-3 (R-3)

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LUNGWORM IN CATTLE

SYNOPSIS

Etiology The nematode *Dictyocaulus viviparus* (the bovine lungworm).

Epidemiology Disease seen mostly in dairy calves; immunity develops relatively quickly, but cattle will succumb if exposed to overwhelming numbers of infective larvae while grazing.

Signs Coughing, tachypnea, dyspnea.

Clinical pathology Characteristic larvae in feces (but not present during all stages of disease); eosinophilia; enzyme-linked immunosorbent assay (ELISA) tests for serum antibodies.

Lesions Large volumes of consolidation in diaphragmatic lobes of lung, emphysema, worms up to 8 cm long in bronchi (only in patent phase of disease).

Diagnostic confirmation Clinical pathology as noted; at necropsy, distribution of lesions in lungs and demonstration of worms in bronchi.

Treatment Eprinomectin, avermectins/milbemycins and benzimidazoles are active against all parasitic stages of *D. viviparus;* eprinomectin and avermectins also have a persistent protective effect; levamisole also used.

Control Vaccination; early-season anthelmintic prophylactic programs using suitable intraruminal boluses or multiple doses of avermectins/milbemycins; keep susceptible animals off potentially dangerous pasture.

ETIOLOGY

The nematode *Dictyocaulus viviparus* is the only lungworm of cattle. The disease it causes has many local names, including the following:

- Parasitic bronchitis
- Verminous pneumonia
- Verminous bronchitis
- Husk
- Hoose

Bovine lungworm has a very wide distribution through temperate and cold areas and, depending on climatic conditions and season, can cause serious losses.[1] The disease reaches its greatest importance in mild, damp regions of the British Isles and parts of western Europe. *D. viviparous* is also carried by the European bison, in which it causes a disease with similar pathologic lesions to those in cattle.[2,3] Deer carry similar parasites, including *D. eckerti* and *D. capreolus*. It is uncertain whether deer play a role in the transmission of *D. viviparus* but lungworm species are generally host specific.

LIFE CYCLE

Adult lungworms live in the trachea and bronchi. The females are prolific egg producers, and it has been estimated that a single infested calf may contaminate a pasture with 33 million larvae. The eggs are coughed up and swallowed. They hatch in the air passages or alimentary canal, and larvae are passed in the feces. These develop in the dung pat through to the infective third stage, which is protected by cuticles retained from both first and second molts. Because the ensheathed larvae cannot feed, glycogen granules are stored in the intestinal cells. Moisture is essential for the survival and development of the larvae, and a moderate temperature of 18° to 21°C (65–70°F) permits their full development to the infective state in 3 to 7 days. Larvae survive best in cool, damp surroundings, especially when the environment is stabilized by the presence

of long herbage or free water. Under optimum conditions, larvae may persist for over 1 year. They can overwinter in climates as cold as Canada and Germany. When warmer spring weather arrives, the larvae resume their motility but quickly die once their food stores are depleted.

Transmission occurs when cattle ingest third-stage larvae while grazing. These migrate through the intestinal wall to reach the mesenteric lymph nodes. From here they pass via the lymphatics to the venous bloodstream and through the heart to the lungs, where they break into the alveoli. They migrate up the bronchioles to their predilection site in the larger air passages and start to lay eggs some 3 to 4 weeks after infestation. Most adult worms succumb to immune expulsion within a few weeks. These events determine the progression of the clinical syndrome and their approximate timing is as follows:

1. Penetration phase (ingestion to arrival of larvae in lung), days 1 to 7
2. Prepatent phase (larvae in lung), days 7 to 25
3. Patent phase (mature worms in lung) days 25 to 55
4. Postpatent phase (lungworms disappearing from lung), days 55 to 70

EPIDEMIOLOGY

Bovine parasitic bronchitis is a sporadic and largely unpredictable disease. This is because immunity develops more quickly than is the case with many other nematode infections, but nevertheless can remain incomplete for many weeks and may wane in the absence of reinfection. In most grazing seasons, immunity will develop fast enough to protect calves against the accumulating numbers of infective larvae on the grass. The farmer may not even realize that the land is contaminated. Clinical outbreaks occur when weather patterns, management, or other factors result in sudden exposure to a pasture challenge sufficient to overwhelm any immunity that has already developed. In comparison with the gastrointestinal nematodes of cattle, relatively few worms (i.e., a few hundred or thousand) are required to produce clinical signs. Thus the disease is almost entirely confined to grazing cattle and occurs most frequently in young animals in their first year on grass, although outbreaks are becoming more common in adults.[4] The epidemiology of lungworm disease is largely concerned with factors determining the number of infective larvae on the pasture and the rate at which they accumulate.

Infective *D. viviparus* larvae are relatively inactive and are incapable of traveling more than 5 cm from the dung pat. Factors that disperse the larvae more widely over the pasture include mechanical spread by the following:

- Rain
- Earthworms
- Wheeled vehicles
- Human and animal feet

A fungus, *Pilobolus,* plays a particularly important role in this process and can transfer larvae across field boundaries. Fungal spores on grass pass through the grazing animal and germinate in the feces. *Dictyocaulus* larvae climb onto the sporangium (fruiting body), which fills with water and bursts, propelling the fungal spore and the lungworm larvae for distances of up to 3 m.[5]

Dairy calves are most vulnerable to lungworm disease because they are often reared indoors until 4 to 5 months of age and then placed on paddocks grazed each year by successive calf crops. If the paddocks are heavily contaminated, acute disease may occur in 1 week or so. Usually, however, only sufficient larvae survive the winter to induce low-grade asymptomatic infections in the susceptible calves, which then start to recontaminate the pasture and recycle the infection. With the high stocking densities commonly used, pasture challenge can reach pathogenic levels within 2 to 4 months. This model does not satisfactorily explain all outbreaks, and it has been suggested that larvae may be washed into the soil to emerge later (e.g., onto hay aftermath). Beef calves at grass with their dams are less likely to be affected as this system provides fewer opportunities for large numbers of larvae to accumulate, but outbreaks can occur particularly after weaning in the autumn.[6]

In older animals, larvae ingested in the autumn become hypobiotic and resume their development in the following spring. This event occasionally causes disease in housed cattle[6] but such infections are usually asymptomatic and provide a source of pasture contamination when these carrier animals are put out to graze. This is thought to be the main source of infection in more severe climates where overwintering larvae may not survive on the pasture, but carrier animals have also been incriminated in disease outbreaks in, for example, Louisiana in the United States.

Immunity to reinfestation occurring after initial exposure to *D. viviparus* is variable in degree and duration. It normally provides protection during the first grazing season and is boosted by exposure to overwintered larvae at the beginning of each subsequent grazing season. Cattle removed from infected pastures for long periods can suffer clinical disease when reexposed. Recently the number of outbreaks of parasitic bronchitis in yearling and adult cattle in the United Kingdom, Denmark and some other countries has been rising. Reasons for this are speculative but include the following:

- A decline in the use of vaccination
- Changes in weather patterns and management systems
- Use of highly effective anthelmintic strategies in the first grazing season that may prevent adequate antigenic exposure

PATHOGENESIS

Migrating *D. viviparus* larvae provoke little damage until they reach the lungs. Thereafter, passage of larvae up the bronchioles causes them to become blocked by mucus, eosinophils, and other inflammatory cells, leading to collapse of the alveolae that they supply. Coughing and dyspnea occur if a sufficiently large volume of lung tissue is affected. This is accompanied by pulmonary edema and interstitial emphysema. As no structural damage has yet occurred, treatment at this stage in the disease produces an immediate clinical response. Later, however, when mature parasites are in the major bronchi, eggs and fragments of worms killed by immunity are aspirated and provoke a foreign-body pneumonia. Secondary bacterial infections establish and sequelae such as bronchiectasis occur. Such lesions are slow to resolve, and treated animals will require a long recovery period. Later still, once all or most of the worms have been expelled, the alveolar lining cells of some 25% of recovering animals become cuboidal and nonfunctional. The reason for this is unknown but may be a response to substances released by the dead worms. Because this reaction is irreversible, many animals affected in this way will die.

The response of the lung varies widely depending on the number of larvae ingested, the nutritional status and age of the host, and whether or not it is exposed to lungworm infection for the first time. Vaccinated animals or those that have recovered from clinical or subclinical infection may cough and even become tachypneic if grazed on contaminated pasture. This is known as the "reinfection syndrome" and occurs as many larvae reach the lungs before succumbing to the immune response. Exposure of older previously infected animals to massive challenge may invoke a severe or fatal hypersensitivity reaction.

CLINICAL FINDINGS

Outbreaks vary in severity from sporadic coughing with no apparent production loss to acute cases with a rapidly fatal outcome. Individuals within a group are usually affected to varying degrees. Poorly nourished animals appear less able to withstand lungworm infection. Nevertheless, it is not unusual for severe infestations to be fatal in well-fed calves.

Acute cases have rapid shallow abdominal breathing of sudden onset that may reach a rate of 60 to 100 breaths/min. There is a frequent bronchial cough, a slight nasal discharge, a temperature of 40 to 41°C (104–105°F) and a heart rate of 100 to 120 bpm. The animal is bright and active and will attempt to eat, although respiratory distress often prevents this. Progress of the disease is

rapid, and within 24 hours dyspnea may become very severe, accompanied by mouth breathing with the head and neck outstretched, a violent respiratory heave and grunt, cyanosis, and recumbency. On auscultation, lung consolidation is evidenced by loud breath sounds, and crackles are heard over the bronchial tree. The crackling of interstitial emphysema commences over the dorsal two-thirds of the lung but is never as evident as in less acute cases. Fever persists until just before death, which usually occurs in 3 to 14 days and is greatly hastened by exercise or excitement. The case-fatality rate in this form of the disease is high, probably of the order of 75% to 80%.

Subacute disease is more common in calves than the very acute form. The onset is usually sudden, the temperature is normal or slightly elevated and there is an increase in the rate (60-70 breaths/min) and depth of respiration. An expiratory grunt is heard in severe cases and expiration may be relatively prolonged. There are frequent paroxysms of coughing. The course of the disease is longer, 3 to 4 weeks, and auscultation findings vary widely with the duration of the illness and the area of lung involved. In general, there is consolidation and bronchitis ventrally and marked emphysema dorsally. Affected animals lose weight very quickly and are very susceptible to secondary bacterial bronchopneumonia. The mortality rate is much less than in the acute form, but many surviving calves have severely damaged lungs. Consequently, they may remain stunted for long periods, and breathing may be labored for several weeks. Some surviving calves may show a sudden exacerbation of dyspnea around 7 to 8 weeks after the initial onset of disease. In these relapsed cases the prognosis is grave.

Adult dairy cattle are usually immune but sporadic outbreaks do occur as a result of waning immunity. Mortality is low but morbidity can be high, with reduced milk yields causing significant economic loss.[7-9] Coughing is a constant feature, but other clinical signs are variable and may include dyspnea, nasal discharge, and weight loss.[7] Sudden exposure of immune adults to massive challenge can cause severe interstitial pneumonia.

CLINICAL PATHOLOGY

The presence of *D. viviparus* larvae in feces confirms lungworm infestation, but their absence does not necessarily exclude the possibility of parasitic bronchitis. No larvae will be passed in the early stages of disease when the causal worms are still immature, nor will they be a constant finding when partially immune animals (e.g., dairy cows) succumb to challenge. In general, larvae can be found about 12 days after signs appear (i.e., around 24 days after infestation occurs). They are few in number at first but may become more numerous later.

Enzyme-linked immunosorbent assay (ELISA) tests using adult or larval worm antigen for the detection of *D. viviparous*-specific antibodies in serum and in milk (including bulk tank milk) have been developed.[10,11,12] Care is required with interpretation because antibodies to adult antigen may not be detectable until several weeks after primary challenge and do not correlate with the immune status of the animal. Eosinophilia is a fairly consistent finding but not pathognomonic.

An alternative method, if disease is suspected but the lungworms are still in the prepatent stage, is to examine pasture clippings for larvae. This is a laborious procedure because large amounts of herbage (0.5–1 kg) must be used, and the yield of larvae is low.

NECROPSY FINDINGS

Adult *D. viviparus* are up to 8 cm long and easily seen when the trachea and bronchi are cut open. Worms may also be recovered by lung perfusion. Up to several thousand may be present in severely affected animals. In prepatent disease, however, careful microscopic examination of bronchial mucus is necessary to find larvae. Adult worms may be few or absent if the case is of sufficient duration for immune expulsion to have taken place.

In acute cases, morphologic changes include the following:

- Enlargement of the lungs as a result of edema and emphysema
- Widespread areas of collapsed tissue of a dark pink color
- Hemorrhagic bronchitis with much fluid filling all the air passages
- Enlargement of the regional lymph nodes

Histologically, the characteristic signs are as follows:

- Edema
- Eosinophilic infiltration
- Dilation of lymphatics
- Filling of the alveoli and bronchi with inflammatory debris
- Larvae in the bronchioles and alveoli

In subacute cases, interstitial emphysema is usually gross. Areas of dark pink consolidation are present in the diaphragmatic lobe and may also occur in other lobes. They can occupy two-thirds of the lung volume. There is froth in the bronchi and trachea. The regional lymph nodes are enlarged. Histologically, eggs and larvae can be seen in the air passages, the bronchial epithelium is much thickened, the bronchioles are obstructed with exudate, and the alveoli show epithelialization and foreign-body giant-cell reaction.

The reinfection syndrome is characterized by the presence of numerous 5-mm gray-green nodules formed by lymphoreticular cells clustering around dead larvae.

DIAGNOSTIC CONFIRMATION

D. viviparus larvae may be demonstrated by placing feces on a fine sieve or dental gauze on the top of a water-filled funnel (the Baermann technique). The larvae that swim into the water and collect at the bottom of the funnel are less than 0.5 mm long, sluggish, and often appear curved or coiled. Their most important diagnostic feature is the presence of easily visible refractile granules in the intestinal cells. Because not all animals will be shedding larvae, samples should be taken from all, or at least a representative proportion, of the group. Grass samples are washed in water with a surfactant and the sediment Baermannized as described previously. A technique that effectively separates larvae from plant debris by migration through agar gel has been reported. Gathering grass close to dung pats maximizes chances of finding larvae. Cattle with parasitic bronchitis are likely to have eosinophilia, and serologic tests can be used to rule out some other respiratory diseases, such as infectious bovine rhinotracheitis (IBR).

In view of the uncertainties associated with laboratory tests for parasitic bronchitis and the need for prompt treatment, diagnosis often has to be based on clinical history, signs, and auscultation. Affected animals have usually grazed alongside potential carriers or had access to pasture previously used by susceptible calves or older carrier animals. The timing of the outbreak may coincide with that expected from recycling of an infection initiated by overwintered larva (often 2-4 months after turnout) or recent exposure to heavily contaminated land. Many of the clinical signs of parasitic bronchitis are common to pneumonias of bacterial and viral origin. One feature that may be of value in differentiation is the relative softness and paroxysmal nature of the cough in parasitic infection.

DIFFERENTIAL DIAGNOSIS

- Bacterial bronchopneumonia
- Acute and chronic interstitial pneumonia
- Viral pneumonia
- Acute interstitial pneumonia (fog fever)
- Heavy infestations with ascarid larvae on pastures contaminated with pig feces

In adult cattle, the major problem in diagnosis is to differentiate the acute form of the disease from acute interstitial pneumonia attributable to other causes. Clinically, the diseases are indistinguishable, and a history of movement onto a new pasture 1 to 2 weeks before the onset of the disease may be common to both. It is necessary to demonstrate *D. viviparus* antibodies in blood, worms at necropsy, and larvae in the herbage or in the feces of animals that previously grazed the pasture.

TREATMENT

TREATMENT AND CONTROL

Treatment

Eprinomectin (0.5 mg/kg TOPp, 0.2 mg/kg SC) (R-1)

Ivermectin (0.2 mg/kg, SC, PO; 0.5 mg/kg, TOPp) (R-1)

Doramectin (0.2 mg/kg, SC, IM) (R-1)

Moxidectin (0.2 mg/kg, PO, SC; 0.5 mg/kg, TOPp) (R-1)

Albendazole (10 mg/kg, PO) (R-2)

Oxfendazole (7.5 mg/kg, PO) (R-2)

Febantel (7.5 mg/kg, PO) (R-2)

Fenbendazole (5 mg/kg, PO) (R-2)

Netobimin (7.5 mg/kg) (R-2)

Levamisole (7.5 mg/kg) (R-3)

Control

Eprinomectin extended-release formulation (1.0 mg/kg, SC) (R-2)

Ivermectin long-acting formulation (0.63 mg/kg, SC) (R-2)

Vaccination

Live irradiated infective *D. viviparus* larvae (1000 larvae/calf, PO) (R-2)

PO, orally; SC, subcutaneously; TOPp, topical pour on formulation.

Anthelmintics may be used prophylactically to prevent disease from occurring, as a curative treatment once disease strikes, or to prevent reinfection following an outbreak. Avermectins and milbemycins are particularly useful for prophylaxis and prevention of reinfection because they are not only highly effective against the lungworms present in the animals at the time of treatment but have prolonged activity against subsequent incoming larvae. The duration of this persistent effect varies with compound and formulation.

Most modern broad-spectrum drugs are active against *D. viviparus*. Dosage rates and label claims vary from country to country according to local conditions and regulatory requirements. Avermectins and milbemycins (macrocyclic lactones) are particularly potent against immature and mature stages; doses of ivermectin, for example, as low as 0.05 mg/kg, are effective. At commercial dose rates, ivermectin by injection or as a pour-on formulation provides residual protection for up to 28 days; corresponding figures are up to 35 days for doramectin by injection and 42 days both for doramectin as a pour-on formulation and moxidectin by either route of administration. These compounds are given at 0.2 mg/kg by injection and 0.5 mg/kg as a pour-on formulation. Eprinomectin is the compound of choice for adult dairy cattle because it has a nil milk withdrawal period[14] and provides residual protection of up to 28 days when given topically (0.5 mg/kg). Albendazole (10 mg/kg), febantel (7.5 mg/kg), fenbendazole (5 mg/kg), netobimin (7.5 mg/kg), and oxfendazole (4.5 mg/kg), which are given orally, are active against all stages of the parasite but have no residual activity. Levamisole (oral or injection—7.5 mg/kg; pour on—10 mg/kg) also has activity against lungworm but no persistent effect.

Sustained-release intraruminal devices ("boluses") provide extended periods of protection. For example, fenbendazole is released for up to 140 days from one bolus. There are also pulse release boluses containing oxfendazole that release five or six anthelmintic doses at 3-week intervals. Most boluses normally protect against disease but may allow some worms to establish (in the case of the fenbendazole bolus) or to reach the lungs between pulses (oxfendazole bolus), which may allow immunity to develop. Formulations of eprinomectin extended-release injection have been developed that have greater than 98% efficacy against *D. viviparus* and provide protection from reinfestation for up 150 days in catlle.[13,14,15,16] Similarly, an ivermectin long-acting injectable formulation has been shown to have up to 100% efficacy against *D. viviparous* in cattle for at least 77 days.[17]

For veterinarians in the field, the outcome of therapeutic treatment is often unpredictable because it depends on the amount of structural damage in the lungs. Best results are obtained early in the course of disease when most pathologic changes can be quickly resolved. In severe cases, treatment may initially exacerbate clinical signs because the death and disintegration of many worms in the air passages releases antigens and adds to the mass of foreign material that can be aspirated. Because of animal welfare considerations and the high risk of mortality, anthelmintic treatments are often combined with an antihistamine or nonsteroidal antiinflammatory drug (NSAID) such as flunixin to reduce the severity of the reaction to the larvae and an antibiotic or sulfonamide to prevent secondary bacterial infection. Severely affected animals should be brought indoors for nursing and all other members of the group removed from the contaminated pasture and placed on clean grazing ground.

CONTROL

Two major strategies of control derive from the premise that the main factor governing the occurrence of disease is the density of *D. viviparus* larvae on pasture grazed by susceptible cattle. First, cattle grazing potentially contaminated pasture can be protected by vaccination or anthelmintic cover. Alternatively, steps can be taken to ensure that pastures are safe for grazing. This is usually achieved by prophylactic anthelmintic programs, but delaying spring turnout until overwintered larvae have died away is a theoretical option on organic farms.

Sensible grazing management is important in all systems but cannot be relied upon, per se, for controlling parasitic bronchitis in view of the unpredictable nature of the disease. Although natural immunity provides adequate protection on many farms, it cannot be accurately measured nor predetermined. With the possible exception of beef suckler systems, calves should not be run with or follow older cattle because these may harbor asymptomatic patent infections and contaminate the pasture. An important consideration is that clean pasture can be contaminated by larvae from neighboring fields carried on windborne fungal spores (see epidemiology paragraph earlier). Although the numbers of larvae spread in this way are likely to be small, they can initiate the epidemiologic cycle culminating in disease after some weeks.

Vaccination of calves with two doses of 1000 infective larvae attenuated by irradiation is a long-established and effective method of preventing disease. Only healthy calves should be vaccinated and they should be at least 8 weeks old. The vaccine is given 6 and 4 weeks before turnout. Exposure to lightly contaminated pasture will boost immunity, but low-grade patent infections may develop in some animals. Vaccinated and nonvaccinated calves should not be grazed together because the former may contaminate the pasture, enabling lungworm to cycle through the susceptible animals. The vaccine gives a high level of protection under most conditions, but vaccinated calves should not be put onto heavily contaminated pasture. Coughing may occur when immune responses kill lungworm larvae in the lungs. Overt disease can occur in cases of overwhelming challenge. To avoid such problems on severely affected farms, vaccinated calves should be allowed only gradual access to pasture. An experimental recombinant subunit vaccine, based on the parasite's paramyosin as a recombinant antigen, that overcomes the disadvantages of the attenuated vaccine has been developed.[18]

In some endemic areas, for example, in the south of Ireland, which has mild winters and an early start to the grazing season, the ideal vaccination program described earlier may be inconvenient and it is possible, by cautiously avoiding periods when massive pasture contamination is likely to occur, to vaccinate at pasture. Calves are sometimes vaccinated at less than 8 weeks old to allow spring-born calves to graze during late summer and autumn, but optimal protection may not be afforded in all cases.

Strategic anthelmintic programs provide an alternative to vaccination. The aim is to suppress the infection initiated by overwintered larvae and thereby prevent subsequent contamination of the pasture. This can be done by application of a suitable intraruminal bolus at or just before spring turnout or by giving two or three doses of an avermectin/milbemycin during the early grazing season.

These systems are designed to control parasitic gastroenteritis and lungworm. Clinical field experiments have demonstrated good results with ivermectin, fenbendazole, and oxfendazole boluses and with ivermectin treatments given at 3, 8, and 13 weeks after turnout, or doramectin administered at turnout and again 8 weeks later. Calves may become vulnerable after the period of anthelmintic cover if pasture contamination occurs (e.g., because of fungal spread). An extra anthelmintic treatment may be indicated in regions with a very long grazing season. Calves that are exposed to infection but protected by chemoprophylaxis during their first grazing season generally have substantial resistance to reinfection in their second year. Nevertheless, field experiments have shown that immunity can be compromised to a degree related to the level of protection provided. There is concern that such intensive treatment may provoke anthelmintic resistance, but no resistant strains of *D. viviparus* have yet been reported.

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ATYPICAL INTERSTITIAL PNEUMONIA OF CATTLE (ACUTE BOVINE RESPIRATORY DISTRESS SYNDROME, ACUTE PULMONARY EMPHYSEMA, AND EDEMA)

SYNOPSIS

Etiology Uncertain; a number of etiologies such as D,L-tryptophan in forage, inhalation of toxic gases and fumes, hypersensitivity to molds, mycotoxicosis, and plant poisonings or feed supplementation with melengestrol acetate have been discussed.

Epidemiology Occurs primarily in adult cattle moved from dry to lush pasture and incidentally in feedlot cattle. Outbreaks or incidental cases of AIP in adult cattle moved from dry to lush pasture in autumn. In feedlot cattle incidental cases are observed toward the end of the finishing period.

Signs Outbreaks of acute respiratory distress in pasture form of disease within days of moving to lush pastures; severe dyspnea, open-mouth breathing with extended head and neck, expiratory grunt, subcutaneous emphysema, and rapid death. Subacute form less severe and may survive but develop cor pulmonale later.

Clinical pathology None clinically applicable.

Lesions Enlarged firm lungs that do not collapse, diffuse congestion and edema, interstitial and bullous emphysema, cranioventral consolidation, hyaline membrane formation, alveolar epithelial hyperplasia, fibrosis.

Diagnostic confirmation Lesions at necropsy.

Treatment Symptomatic, no effective treatment available.

Control Grazing management. Use of antimicrobials to control conversion of tryptophan to 3-methylindole in pastured animals.

Atypical interstitial pneumonia (AIP) of cattle has been known for many years under many different terms, including acute interstitial pneumonia, acute pulmonary emphysema and edema (APEE), acute bovine respiratory distress syndrome (ABRDS), bovine pulmonary emphysema, pulmonary adenomatosis, bovine asthma, pneumoconiosis, and "fog fever." The syndrome that is characterized by diffuse or patchy damage to alveolar septa is known to occur in an acute and chronic form. The acute presentation, frequently occurring as an outbreak in adult pastured cattle a few days after they are moved from heavily grazed summer pastures to lush fall pastures, is also referred to as "fog fever." A similarly acute to peracute syndrome in feedlot cattle, primarily affecting animals toward the end of the finishing period, is known as acute or atypical interstitial pneumonia of feedlot cattle. A more chronic form occurring sporadically, often with secondary bacterial involvement, has also been described.

The term *atypical interstitial pneumonia* refers to some clinical characteristics of the syndrome that set it apart from the common acute infectious respiratory tract diseases, especially the viral diseases also causing interstitial pneumonia. Clinically the syndrome is atypical, especially compared with the bacterial pneumonias:

- Presentation can be acute or chronic.
- Acute or chronic respiratory distress in absence of toxemia
- Syndrome is progressive and generally nonresponsive to treatment.
- Pathology consists of varying degrees of pulmonary emphysema, edema, hyaline membrane formation, and alveolar epithelial cell and interstitial tissue hyperplasia.

ETIOLOGY

The precise etiology of the condition is currently not entirely understood, but because of the obvious epidemiologic differences between the conditions occurring in pastured, housed, and feedlot cattle, it is assumed the AIP can have several different etiologies, all leading to the characteristic lung lesions. The etiologies presented in the following subsections have been proposed.

Ingestion of Excessive Amounts of D,L-Tryptophan With the Forage

Clinical cases of AIP are frequently reported in adult cattle that have been moved from a dry to a lush pasture in the autumn season. Specific forages have not been implicated, but affected cattle have often been consuming alfalfa, kale, rape, turnip tops, rapidly growing pasture grass, and several other feeds. The levels of tryptophan in lush pasture are sufficient to yield toxic doses of 3-methylindole, the product of tryptophan fermentation in the rumen. A 450-kg cow eating grass at an equivalent DM intake of 3.5% of BW/day with a tryptophan concentration of 0.3% of DM would ingest 0.11 g tryptophan/kg BW/day. The total amount ingested over a 3-day period would approximate the single oral dose of 0.35 g/kg BW needed to reproduce the disease experimentally. However, pasture levels of tryptophan are not necessarily higher in those associated with the disease compared with normal pastures.

D,L-tryptophan is converted in the rumen to 3-methylindole (3mI), which, when given orally or intravenously, also produces the lesions characteristic for AIP in cattle and goats. In some naturally occurring cases of AIP in beef cows changed from a dry summer range to a lush green pasture, there is a marked increase in the ruminal levels of 3mI, whereas in other cases the levels are not abnormal. Failure to detect abnormally high levels in the rumen and plasma of naturally occurring cases may be related to the rapid metabolism and elimination of 3mI.

Ingestion of D,L-tryptophan has generally been discounted as a possible cause for AIP in feedlot cattle because of its sporadic occurrence and the lack of an epidemiologic association between occurrence of the disease and ration changes. Nevertheless, significantly higher concentration of a 3mI metabolite in the blood of animals affected by AIP compared with healthy control animals were measured in one study, suggesting a possible etiologic role of D,L-tryptophan in AIP in feedlot cattle.[1]

Hypersensitivity to Molds

AIP has also been associated with chronic hypersensitivity to moldy hay based on the presence of serum precipitins of the thermophilic antigens of *Thermopolyspora polyspora, Micropolyspora faeni,* and *Thermoactinomyces vulgaris* in cattle with allergic alveolitis, a condition also termed as "bovine farmer's lung." In Switzerland, a high incidence of serum precipitins against *Micropolyspora faeni* (60%) and moldy hay antigen (80%) was demonstrated in exposed but apparently healthy cattle from an area where the chronic presentation of bovine farmer's lung is common. Outbreaks of acute respiratory disease in adult cattle as a result of acute allergic pneumonitis can occur 15 hours after the introduction of severely moldy hay. Serologic investigation and provocative challenge may reveal a hypersensitivity pneumonitis attributable to allergens of *Micropolyspora faeni.* A hypersensitivity pneumonitis has been produced experimentally in calves by exposure to aerosols of *Micropolyspora faeni* with or without prior sensitization by subcutaneous injection of the antigen.

Although clinically allergic pneumonitis and AIP share a very similar presentation, there are significant pathologic differences indicating that allergic pneumonitis and AIP are different conditions.[2] Although hyaline membrane formation, which is characteristic for AIP, is rarely seen with allergic pneumonitis, the latter is typically associated with microscopic granuloma formation that is not seen with AIP.[2]

Inhalation of Toxic Gases and Fumes

Incidental cases of AIP have been reported in cattle exposed to different noxious gases and fumes, such as silo gas, nitrogen dioxide, chlorine gas, or zinc oxide fumes.[3] The experimental inhalation of nitrogen dioxide gas is capable of causing acute interstitial pneumonia in cattle and severe alveolar edema and emphysema in pigs, but it seems unlikely that animals of either species would be exposed naturally to a significant concentration of the gas for a sufficiently long period to produce such lesions.

Pigs that survived experimental exposure to silo gas did not have the lesions seen in silo-fillers' disease in humans, and experimental exposure of cattle to nitrogen dioxide gas produces lesions that do not occur in naturally occurring AIP. Acute pulmonary emphysema and deaths have occurred in cattle exposed to zinc oxide fumes produced by the welding of galvanized metal in an enclosed barn housing cattle.

Parasitic Infestation

For many years it was thought that massive infestation of the lungs by large numbers of lungworm larvae in a lungworm-sensitized animal could cause an allergic reaction resulting in the development of AIP. The possibility of such hypersensitivity as being associated cannot be totally ignored, but at the present time there is no evidence to support such a theory. Such hypersensitivity may occur when the level of larval infestation of pasture is extremely high, but it is not involved in the great majority of cases. In most cases of naturally occurring AIP, there is no laboratory evidence of lungworm infestation of affected and in-contact animals. Reinfection of cattle with lungworm will occur 2 to 3 weeks following introduction to an infected pasture and cause acute respiratory distress that may be indistinguishable clinically from AIP.

The migration of abnormal parasites, particularly *Ascaris suis,* has been observed to cause an acute interstitial pneumonia in cattle that were allowed access to areas previously occupied by swine.

Mycotoxicosis and Plant Poisonings

The ingestion of sweet potatoes infested with the mold *Fusarium solani* has been incriminated as a cause of AIP in cattle. Growth of the mold on the potatoes produces the toxins ipomeamarone and ipomeamaronol and a lung edema factor. The latter is a collective term for a group of substances capable of causing death associated with severe edema and a proliferative alveolitis of the lungs of laboratory animals. It produces a respiratory syndrome that is clinically and pathologically indistinguishable from AIP.

The fungus *Fusarium semitectum* growing on moldy garden beans, *Phaseolus vulgaris,* which were discarded on pasture, was associated with acute pulmonary emphysema in cattle that consumed the beans and their vines. The fungus produces a pulmonary toxin. The pulmonary toxin 4-ipomeanol (ipomeanol) accumulates in mold-damaged sweet potatoes and induces pulmonary edema, bronchiolar necrosis, and interstitial pneumonia in many mammalian species. Outbreaks have occurred in lactating cows following ingestion of sweet potatoes damaged by *Myzus tersicae.* Other *Fursarium* spp. have been found in peanut-vine hay, which has been associated with acute respiratory distress and atypical interstitial pneumonia in adult beef cattle. The population mortality rate as a result of respiratory disease was about 12% and the case-fatality rate 77%. Clinical signs occurred within a few days to 2 months after the animals were fed the peanut-vine hay.

A weed, *Perilla frutescens,* is considered to be a cause of the disease in cattle in the United States and wherever the plant is found. High morbidity and high case-fatality rates are characteristic, and the plant contains a perilla ketone that can be used to produce the disease experimentally.

Turf-quality perennial ryegrass straw (*Lolium perenne*) infected with the endophyte (*Acremonium lolii*), which yields toxic substances, including lolitrem-B, has been associated with atypical pneumonia in weaned beef calves. However, feeding the suspect hay resulted in typical ryegrass staggers but not atypical interstitial pneumonia.

Melengestrol Acetate

Melengestrol acetate (MGA), a feed additive commonly fed to feedlot heifers to suppress estrus, has been associated with AIP based on epidemiologic evidence. Data obtained from Canadian feedlots indicated that the great majority of cases of AIP occurred in heifers and that discontinuing MGA treatment resulted in a reduced number of emergency slaughters, most of which were attributable to AIP.[2] Further work did not reveal any effect of oral MGA administration on plasma 3mI concentration. If MGA does play a role in the etiology of AIP, the mechanism through which this occurs is not clear.

Bacterial and *Mycoplasma* spp. Infections

There is no evidence that any of the common bacterial pathogens of cattle such as *Mannheimia haemolytica, Pasteurella multocida, Histophilus (Haemophilus) somni,* or *Mycoplasma* spp. are primarily associated with AIP. In a series of feedlot cattle with clinical findings consistent with AIP, the pathogens were present in the lung tissues of some animals at necropsy, but their presence was not considered as a primary cause of the pneumonia but rather secondary to the initial injury of the lung that was undetermined.

Viral Infections

Certain viral infections of the lung may result in interstitial pneumonia. In the interstitial pneumonias caused by the bovine respiratory syncytial virus (BRSV) there is a bronchiolitis and alveolitis, and these should be termed bronchiointerstitial pneumonias. The BRSV is an important cause of outbreaks of acute interstitial pneumonia in beef calves 2 to 4 weeks after weaning. Pathologic evaluation of the lung tissues of feedlot cattle that had acute interstitial pneumonia found that BRSV was not a causative agent. In a series of cases of interstitial pneumonia in feedlot cattle in Saskatchewan, the presence of the BRSV antigen was demonstrated in only 7% of cases, and there was more severe bronchiolar epithelial necrosis than in the other cases that were negative for the virus.

EPIDEMIOLOGY

AIP occurs primarily in adult cows and bulls, usually 4 to 10 days after they are moved abruptly from a dry or overgrazed summer pasture to a lush autumn pasture. The new pasture may or may not have been grazed during that summer, and the species of grass or plants does not seem to make a difference, but usually there is some lush regrowth of

grass, legume, or other palatable plants. Merely changing pasture fields in the autumn has precipitated the disease.

AIP in pastured animals usually occurs in outbreaks, with the morbidity ranging from 10% in some herds up to 50% and higher in others, with a case fatality ranging from 25% to 50%. It is not unusual to observe a mild form of the disease in about one-third of the adults at risk, but only 10% of those at risk may be severely affected. Often, a number of cows are found dead without premonitory signs; many others are severely ill and die within 24 hours. Calves and young growing cattle up to 1 year of age grazing the same pasture are usually unaffected.

A retrospective analysis and random sample survey of cattle ranches in northern California found that the type of forage management has a significant effect on the occurrence of the disease. The greatest occurrence of the disease was in herds where the cattle were moved from summer ranges to second-growth hay fields or to irrigated pastures or from one irrigated field to another. The adult morbidity rate was 2.6% and the case-fatality rate about 55%. The disease did not occur on ranches with limited or no movement of cattle from summer ranges to lush autumn pastures. The timespan over which the disease occurs during the autumn months is only 2 to 4 weeks. The incidence of the disease declines rapidly following the first frost. The disease has also occurred in the same herd on the same pasture in successive years.

AIP in pastured cattle has been recorded in Canada, the United States, Great Britain, Holland, New Zealand, and other countries. The disease is rare in Australia. The disease has been recognized in France for many years as an aftermath disease or aftermath emphysema, especially in the Normandy region.

Some reports have suggested a breed predilection, with Herefords being more commonly affected than the Jersey, Holstein, Shorthorn, and Angus breeds, but there are few exact epidemiologic data to support the observation.

AIP in feedlot cattle is recognized as important cause of economic loss in feedlot cattle in western Canada and the United States. The disease occurs sporadically, and the incidence is about 2.8% of all cattle placed in feedlots.[4] Cases occur most commonly during the summer and fall months, with a higher incidence rate toward the end of the finishing period. In southern Alberta, the disease is most common during hot, dry, and dusty spring and summer days, and typically affects animals expected to be ready for slaughter within 15 to 45 days. Some feedlot operators have observed that the disease is more common in cattle exposed to excessive dust from bedding.

In southern Alberta feedlots, the disease occurred late in the finishing period, when animals had been on feed an average of 114 days and weighed 475 kg. All confirmed cases were in heifers, and plasma concentrations of 3mI metabolites (adducts) were higher in heifers with AIP than in controls. Most of the heifers were receiving melengestrol (MGA) orally to suppress estrus. The odds of an animal with acute interstitial pneumonia being a heifer were 3.1 times greater than the odds that an animal with the disease was a steer. In some large feedlots the estimated relative risk was 4.9.

Other types of atypical interstitial pneumonia occur sporadically and may affect only a single animal or several over a period of time. There is not necessarily a seasonal incidence except in areas where cattle are housed and fed dusty and moldy hay during the winter months. AIP has been reported to occur in weaned beef calves about 4 weeks after weaning.

PATHOGENESIS

Because of the number and variety of circumstances in which acute or chronic interstitial pneumonia occurs, it is difficult to suggest a basic underlying cause, or to explain the mechanisms for the development of the lesions and the variations that occur from one circumstance to another.

The L-isomer of tryptophan contained in feed is metabolized by ruminal microorganisms to indoleacetic acid, which is then converted to 3-methylindole (3mI). The conversion of L-tryptophan to 3mI is maximal at a ruminal pH near neutrality. The 3mI is absorbed from the rumen and metabolized by a mixed-function oxidase system to an active intermediate, which has pneumotoxic properties.

Bioactivation of 3mI by alveolar Clara cells leads to profound cellular injury in Clara and type-1 alveolar epithelial cells and, ultimately, atypical interstitial pneumonia. It is postulated that the compound responsible for causing the injury is the electrophilic metabolite of 3mI, 3-methylenedolenine (3mEIN), which forms stable adducts with cellular macromolecules. (Adducts are compounds formed by an addition reaction.)

Concentrations of 3mEIN in lung tissue and blood were higher in feedlot cattle that had died of AIP than in healthy feedlot cattle. However, lung tissue concentrations of 3mEIN were similar in samples from cattle with interstitial pneumonia and bronchopneumonia. Mean concentration of 3mEIN-adduct increased to a maximum value on day 33 and then decreased to a minimum on day 54 after arrival in the feedlot. Plasma 3mI concentrations initially decreased and remained low until after day 54. Neither 3mEIN-adduct concentrations nor plasma 3mI concentrations were associated with deleterious effects on weight gains.

The reaction that occurs is a nonspecific but fundamental reaction of the pulmonary parenchyma to a wide variety of insults that may be ingested, inhaled, or produced endogenously. Pulmonary edema is the first morphologic change occurring in ruminants given 3mI. The edema is preceded by degeneration, necrosis, and exfoliation of type I alveolar septal cells. During the **acute stage**, there is flooding of the alveoli with serofibrinous exudate, congestion, edema of alveolar walls, and hyaline membrane formation. Varying degrees of severity of interstitial emphysema also occur. The interstitial emphysema may spread within the lymphatics to the mediastinum and into the subcutaneous tissues over the withers, over the entire dorsum of the back, and, occasionally, over the entire body, including the legs. If the acute phase is severe enough, there is marked respiratory distress and rapid death from hypoxemia. Unlike the bacterial pneumonias, the emphasis is on edema and proliferation rather than on necrosis.

The lesions have been produced experimentally in cattle, sheep, and goats following oral or IV administration of 3mI. Calves appear be more resistant to experimental toxicity with 3mI than adults, which supports the observation that the naturally occurring disease is uncommon in calves grazing the same pasture in which adults are affected.

In case the animal survives the acute stage a **proliferative stage** follows that is marked by proliferation of alveolar type II cells. There is alveolar epithelialization and interstitial fibrosis, the latter being progressive and irreversible. The central features of chronic interstitial pneumonia are intraalveolar accumulation of mononuclear cells, proliferation and persistence of alveolar type 2 cells, and interstitial thickening by accumulation of lymphoid cells and fibrous tissue. Diffuse fibrosing alveolitis is a form of chronic interstitial pneumonia of uncertain etiology, but it is possibly the chronic form of AIP.

AIP has been recorded **in sheep,** and there was extensive alveolar epithelialization. In Norway, an acute respiratory distress syndrome has occurred in lambs moved from mountain pastures onto lush aftermath pasture. The lesions were those of AIP and alveolar epithelial hypersensitivity to molds in the grass is being explored. The experimental oral administration of 3mI to lambs will result in acute dyspnea and lesions similar to those that occur in cattle and adult sheep following dosing with 3mI. However, the lesions in experimental lambs are different from those that occur in lambs affected with the naturally occurring disease.

CLINICAL FINDINGS

This acute form of **AIP in pastured cattle** is usually obvious. Within 4 to 10 days after adult cattle have been moved onto a new pasture, they may be found dead without any premonitory signs. In the experimental disease, the typical clinical signs of respiratory disease appear within 24 to 36 hours

after the oral administration of L-tryptophan to adult cattle and within 4 days, 50% of the dosed cows will die. One or several cattle may exhibit labored breathing, often with an expiratory grunt, open-mouthed breathing, head and neck extended, frothing at the mouth, and anxiety. Severely affected cattle do not graze, stand apart from the herd, and are reluctant to walk. If forced to walk, they may fall and die within a few minutes. Moderately affected cattle continue to graze, but their respirations are increased above normal. Coughing is infrequent regardless of the severity. The temperature is normal to slightly elevated (38.5–39.5°C [102–103°F]) but may be up to 41 to 42°C (106–108°F) during very warm weather. There is a similar variation in the heart rate (80-120/min), and those with a rate of more than 120/min are usually in the terminal stages of the disease. Bloat and ruminal atony are common in severe cases. Subcutaneous emphysema is common over the withers and may extend to the axillae and ventral aspects of the thorax. The nostrils are flared, and the nasal discharge is normal. Diarrhea may occur but is mild and transient.

Loud, clear breath sounds audible over the ventral aspects of the lung, indicating consolidation without bronchial involvement, are the characteristic findings on auscultation in the early stages of the acute disease. The intensity of the breath sounds may be less than normal over the dorsal parts of the lung if involvement is severe, but in animals that survive for several days the loud crackles characteristic of interstitial emphysema are of diagnostic significance. Most severely affected cases will die within 2 days of onset, but less severe cases will live for several days and then die of diffuse pulmonary involvement. Those that survive longer than 1 week will often have chronic emphysema and remain unthrifty. Of those moderately affected cattle that recover in a few days, some will develop congestive heart failure a few months later, as a result of chronic interstitial pneumonia (cor pulmonale). Calves running with their adult dams will usually not be affected.

AIP in nonpastured cattle such as feedlot cattle usually occur sporadically, but several animals may be affected over a period of time. There may or may not be a history of a change of feed or the feeding of moldy or dusty feed. In many cases, a few days will elapse after the appearance of signs before the owner is aware of the affected animals. The animal may have been treated with an antimicrobial for a bacterial pneumonia with little or no response. Dyspnea, increased respiratory effort sometimes with a grunt, deep coughing, a fall in milk production, an absence of toxemia, a variable temperature (38.5-40°C [102-104°F]) and a variable appetite are all common. On auscultation there are loud breath sounds over the ventral aspects of the lungs and crackles over both dorsal and ventral aspects. The presence of moist crackles suggests secondary bacterial bronchopneumonia. Subcutaneous emphysema is uncommon in these, and most will become progressively worse.

Yearling cattle with acute interstitial pneumonia that may be viral in origin may become much worse and die in a few days in spite of therapy. Mature cattle affected with the chronic form of AIP will survive in an unthrifty state with the chronic disease for several weeks and even months.

The major clinical features of all these other interstitial pneumonias are obvious respiratory disease, lack of toxemia, poor response to treatment, progressive worsening, and abnormal lung sounds distributed over the entire lung fields.

DIFFERENTIAL DIAGNOSIS

Atypical interstitial pneumonia (AIP) is usually obvious when presented with an outbreak of acute respiratory disease in adult cattle that have recently been moved onto a new pasture. The onset is sudden; several cattle may have been found dead, and many are dyspneic.

Clinical differential diagnoses for AIP include:

- **Pneumonic pasteurellosis** (shipping fever, enzootic pneumonia of calves) that is characterized by fever, toxemia, mucopurulent nasal discharge and less dyspnea; young cattle are more commonly affected, and there is a beneficial response to therapy within 24 hours.
- **Organophosphatic insecticide poisoning** may resemble AIP because of the dyspnea, but additionally there is pupillary constriction, mucoid diarrhea, muscular tremor and stiffness of the limbs, and no abnormal lung sounds.
- **Nitrate poisoning** may occur in cows moved into a new pasture with high levels of nitrate. Many cows are affected quickly, they are weak, stagger, gasp, fall down, and die rapidly. The chocolate brown coloration of the mucous membranes, the lack of abnormal lung sounds, and the response to treatment are more common in nitrate poisoning.
- **Other interstitial pneumonias** in cattle are generally not associated with a change of pasture in the autumn and are difficult to diagnose clinically and pathologically, especially when they occur in a single animal. The chronic and subacute types of interstitial pneumonia are difficult to differentiate from each other and from other pneumonias of cattle.
- **Extrinsic allergic alveolitis (bovine farmer's lung)** occurs in housed cattle exposed to dusty or moldy feeds for a prolonged period and is characterized by a history of chronic coughing, weight loss, poor milk production, occasionally green-colored nasal discharge, and dry crackles over most aspects of the lungs. Not infrequently, acute cases occur, and animals die within a week after the onset of signs.
- **Verminous pneumonia** caused by *Dyctiocaulus viviparus* occurs in young cattle on pasture in the autumn months and causes subacute or acute disease that may resemble AIP clinically but not epidemiologically. Identification of the larvae in the feces or tissues of affected animals should be attempted.
- **Verminous pneumonia** caused by aberrant migration of *Ascaris suis* larvae may be indistinguishable from acute interstitial pneumonia, but a history of previous occupation of the area by pigs may provide the clue to the diagnosis, which can only be confirmed on histologic examination of tissues.

CLINICAL PATHOLOGY

There are no abnormalities of the hemogram or serum biochemistry that have any diagnostic significance. Examination of feces and forage for lungworm larvae will aid in differentiation from verminous pneumonia if past the prepatent period.

NECROPSY FINDINGS

In AIP the lungs are enlarged and firm and do not collapse on cutting. In the early stages of acute cases they contain much fluid that is more viscid than usual edema fluid. The pleura is pale and opaque and appears to be thickened. In peracute cases, the entire lungs are homogeneously affected in this way. Such cases usually have edema of the larynx.

In the more common acute case, the lung has a marbled appearance. Adjacent lobes may be affected with any one of four abnormalities. Areas of normal, pink lung are restricted to the dorsal part of the caudal lobes. There are areas of pale tissue indicative of alveolar emphysema, areas of a dark pink color affected by early alveolar exudation, yellow areas in which the alveoli are filled with coagulated protein-rich fluid, and dark red areas where epithelialization has occurred. The latter two lesions are firm on palpation and resemble thymus or pancreas. They are more common in the ventral parts of the cranial lobes.

In chronic cases, as a sequela to the acute form described previously, the obvious differences in the age of the lesions suggest that the disease progresses in steps by the periodic involvement of fresh areas of tissue. In all cases there is usually a frothy exudate, sometimes containing flecks of pus, in the bronchi and trachea, and the mucosa of these passages is markedly hyperemic.

Histologically, the characteristic findings are an absence of inflammation, except in the case of secondary bacterial invasion, and the presence of an eosinophilic, protein-rich

fluid that coagulates in the alveoli or may subsequently be compressed into a **hyaline membrane.** This is more apparent in acute cases, and if animals live for a few days, there is evidence of epithelialization of the alveolar walls. In longstanding cases, there is extensive epithelialization and fibrosis. There is a lack of obvious lesions of the small airways, which differentiates interstitial pneumonia from bronchopneumonia.

Bacteriologic examination of the lungs is often negative, although in longstanding cases in which secondary bacterial pneumonia has developed, *Pasteurella multocida, Mannheimia haemolytica, Streptococcus* spp., and *Trueperella* (formerly *Arcanobacterium*) *pyogenes* may be found. A careful search should be made for nematode larvae.

TREATMENT

The treatment of AIP in cattle is empirical and symptomatic because there is no specific therapy available. The lesion is irreversible in severe cases, and treatment is unlikely to be effective. When outbreaks of the disease occur on pasture, the first reaction is to remove the entire herd from the pasture to avoid the development of new cases. However, almost all new cases will usually occur by day 4 after the onset of the outbreak, and removal from the pasture usually will not prevent new cases. Conversely, leaving the herd on pasture usually will not result in additional cases. Severely affected cattle should be removed from the pasture with extreme care, very slowly, and only if necessary, and they should be moved to shelter from the sun. Immediate slaughter for salvage may be indicated in severe cases. Mild or moderately affected cases will commonly recover spontaneously without any treatment if left alone and not stressed, a fact that has not been given due consideration when claims are made for the use of certain drugs. Several different drugs have been advocated and used routinely for the treatment of AIP in cattle. However, none has been properly evaluated, and definitive recommendations cannot be made.

Treatment of the chronic interstitial pneumonias is unsatisfactory because the lesion is progressive and irreversible.

TREATMENT AND CONTROL

Treatment

No specific treatment is available for AIP.

Control

AIP in pastured cattle

Monensin (200 mg/head PO q24h from 1 day before pasture change for at least 4 days after moving to fall pasture) (R-2)

Chlortetracycline (2.5 g/head PO q24h from 1 day before pasture change for at least 4 days after moving to fall pasture) (R-2)

Lasalocid (200 mg/head PO q24 from 1 day before pasture change for at least 14 days)

CONTROL

There are no known reliable methods for the prevention of AIP in pastured cattle, but there are some strategies that merit consideration.

Grazing Management

If lush autumn pasture contains toxic levels of the substance that causes the acute disease, it would seem rational to control the introduction of cattle to the new pasture. This can be done by controlling the total grazing time during the first 10 days: allow the cattle to graze for 2 hours on the first day, increasing by increments of 1 hour per day, and leave them on full time at the end of 10 to 12 days. If possible, this may be accomplished by rotating cattle back and forth, either between the summer and fall pastures or between the fall pasture and a drylot where ample supply of dry, mature hay is available. Dry, mature hay may be offered ad libitum to adult cattle in the morning before going on pasture for at least 4 days into the grazing period to reduce consumption of pasture forages. Such a management procedure is laborious and may not be practical depending on the size and terrain of the pasture and the holding yards that are available.

Inhibition of 3-Methylindole Production in Rumen

Controlling the conversion of D,L-tryptophan in forage to 3mI is a plausible control strategy. Experimental tryptophan-induced AIP can be prevented by oral administration of chlortetracycline or polyether antibiotics such as monensin. The daily oral administration of 2.5 g/head of chlortetracycline beginning 1 day before and for 4 days following administration of a toxin of L-tryptophan will prevent clinical signs.

The daily oral administration of monensin at the rate of 200 mg/head/day beginning 1 day before and for 7 days after an abrupt change from a poor-quality hay diet to a lush pasture reduced the formation of 3mI during the 7 days of treatment, but the effect of the drug was diminished on day 10, 3 days after its withdrawal. Because the effects of monensin on ruminal 3mI are diminished within 48 hours after withdrawal of the drug, effective prevention of acute pulmonary edema and emphysema may require continuous administration of monensin for the critical period of approximately 10 days after the mature animals are exposed to the lush pasture. The daily feeding of monensin in either an energy or protein supplement will effectively reduce ruminal 3mI formation in pasture-fed cattle.

Lasalocid at a dose of 200 mg per head once daily in ground grain for 12 days reduced the conversion of tryptophan to 3mI and prevented pulmonary edema.

Any combination of these management practices may reduce 3mI production to a greater extent than just providing monensin or implementing grazing management techniques alone.

Other Forms of AIP

The control of nonpasture cases of AIP depends on the suspected cause and removal of it from the environment of the animals. Every attempt must be made to control the concentration of dust and moldy foods to which cattle are exposed. Feed supplies must be harvested, handled, and stored with attention to minimizing dust and molds. In the preparation of mixed ground feed for cattle, the fineness of grind must be controlled to avoid dusty feed particles that may be inhaled. Because of the creation of dust, the grinding and mixing of dry feeds such as hay, straw, and grains should not be done in the same enclosed area in which cattle are housed. If dusty feeds must be used, they should be wetted to assist in dust control.

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Diseases of the Ovine and Caprine Respiratory Tract

ENZOOTIC NASAL ADENOCARCINOMA OF SHEEP AND GOATS (ENZOOTIC NASAL TUMOR)

Intranasal adenocarcinoma has been recorded as a sporadic disease of sheep and goats for many years and is now recognized as a contagious neoplasm in these species.[1] The disease in sheep and goats is associated with related but different retroviruses, the ovine nasal adenocarcinoma virus (ENT-1) and the caprine adenocarcinoma virus (ENT-2), respectively. These retroviruses are highly conserved, with North American and European isolates being 96% homologous.[2] They are homologous with the retrovirus that causes jaagsiekte (JSVR) but can be distinguished by unique sequences of the genome. Nasal adenocarcinoma is not a

component of the disease jaagsiekte, nor are pulmonary tumors present in sheep and goats with nasal adenocarcinoma. Infections with the viruses of enzootic nasal adenocarcinoma and jaagsiekte can occur in the same sheep, and this can potentiate the proliferation of jaagsiekte virus in the infected sheep.

Enzootic nasal adenocarcinoma is recorded in the United States, Canada, Europe, Japan, India, China, and Africa. It is believed to occur on all continents except Australia and New Zealand, but it is not present in the United Kingdom. The disease occurs sporadically but is often clustered in certain flocks and herds, and it is assumed to transmit by the respiratory route. The prevalence in affected flocks varies in different countries. It is generally less than 2% but can be as high as 10% to 15%.

There is no seasonal occurrence and no apparent breed or genetic predisposition.

There is no apparent influence of nasal myiasis on the prevalence of nasal adenocarcinoma in infected flocks.

Clinical disease is recorded occurring as early as 7 months of age, but most occurs in mature sheep between 2 and 4 years of age. Affected animals are afebrile, have a profuse seromucous or seropurulent nasal discharge, and sneeze and shake their heads frequently. There is depilation around the nostrils. The tumor may be unilateral or bilateral.

As the disease progresses, there is dyspnea, stertorous breathing with flaring of the nostrils at rest, and open-mouthed breathing following exercise. Some animals develop facial deformity and protrusion of one or both eyes from tumor growth, and the tumor may protrude from the nostril (Fig. 12-17). There is progressive loss of weight, emaciation, and death after a clinical course of 3 to 6 months. There is no detectable immune response in affected animals.

At **postmortem**, the tumor masses are in the ethmoid turbinates, with metastasis to regional lymph nodes in some cases. The tumors may be unilateral or bilateral and are gray or pink in color with a granular surface. The tumors originate in the serous glands of the turbinates and have the histologic features of adenocarcinoma.

The disease has been transmitted experimentally in both goats and sheep, with challenge of young kids resulting in disease at 12 to 16 months of age.

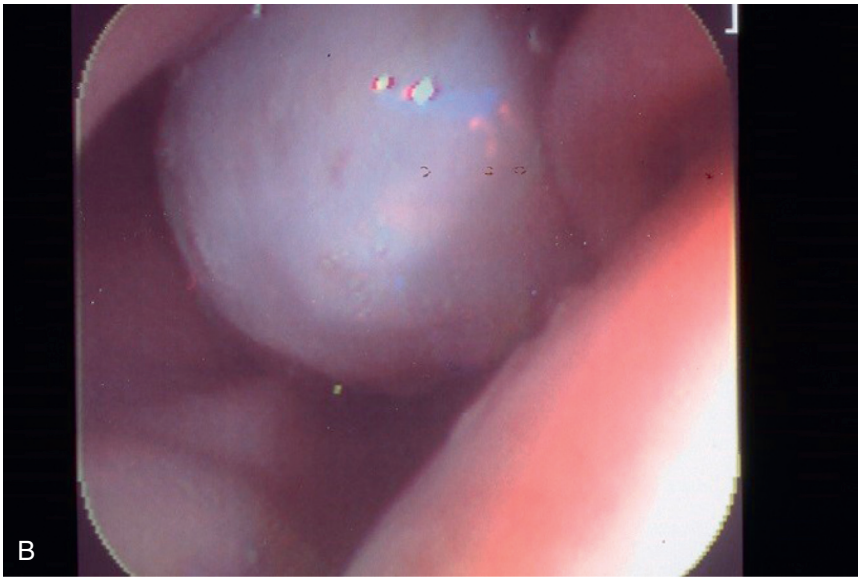

Fig. 12-17 A, Nasal adenocarcinoma in a Suffolk ewe. Notice the seromucous to seropurulent nasal discharge (left greater than right). Very little air movement was detected from the right nostril. B, Endoscopic view of a nasal adenocarcinoma (dorsal pink gray spherical mass) in a Suffolk ram with clinical signs of an upper respiratory tract obstruction.

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CONTAGIOUS CAPRINE PLEUROPNEUMONIA

SYNOPSIS

Etiology *Mycoplasma capricolum* subsp. *capripneumoniae*.

Epidemiology Highly contagious disease of goats, outbreaks in wild small ruminants do occur

Clinical findings Pleuropneumonia.

Lesions Pleuropneumonia with no enlargement of the interlobular septa.

Diagnostic confirmation Culture, polymerase chain reaction (PCR) on pleural fluid. Latex agglutination test.

Treatment Antimicrobials.

Control Herd biosecurity; vaccination provides strong immunity but of short duration.

Table 12-9 Summary of systemic mycoplasmoses of sheep and goats

Bacterial species	Animals affected	Diseases caused	Pathogenicity
M. agalactiae	Sheep/goats	Contagious agalactia, arthritis, pneumonia, granular vaginitis, pinkeye	High
M. arginini	Sheep/goats	Pneumonia, arthritis, vaginitis, pinkeye, mastitis	Low
M. capricolum subsp. *capricolum*	Sheep/goats	Mastitis and agalactia, pneumonia, arthritis	High
M. mycoides subsp. *capri* (formerly *M. mycoides* subsp. *mycoides*)	Goats	Contagious agalactia, pneumonia, arthritis, high mortality in young kids	Moderate
M. ovipneumoniae	Sheep/goats	Pneumonia	Commonly precursor to pneumonic pasteurellosis
M. putrefaciens	Goats	Mastitis and arthritis	High
Ureaplasma sp.	Goats	Vaginitis	Low
Mycoplasma capricolum subsp. *capripneumoniae* (formerly strain F38)	Sheep/goats	Contagious caprine pleuropneumonia	High

ETIOLOGY

Contagious caprine pleuropneumonia (CCPP) is a classical disease of goats, associated with *Mycoplasma capricolum* subsp. *capripneumoniae* and commonly confused with other serious pneumonias of goats and sheep (first isolated in 1976 and previously known as mycoplasma strain F38) (Tables 12-6 and 12-9). The disease was first described in Algeria in 1873 and presents primarily as a pleuropneumonia. The organism is difficult to grow, which can lead to poor differentiation of the CCPP from pneumonic disease induced by other mycoplasmas (*M. capricolum* subsp. *capricolum* and *M. mycoides* subsp. *capri*). Highly specific PCR tests can differentiate infections within the *M. mycoides* cluster of goats and will provide more information on the distribution and epidemiology of these diseases.

EPIDEMIOLOGY

Occurrence

CCPP is one of the most serious fatal diseases of goats in Africa and Asia and has serious socioeconomic effects for subsistence goat herders. It is known as Abu Nini in the Sudan. The exact distribution is uncertain, but clinical disease has been reported from 38 countries, mostly from Africa and Asia, with recent detections in Mauritius (2009), Turkey (2009), and China (2012).[1,2] However, the causative organism has only been isolated in some of these countries because of the difficulty growing it and lack of mycoplasmal laboratories. CCPP has many similarities clinically and at necropsy to contagious bovine pleuropneumonia, caused by *M. mycoides* subsp. *mycoides* SC, but it is not transmissible to cattle. Sheep can be infected experimentally and seroconvert, and they have been reported with respiratory disease in Eritrea. Captive and free-ranging wild ungulates, including deer, gazelles, and ibex, can also become infected and suffer disease.[1,2] *M. capricolum* subsp. *capripneumoniae* is highly infectious. In newly affected flocks the illness is acute and severe following a brief incubation period (generally 6-10 days, but up to 28 days), with morbidity rates of 90% and case mortality rates of 60% to 100%. The disease is less severe and more sporadic in endemically exposed flocks.

Transmission

The disease is readily transmitted by inhalation, but the organism does not survive for long in the environment. Infection is brought into the flock by asymptomatic carrier or clinically affected animals.

Agent

Mycoplasmas are among the fastest evolving bacteria, with high mutation rates.[3] *Mycoplasma capricolum* subsp. *capripneumoniae* shows a degree of heterogeneity not found among other members of the *M. mycoides* cluster. Based on sequencing of several genes, including 16SrRNA and H2 locus and other proteins, 24 haplotypes were identified in 25 strains of *Mycoplasma capricolum* subsp. *capripneumoniae* and placed within six genotyping groups (A to F), with two distinct evolutionary lineages identified.[3] Lineage 1 contains two groups with strains from East Africa, Qatar, Niger, and Mauritius; lineage 2 is subdivided into three groups with strains from the United Arab Emirates, China, and Tajikistan.

CLINICAL FINDINGS

All ages and sexes are affected. Clinical findings are restricted to the respiratory system and include cough, dyspnea with an extended neck, painful cough, and fever (40.5–41.5° C [104.5–106° F]). Animals often lay down, although they can stand and walk, and continue to eat and ruminate. In the terminal stages there is rapid respiratory rate, mouth breathing, tongue protrusion, and frothy salivation, followed by death in 2 or more days. In less acute infections the clinical signs are milder, and coughing may only occur following exercise. Under adverse climatic conditions or in kids the disease may occur in a septicemic form, with little clinical or postmortem evidence of pneumonia.

Outbreaks of CCPP in gazelle in the Middle East had similar clinical and pathologic signs to goats, although there was often sudden death.[2]

CLINICAL PATHOLOGY

Antigen can be detected in lung tissue and pleural fluid by PCR based upon the 16S rRNA genes. A real-time PCR offers advantages over conventional PCR including speed, greater specificity and sensitivity, and elimination of post-PCR processing.[4] Serologic tests used to identify carrier animals include complement fixation, ELISA, and a latex agglutination test. The latter is robust, available commercially, and suitable for field use. Monoclonal antibody is used in serologic tests to identify caprine isolates by the disc growth inhibition method, which will include *M. agalactiae, M. capricolum* subsp. *capricolum,* and the other members of the *M. mycoides* cluster associated with goats. A competitive ELISA using monoclonal antibodies is highly specific for CCPP.[5]

NECROPSY FINDINGS

The necropsy findings are similar to those of contagious bovine pleuropneumonia except that there is no thickening of the interlobular septa. Lesions are restricted to the lungs (often one lung) and pleura, with hepatization, increased pleural fluid, and a fibrinous pleuritis, which differentiates the disease from that caused by *M. mycoides* subsp. *capri.* Histologically, there is acute serofibrinous to chronic fibrino-nectoric pneumonia with interstitial intralobular edema, rather than a thickening of the interlobular septa that occurs with other mycoplasmal infections. Inflammatory exudate consists mainly of neutrophils. There is also peribronchiolar lymphoid hyperplasia.

Samples for Confirmation of Diagnosis

- **Bacteriology**—Pleural fluid and lung from the interface of the hepatized and normal lung tissue. These mycoplasmas are fragile and should be freeze dried or placed in transport medium if there is to be a significant transport time (>2 days). Conventional or real-time PCR can be performed on samples of pleural fluid dried on filter paper.
- **Serology**—CFT, Latex agglutination, competitive ELISA[5]

DIFFERENTIAL DIAGNOSIS

The other pulmonary mycoplasmoses from which this disease needs to be differentiated are those associated with *Mycoplasma mycoides* subsp. *capri* (formerly *M. mycoides* large colony type) and *M. capricolum* subsp. *capricolum.*

TREATMENT

Clinical cases respond to a range of antibiotics, including intramuscular tylosin (10 mg/kg BW), oxytetracycline (15 mg/kg/d), or tilmicosin, marbofloxacin, and danofloxacin.[6-8] The severity of the disease is reduced, but treated animals are still sources of infection.

CONTROL

Effective biosecurity measures are needed to prevent the introduction of the disease into a flock via contact with infected carriers. Killed vaccines effectively reduce morbidity and mortality rates. These have been widely used in many countries, although they can be of variable quality.[2] Immunity is generally short lived, and so a booster 1 month after the first vaccination provides additional protection. There is little evidence that maternal antibody interferes with the development of immunity, but kids born to does that have been vaccinated while pregnant are often not vaccinated before 10 to 12 weeks of age. Live attenuated vaccines have been trialed but are not yet commercially available and may not be permitted in some jurisdictions.[9]

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CHRONIC ENZOOTIC PNEUMONIA OF SHEEP (CHRONIC NONPROGRESSIVE ATYPICAL PNEUMONIA, SUMMER PNEUMONIA, PROLIFERATIVE EXUDATIVE PNEUMONIA)

SYNOPSIS

Etiology Multifactorial, with *Mycoplasma ovipneumoniae,* viruses and secondary bacterial infections implicated.

Epidemiology Affects sheep under 12 months of age. Seasonal occurrence, summer and autumn in southern hemisphere. Common disease affecting most flocks, but severity varies between farms.

Clinical findings Insidious onset. Coughing, nasal discharge and uneven weight gain in a mob.

Lesions Consolidation of anterioventral lobes of lung. Pleuritis.

Diagnostic confirmation Postmortem lesions.

Treatment Antimicrobials for severely affected individual sheep.

Control No effective control procedure established.

Enzootic pneumonia is defined here as the common, lowly pathogenic disease of sheep, particularly lambs, which is common in all sheep populations. The disease is recognized by different names in different areas of the world. It can be differentiated from the acute fibrinous pneumonia and pleurisy associated with *Mannheimia (Pasteurella) haemolytica,* which is often called enzootic pneumonia in the British literature, and from the chronic progressive pneumonias, maedi and jaagsiekte.

ETIOLOGY

Although the disease is well known, its cause is not well defined. This is partly because of its nonfatal character, which leads to incomplete examination of early cases; most of those submitted for examination or necropsy are distorted by secondary bacterial infections. It has a multifactorial etiology, with a mix of (mainly) *Mycoplasma ovipneumoniae, Bordetella parapertussis,* chlamydia, parainfluenza-3 (PI-3) virus, adenovirus, a respiratory syncytial virus, and reovirus nominated as causes. *M. haemolytica* is a common secondary infection and may lead to more acute, suppurative respiratory disease. The disease, which might be most accurately identified as chronic undifferentiated enzootic pneumonia of sheep, is probably a collection of etiologically specific diseases.

M. ovipneumoniae

M. ovipneumoniae is now considered to be one of the more important parts of the disease complex, and may be the initiating cause.[1] It is commonly isolated in large numbers from the lungs of affected sheep, but it can also be isolated from the nasal cavity of some normal sheep and less occasionally from normal lung.[1] Experimental challenge with pure cultures of the organism produces minimal lesions, but aerosol or intrabronchial challenge with homogenates of affected lung that contain the organism produces proliferative interstitial and lymphoid pneumonic lesions indistinguishable from the natural disease. *M. ovipneumoniae* is a facultative pathogen that requires compromised lung defense mechanisms to initiate lesions; infection with this organism subsequently predisposes the lung to secondary infection with organisms such as *Past. haemolytica.* There is considerable heterogeneity in *M. ovipneumoniae,* and several different strains may be isolated from a pneumonic lung.[2] Differences between strains in pathogenicity are not determined. Other mycoplasma, including *M. mycoides* subsp. *mycoides, M. mycoides* subsp. *capri, M. putrifasciens,* and *M. argininii,* may be associated with chronic enzootic pneumonia in tropical zones, but *M. argininii* is considered to have no role in atypical pneumonia of lambs in the United Kingdom.[3]

B. parapertussis

B. parapertussis is a common isolate from the nasal cavities and lungs of sheep with chronic enzootic pneumonia in New Zealand and is also believed to have an initiating role in the disease. It produces a cytotoxin that damages ciliated epithelium in the trachea and experimental challenge of colostrum-deprived lambs produces mild pulmonary lesions similar to those seen early in the natural disease. *B. parapertussis* also can predispose pneumonic pasteurellosis.

Parainfluenza-3 (PI-3) Virus

PI-3 is a cause of a mild undifferentiated pneumonia in sheep, and surveys around the world have shown that it is a widespread infection. The disease is clinically mild and marked by the presence of interstitial pneumonia. Antibodies to PI-3 are present in lambs soon after birth, but the half-life is short, and lambs are susceptible by the time they are weaned and mixed with other lambs, which is when clinical disease often occurs. In the experimentally produced

disease in lambs there is a slight seromucosal nasal discharge, coughing, increased sensitivity to tracheal compression, and fever of 40° to 41°C (104–106°F). At necropsy there is obvious hyperemia of the upper respiratory mucosa, including the trachea; the bronchial lymph nodes are enlarged; and there are small foci of catarrhal inflammation of pulmonary parenchyma of the apical and cardiac lobes. However, challenge of lambs at 2 weeks of age with this virus and *M. haemolytica,* although producing disease, did not result in prolonged disease lasting to slaughter, and it was concluded that these agents, without other factors, were not the cause of enzootic pneumonia. This conclusion is supported by the results of vaccine trials with PI-3 against enzootic pneumonia.[4]

Bovine Respiratory Syncytial Virus

BRSV has resulted in pneumonia following experimental challenge of sheep and is evidenced clinically by fever and hyperpnea and pathologically by multifocal pulmonary consolidation and necrosis of epithelial cells. There is little evidence for BRSV as a cause of significant respiratory disease in sheep.

Other Agents

Adenovirus and a type-3 *reovirus* have been used experimentally to produce pneumonic lesions, and a vaccine has been produced to protect lambs against the adenovirus infection. Similarly, sheep herpesvirus, *caprine herpesvirus-1,* will produce an interstitial pneumonia in experimentally challenged SPF lambs, but there is no evidence of a causal association with chronic enzootic pneumonia.

Autoantibodies to upper respiratory cilia have been detected in sheep colonized with *M. ovipneumoniae,* and it is suggested that they contribute to the pathogenesis of coughing in this disease.

EPIDEMIOLOGY

Occurrence

Enzootic pneumonia affects animals up to 12 months but may commence as early as 6 weeks of age. The disease can occur in both lambs at pasture and housed lambs. In many affected flocks, 80% of 4- to 5-month-old lambs have clinical signs and lesions, and the disease is credited with causing a significant depression in growth rate after weaning in lamb flocks with a high prevalence. This has been confirmed in controlled studies on the effect of the experimentally produced disease on weight gain in housed and pasture-fed lambs.

Enzootic pneumonia has a seasonal pattern that differs according to locality and management. In Australia and New Zealand, the period of peak prevalence is in the late summer and autumn. In a longitudinal slaughter study of lambs in New Zealand, the prevalence of pneumonic lesions was found to increase from early summer to early autumn, with an overall prevalence of pneumonia of 42%. There were significant differences in prevalence between different regions of the country. Factors such as comingling sheep from different sources and environmental stress can precipitate clinical disease.

Environmental Risk Factors

In Australia and New Zealand, clinical outbreaks of enzootic pneumonia in lambs aged 5 to 8 months are often associated with heat stress, yarding after weaning, use of plunge or shower dips, and transport or mustering of sheep in hot dry conditions. Cases commence within 1 to 3 weeks after transport. In contrast, in the United Kingdom and Europe this disease occurs primarily in the late winter and early spring; in the more intensive production systems of the northern hemisphere, the disease is commonly associated with environmental problems of housing. In Ireland, an association has been made between the occurrence of lesions at slaughter and the extent of rain and windchill experienced by the sheep in the 2 months before slaughter.

Economic Importance

Death loss from this disease is minor, but economic loss is considerable and includes reduced growth rate, prolonged periods on the farm before reaching slaughter weight, the drug and labor costs associated with treatment, slaughterhouse wastage, and downgrading of carcasses with pleural adhesions and an effect on carcass quality. The situation is similar to that with enzootic pneumonia of pigs.

CLINICAL FINDINGS

The disease is insidious in onset and can persist in a group of lambs for 4 to 7 months. The disease has mild clinical manifestations, with the primary signs being poor and uneven weight gains, an increased nasal discharge, coughing, increased respiratory rate, and respiratory distress with exercise. Increased intensity and a higher pitch of breath sounds are heard on auscultation over the region of the bronchial hilus, and sounds of fluid in the airways are heard in some cases at rest but can usually be elicited by inducing the lamb to cough. There may be periods of fever.

There is a relationship between the proportion of the lung affected with pneumonia and average daily gain, and in one study weight gain was reduced by 50% when greater than 20% of the lung was affected. The weight loss is most apparent clinically soon after the disease commences.

NECROPSY FINDINGS

At postmortem there are clearly demarcated areas of consolidation in the anterioventral lobes, and there may be pleuritis with pleural adhesions. The diagnosis is on gross lesions and the presence of typical lesions on histologic examination.

TREATMENT AND CONTROL

Treatment is not usually undertaken unless there is secondary infection to produce acute respiratory disease. Nevertheless, lincomycin (5 mg/kg IM), given twice or three times at intervals of 2 days, and oxytetracycline (20 mg/kg, IM), given twice at 4-day interval, both gave good clinical cure and increased growth rates in a study in Greece.[5] Control is based on the avoidance of stress factors that can exacerbate existing infection.

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OVINE PROGRESSIVE PNEUMONIA (MAEDI, MAEDI-VISNA)

Ovine progressive pneumonia and maedi are North American and European terms for slow virus diseases of sheep in which a chronic progressive pneumonia is a major manifestation. The name maedi is derived from the Icelandic term for dyspnea. Maedi-visna virus can also produce visna, which is a disease of the nervous system and is discussed elsewhere under that heading. Additional manifestations of infection are arthritis, indurative mastitis, and ill-thrift. These diseases have a close relationship with caprine arthritis encephalitis. La Bouhite and Graff–Reinert disease are local names for maedi in France and South Africa, respectively. In the United States it was originally described as Montana progressive pneumonia, and in Holland as zwoergersiekte.

SYNOPSIS

Etiology Ovine retroviruses

Epidemiology Most sheep infected as lambs. Persistent infection. High prevalence of infection in many countries but low prevalence of clinical disease. Transmission is via infected colostrum and milk, but lateral transmission also occurs.

Continued

Clinical findings Clinical disease of mature sheep, long incubation, long clinical course. Dyspnea and respiratory distress, initially with exercise but eventually also at rest. Some sheep also manifest chronic wasting and/or indurative mastitis.

Necropsy findings Lungs uniformly increased in bulk with enlargement of bronchial and mediastinal lymph nodes. Lymphocytic interstitial pneumonia. Discrete or diffuse hardening of mammary glands with lymphoid infiltration.

Diagnostic confirmation Clinical signs, pathology and serology. Polymerase chain reaction (PCR) provides confirmation of infection.

Treatment None.

Control Segregated rearing. Test and cull.

ETIOLOGY

Maedi-visna virus (MVV) and ovine progressive pneumonia virus (OPPV) are single-stranded RNA nononcogenic ovine lentiviruses within the retrovirus family. They have a tropism for monocytes, macrophages, and dendritic cells, but not T-lymphocytes. This is an important determinant of their pathogenesis because they induce a persistent infection in sheep that can cause lymphoproliferative changes in the lung, mammary tissues, brain, and joints. There is a high degree of relatedness with the lentivirus associated with caprine arthritis encephalitis (CAE), and these ovine and caprine lentiviruses share nucleotide homology and serologic properties. Consequently, MVV, OPPV, and CAE viruses are now regarded as a viral continuum and referred to as small ruminant lentiviruses (SRLV).[1]

Isolates of MVV from naturally infected sheep are genetically heterogeneous, antigenic drift is common, and antigenic variation of the surface protein facilitates the persistence of the virus in the host, both as latent and chronic infections. There is evidence for variation in pathogenic potential between isolates and hosts, and it is estimated that only about 30% of infected animals develop disease.[2]

There is some evidence that the North American strains of ovine lentivirus may have originated from cross-species transmission of caprine arthritis–encephalitis virus rather than from maedi lentivirus. However, the similarity in clinical manifestation of maedi and ovine progressive pneumonia and the evidence that the causative viruses are part of a continuum permit the discussion of these diseases as a single entity.

EPIDEMIOLOGY

Occurrence

The earliest reports of the disease were from South Africa and the United States, but it now occurs in all major sheep-producing countries with the exception of Australia, New Zealand, and Iceland. Maedi virus was present in Iceland, being introduced in 1933 through the importation of infected Karakul sheep, but was eradicated in 1965. Because of the susceptibility of the local sheep and management practices that favored transmission, it developed to a problem of major national significance. In individual flocks the annual mortality was often 15% to 30%, and in these circumstances sheep farming was not economically viable. Approximately 105,000 sheep are believed to have died of the disease, and 650,000 sheep had to be slaughtered to eradicate it from the country.

The international movement of sheep has facilitated the spread of the disease, and it is believed to have been introduced into Denmark, Norway, Sweden, and Great Britain since the 1970s through the importation of infected sheep. However, as yet no other country has experienced the severity of disease that occurred in Iceland.

Host Range

Sheep and goats are the only species known to be susceptible to MVV, and infection cannot be established by experimental challenge in cattle, deer, pigs, dogs, horses, chickens, mice, and rats. Rabbits are susceptible, but infection is limited to the acute stage before the production of antibody; chronic infection, as seen in sheep and goats, does not occur. Hybrid mouflon–domestic sheep and calves have been experimentally infected with CAE virus, although the infection was cleared naturally in the latter, and SRLV nucleic acid detected in wild ibexes in close contact with goats.[2]

A serologic survey of wildlife in the United States found no evidence of infection in bighorn sheep, elk, white-tail deer, or antelope. All breeds of sheep appear to be susceptible to infection, but there may be differences in breed susceptibility, based on differences in seroprevalence in flocks with more than one breed of sheep. These differences are not consistent and so they may reflect differences in the susceptibility of family lines within a breed. Variations in the gene encoding an ovine transmembrane protein (TMEM 154) are associated with increased or decreased susceptibility of sheep to SRLV; sheep with glutamate at position 35 have increased susceptibility, whereas those with lysine or a deletion have decreased susceptibility.[3] The average frequency of highly susceptible alleles across 74 sheep breeds was 0.51, with more than 25% of mainstream breeds being greater than 0.8.[4] In contrast, frequency of highly susceptible alleles across 3 hill breeds was 0.26 to 0.42, suggesting that they would be less affected by MVV infection.[5]

Prevalence

The prevalence of infection varies between farms, breeds, and countries. In the United States, infection is more common in the western and midwestern and uncommon in the southern states. An estimate of flock seroprevalence (flocks with 1 or more positive sheep) of 48%, and overall seroprevalence of 24%, was recorded in samples collected in 2001 from sheep in 29 states. More recently, samples collected in Wyoming in 2011 found a flock prevalence for OPPV of 47.5% and overall seroprevalence of 18%, with open range (unfenced) flocks at significantly higher risk compared those that were fenced in (an odds ratio of 3.5).[6] In Canada, a random survey found a flock prevalence of 63%, with 19% of sheep over 1 year of age being seropositive, and more recently a flock prevalence of 25% in Manitoba. Serologic surveys that use the AGID test will markedly underestimate prevalence, demonstrated by a study in Alberta where 27% of culled ewes were positive on histopathology, but using the AGID there was a seroprevalence of only 13%.[7]

In the United Kingdom, MVV was introduced more recently, and so this country has lower flock and within-flock prevalence rates (estimated at 3% and up to 15%, respectively, in 2010). However, there is concern that the prevalence of maedi-visna may be increasing, demonstrated by a steady increase in the number of introduced infections in approximately 2600 flocks that participate in a maedi-visna accreditation scheme. These breakdowns are usually caused by flocks not adhering to the biosecurity rules of the scheme, such as introducing sheep into a nonaccredited flock on the same holding.[8]

There is considerable variation in the prevalence of seropositive sheep between flocks. Rates of seropositivity increase with age, and so within-flock seroprevalence is influenced by the average age of the flock. Flock seroprevalence also has been positively associated with the use of foster ewes, allowing lambs older than 1 day to have contact with other lambing ewes, flock size, close contact during confinement for lambing, stocking density on pasture, and the length of time that the flock has been in existence. Within flock seroprevalence is much higher in flocks that are also infected with pulmonary adenomatosis compared with those that are not.

Transmission

The disease is spread most commonly by inhalation of infected aerosols and the ingestion of infected colostrum or milk. Vertical transmission following in utero infection is possible but relatively uncommon. Virus is also shed in the semen of infected rams if there are leukocytes in the semen, and so this risk may be increased in rams that are also infected with *Brucella ovis*.

Lambs may contract the infection at or shortly after birth, either from contact with infected ewes or from ingestion of infected colostrum and milk. The virus then infects

dendritic cells at the mucosal surface, and these migrate to local lymph nodes, where virus is transferred to macrophages and spreads systemically.[2] Alveolar macrophages play a similar role when infection occurs via the respiratory route. Lambs born to seropositive ewes have a significantly greater risk of infection than those from seronegative ewes, and lambs born to ewes that have been infected for a long time are at greater risk of infection. The chance of transmission to lambs from infected ewes increases with the period of contact, but it can occur within the first 10 hours of life.

Lateral transmission can occur in older sheep, and this was probably important in the transmission of the disease in Iceland and the Basque area of Spain, and it has been a significant component of the spread of infection of the virus in flocks in the United Kingdom. In some flocks, the spread of infection can be rapid, and the majority of the flock can seroconvert within a few years of the introduction of infected sheep. The spread of infection is often rapid in flocks that are concurrently infected with the retrovirus causing pulmonary adenomatosis. There are many macrophages in the lungs of these sheep, and so these cells will also be infected with MVV if it is present. In dual-infected sheep, the copious lung fluid produced by sheep with pulmonary adenomatosis contains MVV and can increase the risk of lateral transmission of maedi-visna.

Economic Importance

Economic loss from this disease is associated with increased mortalities, decreased longevity, decreased value of cull animals, and reduced productivity associated with subclinical infections, such as failure to thrive or to rear lambs. Losses are usually more severe in intensive housed operations, and they can be catastrophic in flocks that derive a large proportion of income from the sale of breeding animals (stud or seedstock flocks).

Clinical disease occurs in sheep 2 years old or older, usually in sheep 3 to 4 years of age. Severe disease is more likely when the within-flock prevalence exceeds 50% and has a case-fatality rate of 100%.[9] Up to 30% of infections are subclinical, and so clinical disease may not be obvious or common in flocks that have a low prevalence. A high proportion of infected sheep, premature culling, and high mortality rates have occurred in flocks in Iceland, the Netherlands (particularly the Texel breed), the United States, the United Kingdom, and other European countries, especially in intensively managed dairy flocks.

It is possible that the major economic loss associated with infection with these viruses rests with the effects of subclinical infection on productivity of infected flocks. Subclinical infection of breeding ewes in some flocks has been associated with a reduction in conception rate and lowered birth weights and/or reduced growth rates in their lambs. The reduction in growth rate of the lamb is associated with indurative mastitis and a lowered milk intake. This may be expressed by a decreased growth rate of lambs from only older ewes. In other flocks there has been no evidence of effect on the birth weight or growth rate of lambs born of infected ewes. Subclinical infection has no effect on mature ewe body weight or greasy fleece weight.

PATHOGENESIS

SRLV infections are classed as immunopathologic diseases, whereby the host immune response is responsible for most of the pathology rather than the virus itself.

The virus infects cells of the monocyte/macrophage lineage and attaches to cells by the binding of its envelope glycoprotein to specific receptors on the cell surface. The virus replicates its RNA genome via a DNA intermediate provirus that is integrated into the chromosomal DNA of infected cells. With initial infection there is virus replication; this is followed by an immune response that restricts viral replication but fails to eliminate the virus completely. The immune response occurs between 2 and 8 weeks after infection, with antibody to different viral antigens emerging at different times during this period, although some sheep do not develop an antibody response until several months after infection. The ability to establish latent infection of monocytes, which then transfer virus to other organs, may be related to the fact that SRLV are relatively poor at inducing type 1 interferon (IFN), an important mediator of immunity to viral infection.[2]

In monocytes, replication is restricted and does not proceed beyond the synthesis of provirus in most infected cells. The principal site of virus replication is the macrophage, and pulmonary secretions and milk containing infected macrophages are the main source of virus for natural transmission. Diseases such as pulmonary adenomatosis, which increase the number of macrophages in lung secretions, will facilitate transmission of ovine progressive pneumonia virus via aerosols.

The replication of virus initiates viral-specific immune responses (immune activation), and immune-mediated lesions develop in various organs. Production of viral antigen attracts more monocytes, which become latently infected, and so a cycle of latent infection and immune activation, with lymphocytic hyperplasia, is established.[2] The infected macrophages in the affected tissues are surrounded by a slowly progressing inflammatory response, creating a focus of mononuclear cell aggregation. Many tissues can be involved, but the lungs, mammary gland, central nervous system and joints are most affected. Any or all of these organs can be affected in a single sheep, but genetic differences in host susceptibility and the virus often lead to a predominance of a single syndrome in a flock. For example, Border Leicesters in the United States and Texels in Holland appear more susceptible to lung infection (maedi), whereas Icelandic sheep are more susceptible to the central nervous disease (visna).

In the lung there is a gradual development of a lymphocytic interstitial pneumonia dominated by CD8+ T lymphocytes, hyperplasia of smooth muscle, and fibrosis.[2] There is no healing or shrinkage of tissue, so the lungs increase in size and weight, alveolar spaces are filled, and dyspnea and anoxia gradually develop. The pathologic lesions develop very slowly during the preclinical and clinical stages of the disease, so that they are widespread and there is little ability to compensate when clinical signs do appear. In the central nervous system there is infiltration of the meninges and white matter with lymphocytes. The demyelination that occurs in visna is believed to result from the direct effect of the virus on oligodendrocytes and astrocytes and is believed to be the result of an inflammatory response provoked by the presence of viral antigen in these cells. Similar infiltrations occur in the udder. Lymphoid follicles are found in the alveolar parenchyma, often with atrophy of the alveolar tissue. Numerous lymphocytic follicles also occur around the lactiferous ducts, some of which may be occluded by lymphocytic aggregates protruding into their lumens.

CLINICAL FINDINGS

There is a long incubation period. Clinical disease, if it occurs, does not develop before 2 years of age, and most clinically affected sheep are older than 3 years. The clinical signs develop insidiously and progress slowly, and there is a long clinical course.

The earliest signs are usually listlessness and loss of body condition that progresses to emaciation. The presenting syndrome can be one of an increased cull rate of ewes in poor condition. Signs of respiratory involvement are not evident in the initial stages of the disease, but there is exercise intolerance, and affected sheep will fall back behind the flock when the flock is moved. Dyspnea, with an increase in respiratory rate (80–120/min at rest) and flaring of the nostrils, or open-mouth breathing, develops later, but there is no evidence of excess fluid in the lungs. There may be coughing and some nasal discharge, but in most instances this occurs in sheep with secondary bacterial pneumonia. The body temperature is in the high normal range, and there may be inflammation of the third eyelid. Clinical illness lasts for 3 to 10 months, and the disease is always fatal. Clinically affected sheep are more prone to other diseases, such as pregnancy toxemia. In some sheep, clinical respiratory disease is minimal, and the major manifestation is wasting and the thin ewe syndrome.

Indurative mastitis ("hard bag" or "hard udder") also has an insidious onset, with ewes usually in their third or later lactation before the disease becomes clinically obvious, although histologic changes will be apparent far sooner. In early stages, hardening of the udder is more easily detected at drying off. In advanced cases the udder is enlarged and uniformly very firm, but the teats are limp. There is very little milk in the teat cistern, although it appears normal.[9] Mammary involvement may occur, along with signs of respiratory infection, or affected ewes may show no other clinical abnormality. The lambs of ewes with less severe involvement may have a reduced growth rate.

Arthritis is occasionally seen in naturally infected sheep, usually when they are from 1 to 6 years of age. These sheep become lame and emaciated, with obvious swelling of the carpal joints.

CLINICAL PATHOLOGY

There is a progressive, moderate hypochromic anemia, with hemoglobin levels falling from 12 to 14 g/dL to 7 to 8 g/dL, and some depression of the red cell count. There is a tendency to leukocytosis, which in experimental infections is quite marked between exposure and the onset of clinical disease, but the count returns to normal when clinical signs appear. There is also hypergammaglobulinemia. There is an increase in the number of lymphocytes and neutrophils in bronchoalveolar lavage fluid, with more CD8+, fewer CD4+, and an inversion of the CD4+/CD8+ cell ratio.

In clinical cases, diagnosis is by the presence of the appropriate clinical syndrome, supported by the presence of a positive serologic test for the virus. A positive serologic test, by itself, has limited value for the diagnosis of individual sheep because there is a high prevalence of seropositivity in many flocks, especially in older animals. A positive test indicates that the animal is infected, but does not indicate that signs or lesions are attributable to infection with the virus.

Detection of Antigen

PCR is a sensitive method for detection of small amounts of viral nucleic acid, but it may not be available for routine diagnosis in some jurisdictions. It has been used to detect antigen in the third eyelid of infected sheep.

Serologic Tests

Assessing flock status (the presence or absence of infection) and the status of an individual sheep currently relies on serologic testing. The agar gel immunodiffusion (AGID) and ELISA tests are used in most countries. The AGID test is easy to perform and inexpensive, and thus it is often the most commonly used routine diagnostic test. It has a high specificity but often a lower sensitivity than the indirect and competitive ELISA tests, which may vary depending on the antigen used.[10] Sensitivities of these tests vary from 64% to 97%, and thus they will be unsuited for diagnosis of infection in individual animals or use in test and cull programs if at the lower end of this range. The value of serologic testing rests primarily with the establishment of the infection status of the flock. A negative test in an individual sheep could mean that the sheep is free of infection, but this result can also occur in an infected animal that has not yet responded to infection.

A commercial indirect ELISA using a recombinant core protein and a synthetic transmembrane protein as antigens was developed in the Netherlands. Although it had high sensitivity and specificity, it was labor intensive and expensive, and so a pooling procedure that required modifications to the test was developed.[11] Subsequently, testing of bulk milk tank samples by ELISA or PCR was confirmed as a cost-effective alternative for flock testing and capable of detecting early infection in dairy flocks. The ELISA detected a within-herd prevalence of less than 1% when samples were tested undiluted and less than 3% when using samples diluted 1 in 10.[12] All the bulk milk samples from known SRLV-free flocks (138) tested negative, whereas 50% of samples from flocks with an unknown SRLV status (111) were positive.[12] Agreement between the ELISA and two real-time PCR tests on a subsample of 59 milk samples was 90% for the LTPCR and 98% for the leader-gag PCR.[12]

NECROPSY FINDINGS

Lesions may be present in the lungs and associated lymph nodes, brain, joints, mammary gland, and blood vessels, but gross lesions in most sheep are confined to the lungs and, in some cases, the mammary glands. In advanced cases, the lungs are larger and 2 to 4 times as heavy as normal lungs. They collapse much less than normal when the chest is opened, and are gray–blue to gray–yellow in color. There is a diffuse thickening of both lungs, with abnormal color and consistency in all lobes and consistent enlargement of the bronchial and mediastinal lymph nodes. Histopathologic changes are characteristic of a chronic interstitial pneumonia, with proliferation of lymphoid tissue and the presence of numerous lymphoid follicles. There is infiltration of lymphocytes and macrophages in the interalveolar septa, which are thickened, and the bulk of the alveolar space is replaced by the thickened alveolar walls. Larger airways are unaffected. There is a complete absence of healing, consistent with the progressive nature of the disease, and vasculitis is often present.

Lesions of arthritis, encephalitis, and mastitis are often present. The mastitic lesion comprises an interstitial accumulation of lymphocytes and the presence of periductal lymphoid nodules with atrophy of alveolar tissue. Culture of the virus is difficult, and confirmation of the diagnosis is often limited to the presence of characteristic microscopic lesions, preferably supported by a positive serologic titer to the virus. Immunohistochemistry is highly specific, but it may not be routinely available.

Samples for Confirmation of Diagnosis

- **Virology**—lung, mammary gland, synovial membrane, brain (PCR, ISO)
- **Bulk or individual milk**—(PCR, ELISA)
- **Serology**—heart blood serum (AGID, ELISA, PCR)
- **Histology**—formalin-fixed lung, bronchial lymph node, mammary gland, synovial membrane, half of brain section midsagittally (LM, IHC)

DIFFERENTIAL DIAGNOSIS

There are several chronic pneumonias requiring differentiation from maedi:

- Jaagsiekte
- Parasitic pneumonia
- Chronic suppurative pneumonia
- Caseous lymphadenitis
- Postdipping pneumonia
- Enzootic pneumonia
- Melioidosis
- Chronic wasting conditions:
- Johne's disease
- Caseous lymphadenitis

TREATMENT

No treatment has been successful. Secondary bacterial infections can be treated with commonly used antibiotics, such as tetracyclines, but there will be no improvement in the underlying chronic pneumonia.

CONTROL

In the past, the only control attempted was eradication by complete destruction of all sheep in a flock or area and subsequently restocking. However, it is possible to greatly reduce the prevalence, and even eradicate the disease, by either (a) testing all sheep and removing seropositive sheep from the flock, or (b) by removal of lambs at birth and rearing them in isolation from other sheep.

Many jurisdictions have developed accreditation programs for flocks to establish that they have a low risk of infection with MVV. Once flocks are seronegative they are subjected to testing at various intervals, typically 1 to 3 years depending on an assessment of the biosecurity risk and the presence of untested sheep on the same farm holding.

Test and Cull

Test and cull involves the detection and culling of seropositive animals and is the preferred method when lateral transmission is the dominant mode of transmission in the flock. All sheep (and goats) on the farm are serologically tested once or twice a year, and seropositive animals and their progeny of less than 1 year of age are removed (culled), preferably for slaughter. If immediate slaughter is not feasible, the seronegative flock must be kept isolated from infected sheep and clothing and equipment that have been in contact with any seropositive animals. Testing is continued semiannually or annually until there are at least two consecutive negative tests. The offspring of older seronegative ewes are kept for replacements. Using this approach, an initial seroprevalence to MVV of 66% in a Spanish dairy flock was reduced to 0.2% within 2 years and remained below 2.2% for the next 4 years, and the seronegative flock returned to pretest numbers within 8 years.[13]

Testing all animals, not just those greater than 1 year old, using a combination of serology and real-time PCR assay to detect proviral DNA, combined with a shorter testing interval of 3 months, may be able to accelerate eradication.[14] Using this system, antibody and proviral DNA–negative ewes, proviral DNA–negative lambs, and antibody and proviral DNA–negative yearling ewes were retained as breeders. The PCR test can discriminate lambs that are not infected but serologically positive as a result of maternal antibodies.

Segregated Rearing

Lambs must be separated from the ewes at birth and receive no colostrum from their dam. They can be given bovine colostrum, or colostrum from a known seronegative flock, then reared on milk replacer completely separate from other sheep. This method may be of particular value when lines of sheep of high genetic merit are desirable to maintain. The disadvantage is that it is labor-intensive and expensive, and there is no cash flow unless the infected sheep are maintained in production pending the establishment of a mature infection-free flock. However, retaining infected sheep creates considerable potential for reinfection of the artificially reared flock, either via accidental contact or fomites.

With either method, any future introductions into the flock should be sourced from a known seronegative flock.

Flock Biosecurity and Other Control Methods

Once infection is introduced it is difficult and expensive to eradicate; thus, establishing and maintaining good biosecurity is a cost-effective way of preventing the introduction of maedi-visna and other important infectious diseases, such as foot rot. Unfortunately, the specificity and sensitivity of most currently available serologic tests are inadequate to reliably determine the infection status of an individual. Consequently, the results of flock tests from a potential source of replacement sheep should be examined, along with postmortem and other animal health records if these are available. Rams and replacement ewes should be acquired from accredited free flocks in countries where these programs exist and should be transported directly from the source farm rather than through markets or farms of unknown status.

Other control procedures that attempt to limit or delay the spread of infection and, consequently, the occurrence of clinical disease within an infected flock have limited success. Lambing in sheds and close confinement paddocks is conducive to spread of disease, and so ceasing or modifying this practice is recommended for infected flocks. In flocks that have a high incidence of clinical disease, culling animals before the age at which they develop clinical signs can reduce the economic impact of the disease.

In countries where the disease is endemic, there is often a great deal of movement of animals between farms, especially rams but also replacement ewes. Thus restricting movement of animals between farms and preventing comingling in common grazed areas should help limit the spread of the disease.

Vaccination and Genetic Selection

There is currently no effective vaccine against the SRLVs, including MVV and OPPV, and in some cases candidate vaccines have enhanced viremia and/or the immune-mediated pathology of the disease.[2] The difficulty in developing effective vaccines is common among the lentiviruses, with various approaches, including attenuated vaccines, vector vaccines, and proviral DNA vaccines, having little success. The reasons are obscure but probably relate to the underlying dysfunction in T-cell-mediated immune responses.

However, marker-assisted genetic selection, to identify those sheep less susceptible to infection, has the potential to supplement existing control measures. For example, in a trial involving 187 lambs, the probability of infection following natural exposure to OPPV was 3.6 times greater in crossbred lambs with susceptible or heterozygous diplotype to ovine transmembrane protein gene 154 (TEM154 diplotype "1 3" or "3 3") compared with lambs with diplotype "1 1."[15] This is an active research area, and it is expected that additional markers will be identified with additional investigations.

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OVINE PULMONARY ADENOCARCINOMA (JAAGSIEKTE, PULMONARY ADENOMATOSIS)

SYNOPSIS

Etiology Jaagsiekte sheep retrovirus.

Epidemiology Disease of mature sheep with geographic clustering but low prevalence. Spread probably mainly by respiratory route

Key signs Dyspnea, profuse watery pulmonary discharge, loud fluid sounds on auscultation, long clinical course with progressive emaciation.

Pathology Tumors in lung.

Diagnostic confirmation Histologic changes are diagnostic and histopathologic confirmation, including immunohistochemistry, is the only method currently available.

Treatment None.

Control Culling and strict biosecurity.

Jaagsiekte is Afrikaans for "driving disease" because of the tendency for affected sheep to show clinical signs when driven. The disease manifests clinically as a chronic progressive pneumonia and is a contagious disease of sheep resulting from the development of a bronchioalveolar adenocarcinoma in the lungs.

ETIOLOGY

The disease is associated with an infectious beta-retrovirus, jaagsiekte sheep retrovirus (JSRV) of the family Retroviridae. JSRV has two forms, an exogenous infectious form that alone can produce the disease and an endogenous RSRV-related provirus that is present in all sheep genomes.[1] The disease has been transmitted experimentally with partially purified retrovirus from infected lungs, by infection with cloned JSRV, and supportive evidence for retrovirus as the causative agent includes an inverse dose relationship between reverse-transcriptase activity in the infectious inoculum and the incubation period of the experimental disease.

The presence of retrovirus has been demonstrated in the lungs of sheep with jaagsiekte in different countries, there is serologic cross-reactivity, and strains from different countries have been sequenced.

A herpesvirus has also been isolated in several countries from the lungs of sheep with jaagsiekte, but epidemiologic studies show that it is not the causative agent.

EPIDEMIOLOGY

Occurrence

The disease has worldwide distribution and is recorded in most countries that have significant sheep populations, with the exception of Australia and New Zealand. Until recently there has been no practical method to detect infected sheep, and estimates of the prevalence of jaagsiekte are largely based on clinical or postmortem observations. The prevalence of the disease appears to vary depending on the breed of sheep and the type of flock management. In most endemically infected flocks, annual losses attributable to jaagsiekte are between 2% and 10%, although the tumor is present in a much higher proportion of the flock, and infection without lesions is also common. Annual mortality can be higher in flocks where the infection has recently been introduced and before the disease becomes endemic. PCR analysis of peripheral blood leukocytes of sheep in infected flocks shows significantly higher rates of nonclinical infection.

Prevalence varies between countries, and there can be areas of high prevalence within countries; in Britain, the Borders and the east coast of Scotland, and East Anglia in England, appear to be foci of infection from which other outbreaks arise. The prevalence may be higher than generally recognized; in a biased sample, histologic evidence of jaagsiekte was detected in 25% of cases of pneumonia in sheep submitted to a diagnostic laboratory in Scotland over a 6-year period. In a more recent study, ovine pulmonary adenocarcinoma was confirmed in 0.8% of fallen (culled) adult sheep at a slaughterhouse.[2]

The disease is also a significant cause of mortality in adult sheep in South Africa and Peru, but it is a minor disease in the United States and Canada. It occurred in epizootic proportions in Iceland during the same period of time as the maedi-visna epizootic but has been eradicated by a rigorous slaughter policy.

Animal and Environmental Risk Factors

Mature sheep, 2 to 4 years of age, are most commonly affected, but the disease can occur in younger animals. There are reports of the occurrence of jaagsiekte in goats at very low prevalence rates in India and Greece, and the disease has been experimentally transmitted to goat kids. The lesions produced were small and circumscribed, and goats have low susceptibility to infection.

Jaagsiekte has a prolonged clinical course and is uniformly fatal. In some reports there is a greater prevalence of onset of clinical disease in the winter months, but in others there is no seasonal variation in clinical onset. Ewes may show a sudden onset of clinical disease in late pregnancy.

The incubation period in natural cases is 1 to 3 years, but it may be as short as 5 to 12 months after experimental transmission. Clinical disease is rare in sheep younger than 2 years and is most common at 3 to 4 years of age. Very rarely, cases occur in lambs 3 to 6 months old, and disease can be reproduced in lambs of this age by challenge of very young lambs. A genetic or familial susceptibility to the disease is suspected.

Because of the method of spread, the disease is likely to assume more importance in systems of sheep husbandry where there are significant periods of close contact, as, for example, occurs with intensified lamb-rearing systems. Close housing during the winter is a potent predisposing cause and probably accounted for the occurrence of the disease in epizootic form in Iceland. However, the disease occurs commonly in range sheep in other countries. Sheep that have a combined infection with jaagsiekte and the maedi-visna lentivirus have an increased ability to transmit maedi-visna infection, and flocks with the combined infection can suffer high losses from pneumonic disease.

Transmission

Experimental transmission has been effected by pulmonary or IV injection, or by intratracheal inoculation of infected lung material. The incubation period of the experimental disease in young lambs is much shorter than that in mature sheep. The disease has also transmitted by inhalation of infected droplets when sheep are kept in close contact, and it is assumed that the natural mode of transmission is by droplet infection from respiratory secretions, which are copious in sheep with clinical disease. A longitudinal study of the natural transmission showed that infection established readily and rapidly in young lambs and also horizontally in adult sheep, but that the majority of infected sheep did not show clinical disease during their commercial life span.

PATHOGENESIS

The virus replicates in the type II pneumocytes in the alveolus. Type II pneumocytes and Clara cells in the terminal bronchioles are transformed, and their growth produces intraalveolar and intrabronchiolar polypoid ingrowths. These cells are surfactant-producing secretory cells, and there is also copious production of fluid. The excessive surfactant-like protein produced in the tumor provides a stimulus for the accumulation of macrophages seen in association with this disease. The adenomatous ingrowths of alveolar epitheliums encroach gradually upon alveolar airspace so that anoxic anoxia occurs. The lesions produced by experimental inoculation are identical with those of the naturally occurring disease.

CLINICAL FINDINGS

Affected sheep are afebrile and show progressive respiratory distress with loss of weight. Clinical signs are not evident until a significant proportion of the lung is compromised by the tumor.[1] Occasional coughing and some panting after exercise are the earliest signs, but coughing is not a prominent sign in this disease unless there is concurrent parasitic pneumonia. Subsequently there is emaciation, dyspnea, lacrimation, and a profuse watery discharge from the nose, with death from 6 weeks to 4 months later. A diagnostic test, colloquially known as the wheelbarrow test, in this disease is to hold the sheep up by the hindlegs: in affected animals a quantity of watery mucus (up to about 200 mL) runs from the nostrils. Moist crackles are audible over the affected lung areas and may be heard at a distance, so that a group of affected animals are said to produce a sound like slowly boiling porridge. There is no elevation of body temperature unless there is secondary infection, and the appetite is normal. Advanced cases may have cor pulmonale. Pasteurellosis (*Mannheimia haemolytica*) is a common complication and often the cause of death.

CLINICAL PATHOLOGY

No immune reaction can be detected in affected animals, and there is no serologic test. Sheep in advanced stages of the disease may show neutrophilia and lymphocytopenia. The pulmonary fluid contains round or spherical clusters of epithelial cells, which have the hyperplasic adenomatous epithelium typical of pulmonary lesions and increased numbers of macrophages. Earlier reports of a consistent elevation in circulating immunoglobulin concentrations have not been substantiated. JSRV can be detected by exogenous JSVR-specific PCR in peripheral blood leukocytes and can be used to demonstrate that JSVR is not present in flocks or regions.[3]

NECROPSY FINDINGS

Lesions are usually restricted to the thoracic cavity. As in maedi, the lungs are grossly increased in size and in weight (up to 3 times normal). There are extensive areas of neoplastic tissue, particularly of the anterioventral regions of one or both lungs, with smaller lesions in the diaphragmatic lobes. The affected areas are solid and slightly raised above the adjacent normal lung. This, with the excess frothy fluid in the bronchi, is characteristic. The bronchial and mediastinal lymph nodes are enlarged and hyperplastic, and they occasionally contain small metastases. Pneumonic pasteurellosis is a frequent complication, and secondary pulmonary abscesses and pleurisy may develop. Histologically, the alveolus is lined by cuboidal and columnar epithelial cells that form characteristic adenomatous ingrowths of alveolar epithelium into the alveolar spaces.

Differences between the pathology of classical (progressive) and atypical (nonprogressive) forms of the disease are seen using immunohistochemistry, with an influx of T-cell subsets and expression of MHC class II in the latter.[4]

Samples for Confirmation of Diagnosis

- **Histology**—formalin-fixed lung, bronchial lymph node (LM)

DIFFERENTIAL DIAGNOSIS

Chronic pneumonias requiring differentiation from jaagsiekte:

- Maedi
- Parasitic pneumonia
- Chronic suppurative pneumonia
- Caseous lymphadenitis
- Postdipping pneumonia
- Enzootic pneumonia
- Melioidosis

TREATMENT

No treatment is available.

CONTROL

In Iceland, where the disease assumed epizootic proportions, eradication was achieved in the 1950s by complete slaughter of all sheep in the affected areas. In areas where the prevalence is lower, the disease can be satisfactorily controlled, but not eradicated, by slaughter of clinically affected sheep. There is evidence that the disease is spreading in sheep populations in some countries, such as the United Kingdom, and flocks that are free of disease should attempt to obtain replacement sheep from flocks that are free of jaagsiekte. Infected flocks can reduce the prevalence of disease by culling sheep at the onset of clinical signs and also culling the progeny of affected ewes. PCR can detect infection in the preclinical stages, but there has been no trial to establish if eradication from a flock can be achieved with this technology.

Exclusion of the disease from unaffected flocks requires strict biosecurity measures.

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NASAL BOTS INFESTATION

Infestation of sheep and goats with larvae of the nasal bot fly (*Oestrus ovis*) has a serious effect on the productivity and welfare of both sheep and goats. Adult activity induces stress responses and significant behavioral change. Larval infestation induces moderate to severe pathology that reduces productivity.

Similar flies are known to affect horses, donkeys, and mules (*Rhinoestrus* spp.) in the Mediterranean region and to affect camels (*Cephenemyia titillator*) in Africa and Australia. Wild ungulates are affected by nasal bots (e.g., *Cephenemyia* spp.). Very little is known about the pathology and impact of these later groups of flies, but similarities in life history suggest their effects will be similar to those discussed here.

SYNOPSIS

Etiology *Oestrus ovis* inhabits the nasal passages and sinuses of sheep and goats. Similar species affect horses and camels.

Epidemiology Larvae are sprayed onto the nares of hosts by passing females. Flies are active during spring and summer, inducing behavioral changes in hosts under attack. In temperate climates there is only a single generation per year, but in warmer climates two generations occur. First instars in the nasal passages undergo hypobiosis during winter or hot summer when survival of pupae or adults is low, resuming development when conditions are more favorable.

Clinical signs Shortly after arrival of the larvae an increase in nasal discharge and sneezing are evident. As the infestations develop the amount of discharge increases and the nostrils may become caked with dust and debris, forcing the infested animals to breathe through their mouth.

Clinical pathology Changes are noted to the mucosa of the ethmoid and sinus regions. Inflammation of these surfaces tissues is evident and increases as the larvae become mature. Changes to the epithelial structure are noted, including the erosion of the surface ciliary covering and a breakdown in epithelial cell integrity. Abrasive action of the body armature and the activity of proteolytic enzymes excreted/secreted by the larvae are responsible for the pathology. Secondary effects Include the induction of lung lesions and the activation of latent "orf" infections. Diagnostic confirmation. Behavioral changes during fly season and nasal discharge

Differential diagnosis Unthriftiness usually caused by helminth infection.

Treatment Macrocyclic lactone endectocides, clorsulon.

Control Treatment given when fly activity has ceased.

ETIOLOGY

The sheep nose bot affects sheep and goats in most regions, but it is particularly significant in the Mediterranean basin, central America, southern Africa, and eastern Europe. The larvae inhabit the nasal passages and sinuses, eventually being expelled through the nares. Goats are less dramatically affected than sheep. The slightly dorsoventrally flattened, segmented larvae are light cream in color, but as they reach maturity dark bands appear on each segment. Species affecting horses and camels have distributions that are similar.

LIFE CYCLE AND EPIDEMIOLOGY

The adult fly is stout, mottled gray in color, and about 1cm long. Its mouthparts are rudimentary, and it does not feed. In North America, flies emerge in the late spring and mate, and the females begin larviposition activities approximately 2 to 3 weeks later. Adult flies attempting to deposit larvae on the nares annoy the sheep and cause them to bunch or seek shelter. Stamping of the feet and shaking of the head are common. Sheep may bunch together and press their heads into the fleece of others. Fly activity occurs primarily during the warmer parts of the day but still may result in the loss of a good deal of grazing time. Behavioral changes in goats are less dramatic, presumably because of their browsing habit.

Larval development takes place in in the dorsal turbinates and frontal sinuses. The period of development can vary 3 weeks to several months, after which they migrate to the nostrils. Larvae feed on the mucosal secretions and cells eroded from the mucosal epithelium. The larvae are thick, yellowish-white in color, and when mature there is a dark dorsal band on each segment. The ventral surface has rows of small spines on each segment. Mature larvae exit the host, usually during a bout of sneezing, and

actively burrow beneath the upper layers of soil and ground litter. Pupation occurs at these locations, and development of the adults requires 4 to 5 weeks but may take longer at low temperatures. In temperate areas there may be one or two generations per year, but several generations may be completed in hot areas. *O. ovis* are adapted to the various climates prevailing wherever sheep and goats are kept. When winters are cold, the larvae can overwinter by remaining dormant in the first instar (hypobiosis), but in warmer climates development may continue throughout the winter. In those regions where summer temperatures are extreme, the larvae will also undergo hypobiosis.

O. ovis are an important zoonosis because the females may larviposit in the eye, in the nose, or on the lips of humans. In some countries ophthalmomyiasis or infection of the upper respiratory tract is a common occurrence.

PATHOGENESIS

The stress of the larviposition attacks can be significant with reduced grazing time and overheating resulting from bunching. Herdsmen find the animals are more nervous and difficult during the fly activity periods.

Larvae induce a gradually increasing rhinitis and sinusitis as the infestation persists. Marked changes in the structure of the epithelial tissues are noted. with a marked cellular degeneration and a loss of the ciliary layer. The changes are a result of both mechanical activity of the larval spines and mouthhooks and the effect of proteolytic enzymes excreted or secreted.[1,2] Varying degrees of mucous discharge are observed in the later stages of the infestation. This can lead to the nostrils being occluded by adherent straw and dust.

CLINICAL FINDINGS

Early in the infestation there is a distinct rhinitis accompanied by a muco- to mucopurulent discharge.[1] Later as larvae mature a sinusitis is evident. Presence of mature larvae in nasal cavities may induce excessive sneezing, which assists larval exit.

Activity of the larvae in the nasal cavities, and the changes they induce lead to an increases incidence of secondary pathology. The number and severity of lung abcess are more significant in nose bot–infested sheep. The presence of bots also is correlated with increased carcinomas and may lead to reactivation of latent "orf" symptoms.

DIAGNOSIS

The behavioral changes during fly activity, including bunching and burying of noses in neighbors' fleeces, is a reliable indicator of fly attack. Nasal discharge and excessive sneezing are highly suggestive but not definitive. Infested sheep and goats develop some level of immunity from exposure to larval antigens but is unlikely to be used on the farm.[2] An ELISA for detection of antibodies to larvae secretions has been developed but is not currently used.[2]

TREATMENT

Closantel 5 mg/kg and ivermectin 0.2 mg/kg, in addition to other macrocyclic lactones, are effective, and the use of these compounds for fluke or worm control also controls nasal bots.

CONTROL

Treatment should preferably be applied after the cessation of fly activity, although it may be necessary to apply treatments during prolonged fly activity to give relief.[1]

RECOMMENDATION

Treatment should be applied once or twice a year. This is not absolutely necessary but will increase both endurance and the animal's well-being. Population control of the flies is probably not likely.

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LUNGWORM INFESTATION IN SHEEP AND GOATS

SYNOPSIS

Etiology The nematode parasites *Dictyocaulus filaria, Muellerius capillaris,* and *Protostrongylus rufescens.*

Epidemiology Infective *D. filaria* larvae are found on grass, but *M. capillaris* and *P. rufescens* are transmitted when molluscan intermediate hosts are accidentally ingested by grazing animals.

Signs *D. filaria* and *P. rufescens* can cause bronchitis and loss of condition. *M. capillaris* is asymptomatic in sheep but may be pathogenic in goats.

Clinical pathology Characteristic larvae in feces.

Lesions *D. filaria* and *P. rufescens:* scattered patches of consolidation; *M. capillaris:* small fibrous nodules up to 5 mm in diameter.

Diagnostic confirmation Characteristic larvae in feces.

Treatment Avermectins/milbemycins, benzimidazoles or levamisole.

Control No specific measures available.

ETIOLOGY

Infestations with the nematode *Muellerius capillaris* are ubiquitous. *Dictyocaulus filaria* and *Protostrongylus rufescens* are encountered sporadically. *Cystocaulus ocreatus* and *Neostrongylus linearis* have been recorded in some countries.

LIFE CYCLE

D. filaria has a direct life cycle like that of *D. viviparus* in cattle. The life cycles of the other (protostrongylid) species are similar except that they have different predilection sites in the lung and have indirect life cycles with molluscan intermediate hosts. Transmission occurs when infected slugs or snails are accidentally ingested during grazing.

EPIDEMIOLOGY

D. filaria infestations in sheep appear to follow the same pattern as those of *D. viviparus* in calves, but the number of lungworms is usually low. The third-stage larvae are long-living in damp, cool surroundings. The lambs of one season are the main source of infection for the next season's lambs, but larvae passed by ewes and yearlings also contribute to pasture contamination. The prevalence of infection is low in spring and summer but rises rapidly in the autumn and winter, when most clinical cases are seen. Warm, wet summers give rise to heavier burdens in the following autumn and winter. Immunity after natural exposure is strong and durable in sheep but less so in goats.

M. capillaris infestations in sheep have been recorded from most parts of the world,[1,2,3] and in many temperate areas almost all sheep are infected.[4,5] Massive invasion with larvae is uncommon because the intermediate hosts are not usually ingested in large numbers nor are they grossly infested with larvae. Massive infestations with this worm do not develop acutely, and heavy infestations, when they occur, appear to develop over a long period of time. Infected sheep carry patent infection from 1 year to the next.

PATHOGENESIS

The relative pathogenicity of each lungworm is dependent on its predilection site. *D. filaria* lives in the trachea and bronchi so aspirated eggs, larvae, and debris can affect a large volume of lung tissue. It is therefore the most pathogenic species and provokes changes resembling those described for *D. viviparus.* The volume of damaged lung is however usually insufficient to cause severe dyspnea. Adult *P. rufescens* are found in smaller bronchioles, and so associated lesions are much smaller. *M. capillaris* is found in the lung parenchyma, where it becomes encysted in fibrous nodules. Lesions are thereby confined to its immediate surroundings. Consequently, this worm is generally considered to be relatively innocuous. Heavy mixed protostrongylid infections can impair pulmonary gaseous exchange.

CLINICAL FINDINGS

Lambs 4 to 6 months of age are most severely affected with lungworms, but sheep of all

ages are susceptible. Clinically *D. filaria* is associated with bronchial irritation that results in coughing, moderate dyspnea, and loss of condition. There may be added fever and evidence of toxemia if secondary bacterial infection occurs. It is highly pathogenic in young goats. *P. rufescens* infestations in sheep and goats cause clinical signs similar to those of *D. filaria.*

CLINICAL PATHOLOGY

Laboratory diagnosis depends on the detection of first-stage larvae in the feces by the Baermann technique. *D. filaria* larvae have refractile granules in their intestinal cells and a conical tail. *P. rufescens* has a wavy tail as does *M. capillaris,* which, in addition, has a spine just anterior to the tail.

NECROPSY FINDINGS

D. filaria lesions are similar to those of the subacute form of parasitic bronchitis in calves with exudate in the bronchioles, scattered patches of consolidation, and thickening of the alveolar septa,[6-8] but widespread lesions are not common. *M. capillaris* is found in small fibrous nodules up to 5 mm in diameter. Most of these are in the parenchyma of the lung immediately under the pleura. Many of them are calcified and often contain only one live or dead worm. Infestation of goats leads to a diffuse infection quite different from the nodular reaction in sheep and to the production of an interstitial pneumonia. Whether this is attributable solely to *M. capillaris* infection or whether a chlamydial or viral agent is involved has not been determined. However, cases of nodular reaction in goats attributable to *M. capillaris* larvae have been reported.[6]

DIAGNOSTIC CONFIRMATION

The presence of larvae in the feces confirms lungworm infection, but their number is often no indication of the degree of infestation.

DIFFERENTIAL DIAGNOSIS

Lungworm infestation in sheep needs to be differentiated from maedi and jaagsiekte.

TREATMENT

TREATMENT
Ivermectin (0.2 mg/kg, SC) (R-1)
Moxidectin (0.2 mg/kg, SC or PO) (R-1)
Fenbendazole (5 mg/kg PO, every day for 7 days) (R-2)
Albendazole (7.5 mg/kg BW, PO) (R-2)

Ivermectin, moxidectin, the benzimidazoles, and levamisole are effective against *D. filaria* at normal dose rates. Ivermectin, in addition, has a label claim for *P. rufescens.* It is doubtful whether treatment of sheep for *M. capillaris* is ever justified. In goats, one or two doses of ivermectin (0.2 mg/kg, SC o rPO) or elevated doses of benzimidazoles destroys the adult worms but not the immature stage, but regular daily oral doses of fenbendazole (up to 5 mg/kg/d) in the feed for 1 to 2 weeks or albendazole (1 mg/kg in the feed for 2 weeks) are highly effective against all stages. The label dose of albendazole (7.5 mg/kg BW, once in sheep and 10 mg/kg BW once in goats) is effective in treating adult lungworms, but is not effective against the immature stage.

CONTROL

An attenuated vaccine for *D. filaria* is available in a few countries where this worm is a particular problem. With most forms of sheep husbandry, there are few precautionary measures that can be taken, particularly against lungworms with molluscan intermediate hosts.

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Diseases of the Equine Respiratory Tract

ABNORMALITIES OF THE UPPER RESPIRATORY TRACT OF HORSES

Impairment of ventilation by abnormalities of the upper respiratory tract is an important cause of poor performance in athletic horses. Abnormalities that impair athletic capacity are those that reduce the effective diameter of the upper airway, thereby increasing the work needed to maintain the same level of tidal volume and minute ventilation or, as is the case clinically, reducing the minute ventilation achieved by the horse during maximal exercise. In other words, a reduction in effective diameter of the upper airway increases work of breathing at all exercise intensities, and at maximal intensity, when the effort expended on breathing cannot be increased, decreases maximal minute ventilation. The result is diminished oxygenation of arterial blood and delivery of oxygen to muscle and other tissues, exacerbated hypercapnia, and reduced athletic capacity during high-speed exercise.[1-3]

The work of breathing is, simplistically, determined by the volume of air moved and the pressure required to do so. The relationship between pressure and resistance in the airway is described mathematically by a rearrangement of Poiseuille's law:

$$\text{Resistance to flow} = \text{pressure drop}/\text{flow} = 8 \times \text{viscosity of the air} \times \text{length of the airway}/(\pi \times [\text{radius}]^4)$$

Given that the viscosity of air is constant and the length of the airway does not change for an individual horse, the radius of the airway has a huge effect on resistance to flow. Notice that a change in pressure is inversely proportional to the fourth power of the radius (r^4), with the consequence that relatively small changes in radius have large effects on the pressure needed to generate a given flow of air. For this reason, abnormalities of the upper airway that cause only small reductions in airway diameter can have clinically important effects on ventilation during high intensity exercise.

Another consequence of changes in airway diameter and structure is the generation of abnormal airflow patterns that result in production of abnormal respiratory sounds. Such sounds can vary from gurgling through to roaring and can be of diagnostic importance.[4]

Advent of first rigid and then flexible endoscopes allowed greater refinement of diagnosis of disorders of the upper respiratory tract of horses when examined at rest. A further advance was the ability to examine the upper airway during intense exercise. This was first achieved in horses running on a treadmill and has now progressed to examination of horses running over ground. Although there are advantages to each mode of examination (at rest, treadmill, over ground), greatest diagnostic utility is achieved by examination of horses exercising over ground and performing their customary activity wearing their usual tack and with their rider.[5-8]

Laryngeal hemiplegia caused by recurrent laryngeal neuropathy is a well-recognized abnormality of the upper airway associated with impaired performance by racehorses. In many of its forms, it is readily identified in horses at rest. However, more subtle abnormalities or those that develop as the horse fatigues are best detected, or can only be detected, on examination during strenuous exercise. It is now clear that most abnormalities of the upper airway of horses, with the exception of laryngeal hemiplegia, can only reliably be detected by examination of exercising horses.[9]

Abnormalities developing during strenuous exercise by horses are best referred to as "dynamic" abnormalities. This term should not be used to denote the mode of examination (ie, "dynamic endoscopy"), which should be specified as "over ground" or "treadmill." Terms describing abnormalities detected during endoscopic examination of the upper airway of horses have recently been standardized (Table 12-10).[10]

The use of endoscopy during exercise has revealed that dynamic abnormalities of the upper airway are often complex and involve

Table 12-10 Preferred terms for describing findings on endoscopic examination of the upper airway of horses (modified from [10])

Preferred term	Preferred abbreviation	Also known as
Recurrent laryngeal neuropathy	RLN	Laryngeal paralysis, laryngeal hemiplegia
Dynamic laryngeal collapse	DLC	Bilateral arytenoid cartilage collapse
Intermittent dorsal displacement of the soft palate	iDDSP	
Persistent dorsal displacement of the soft palate	pDDSP	Permanent DDSP
Palatal instability	PI	
Vocal fold collapse	VFC	Vocal cord collapse
Medial deviation of the aryepiglottic fold	MDAF	Aryepiglottic fold collapse, Axial deviation of the aryepiglottic fold
Nasopharyngeal collapse	NPC	Nasopharyngeal obstruction, pharyngeal wall collapse
Ventromedial luxation of the apex of the corniculate process of the arytenoid	VLAC	Collapse of the apex of the corniculate process of the arytenoid
Cricotracheal ligament collapse		
Collapse of the margins of the epiglottis		
Epiglottic retroversion		
Rostral deviation of the palatopharyngeal arch	RDPA	
High speed treadmill endoscopy	HSTE	
Overground endoscopy	OGE	Dynamic respiratory endoscopy, telemetric endoscopy

multiple structures.[9,11] Up to 50% of horses examined during high-speed exercise have multiple abnormalities of the upper airway. Furthermore, examination of exercising horses has revealed abnormalities not apparent during examination of resting horses, including the full spectrum of manifestations of recurrent laryngeal neuropathy, palatal instability including intermittent dorsal displacement of the soft palate, vocal fold collapse, aryepiglottic fold collapse, axial deviation of the aryepiglottic folds, dynamic nasopharyngeal collapse, collapse of the corniculate process of the arytenoid cartilage, bilateral arytenoid and vocal fold collapse, and epiglottic retroversion.[9]

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PALATAL DYSFUNCTION (INSTABILITY, DORSAL DISPLACEMENT OF THE SOFT PALATE)

The soft palate of equids is unique in that it provides an airtight seal between the oropharynx and nasopharynx during respiration, rendering equids obligate nasal breathers. During swallowing the soft palate is transiently displaced dorsally to permit passage of the feed bolus as part of the normal act of deglutition. Abnormalities of the soft palate that result in alteration of its anatomic position (displacement) or inability to maintain normal tone during exercise are associated with impaired respiration and exercise intolerance.[1-3] Displacement of the soft palate other than during deglutition is abnormal and can be intermittent, which is usually associated with exercise, or persistent, which is usually associated with disruption of the nerve supply to the pharynx.

Palatal Instability and Intermittent Dorsal Displacement of the Soft Palate During Exercise

Dysfunction of the palate during exercise results in a range of structural abnormalities that reduce the functional area of the rima glottidis (the opening of the larynx) and thus impair ventilation during high-speed exercise. Dysfunction during exercise ranges from palatal instability to intermittent dorsal displacement of the soft palate.[4] Palatal instability and dorsal displacement of the soft palate cause an expiratory obstruction to airflow through the larynx and pharynx.[4]

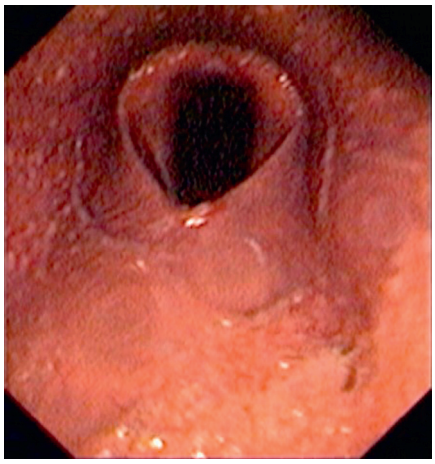

Fig. 12-18 **Endoscopic view of dorsal displacement of the soft palate in a resting Thoroughbred racehorse.**

Palatal instability is evident as dorsoventral billowing of the soft palate during high speed exercise and flattening of the epiglottis against the palate. Palatal instability is significantly associated with dorsal displacement of the soft palate during exercise.[1] Dorsal displacement of the soft palate is an extreme of palatal instability and results when the caudal border of the soft palate displaces dorsal to the epiglottis (Fig. 12-18). Palatal instability is also associated with axial deviation of the aryepiglottic folds and abnormalities in the conformation of the epiglottis.[1,5]

Estimates of the prevalence of the disease in the wider population are unreliable because of the transient nature of the instability and displacement and the fact that it only occurs during exercise. Additionally, examination of large numbers of horses to determine prevalence in populations of horses has not been performed, with most reports being prevalence rates in horses selected for high-speed endoscopic examination. It is estimated to occur in 0.5% to 1.3% of Thoroughbred racehorses, and of 52 Thoroughbred racehorses examined using over ground endoscopy, 25% had dorsal displacement of the soft palate, 40% had axial deviation of the aryepiglottic fold, 35% had vocal fold collapse, and 33% had abnormal arytenoid function.[6] Forty-eight percent of the horses had multiple abnormalities. Nineteen of 57 Thoroughbred yearlings had intermittent dorsal displacement during a single examination using over ground endoscopy.[7] Dorsal displacement was detected in 10 of 46 Standardbred racehorses examined using overground endoscopy during racing—these horses were presumably considered healthy before examination.[8] Three

percent of performance horses (nonracing) had dorsal displacement of the soft palate as the sole abnormality during exercise.[9]

Palatal instability and intermittent dorsal displacement of palate are a common part of complex dynamic abnormalities of the upper respiratory tract in harness horses, with 70% of examined horses having a complex disorder.[5] Similarly, 19% of performance horses (nonracing horses) had complex upper respiratory tract abnormalities during exercise.[9]

The cause of intermittent displacement of the soft palate during exercise is unknown, although a number of mechanisms, including palatal myositis, ulcers of the caudal border of the soft palate, caudal retraction of the larynx, and lower respiratory disease, are suggested. Retropharyngeal lymphadenopathy can cause neurogenic paresis of the pharyngeal and palatal muscles, with dorsal displacement of the soft palate the most obvious sign of pharyngeal collapse during exercise. The immediate cause of the displacement is the high turbulent flow and negative intrapharyngeal pressure generated during exercise.[10]

Displacement of the soft palate during strenuous exercise places the soft palate dorsal to the epiglottis, a position in which it impedes flow of air during expiration. Peak expiratory airflow, minute ventilation, tidal volume, and rate of oxygen consumption are all decreased in horses with dorsal displacement of the soft palate, whereas inspiratory flow and breathing rate are not affected.[4]

Clinical Signs

The clinical signs include exercise intolerance and intermittent production of a gurgling noise during strenuous exercise. **Endoscopic examination** of resting horses usually demonstrates a normal pharynx and larynx. Brief nasal occlusion (30–60 s) that induces displacement of the soft palate (Fig. 12-18), in combination with a history of respiratory noise during exercise, increases the likelihood of the disorder.

Endoscopic examination of affected horses during exercise is the gold standard for diagnosis and reveals signs of palatal instability or dorsal displacement of the soft palate and related abnormalities.

Detection of palatal instability and associated abnormalities is described as follows:[1]

Axial deviation of the aryepiglottic folds: Graded as none, mild, moderate or severe.

1. **Mild** ADAF, defined as axial collapse of the aryepiglottic folds with the folds remaining abaxial to the vocal cords.
2. **Moderate** ADAF, defined as axial deviation of the aryepiglottic folds less than halfway between the vocal cord and the midline.
3. **Severe** ADAF, defined as collapse of the aryepiglottic folds more than halfway between the vocal cord and the midline.

Epiglottic conformation: Epiglottic conformation is categorized into three groups.

1. Convex epiglottic appearance when the epiglottis maintained a convex shape during exercise; typically only the tip of the epiglottis is in contact with the soft palate.
2. Flattened epiglottis where the epiglottis loses its convex shape and appears to lie flat or slightly concave on the surface of the soft palate, but the tip of the epiglottis remains ventral to the base.
3. A tipped up appearance when the epiglottis has a flattened or concave appearance and during inspiration the tip of the epiglottis is at the same level as or higher than the base of the epiglottis.

Obstruction of the rima glottidis *by the soft palate (soft palate stability):* The stability of the soft palate is graded according to whether the *rima glottidis* is obscured by the billowing soft palate.

1. The soft palate is considered stable when there is no movement or lifting of the soft palate was observed (Fig. 12-19).
2. Palatal instability with no *rima glottidis* obstruction when the soft palate lifts up to the level of the base of epiglottis but the *rima glottidis* is not obscured (Fig. 12-20).
3. Palatal instability with *rima glottidis* obstruction when the soft palate lifts so that the *rima glottidis* becomes obscured (Fig. 12-21).

Soft palate conformation: The soft palate of horses with palatal instability is either flaccid, billowing dorsally in front of the epiglottis or billowing dorsally either side of the epiglottis. The presence or absence of a sling appearance to the ventrolateral pharyngeal walls at the level of the guttural pouch ostia should be noted. The caudal soft palate should be assessed as to whether a concave appearance was present and if so should be graded as absent, small, or large during each of inspiration and expiration.

Radiographic examination of the pharynx reveals a shortened epiglottis (<7 cm) in some affected horses.

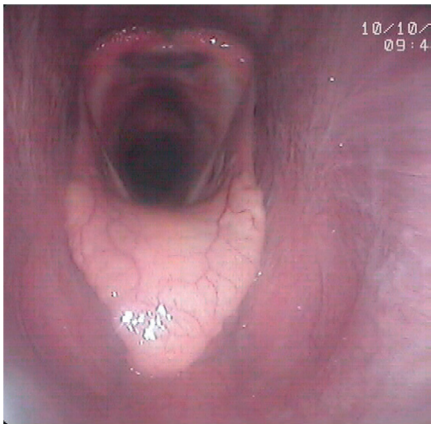

Fig. 12-19 Normal airway of a horse during exercise. (Reproduced with permission.[1])

DIFFERENTIAL DIAGNOSIS

Differential diagnoses for exercise intolerance and respiratory noise include **laryngeal hemiplegia, subepiglottic cysts, arytenoid chondritis, and aryepiglottic fold entrapment**. The important differentiating factor is that the noise occurs predominantly during expiration, and it has a more gurgling sound to it than does the noise produced by horses with laryngeal hemiplegia.

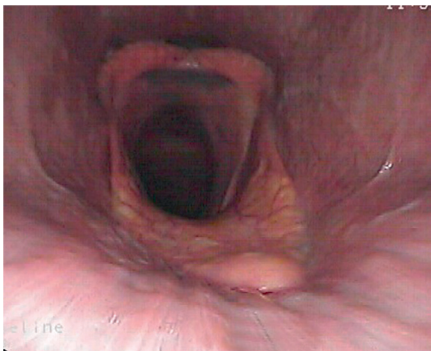

Fig. 12-20 Palatal instability with no rima glottidis obstruction. The epiglottis has a flattened appearance, and the soft palate appears flaccid with no concave depression caudally. (Reproduced with permission.[1])

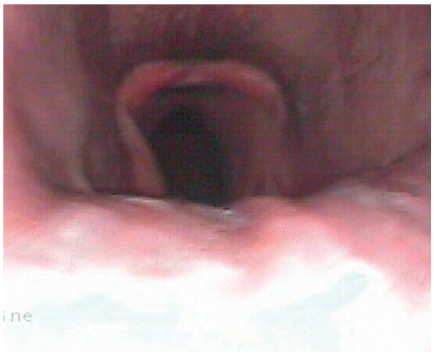

Fig. 12-21 Palatal instability with obstruction of the rima glottidis. The soft palate is billowing in front of the rima glottidis. (Reproduced with permission.[1])

Treatment

There is no definitive treatment and no evidence that any one treatment is superior.[11] Usual methods of surgical intervention include augmentation of the epiglottis by injection of polytetrafluoroethylene (Teflon) paste, resection of the caudal edge of the soft palate or sternothyrohyoideus myectomy, and palatal sclerotherapy,[12] although some of these interventions may have deleterious effects on upper airway airflow. A newer surgical technique involves the "laryngeal tie-forward" procedure.[13,14] Reports of success of surgical treatment of the disease are not definitive, in part because horses with the disorder that went untreated are not examined. It is plausible that the response to surgical treatment could be the result of enforced rest rather than the manipulation. Treatment of retropharyngeal lymphadenopathy may be beneficial. Nonsurgical treatment includes the use of antiinflammatory drugs, tongueties,[15] a variety of bits, and a laryngohyoid support apparatus.

Persistent Dorsal Displacement of the Soft Palate

Persistent dorsal displacement of the soft palate is usually the result of damage to the innervation of the pharyngeal and palatal muscles as a result of the following:

- Guttural pouch mycosis
- Guttural pouch empyema
- Retropharyngeal lymph node abscessation
- Equine protozoal myeloencephalitis
- Otitis media
- Myositis or muscle disease, such as white-muscle disease
- Botulism
- Idiopathic in young foals.[16]

Blockade of the pharyngeal branch of the vagus nerve by injection of local anesthetic causes persistent dorsal displacement of the soft palate, whereas blockade of the hypoglossal and glossopharyngeal nerves does not.

Clinical Signs

Persistent dorsal displacement of the soft palate causes dysphagia and stertorous respiration. Food material discharges from the nares and there is frequent coughing, probably secondary to the aspiration of feed material. Affected horses may develop aspiration pneumonia. If the condition persists, there is dehydration and weight loss. **Endoscopic examination** of the upper airways reveals dorsal displacement of the soft palate and may reveal other abnormalities, such as guttural pouch mycosis, that provide a cause for the disease.

Treatment

Treatment should be directed toward resolution of the underlying disease and provision of food and water. It is often necessary to feed affected horses through a nasogastric tube.

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DISEASES OF THE GUTTURAL POUCHES (AUDITORY TUBE DIVERTICULUM, EUSTACHIAN TUBE DIVERTICULUM)

The guttural pouches are diverticula of the auditory (or eustachian) tubes found in equids and a limited number of other species. The function of the guttural pouch is unclear, although it could have a role in regulation of cerebral blood pressure, swallowing, and hearing. It is unlikely to have a role in brain cooling. Each guttural pouch of an adult horse has a volume of approximately 300 mL and is divided by the stylohyoid bone into lateral and medial compartments.

The medial compartment of the guttural pouch contains a number of important structures including the internal carotid artery and glossopharyngeal, hypoglossal, and spinal accessory nerves in addition to branches of the vagus nerve and the cervical sympathetic trunk. Retropharyngeal lymph nodes lie beneath the mucosa of the ventral aspect of the medial compartment, an important factor in the development of guttural pouch empyema.

In the lateral compartment the external carotid artery passes along the ventral aspect as do the glossopharyngeal and hypoglossal nerves. Involvement of any of the aforementioned structures is important in the pathogenesis and clinical signs of guttural pouch disease and may result in abnormalities, such as Horner's syndrome, that are not readily recognized as being caused by guttural pouch disease.

The common diseases of the guttural pouch are described here.

GUTTURAL POUCH EMPYEMA

ETIOLOGY

Empyema is the accumulation of purulent material in one or both guttural pouches. Initially, the purulent material is liquid, although it is usually viscid, but over time it becomes inspissated and is kneaded into ovoid masses called **chondroids** (Fig. 12-22). Chondroids occur in approximately 20% of horses with guttural pouch empyema. The condition is most commonly associated with *S. equi* var. *equi* infection and is a recognized sequela to strangles. Therefore any horse with guttural pouch empyema should be isolated and treated as if it were infected with *S. equi* var. *equi* until proven otherwise. The empyema can be associated with other conditions of the guttural pouches, especially if there is impaired drainage of the pouch through the pharyngeal opening of the eustachian tube.

Fig. 12-22 Chondroids removed at postmortem from the guttural pouch of a horse.

EPIDEMIOLOGY

The epidemiology, apart from its association with strangles, has not been defined. The disease occurs in all ages of horses, including foals, and all equids, including asses and donkeys. The case-fatality rate is approximately 10%, with one-third of horses having complete resolution of the disease. Guttural pouch empyema occurs in approximately 7% of horses with strangles. The recovery rate for horses with uncomplicated empyema treated appropriately is generally considered to be good, although the presence of chondroids worsens the prognosis.

PATHOGENESIS

The pathogenesis of guttural pouch empyema is unclear, although when secondary to strangles it is usually attributable to the rupture of abscessed retropharyngeal lymph nodes into the medial compartment. Continued drainage of the abscesses presumably overwhelms the normal drainage and protective mechanisms of the guttural pouch, allowing bacterial colonization, influx of neutrophils, and accumulation of purulent material. Swelling of the mucosa, especially around the opening to the pharynx, impairs drainage and facilitates fluid accumulation in the pouch. The accumulation of material in the pouch causes distension and mechanical interference with swallowing and breathing. Inflammation of the guttural pouch mucosa may involve the nerves that lie beneath it and result in neuritis with subsequent pharyngeal and laryngeal dysfunction and dysphagia.

CLINICAL FINDINGS

Clinical findings include the following:

- Purulent nasal discharge
- Swelling of the area caudal to the ramus of the mandible and ventral to the ear
- Lymphadenopathy
- Carriage of the head with the nose elevated above its usual position
- Dysphagia and other cranial nerve dysfunction
- Respiratory stertor

The nasal discharge is usually unilateral, as is the disease, intermittent, and white to yellow. Guttural pouch empyema is not usually associated with hemorrhage, although the discharge may be blood tinged. Bilateral disease, and the resultant neuritis and mechanical interference with swallowing and breathing, may cause discharge of feed material from the nostrils, dysphagia, and respiratory stertor.

Endoscopic examination of the pharynx reveals drainage of purulent material from the pharyngeal opening of the eustachian tube of the affected side. The guttural pouch contains a variable quantity of purulent material, although in severe cases the quantity of fluid may be sufficient to prevent adequate examination of the pouch with an endoscope.

Radiographic examinations demonstrate the presence of radiodense material in the guttural pouch, sometimes the presence of an air–gas interface (fluid line) within the pouch and distension of the pouch with impingement into the nasopharynx. Chondroids are evident as multiple circular radiodensities. Passage of a **catheter** into the guttural pouch via the pharyngeal opening permits aspiration of fluid for cytology and bacterial culture.

CLINICAL PATHOLOGY

Hematologic examination may reveal evidence of chronic infection, including a mild leukocytosis, hyperproteinemia, and hyperfibrinogenemia. Fluid from the affected guttural pouch contains large numbers of degenerate neutrophils and occasional intracellular and extracellular bacteria. Bacterial culture yields *S. equi* in approximately 30% of cases and *S. zooepidemicus* in approximately 40% of cases.

NECROPSY FINDINGS

Lesions of guttural pouch empyema include the presence of purulent material in the guttural pouch and inflammation of the mucosa of the affected guttural pouch.

DIAGNOSTIC CONFIRMATION

Diagnostic confirmation in a horse with clinical signs of guttural pouch disease is achieved by demonstration of purulent material in the guttural pouch by endoscopic or radiographic examination and examination of the fluid.

DIFFERENTIAL DIAGNOSIS

Differential diagnosis of guttural pouch empyema includes the following conditions:

- Abscessation of retropharyngeal lymph nodes
- Guttural pouch tympany
- Guttural pouch mycosis

Guttural pouch empyema should also be differentiated from other causes of nasal discharge in horses, including the following:

- Sinusitis
- Recurrent airway obstruction (heaves)
- Pneumonia
- Esophageal obstruction
- Dysphagia of other cause

Infection by *Mycobacterium avium* complex organisms causes nasal discharge and granulomatous lesions in the guttural pouch.

TREATMENT

The principles of treatment are removal of the purulent material, eradication of infection, reduction of inflammation, relief of respiratory distress, and provision of nutritional support in severely affected horses.[1]

Removal of purulent material can be difficult but can be achieved by repeated flushing of the affected guttural pouch. The guttural pouch can be flushed through a catheter (10–20 French, 3.3- to 7-mm male dog urinary catheter) inserted as needed via the nares, or a catheter (polyethylene 240 tubing) with a coiled end inserted via the nares and retained in the pouch for several days. The pouch can also be flushed through the biopsy port of an endoscope inserted into the guttural pouch.

The choice of fluid with which to flush the guttural pouch is arbitrary, but frequently used fluids include normal (isotonic) saline, lactated Ringer's solution, or 1% (v/v) povidone–iodine solution. It is important that the fluid infused into the guttural pouch be nonirritating because introduction of fluids such as hydrogen peroxide or strong solutions of iodine (e.g., 10% v/v povidone iodine) will exacerbate the inflammation of the mucosa and underlying nerves and can actually prolong the course of the disease.[2] The frequency of flushing is initially daily, with reduced frequency as the empyema resolves.

Infusion of antibiotics into the guttural pouches is probably without merit although this is debated. Because of the viscid nature of the empyema fluid, it is necessary to infuse large volumes of lavage solution (1–2 L) on consecutive days. It may be necessary to treat for 7 to 10 days. The infusion of **acetylcysteine** (60 mL of a 20% solution) into the pouch after lavage with 1 to 2 L of saline has been reported to be effective in aiding the removal of purulent material. Removal of **chondroids** usually requires surgery, although dissection and removal of chondroids through the pharyngeal opening has been described. A stone remover inserted through the biopsy channel of the endoscope can be useful for removal of small numbers of chondroids, but is tedious if there are large numbers of them. A rule of thumb is that if the chondroids occupy more than one-third of the volume of the guttural pouch, then removal should be carried out surgically.

Systemic antimicrobial administration is recommended for all cases of guttural pouch empyema because of the frequent association of the disease with bacterial infection and especially *S. equi* and *S. zooepidemicus* infection of the retropharyngeal lymph nodes. The antibiotic of choice is **penicillin G** (procaine penicillin G, 20,000 IU/kg intramuscularly every 12 hours for 5-7 days), although a combination of sulfonamide and trimethoprim (15-30 mg/kg orally every 12 hours for 5-7 days) is often used. **Topical application of antimicrobials** into the guttural pouch is probably ineffective because they do not penetrate the infected soft tissues of the pouch and retropharyngeal area.

NSAIDs such as flunixin meglumine (1 mg/kg intravenously or orally every 12 hours) or phenylbutazone (2.2 mg/kg intravenously or orally every 12 hours) are used to reduce inflammation and pain. Severely affected horses may require relief of respiratory distress by tracheotomy. Dysphagic horses may need nutritional support, including administration of fluids.

Chronic cases refractory to treatment might require fistulation of the guttural pouch into the pharynx.

CONTROL

Prevention of guttural pouch empyema is based on a reduction in the frequency and severity of *S. equi* infection in horses (see "Strangles").

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GUTTURAL POUCH MYCOSIS

ETIOLOGY

Mycosis of the guttural pouch is caused by infection of the dorsal wall of the medial compartment of the pouch, caudal and medial to the articulation of the stylohyoid bone and the petrous temporal bone. The most common fungi isolated from the lesions are *Aspergillus (Emericella) nidulans, Aspergillus fumigatus,* and, rarely, *Penicillium* spp. and *Mucor* spp., although spores of these fungi are present in the guttural pouches of normal horses. Other fungal species isolated include *Fusarium, Trichosporon, Acremonium,* and *Rhodotorula.*[1]

EPIDEMIOLOGY

The disease occurs in horses of both sexes and all breeds. Horses are affected at all ages, with the youngest recorded case being a 6-month-old foal. The overall prevalence is low, although precise figures are lacking. Among horses left untreated the case-fatality rate is ~50% to 60%, whereas in those treated medically it is ~45%, and in horses treated surgically the case-fatality rate is 33%.[1,2]

PATHOGENESIS

The pathogenesis of the disease is unclear, although it is likely that fungal spores gain access to the guttural pouch through the pharyngeal opening. The spores then germinate and proliferate in the mucosa of the dorsal, medial aspect of the medial compartment of the guttural pouch. The location of the lesion is consistent, but the reason for the disease occurring in this particular position is unclear. Factors that predispose to the development of mycotic lesions have not been determined, although it appears unlikely that fungal infection is the initial insult to the mucosa. Invasion of guttural pouch mucosa is followed by invasion of the nerves, arteries, and soft tissues adjacent to it. Invasion of the nerves causes glossopharyngeal, hypoglossal, facial, sympathetic, or vagal dysfunction. Invasion of the internal carotid artery, and occasionally the maxillary or external carotid, causes weakening of the arterial wall and aneurysmal dilatation of the artery, with subsequent rupture and hemorrhage. Death is caused by hemorrhagic shock or, in horses with dysphagia, aspiration pneumonia or starvation.

Guttural pouch mycosis is usually **unilateral**, although in approximately 8% of cases there is erosion of the medial septum and spread of infection into the other pouch. There is no predisposition for either the left or right pouch. Guttural pouch mycosis presents as either **epistaxis** that is not associated with exercise or as **cranial nerve disease**.

CLINICAL FINDINGS

The clinical signs of guttural pouch mycosis include epistaxis (75% of cases), dysphagia (15%), and purulent nasal discharge (10%).[1-3] **Epistaxis** is usually severe and frequently life-threatening. There is profuse bleeding of bright red blood from both nostrils during an episode, and between episodes there may be a slight, serosanguineous nasal discharge. There are usually several episodes of epistaxis over a period of weeks before the horse dies. Most horses that die of guttural pouch mycosis do so because of hemorrhagic shock.

Signs of cranial nerve dysfunction are common in horses with guttural pouch mycosis and can precede or accompany epistaxis.

- **Dysphagia** is the most common sign of cranial nerve disease and is attributable to lesions of the glossopharyngeal and cranial laryngeal (vagus) nerves. Dysphagic horses may attempt to eat or drink but are unable to move the food bolus from the oral cavity to the esophagus.
- Affected horses frequently have nasal discharge that contains feed material and often develop aspiration pneumonia.
- Lesions of the recurrent laryngeal nerve cause **laryngeal hemiplegia.**
- **Horner's syndrome** (ptosis of the upper eyelid, miosis, enophthalmos, and prolapse of the nictitating membrane) is seen when the lesion involves the cranial cervical ganglion or sympathetic nerve trunk.
- **Facial nerve dysfunction**, evident as drooping of the ear on the affected side, lack of facial expression, inability to close the eyelids, corneal ulceration, and deviation of the muzzle away from the affected side, also occurs.

Signs of cranial nerve and sympathetic trunk dysfunction may resolve with eradication of the infection, but they are frequently permanent.

Guttural pouch mycosis is also associated with **pain** on palpation of the parotid region, **head shyness** and **abnormal head position**. The infection may spread to the atlanto-occipital joint, causing pain on movement of the head, or to the brain, causing encephalitis.[4]

Endoscopic examination of the guttural pouch reveals a plaque of dark yellow to black necrotic material in the dorsal aspect of the medial compartment. A sample of the material can be collected through a biopsy port of the endoscope and submitted for culture. The mycotic plaque cannot be easily dislodged by manipulation with biopsy instruments or the end of the endoscope. In cases with ongoing or recent hemorrhage, the presence of large quantities of blood might prevent identification of the mycotic plaque. Both pouches should always be examined because of the occasional occurrence of bilateral disease or extension of the disease through the medial septum. Caution should be exercised in performing endoscopic examination of the guttural pouch of horses with acute or ongoing hemorrhage because of the risk of exacerbating the hemorrhage. These horses are usually referred for urgent surgical intervention.

Radiographic examination of the guttural pouches may reveal the presence of a lesion in the appropriate position, but it is frequently unrewarding.

CLINICAL PATHOLOGY

There are no characteristic findings on the hemogram, nor are there serum biochemical abnormalities. Horses with repeated hemorrhage are often **anemic**. Immunoblot may identify the presence of serum antibodies specific for *A. fumigatus* in infected horses, although the diagnostic usefulness has not been determined. **Culture** of a sample of the necrotic tissue will frequently yield one of the causative fungi.

NECROPSY FINDINGS

Lesions of guttural pouch mycosis include the presence of a clearly demarcated, yellowish-brown to black, dry plaque of necrotic tissue in the dorsal aspect of the medial compartment of the guttural pouch. The plaque of tissue is firmly adherent to underlying tissues and may perforate the medial septum and invade the other pouch. The infection may involve the adjacent nerves and blood vessels and spread to soft tissues and bone. Histologic examination reveals the presence of inflammatory cells in nerves and tissues surrounding the gross lesion. There is chromatolysis and degeneration of neurones in affected nerves. The internal carotid artery may have an aneurysmal dilatation, or there may be rupture of the arterial wall without dilatation. There is usually partial thrombosis of the arterial wall.

DIFFERENTIAL DIAGNOSIS

Differential diagnoses for epistaxis not associated with exercise include **ethmoidal hematoma** or **guttural pouch empyema**, **neoplasia**, **rupture of the longus capitis muscle,** and penetration by a **foreign body.**

TREATMENT

Treatment of guttural pouch mycosis involves prevention of death from hemorrhage and administration of antifungal agents.

Prevention of hemorrhage from the internal carotid or maxillary artery is achieved by surgical ligation, transarterial coil embolization, or occlusion with intra-arterial balloons of one or more of the external carotid, internal carotid, or maxillary artery.[5-8] Because of the high rate of death from hemorrhage in horses with guttural pouch mycosis, some authorities recommend that all horses with the disease have the internal artery ligated or occluded. Medical treatment of horses with hemorrhage secondary to guttural pouch mycosis is rarely successful.

Administration of antifungal agents by instillation into the guttural pouch through a catheter or endoscope has been reported, although there is disagreement about the need for such treatment in horses that have had the problematic arteries ligated or occluded. Oral administration of antifungal agents is generally ineffective or prohibitively expensive, although itraconazole (5 mg/kg orally once daily) might be useful. Agents reported to be usefully given by topical administration include enilconazole (60 mL of 33 mg/mL solution once daily for 3

weeks), miconazole (60 mL of 1 mg/mL solution), natamycin, and nystatin. Topical therapy is laborious because it must be continued for weeks and involves placement and maintenance of a catheter in the guttural pouch or instillation of medication by daily endoscopy.

Horses with signs of cranial nerve or sympathetic trunk damage may not recover completely even if cured of the fungal infection because of irreparable damage to the affected nerves. Provision of supportive care, including fluid and nutrient administration to dysphagic horses and administration of antibiotics to prevent or treat aspiration pneumonia, may be indicated.

CONTROL

There are no recognized effective measures to control or prevent the disease.

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GUTTURAL POUCH TYMPANY

ETIOLOGY AND EPIDEMIOLOGY

Guttural pouch tympany refers to the gaseous distension of one, rarely both, guttural pouches of young horses. Tympany develops in foals up to 1 year of age but is usually apparent within the first several months of life. **Fillies** are more commonly affected than are colts by a ratio of 2 to 4 : 1, and the disease has a heritability of 0.8. The cause is not known, although a polygenic cause has been proposed for Arabians, and genome-wide association studies have identified linked regions in Arabian and Warmblood horses.[1] The presence of a quantitative trait loci on ECA2 in fillies and ECA15 in colts supports the reported sex distribution of the disease.[2]

CLINICAL FINDINGS

Clinical findings include marked swelling of the parotid region of the affected side, with lesser swelling of the contralateral side. The swelling of the affected side is not painful on palpation and is elastic and compressible. The disease is usually unilateral but can be bilateral. There are stertorous breath sounds in most affected foals as a result of impingement of the distended pouch on the nasopharynx. Respiratory distress can develop. Severely affected foals are dysphagic and develop aspiration pneumonia.

Endoscopic examination of the pharynx reveals narrowing of the nasopharynx by the distended guttural pouch. The guttural pouch openings are usually normal. There are usually no detectable abnormalities of the guttural pouches apart from distension. Radiographic examination demonstrates air-filled pouches, and dorsoventral images permit documentation of which side is affected. There are no characteristic changes in the hemogram or serum biochemical profile.

There are no characteristic lesions, and necropsy examination usually does not demonstrate a cause for the disease.

TREATMENT

Treatment consists of surgical fenestration of the medial septum allowing drainage of air from the affected pouch into the unaffected side. The usual approach is through Viborg's triangle. Creation of salpingopharyngeal fistulas using transendoscopic laser fenestration is reported in a foal with bilateral disease.[3] The prognosis for long-term resolution after surgery is approximately 60%.

A nonsurgical option for treatment involves placement of Foley catheters (22 or 24 French) into the guttural pouches. Placement is achieved by endoscopic guidance via the ipsilateral nostril and resulted in resolution of the tympany in 5 of 8 foals over a period of weeks to months. The catheters are sutured in position and remain in place for weeks.[4]

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OTHER GUTTURAL POUCH DISEASES

Rupture of the longus capitis muscle or avulsion of its insertion on the basisphenoid bone causes epistaxis and is usually associated with trauma to the head, such as is caused by rearing and falling over backward. **Endoscopic examination** reveals the following:

- Compression of the nasopharynx that is asymmetric
- Blood in the guttural pouch
- Submucosal hemorrhage and swelling of the medial aspect of the medial compartment of the guttural pouch

Radiographic examination reveals ventral deviation of the dorsal pharynx and loss of the usual radiolucency associated with the guttural pouch (Fig. 12-23). **Treatment is conservative** and consists of supportive care, monitoring the hematocrit, and administration of broad-spectrum antibiotics if there is concern of the development of secondary infection. The prognosis for complete recovery is guarded.

Traumatic injury to the guttural pouch can occur during attempts to pass a nasogastric tube.[1] Clinical signs include swelling of the throatlatch area, pain on palpation, crepitus, and pain on swallowing. Endoscopic and radiographic examination is diagnostic. Treatment is supportive, including administration of antimicrobials and analgesics and ensuring maintenance of hydration and nutrition.

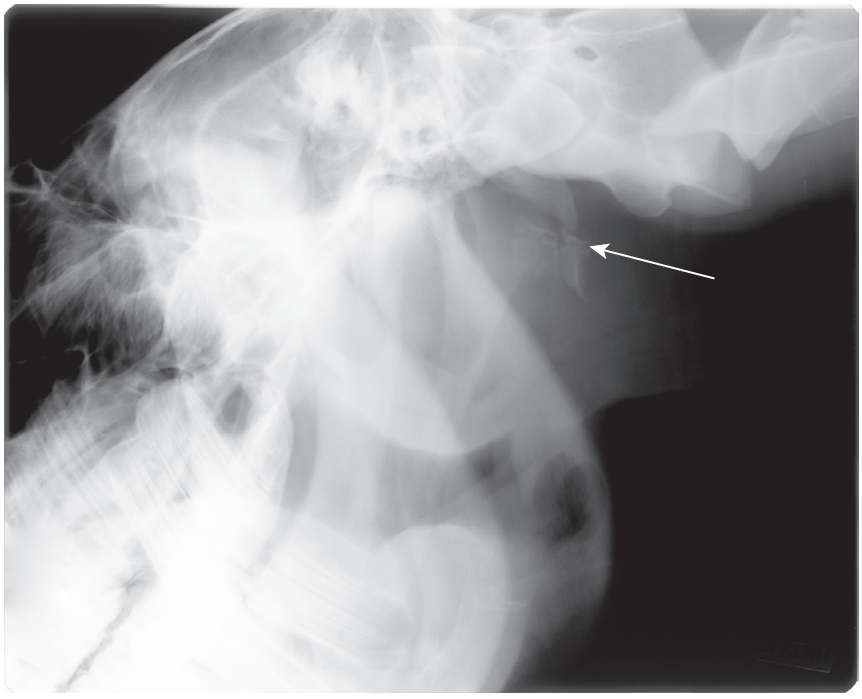

Fig. 12-23 **Lateral radiograph of the head of a horse with rupture of the longus capitis muscle after rearing and falling over backward. There is loss of radiolucency of the guttural pouches and evidence of an avulsion fracture of the basisphenoid bone (white arrow).**

Various neoplasms have been recorded as involving the guttural pouches. The presenting signs are swelling of the parotid region, epistaxis, dysphagia, or signs of cranial nerve disease. Neoplasms include melanoma, lymphosarcoma, hemangiosarcoma, squamous-cell carcinoma, and sarcoma. Diagnosis is made by physical, endoscopic, and radiographic examination and biopsy. The prognosis is very poor to hopeless.

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DISEASES OF THE EPIGLOTTIS AND ARYTENOIDS

Aryepiglottic Fold Entrapment (Epiglottic Entrapment)

Entrapment of the epiglottis in the fold of tissue that extends from the arytenoid cartilage to the ventrolateral aspect of the epiglottis causes exercise intolerance and respiratory noise during exercise in racehorses. The disorder occurs in both young and mature racehorses, and it is found in approximately 1% of Thoroughbred racehorses. The entrapment is often detected during rhinolaryngoscopic examination of racehorses, although it might not be the cause of poor performance. The condition occurs in nonracehorses (13 cases in 23 adult horses with epiglottic disease).[1] The presence of aryepiglottic fold entrapment causes a predominantly expiratory obstruction to airflow across the larynx during exercise. The interference with airflow, if any, does not appreciably impair performance in all horses.

Clinical Signs

Clinical signs are of exercise intolerance and respiratory noise during exercise. Acute cases can be associated with epiglottitis, whereas chronic cases are usually an incidental finding during endoscopic examination of the upper airway.

Endoscopic examination of the upper airway reveals the border of the epiglottis to be obscured by the aryepiglottic folds (Fig. 12-24). Normally, the serrated margin of the epiglottis and dorsal blood vessels extending to the lateral margins of the epiglottis are readily apparent, but when the epiglottis is entrapped, these features are no longer visible. Because of the frequently intermittent nature of the entrapment, the horse should be examined on several occasions and preferably immediately after strenuous exercise. Radiography of the pharynx reveals the entrapped epiglottis.

Treatment

Treatment consists of surgical revision of the aryepiglottic fold.[2,3] Surgery is reported to have both a high success rate and,[2] in some reports, a complication rate for surgical correction of 60%, indicating that careful consideration should be given to not attempting surgical repair, especially in animals performing to expectation. Entrapment associated with acute epiglottitis should include administration of antimicrobials and antiinflammatory agents to resolve the epiglottitis.

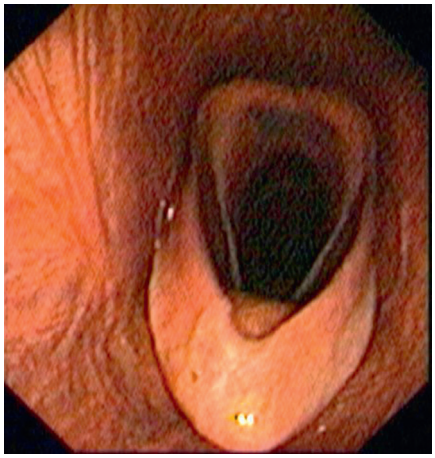

Fig. 12-24 Endoscopic view of the pharynx and larynx of a horse with entrapment of the epiglottis by the aryepiglottic folds.

Epiglottic Retroversion During Exercise

Retroversion of the epiglottis occurs during exercise in horses. The condition is rare and is associated with poor performance and an inspiratory "grunt" during exercise. Endoscopic examination at rest might reveal that the tip of the epiglottis is pointed more dorsal than normal, but this finding is not conclusive evidence of the disorder. Endoscopy during high-speed exercise demonstrates that the tip of the epiglottis is pointed dorsally or, in severe cases, points caudally through the rima glottidis. Retroversion occurs only during inspiration. Poll flexion during exercise exacerbates the condition. Frequency of retroversion increases as the intensity of exercise increases. There is no definitive treatment, and the prognosis for successful return to athletic performance is poor.[4]

Epiglottitis

Epiglottitis is usually a disease of racehorses, although animals of any age can be affected. The clinical signs are exercise intolerance, respiratory noise, and coughing. The disease can readily be mistaken for epiglottic entrapment. The epiglottis is thickened and ulcerated, and these changes are apparent on endoscopic examination. Treatment includes topical application of a mixture of nitrofurazone, dimethyl sulfoxide, glycerin, and prednisolone, and systemic administration of antiinflammatory drugs. The prognosis for recovery is excellent.

Subepiglottic Cysts

Fluid-filled cysts in the subepiglottic, dorsal pharyngeal, or soft palate tissues cause exercise intolerance and abnormal respiratory noise in exercising adult horses and mild dysphagia, chronic cough, and nasal discharge in foals. Most cysts are asymptomatic.[5] The cysts are usually embryonic remnants, although cysts may be acquired in adult horses by obstruction or inflammation of mucous glands. Endoscopic examination of the upper airway reveals the presence of smooth-walled cysts. Subepiglottic cysts may only be apparent on careful examination of the epiglottis, although most will cause the epiglottis to assume a more upright posture than is normal. Treatment is surgical removal or intralesion injection with formalin.[6]

Arytenoid Chondritis

Arytenoid chondritis is a progressive disease of the arytenoid cartilages in which there is distortion of the cartilage with consequent partial occlusion of the lumen of the larynx. The cause of the disease is not known, but it is most common in racehorses in heavy work. Distortion and swelling of the cartilage, combined with restricted abduction, increase resistance to airflow through the larynx and cause respiratory noise during exercise and exercise intolerance. In severe cases respiratory noise and increased respiratory effort are apparent at rest. The disease can occur as a progression of idiopathic mucosal ulceration of the axial aspect of the arytenoid cartilages.

Endoscopic examination reveals the cartilage to be enlarged and distorted, and there may be luminal projections of cartilage and granulation tissue. In less severe cases there is mild swelling of the cartilage and ulceration of the mucosa covering the cartilage. Bilateral disease is uncommon. Ultrasonographic examination is useful in determining the extent of the lesion.[7] Affect cartilages are increased in size and echogenicity and have an abnormal contour. The cartilage contains areas of necrosis, dystrophic mineralization, and granulation tissue.

Treatment

Treatment requires surgical removal of the affected cartilage, although progression of the disease can be achieved in horses with mild lesions by administration of antimicrobials and antiinflammatory drugs.

The disease also occurs in calves, in which it can be treated by partial arytenoidectomy.[8]

Mucosal Lesions of the Arytenoid Cartilages

Lesions of the mucosa of the axial aspect of the arytenoid cartilages are observed in Thoroughbred racehorses.[9] The condition occurs in approximately 2.5% of Thoroughbred racehorses and 0.6% of Thoroughbred yearlings. The pathogenesis is unknown. The disorder is recognized during endoscopic examination of the horses for other reasons

(before sale, examination for exercise-induced pulmonary hemorrhage). Endoscopic appearance of the lesion is that of a roughly circular lesion of the mucosa of the axial surface of the arytenoid cartilage, with or without visual evidence of inflammation, and without deformity of the underlying cartilage. The lesions can progress to arytenoid chondritis, although most do not.[9] Because of the risk of progression, medical therapy, including systemic or local administration of antimicrobial and antiinflammatory drugs, is indicated. The prognosis for full recovery is excellent.

Axial Deviation of the Aryepiglottic Folds

Axial deviation of the aryepiglottic folds is one of the most common abnormalities detected during laryngoscopic examination of horses running on a treadmill and is part of the pharyngeal instability complex of diseases in horses.[10-14] The disorder can only be detected in horses by endoscopic examination of the larynx while the horse is performing strenuous exercise. Collapse of the axial portion of the aryepiglottic folds causes obstruction of the laryngeal airway during inspiration. Treatment is symptomatic.

Retropharyngeal Lymphadenopathy

Lymphadenopathy of the retropharyngeal lymph nodes is usually associated with *S. equi* var. *equi* infection and is often a sequela to strangles (see "Strangles" in this chapter).[15] Shedding of *S. equi* from clinically inapparent retropharyngeal lymph node abscesses is an important source of new infections in horse barns. Retropharyngeal lymphadenopathy is also caused by trauma to the pharynx, brachial cysts remnants,[16] neoplasia (predominantly lymphosarcoma),[17] and infection by *Actinomyces* spp.[18] Enlargement of the retropharyngeal lymph nodes compresses the nasopharynx, increases resistance to airflow, and may impair swallowing (Fig. 12-25).

Clinical Signs

Clinical signs are swelling of the parotid region, although this may be slight even in horses with marked respiratory distress, pain on palpation of the parotid region, stertorous respiratory noise, respiratory distress, and dysphagia evident as food material discharging from the nostrils. Affected horses are frequently depressed, inappetent, and pyrexic.

Endoscopic examination of the upper airway will reveal ventral displacement of the dorsal wall to the pharynx and narrowing of the nasopharynx. There may be deviation of the larynx to the side away from the mass. Guttural pouch empyema often coexists with retropharyngeal lymph node infection, and the guttural pouches should be examined. Radiography will reveal the presence of a soft tissue density in the retropharyngeal region with compression of the guttural pouches and pharynx. Hematologic examination often demonstrates a mature neutrophilia and hyperfibrinogenemia.

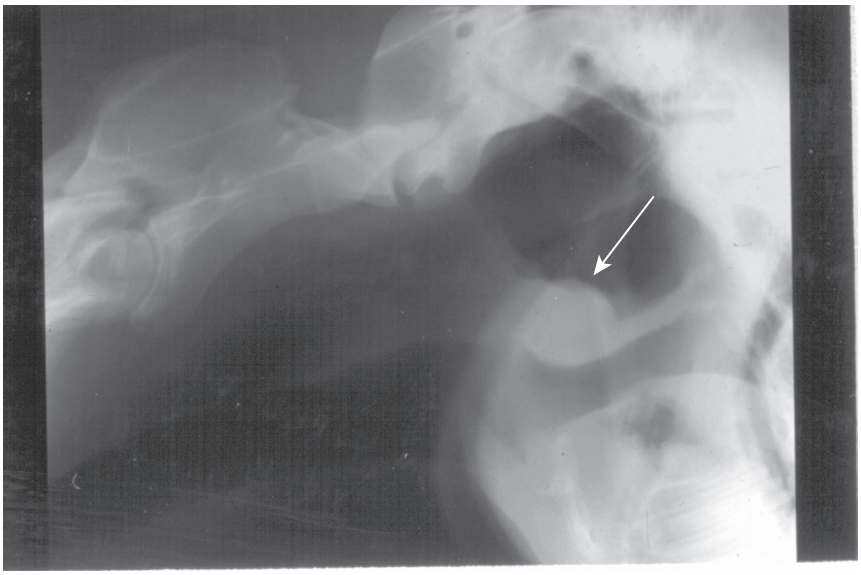

Fig. 12-25 Lateral radiograph of the head of a yearling horse demonstrating presence of a retropharyngeal mass (identified by white arrow).

Treatment

Treatment consists of administration of penicillin (procaine penicillin 20,000 IU/kg, intramuscularly every 12 hours) until signs of the disease resolve, followed by administration of a combination of sulfonamide and trimethoprim (15-30 mg/kg orally every 12 hours for 7-14 days). Administration of antiinflammatory drugs such as **phenylbutazone** (2.2 mg/kg intravenously or orally every 12 hours) is important in reducing inflammation and swelling and thereby allowing the horse to eat and drink. Horses that have severe respiratory distress may require a tracheotomy. Dysphagic horses might require fluid and nutritional support. Surgical drainage of the abscess is difficult and should be reserved for cases with large, cavitating lesions evident on radiographic or ultrasonographic examination.

Control consists of preventing infection of horses by *S. equi* var. *equi* and adequate treatment of horses with strangles.

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RECURRENT LARYNGEAL NEUROPATHY (ROARERS)

Neuropathy of the recurrent laryngeal nerve (usually the left one) causes paresis or paralysis of the cricoarytenoid muscle and failure of abduction of the arytenoid cartilages. This is apparent during exercise as obstruction of the upper airway and abnormal respiratory noise during heavy exercise. The idiopathic disease is referred to as recurrent laryngeal neuropathy, thereby describing the lesion, whereas other causes of laryngeal hemiplegia are referred to more specifically.

ETIOLOGY

The cause of laryngeal hemiplegia is degeneration of the recurrent laryngeal nerve with subsequent neurogenic atrophy of the cricoarytenoid dorsalis and other intrinsic muscles of the larynx. The etiology of **neuronal degeneration** is unknown, but the pathologic changes are typical of a distal axonopathy. There is evidence of a genetic component to recurrent laryngeal neuropathy, with a genome-wide association study in Thoroughbreds demonstrating a major quantitative trait locus near a known locus for body height and explaining 60% of the recurrent laryngeal neuropathy phenotype.[1] This study follows on from two others that in total identified three significant loci (ECA10, 21, and 31) in Warmblood horses.[2,3] To date,

no studies have identified it as a simple mendelian trait.[4]

The disease is usually idiopathic, but occasional cases are caused by guttural pouch mycosis or inadvertent perivascular injection of irritant material, such as phenylbutazone, around the jugular vein and vagosympathetic trunk. **Bilateral laryngeal paralysis** is usually associated with intoxication (organophosphate, haloxon), trauma from endotracheal intubation during general anesthesia, or as a complication of hepatic encephalopathy.[5]

EPIDEMIOLOGY

Prevalence

The disease affects large horses more commonly than ponies, and it is commonly recognized in draft horses, Thoroughbreds, Standardbreds, Warmbloods, and other breeds of large horse. The **prevalence** of laryngeal hemiplegia in Thoroughbred horses in training is between 1.8% and 13% depending, among other factors, on the criteria used to diagnose the condition. Among apparently normal Thoroughbred horses examined after racing, grade 4 laryngeal hemiplegia was detected in 0.3% of 744 horses, grade 3 in 0.1%, and grade 2 in 1.1%. Male horses over 160 cm tall are at most risk of developing the disease. There is evidence of a **familial** distribution of the disease, with offspring of affected parents being more frequently affected (61%) than adult offspring of unaffected parents (40%).

PATHOGENESIS

Axonal degeneration causes preferential atrophy of the adductor muscles of the larynx, although both the adductor (dorsal cricoarytenoid muscle) and adductor (lateral cricoarytenoid muscle) are involved. Fiber-type grouping of laryngeal muscles, evidence of recurrent laryngeal neuropathy, is present in draft foals as young as 2 weeks of age, indicating an early onset of the disease. The disease is progressive in some horses.

Compromised function of the adductor muscles results in **partial occlusion of the larynx** by the arytenoid cartilage and vocal fold during inspiration. The obstruction is most severe when airflow rates through the larynx are large, such as during strenuous exercise. **Laryngeal obstruction** increases the work of breathing, decreases the maximal rate of oxygen consumption, and exacerbates the hypoxemia and hypercarbia normally associated with strenuous exercise by horses. These effects result in a severe limitation to athletic capacity and performance.

CLINICAL FINDINGS

Clinical findings include exercise intolerance and production of a whistling or roaring noise during strenuous exercise. The disease can be detected by analysis of respiratory noise.

Endoscopic examination of the upper airway provides the definitive diagnosis in most cases. Examination of the larynx is performed with the horse at rest and the position and movement of the arytenoid cartilages assessed. Laryngeal function can also be observed during swallowing, brief (30–60 s) bilateral nasal occlusion, and during and after exercise.

The severity of the disease is graded I through IV with five subgrades; there are therefore seven grades of abnormality:

- **Grade I** is normal, there being synchronous, full abduction and adduction of both arytenoid cartilages.
- **Grade II** presents as weakness of the adductors evident as asynchronous movement and fluttering of the arytenoid cartilage during inspiration and expiration but with full abduction during swallowing or nasal occlusion.
  - *Subgrade 1:* Transient asynchrony, flutter, or delayed movements.
  - *Subgrade:* Asymmetry of the rima glottidis as a result of reduced mobility of the affected arytenoid cartilage and vocal fold but with full symmetric abduction achieved and maintained after swallowing or nasal occlusion.
- **Grade III** has asynchronous movement of the arytenoid cartilage during inspiration or expiration; full abduction is not achieved during swallowing or nasal occlusion.
  - *Subgrade 1:* Asymmetry of the rima glottidis as a result of reduced mobility of the affected arytenoid cartilage and vocal fold but with full symmetric abduction achieved but not maintained after swallowing or nasal occlusion.
  - *Subgrade 2:* Obvious arytenoid abductor deficit and arytenoid asymmetry. Full abduction is never achieved.
  - *Subgrade 3:* Marked but not total arytenoid deficit and asymmetry with little arytenoid movement. Full abduction is never achieved.
- **Grade IV** implies marked asymmetry of the larynx at rest and no substantial movement of the arytenoid cartilage during respiration swallowing or nasal occlusion.

Intraobserver agreement (two observers, 80 recordings) of the same recording of an endoscopic examination on standing draft horses was 76% when the recording was graded twice, and it is thus regarded as excellent.[6] Two observers assigned the same grade and subgrade for 63% of recordings differed by one grade for 32% of recordings and by two grades in 5 % of recordings, which is assessed as good agreement. Repeatability of examination in the same horse was low when the same horse was examined twice, 24 to 48 hours apart. Forty-two percent of horses had the same grade assigned on both examinations, 42% differed by one grade, 17% received an improved grade (ie, less severe) and 26% a worse grade, and 13% of horses differed by two grades. One horse improved by four grades.

Endoscopic examination during **strenuous exercise on a treadmill or over ground** can be beneficial in determining the severity of the disease or detecting disease of lesser severity.[7-10] Horses with early or mild degeneration of the recurrent laryngeal nerve and associated laryngeal musculature can have normal laryngeal function at rest. However, the loss of muscle function becomes apparent during exercise, when the laryngeal muscles of affected animals fatigue more rapidly than do those of normal animals, with the result that laryngeal dysfunction can become apparent during or immediately after exercise. Endoscopic examination during exercise is useful in differentiating the disease from **axial deviation of the aryepiglottic folds**.

Ultrasonographic examination of the larynx and musculature is both sensitive (~90%) and specific (~98%) for detection of atrophy of the cricoarytenoid muscle or abnormal laryngeal function.[11-13]

There are no characteristic changes in the hemogram or in serum biochemical variables in resting horses. During exercise there is a marked exacerbation of the normal exercise-induced hypoxemia and the development of hypercapnia in affected horses.

NECROPSY FINDINGS

Lesions are confined to an axonopathy of the recurrent laryngeal nerves and neurogenic muscle atrophy of the intrinsic muscles of the larynx.[14,15] A technique for ultrasound guided biopsy of the cricoarytenoid lateralis muscle is described.[16]

DIAGNOSTIC CONFIRMATION

Diagnostic confirmation is achieved by endoscopic examination of the larynx.

DIFFERENTIAL DIAGNOSIS

Differential diagnoses of exercise intolerance and exercise-induced respiratory noise include the following:

- Dorsal displacement of the soft palate
- Subepiglottic cysts
- Arytenoid chondritis
- Aryepiglottic fold entrapment
- Axial deviation of the aryepiglottic folds

TREATMENT

Treatment requires a prosthetic laryngoplasty with or without ventriculectomy.[17] An alternative to prosthetic laryngoplasty is implantation of a nerve-pedicle graft into the

larynx. Effectiveness of the procedure is assessed by the return of the horse to its previous level of athletic activity.[18-21]

The disease is not life-threatening, and horses that are not required to work strenuously or in which respiratory noise associated with mild exercise is not bothersome to the rider may not require surgery. A complication of surgical repair that includes prosthetic laryngoplasty is that horses can no longer close the glottis and therefore do not have an effective cough. This could be the cause of tracheal inflammation and accumulation of debris in the trachea of these horses.

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EQUINE PLEUROPNEUMONIA (PLEURITIS, PLEURISY)

ETIOLOGY

Pleuropneumonia of horses is almost always associated with bacterial infection of the lungs, pleura, and pleural fluid. The most common bacterial isolates from tracheal aspirates or pleural fluid of horses with pleuropneumonia are as follows:

- **Aerobes or facultative anaerobes.** including *S. equi* var. *zooepidemicus, Pasteurella* spp., *Actinobacillus* spp., Enterobacteriaceae (particularly *E. coli, Klebsiella* spp., and *Enterobacter* spp.), *Pseudomonas* spp., *Staphylococcus* spp., and *Bordetella* spp. *S. zooepidemicus* is isolated from over 60%, Enterobacteriaceae from approximately 40% of cases, and *Pasteurella/Actinobacillus* spp. from approximately one-third of cases. *Corynebacterium pseudotuberculosis* can cause septic pericarditis and pleuritis, although this is an uncommon disease. *Mycoplasma felis* is an unusual cause of pleuritis in horses. *R. equi,* usually a cause of pneumonia in foals, rarely causes pleuropneumonia in immunocompetent adult horses.
- **Obligate anaerobes**, including *Bacteroides* spp. (including *B. fragilis* and *B. tectum*), *Prevotella* spp., *Clostridium* spp., *Eubacterium, Prevotella* spp., *Peptostreptococcus* spp., *Fusobacterium* spp., and *Bacteroides* sp. are isolated from approximately 20%, *Clostridium* sp. from 10%, and *Eubacterium* sp. from 6% of horses with pleuropneumonia. Obligate anaerobes are cultured from approximately 70% of horses with severe pneumonia.

SYNOPSIS

Etiology Most infections are polymicrobial combinations of *S. equi* var. *zooepidemicus, Actinobacillus* sp., *Pasteurella* sp., Enterobacteriaceae, and anaerobic bacteria, including *Bacillus fragilis.* Disease attributable to infection by a single bacterial species occurs. Other causes are *Mycoplasma felis,* penetrating chest wounds and esophageal perforation.

Epidemiology Recent prolonged transport, racing, viral respiratory disease and anesthesia increase the likelihood of a horse developing pleuropneumonia. Aspiration of feed material secondary to esophageal obstruction or dysphagia also causes the disease.

Pathogenesis Overwhelming challenge of oropharyngeal bacteria or reduced pulmonary defense mechanisms allow proliferation of bacteria in small airways, alveoli, and lung parenchyma. Subsequent inflammation and further spread of infection involve the visceral pleura. Impaired drainage of pleural fluid and increased permeability of pleural capillaries cause the accumulation of excessive pleural fluid, which then becomes infected. Fibrin deposition and necrosis of lung causes formation of intrathoracic abscesses. Death is attributable to sepsis and respiratory failure.

Clinical signs Fever, depression, anorexia, respiratory distress, cough, nasal discharge, exercise intolerance, reduced breath sounds on thoracic auscultation, and presence of pleural fluid and pneumonia on thoracic radiology and ultrasonography. Chronic disease is characterized by weight loss, increased respiratory rate, nasal discharge, and exercise intolerance.

Clinical pathology Leukocytosis, hyperfibrinogenemia, hypoalbuminemia, hyperglobulinemia. Pleural fluid leukocytosis, hyperproteinemia and presence of intra- and extracellular bacteria. Similar findings in tracheal aspirate.

Diagnostic confirmation Clinical signs, examination of pleural fluid.

Treatment Systemic administration of broad-spectrum antimicrobials for weeks to months, chronic effective drainage of the pleural space, infusion of recombinant tissue plasminogen activator (tenecteplase) into pleural space, and nursing care.

Prevention Reduce exposure of horses to risk factors including prolonged transportation and viral respiratory disease.

Equine pleuropneumonia is associated with **polymicrobial infections** of the lungs and pleura in 50% to 80% of cases, although disease associated with infection with a single bacterial species occurs. Infections with a single bacterial species are usually by *S. zooepidemicus, Pasteurella/Actinobacillus* sp. or one of the Enterobacteriaceae, whereas almost all infections by anaerobes are polymicrobial. Infection by obligate anaerobic bacteria is associated with disease of more than 5 to 7 days' duration.

Pleuritis is also caused by penetrating chest wounds, perforated esophagus, and tracheobronchial foreign bodies.[1,2] **Cryptococcus spp** can cause the disease in horses and other species. Other diseases, such as congestive heart failure or neoplasia, can cause pleural effusion without inflammation.

EPIDEMIOLOGY

Pleuropneumonia occurs worldwide in horses of all ages and both sexes, although most cases occur in horses more than 1 and less than 5 years of age. Estimates of the incidence or prevalence of the disease are not available. The **case-fatality rate** varies between 5% and 65%, with the higher rate reported in earlier studies.

Risk Factors

The risk of a horse developing pleuropneumonia is increased factorially in the following cases:

- By a factor of 4 if the horse is a Thoroughbred racehorse
- By a factor of 14 if the horse was transported more than 500 miles in the previous week
- By a factor of 10 if the horse has a recent (< 2 week) history of viral respiratory tract disease or exposure to a horse with such disease
- By a factor of 4 if the horse has raced within the previous 48 hours

Other suggested risk factors include general anesthesia, surgery, disorders of the upper airway, exercise-induced pulmonary hemorrhage, esophageal obstruction, and dysphagia.

PATHOGENESIS

Bacterial pleuropneumonia develops following bacterial colonization of the lungs with subsequent extension of infection to the visceral pleura and pleural space. Organisms initially colonizing the pulmonary

parenchyma and pleural space are those normally present in the upper airway, oral cavity, and pharynx, with subsequent infection by Enterobacteriaceae and obligate anaerobic bacteria.

Bacterial colonization and infection of the lower airway is attributable to either massive challenge or a reduction in the efficacy of normal pulmonary defense mechanisms or a combination of these factors. **Confinement** with the head elevated for 12 to 24 hours, such as occurs during transport of horses, decreases mucociliary transport and increases the number of bacteria and inflammatory cells in the lower respiratory tract and probably contributes to the development of lower respiratory tract disease. **Transport** alters the composition of pulmonary surfactant, which can impair the activity of pulmonary defense mechanisms, allowing otherwise innocuous bacterial contamination to cause disease.

Overwhelming bacterial challenge can occur in dysphagic horses, horses with esophageal obstruction, and racehorses that inhale large quantities of track debris while racing. A single bout of **exercise** on a treadmill markedly increases bacterial contamination of the lower airways. Viral respiratory disease can decrease the efficacy of normal lung defense mechanisms.

Bacterial multiplication in pulmonary parenchyma is associated with the influx of inflammatory cells, principally neutrophils, tissue destruction, and accumulation of cell debris in alveoli and airways. Infection spreads both through tissue and via airways. Extension of inflammation, and later infection, to the visceral pleura and subsequently pleural space causes accumulation of excess fluid within the pleural space. Pleural fluid accumulates because of a combination of excessive production of fluid by damaged pleural capillaries (exudation) and impaired reabsorption of pleural fluid by thoracic lymphatics.

Accumulation of parapneumonic pleural effusions has been arbitrarily divided into three stages: exudative, fibrinopurulent, and organizational:

1. The **exudative stage** is characterized by the accumulation of sterile, protein-rich fluid in the pleural space as a result of increased pleural capillary permeability.
2. Bacterial invasion and proliferation, further accumulation of fluid, and deposition of fibrin in pleural fluid and on pleural surfaces occurs if the disease does not resolve rapidly and is referred to as the **fibrinopurulent stage.**
3. The **organizational stage** is associated with continued fibrin deposition, restriction of lung expansion, and persistence of bacteria. The pleural fluid contains much cellular debris and bronchopleural fistulas may develop.

These categorizations are useful diagnostically and therapeutically.

CLINICAL SIGNS OF ACUTE DISEASE

The acute disease is characterized by the sudden onset of a combination of fever, depression, inappetence, cough, exercise intolerance, respiratory distress, and nasal discharge.[3] The respiratory rate is usually elevated, as is the heart rate.

Nasal discharge ranges from serosanguineous to mucopurulent, is usually present in both nares and is exacerbated when the horse lowers its head. The **breath may be malodorous**, although this is a more common finding in horses with subacute to chronic disease. Horses with pleuritis are often reluctant to cough, and if they do, the cough is usually soft and gentle. Ventral edema occurs in approximately 50% of horses with pleuropneumonia.

The horse may appear **reluctant to move** or may exhibit signs of chest pain, including reluctance to move, pawing, and anxious expression, which may be mistaken for colic, laminitis, or rhabdomyolysis. Affected horses often stand with the elbows abducted.

Auscultation of the thorax reveals attenuation of normal breath sounds in the ventral thorax in horses with significant accumulation of pleural fluid. However, the attenuation of normal breath sounds may be mild and difficult to detect, especially in large or fat horses or in horses in which there is only slight accumulation of pleural fluid. Auscultation of the thorax with the horse's respiratory rate and tidal volume increased by having it breathe with a large airtight bag over its nostrils may reveal crackles and wheezes in the dorsal lung fields and attenuation of the breath sounds ventrally. There is often fluid in the trachea detectable as a tracheal rattle.

Percussion of the chest wall may reveal a clear line of demarcation, below which the normal resonant sounds are muffled. This line of demarcation represents the dorsal limit of the pleural fluid. Both lung fields should be examined to identify localized areas of consolidation. Careful percussion of the thorax is a cheap and effective way of identifying the presence and extent of pleural fluid accumulation.

Ultrasonographic examination of the thorax is a very sensitive technique with which to detect accumulation of pleural fluid, determine the character of the fluid including the presence of accumulations of fibrin[4], identify localized areas of fluid accumulation or pulmonary consolidation, identify sites for thoracocentesis, and monitor response to treatment. The examination is best performed using a 3.5 to 5.0 sector scanner. Linear probes, such as those used for routine reproductive examination, are adequate to identify fluid but do not allow good examination of all areas of the chest accessible with sector scanners. The entire thorax should be examined in a systematic fashion. The presence of and characteristics of fluid within the pleural space, presence and location of pulmonary consolidation or abscessation, and potential sites for diagnostic and therapeutic thoracocentesis should be identified. For horses with longstanding disease, the area cranial to the heart should be examined for the presence of cranial thoracic masses (abscesses). This examination requires that the horse's ipsilateral forelimb be placed well forward, usually with the aid of an assistant, to allow adequate visualization of the cranial thorax.

- **Excessive pleural fluid** can be detected by thorough ultrasonographic examination of both hemithoraces. Pleural fluid initially accumulates ventrally in acute cases, but may become localized dorsally in chronic cases with septation of the pleural space and trapping of fluid.
- The pleural fluid may contain **small gas echoes**, an indication of infection with anaerobic bacteria and a poor prognosis, strands of fibrin, or echogenic material consistent with cellular debris. Sterile pleural effusion, such as may be present during the earliest stages of the disease, is clear and homogeneous without fibrin strands. With increasing chronicity the amount of fibrin increases, the parietal and visceral pleura become thickened, and the pleural fluid becomes echogenic consistent with the presence of cellular debris.
- Regions of consolidated or **atelectatic lung** adjacent to the visceral pleura may be evident on ultrasonographic examination, but lung consolidation deeper in the lung is not evident.
- Accumulation of fibrin is associated with a worse prognosis than is accumulation of parapneumonic fluid that does not include fibrin deposits.[4]
- Ultrasonography is more sensitive than radiographic examination in detection of small quantities of pleural fluid.

Radiographic examination of horses with excessive pleural fluid reveals ventral opacity that obscures the ventral diaphragmatic and cardiac silhouettes. Radiographic examination might not reveal the presence of small amounts of excessive pleural fluid.[5] It is not possible on radiographic examination to differentiate accumulation of pleural fluid from consolidation of the ventral lung lobes. Radiographic examination may be useful in demonstrating lesions, such as pulmonary abscesses or consolidation, that are not confluent with the visceral pleura and therefore not able to be detected by ultrasonographic examination.

Collection of pleural fluid by **thoracocentesis** of both hemithoraces and of a

tracheal aspirate is necessary to characterize the nature of the pleural fluid and determine the bacterial species present (see "Clinical Pathology"). Both tracheal aspirates and pleural fluid should be examined in any horse with pleuropneumonia because bacteria may be recovered from one sample but not the other. Examination of bronchiolar lavage fluid is not useful in diagnosing pleuropneumonia in horses.

The **clinical course** of the acute form of the disease may be less than 10 days if effective therapy is instituted before the pleural effusion becomes infected or there is substantial deposition of fibrin in the pleural space. The prognosis for a return to previous function is good in horses that respond. However, most cases, even if appropriate therapy is instituted, progress to at least stage 2 of the disease process, and the disease becomes chronic.

CLINICAL SIGNS IN CHRONIC DISEASE

The chronic disease is characterized by intermittent fever, weight loss, cough, increased respiratory rate, nasal discharge, malodorous breath, exercise intolerance, and depression. Severely affected horses may display signs of respiratory distress. Signs of thoracic pain are less than in the acute disease.

Findings on auscultation of the chest are similar to those of the acute disease, inasmuch as there is attenuation of normal breath sounds ventrally and the presence of crackles and wheezes dorsally. There is frequently ventral edema of the thorax.

Ultrasonographic examination reveals the presence of excessive pleural fluid that is very echogenic, consistent with it containing cellular debris, and contains large amounts of fibrin. The visceral and parietal pleura are thickened, and there may be evidence of lung atelectasis, consolidation, or abscessation. Septation of the pleural space by fibrin and fibrous tissue results in localized accumulation of purulent pleural fluid. Air in the pleural space may indicate the presence of one or more bronchopleural fistulae.

Radiographic examination reveals a combination of ventral opacity, pulmonary consolidation, pneumothorax, and abscessation.

Complications

Complications of pleuropneumonia include the following:[4,6]

- Development of jugular thrombophlebitis (~25% of cases)
- Pulmonary, mediastinal, or pleural abscesses (~10%-20% of cases)
- Cranial thoracic mass (5%–10% of cases)
- Bronchopleural fistula (5%)
- Pericarditis (2%)
- Laminitis (1%–14%)
- Appropriate secondary erythrocytosis[7]

Development of intrathoracic abscesses is evident as chronic disease, weight loss, cough, and fever, readily detected by a combination of ultrasonographic and radiographic examination.

Cranial thoracic masses are evident as an elevation in heart rate, prominent jugular pulse, spontaneous jugular thrombosis, and forelimb pointing. The signs are referable to a mass in the cranial thorax displacing the heart caudally and to the left and impairing venous return to the heart in the cranial vena cava. Ultrasonographic and radiographic examination reveals the presence of the mass.

Bronchopleural fistulae develop when a section of pulmonary parenchyma sloughs, leaving an open bronchiole that communicates with the pleural space. Mild pneumothorax develops. The bronchopleural fistula can be diagnosed by infusion of fluorescein dye into the pleural space and detecting its presence at the nares or by pleuroscopic examination.

Prognosis

The prognosis for life for horses able to be treated aggressively is very good (60%-95%), and the prognosis for return to previous function if the horse survives is reasonable (60%). The prognosis for return to previous function for horses that develop chronic disease and complications is poor (31%). Prognosis for horses with fibrinous pleural effusion is worse than for horses with pleural effusions that do not include ultrasonographically identifiable accumulations of fibrin (100% of 11 cases vs. 62% of 63 cases, respectively).[4]

CLINICAL PATHOLOGY

Acute pleuropneumonia is characterized by **leukocytosis** with a mature neutrophilia, mild to moderate anemia, hyperfibrinogenemia, and hypoalbuminemia. There are similar findings in horses with chronic disease, and hyperglobulinemia is also usually present. Severely affected horses with acute disease often have hemoconcentration and azotemia. Horses with chronic disease that impairs respiratory gas exchange, causing chronic hypoxemia, can have secondary erythrocytosis.[7]

Pleural fluid in acute cases is usually cloudy and red to yellow. It has an increased leukocyte number (>10,000 cells/μL, 10×10 cells/L) comprised principally of degenerative neutrophils, and an increased protein concentration (>2.5 g/dL, 25 g/L), and it may contain intracellular and extracellular bacteria. A Gram stain of the fluid should be examined. The pleural fluid should be cultured for aerobic and anaerobic bacteria. A putrid odor suggests infection by anaerobic bacteria. Sterile pleural fluid has a pH, Po_2 and Pco_2, and lactate, glucose, and bicarbonate concentration similar to that of venous blood. Infected pleural fluid is acidic and hypercarbic and has an increased concentration of lactate and decreased concentrations of bicarbonate and glucose compared with venous blood.

Tracheal aspirates have a leukocytosis comprised of degenerate neutrophils with intra- and extracellular bacteria. Cultures of tracheal aspirates more frequently yield growth than do cultures of pleural fluid (90% vs. 66%).

DIAGNOSTIC CONFIRMATION

The presence of excessive pleural fluid containing bacteria and degenerate neutrophils in combination with clinical signs of respiratory disease provides confirmation of the disease.

DIFFERENTIAL DIAGNOSIS

Diseases that may cause respiratory distress and pleural effusion in horses include the following:

- Intrathoracic neoplasia, including mesothelioma, lymphoma, and extension of gastric squamous-cell carcinoma
- Penetrating chest wounds
- Esophageal perforation
- Diaphragmatic hernia
- Congestive heart failure
- Hemangiosarcoma (causing hemothorax)
- African horse sickness
- Pulmonary hydatidosis
- Pulmonary infarction and pneumonia

NECROPSY FINDINGS

The pneumonia involves all areas of the lungs but is most severe in the cranial and ventral regions. The pleura are thickened and have adherent fibrin tags, and there is excessive pleural fluid. The pleural fluid contains strands of fibrin and is usually cloudy and serosanguineous to yellow. Histologically, there is a purulent, fibrinonecrotic pneumonia and pleuritis.

TREATMENT

Given early recognition of the disease and prompt institution of appropriate therapy, the prognosis for horses with pleuropneumonia is favorable. However, the long course of the disease and the associated expense often limit therapeutic options and make the outcome a decision based on economic rather than medical grounds.

The **principles of treatment** are prompt broad-spectrum antimicrobial therapy; removal of infected pleural fluid and cellular debris, including necrotic lung; relief of pain; correction of fluid and electrolyte abnormalities; relief of respiratory distress; treatment of complications; and prevention of laminitis.

Antimicrobial Treatment

The prompt institution of **systemic, broad-spectrum antimicrobial therapy** is the single most important component of

treatment of horses with pleuropneumonia. Antimicrobial therapy is almost always started before the results of bacterial culture of pleural fluid or tracheal aspirate are received and the antimicrobial sensitivity of isolated bacteria are determined. Use of antibiotics or combinations of antibiotics with a broad spectrum of antimicrobial activity is important because of the polymicrobial nature of most infections and because the wide range of gram-positive and gram-negative bacteria that may be associated with the disease makes prediction of the susceptibility of the causative organisms difficult. Furthermore, superinfection with bacteria, especially Enterobacteriaceae and obligate anaerobes, commonly occurs in horses with disease initially associated with a single bacterial species. Administration of drugs that are effective in the treatment of penicillin-resistant obligate anaerobes is also important.

Recommended doses for antimicrobials used in the treatment of pleuropneumonia are provided in Table 12-11. Antimicrobial therapy should be broad spectrum to include coverage of the likely bacteria involved in the disease. It should therefore provide coverage against *Streptococcus* spp., *Actinobacillus/Pasteurella* spp., Enterobacteriaceae, and anaerobes, including *Bacteroides* spp. **A combination of penicillin G, an aminoglycoside, and metronidazole** provides broad-spectrum coverage and is a frequently used empirical therapy until the results of bacterial culture are known. Results of bacterial culture and subsequent antimicrobial susceptibility testing may aid selection of further antimicrobials. However, superinfection with gram-negative and anaerobic bacteria is common, and there is a sound rationale for continued use of a combination of antimicrobials providing broad-spectrum coverage throughout treatment of the disease.

Antimicrobial therapy will be prolonged in most cases, usually being required for at least 1 month and often several months. As the disease resolves it may be possible to change from parenteral antibiotics to orally administered antibiotics such as a combination of trimethoprim–sulfonamide, although the clinical response to this combination is sometimes disappointing, or doxycycline or enrofloxacin.

Table 12-11 Antimicrobial agents and recommended doses for treatment of pleuropneumonia in horses

Drug	Dose, route, and interval	Comments
Procaine penicillin G	22,000–44,000 IU/kg IM q12h	Effective against *Streptococcus* sp. and most anaerobes, with the exception of *Bacteroides fragilis*. Achieves low plasma concentrations but has prolonged duration of action. Cheap. Synergistic with aminoglycosides. Should not be used as sole treatment.
Sodium or potassium penicillin G	22,000–44,000 IU/kg IV q6h	Effective against gram-positive organisms (except penicillinase-producing bacteria such as *Staphylococcus* spp.) and most anaerobes. Achieves high plasma concentrations. Synergistic with aminoglycosides. Expensive.
Ampicillin sodium	11–22 mg/kg IV or IM q6h	Wider spectrum than penicillin G. Achieves high plasma concentrations.
Ampicillin trihydrate	20 mg/kg IM q12–24h	Synergistic with aminoglycosides Low blood concentrations. Muscle soreness. Not recommended.
Ceftiofur sodium	2.2–4.4 mg/kg IM or IV q12h	Wide spectrum of action against gram-positive and gram-negative organisms and most anaerobes. Can be used as sole treatment, though not recommended. Clinical results sometimes disappointing
Ceftiofur crystalline	7 mg/kg IM q4 days	Prolonged concentration in blood and bronchoalveolar lavage fluid.
Cefotaxime	40 mg/kg IV q6h	Wide spectrum of action against gram-positive and gram-negative organisms and most anaerobes. Can be used as sole treatment, though not recommended.
Cefepime	2.2 mg/kg IV or IM q8h	Wide spectrum of action against gram-positive and gram-negative organisms and most anaerobes. Can be used as sole treatment, although not recommended.
Chloramphenicol	50 mg/kg, PO q6h	Good spectrum of action, including anaerobic bacteria. Poor oral bioavailability and disappointing clinical efficacy. Use prohibited in some countries. Potential human health hazard. Risk of diarrhea
Gentamicin sulfate	7 mg/kg, IV or IM q24h	Active against *Staphylococcus* spp. and many gram-negative organisms. Inactive against anaerobes. Poor activity against *Streptococcus* spp. Synergistic with penicillin
Enrofloxacin	7 mg/kg IV or PO q24h	Active against some gram-positive and gram-negative bacteria. Not good or reliable activity against streptococci. Contraindicated in young animals because of risk of cartilage damage.
Amikacin sulfate	10 mg/kg IV or IM q24h	Wider spectrum of gram-negative activity than gentamicin. Expensive
Trimethoprim–sulfonamides	15–30 mg/kg PO q12h	Theoretical wide spectrum of action. Disappointing clinical efficacy.
Rifampin	5–10 mg/kg PO q12h	Penetrates abscesses well. Active against gram-positive and some gram-negative bacteria. Must be used in conjunction with another antibiotic (not an aminoglycoside).
Doxycycline	10 mg/kg PO q12h	Broad spectrum of activity, but resistance unpredictable. Only moderate blood concentrations. Suitable for prolonged therapy but not treatment of the acute disease.
Ticarcillin–clavulanic acid	50 mg/kg IV q6h	Broader spectrum of gram-negative activity than penicillin G. Expensive.
Metronidazole	15–25 mg/kg PO q6–8h	Active against anaerobes only. Used in conjunction with other antimicrobials (especially penicillin and aminoglycosides). Neurotoxicity rare.

IM, intramuscularly; IV, intravenously; PO, orally; q, dose administered every "h" hours.

The decision to discontinue antimicrobial therapy should be based on lack of fever, nasal discharge, and respiratory distress or cough; lack of evidence of intrathoracic abscesses on ultrasonographic and radiographic examination of the thorax; and resolution of neutrophilia and hyperfibrinogenemia. There should be no appreciable pleural fluid on ultrasonographic examination.

Thoracic Drainage

Chronic, effective drainage of the pleural cavity and intrathoracic abscesses is critical for successful treatment of horses with pleuropneumonia. Horses with sterile pleural fluid may require only a single drainage of pleural fluid. More severely affected horses may require intermittent drainage on each of several days, and most cases will require insertion of a tube into the pleural space to provide continuous drainage for several days to several weeks. Horses with chronic disease may benefit from a thoracotomy that provides continuous drainage and the ability to lavage the chest. Ultrasonographic examination of the chest is very useful in identifying the presence of pleural fluid, the optimal sites for drainage, and the efficacy of drainage.

Intermittent thoracic drainage can be achieved by inserting a bovine teat cannula or similar blunt cannula into the pleural space. This should be done aseptically and under local anesthesia. If ultrasonographic examination is not available, the cannula should be placed in the sixth to eighth intercostal space on the right side or the seventh to ninth on the left side just above the level of the olecranon. Pleural fluid that does not contain large fibrin clots (which clog the cannula) can be drained and the cannula removed. However, the process is slow if large quantities of fluid must be removed. Intermittent drainage is indicated when the quantities of pleural fluid are small (< 5 L), relatively cell-free, or localized. This situation is most likely to occur in horses with acute disease.

Insertion of large plastic chest tubes (20–30 French, 6- to 10-mm outside diameter) facilitates rapid fluid removal, allows drainage of viscid fluid, and provides continuous drainage. The chest tube should be inserted in an aseptic fashion under local anesthesia at sites indicated by ultrasonographic examination or as described previously. A one-way valve should be attached to the external end of the tube to prevent aspiration of air and development of a pneumothorax. A balloon or condom with the end removed is an effective one-way valve. The chest tube is secured to the chest wall with a purse-string suture. The tube may be retained for several days to a week, but it should be monitored frequently (every few hours) and cleared of fibrin clots as needed.

Complications of drainage of pleural fluid include collapse of the animal if the fluid is removed too rapidly, pneumothorax, sudden death as a result of cardiac puncture or laceration of a coronary vessel, and perforation of abdominal viscera. Collapse can be prevented by administering fluids intravenously during pleural fluid drainage and by removing the fluid gradually (over a period of 30 minutes). Some horses develop cellulitis around the chest tube, which requires that the tube be removed.

Thoracotomy may be required in recurrent or chronic cases to provide drainage of intrathoracic abscesses or chronic pleural effusion that is refractory to treatment with antimicrobials. Thoracotomy is an effective intervention in many horses, with 14 of 16 horses treated by thoracotomy surviving and 6 returning to athletic activity.[8] Thoracotomy should not be considered an emergency or heroic procedure in such cases.

Pleural Lavage

Infusion and subsequent removal of 5 to 10 L of warm saline or balance polyionic electrolyte solution into the affected pleural space may be beneficial in the treatment of cases with viscid fluid or fluid containing large amounts of fibrin and cell debris. The fluid can be infused through the chest tube that is used to drain the pleural space. Care should be taken not to introduce bacteria with the infusion.

Fibrinolytic Therapy

Tissue plasminogen activators have been administered to horses in an attempt to increase activity of plasmin and hence the rate of lysis of fibrin in the pleural cavity. Earlier attempts at fibrinolytic therapy used streptokinase or urokinase and were not beneficial. Use of modified compounds, such as alteplase and tenecteplase, is effective in hastening fibrinolysis, enhancing resolution of accumulated pleural fluid, and improving survival.[6,9,10] There does not appear to be an increased risk of prolonged hemostasis. The procedure in one case involved intrapleural infusion of 12 mg of tenecteplase in 500 ml of isotonic saline after drainage of excessive pleural fluid.[10] The treatment was repeated on three occasions over 10 days. Pharmacokinetics of alteplase in horses are described.[11] A recommended protocol is infusion of tenecteplase (2–10 mg in 1–2 L of isotonic, polyionic fluid) q12 to 24h for 3 days, with a dwell time of 4 hours.[6]

Supportive Therapy

Acutely or severely ill horses may be dehydrated and azotemic, and they may have acid-base disturbance. These horses should be treated with appropriate **fluids** administered intravenously.

Pleuropneumonia is a painful disease, and every attempt should be made to relieve the horse's chest pain. **NSAIDs**, including flunixin meglumine (1 mg/kg, orally, intramuscularly, or intravenously, every 8 hours) or phenylbutazone (2.2 mg/kg, orally or intravenously, every 12 hours), often provide effective analgesia and presumably reduce inflammation in the pleural space.

Horses should be provided with good nursing care, including a comfortable stall, free access to palatable water, and a good diet. Affected horses will often not eat adequately and should be tempted with fresh and nutritious fodder.

Attention should be paid to the horse's feet to detect early signs of laminitis and allow appropriate measures to be taken.

CONTROL

Prevention of pleuropneumonia involves reduction of risk factors associated with the disease. The main risk factors are other infectious respiratory diseases and transportation. Every effort should be made to prevent and treat respiratory disease in athletic horses, including institution of effective vaccination programs. Horses with infectious respiratory disease should not be vigorously exercised until signs of disease have resolved.

Transportation of athletic horses is common and essential for their participation in competitive events. It cannot, therefore, be eliminated. Every effort should be made to minimize the adverse effects of transportation on airway health. Recommendations for transport of horses first made in 1917 are still relevant. Updated, these recommendations include the following:

- Not transporting a horse unless it is healthy. Horses with fever should not be transported
- Knowledgeable staff familiar with the horse should accompany it.
- Suitable periods of rest and acclimation should be provided before recently transported or raced horses are transported.
- The time during which horses are confined for transportation should be kept to a minimum. Horses should be loaded last and unloaded first in flights with mixed cargo.
- The route taken should be the most direct and briefest available.
- Horses should be permitted adequate time to rest at scheduled breaks. If possible, on long journeys horses should be unloaded and allowed exercise (walking) and access to hay and water.
- Horses should have frequent, preferably continuous, access to feed and water during transportation.
- Horses should not be exercised after arrival until they are free of fever, cough, or nasal discharge.
- Horses should not be restrained during transportation such that they are unable or unwilling to lower their heads.

- Air quality should be optimal in the vehicle used to transport the horse.

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ACUTE BRONCHO-INTERSTITIAL PNEUMONIA IN FOALS

Acute broncho-interstitial pneumonia is a disease of foals less than 7 months of age, and usually less than 2 months of age, characterized by a rapid onset of respiratory distress. The condition is clinically similar to **acute lung injury** identified in other species.[1] The **etiology** is unclear in many cases, but causes or agents associated with the disease include equine influenza virus infection,[2] *R. equi,* equine herpesvirus-2, equine arteritis virus, or *Pneumocystis carinii.* The disease is likely a result of severe pulmonary injury by any of a number of infectious or toxic agents. The respiratory distress results from loss of pulmonary function because of necrosis of the epithelium of alveoli and terminal bronchioles.

Foals typically present with an acute onset (<4 days) of respiratory distress, pyrexia, and tachycardia. Foals are depressed and reluctant to eat. There is a pronounced respiratory effort with a marked abdominal component in most affected foals. Crackles, wheezes, and increased bronchial breath sounds are auscultable in most foals. Radiographic examination reveals a bronchointerstitial pattern that is always diffuse, although in some foals there is also a focal interstitial pattern. The prognosis is guarded, with approximately 50% of affected foals dying of the disease.

There is a neutrophilic leukocytosis and hyperfibrinogenemia in most cases. Arterial hypoxemia is present in severely affected foals. Tracheal aspirate demonstrates neutrophilic inflammation. Culture of the tracheal aspirate yields *Rhodococcus equi, S. zooepidemicus,* and *Actinobacillus* sp., in addition to other organisms that are of questionable significance. Serology might demonstrate evidence of infection by equine influenza virus or equine herpesvirus-2. Viral isolation can identify equine influenza virus.[2]

NECROPSY FINDINGS

Necropsy examination reveals the presence of diffusely reddened, wet, and firm lungs that fail to collapse. The predominant histologic lesion is necrosis of the epithelium of terminal bronchioles and alveoli.

TREATMENT

Principles of **treatment** are correction of hypoxemia, reduction of inflammation, and removal of inciting causes. Severely affected foals might require nasal insufflation of oxygen to ameliorate or correct hypoxemia. Administration of corticosteroids has been associated with improved survival. Broad-spectrum antibiotics are administered to treat concurrent bacterial infections and prevent secondary infection.

CONTROL

There are no specific control measures, but reduction of exposure of foals to infectious respiratory disease would be prudent.

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CHRONIC INTERSTITIAL PNEUMONIA IN FOALS

Chronic interstitial pneumonia is a sporadic disease of foals less than 10 months of age characterized by respiratory distress of several weeks' duration. The etiology is unknown, but the disease likely represents a common final response to injury caused by any one of a number of infectious or toxic agents (see "Interstitial Pneumonia of Horses" and "Acute Bronchointerstitial Pneumonia of Foals").

Affected foals are bright and alert and have markedly increased respiratory effort. The respiratory rate is elevated, and there is a prominent abdominal component to respiratory effort. Fever is low grade and intermittent. Thoracic auscultation reveals increased intensity of normal breath sounds and the presence of wheezes and crackles in most affected foals. Ultrasonographic examination of the thorax reveals extensive "comet tail" signs in most cases. Radiography demonstrates the presence of moderate to severe interstitial pneumonia that in some cases can include focal opacities suggestive of alveolar disease. The prognosis with appropriate treatment is excellent.

Affected foals have neutrophilic leukocytosis and hyperfibrinogenemia. Serologic examination for antibodies to common respiratory viruses is unrewarding. Culture of tracheal aspirates does not consistently yield growth of known pathogens, although *Nicoletella semolina* is associated with similar clinical signs and presentation.[1] Lung biopsy is not warranted because the characteristic changes on radiographic examination, combined with the clinical signs, are diagnostic for the disease. The risk of adverse events associated with lung biopsy outweighs any diagnostic utility given the good prognosis for complete recovery from the disease.

TREATMENT

Treatment consists of administration of corticosteroids such as dexamethasone phosphate at an initial dosage of 0.1 to 0.25 mg/kg intravenously every 24 hours for 3 to 5 days followed by a declining dose administered orally over 2 to 3 weeks. Prednisolone can be substituted for dexamethasone. Broad-spectrum antibiotics (combination of penicillin and aminoglycoside, trimethoprim-sulfonamide, or doxycycline) should be administered for 1 to 2 weeks.

There are no recognized control measures, although control of infectious respiratory disease in the herd is prudent.

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INTERSTITIAL PNEUMONIA IN ADULT HORSES

Interstitial pneumonia can be associated with other systemic disease, such as infection by influenza virus, equine infectious anemia virus, or intoxication by various plants or minerals (silica), or it can be a primary disease. Equine multinodular pulmonary fibrosis (EMPF) was previously included in this topic but is now discussed separately later in this section on diseases of the equine respiratory tract.[1]

ETIOLOGY

Interstitial pneumonia is a common finding in horses associated with various infectious agents (Hendra virus, equine influenza virus,[2,3] equine infectious anemia,[4] *Rhodococcus equi, Aspergillus* sp., *Cryptococcus* sp. and *Histoplasma* sp., *Pneumocystis carinii, Parascaris equorum,* and *Dictyocaulus arnfiedli*). Intoxication with perilla ketone, derived from *Perilla frutescens,* causes acute restrictive lung disease of horses. Similarly, ingestion of *Eupatorium* sp. in Australia and Hawaii causes interstitial pneumonia in horses. Ingestion of *Crotalaria* spp. causes interstitial pneumonia in donkeys.[5] Inhalation or ingestion of agricultural chemicals or environmental toxins (e.g., paraquat) has the potential to cause interstitial pneumonia in other species, but this has not been demonstrated in horses.[1] Silicosis causes interstitial pneumonia in horses in California.[6] Lipid pneumonia has an interstitial component.[7]

Chronic **pleuropulmonary fibrosis and elastosis** occurs in aged donkeys in the United Kingdom.[8,9] The disease was present in 32% of over 1100 postmortem examinations of donkeys.[9] The etiology of the disease is unknown.

Hypersensitivity reactions may cause severe respiratory disease in horses. Incriminating allergens include fungi (unspecified)

and chicken dust. Interstitial pneumonia has also been reported subsequent to administration of an immunostimulant containing mycobacterial cell-wall extract.

Chronic eosinophilic pneumonia of adult horses is idiopathic.[10]

EPIDEMIOLOGY

The disease occurs in adult horses, without apparent breed, sex, or age predisposition. In cases in which the cause is infectious, the epidemiology of the disease is characteristic of that of the causal organism.

PATHOGENESIS

The initial insult causes injury to parenchymal cells and an acute alveolitis. Alveolitis results from damage to epithelial and endothelial cells by toxic, metabolic (free radicals), or infectious agents. This is followed by a phase of cellular proliferation of type 2 pneumocytes and fibroblasts with connective tissue deposition. At this time there is an influx of inflammatory cells, the exact type depending to some extent on the cause of the disease. Infiltration of neutrophils, lymphocytes, and macrophages is common. Continued injury to the lung results in development of severe interstitial fibrosis and destruction of gas exchange units.

Interstitial pneumonia results in altered pulmonary function including reduced compliance, impaired pulmonary gas exchange, and a reduction in total and vital lung capacity. The work of breathing is increased.

CLINICAL SIGNS

Horses with interstitial pneumonia have various combinations of: weight loss, recurrent cough, depression, anorexia, fever, or respiratory distress. Signs of respiratory distress are variable between cases and depend on the severity of the disease. The usual history of is a gradual onset of increased respiratory effort. Heart and respiratory rates are usually elevated. Pyrexia is not a constant finding. There may be a nasal discharge. Thoracic auscultation may reveal only increased intensity of normal breath sounds or the presence of occasional crackles and wheezes.

Thoracic radiography reveals pulmonary disease, usually apparent as severe, diffuse interstitial disease. Ultrasonographic examination may reveal the presence of multiple "comet tail" signs in the lung parenchyma confluent with the pleural surface. There is no excess pleural fluid.

Intradermal skin testing may be useful to identify the inciting allergen in cases of allergic interstitial pneumonia.

CLINICAL PATHOLOGY

Hematologic and serum biochemical abnormalities vary with the inciting cause of the disease. Bronchoalveolar lavage, which is preferred over collection of tracheal aspirates, demonstrates changes consistent with the underlying disease, which is usually inflammatory.

NECROPSY FINDINGS

The lungs do not deflate as anticipated, and there may be indentations from the ribs on the surface of the lungs. The histologic changes depend on the etiology of the disease.

TREATMENT

Treatment should be directed toward any cause of the disease that is identified, such as administration of anthelmintics to horses with parasitic disease. Bronchodilating drugs, such as clenbuterol, can be considered, but bronchoconstriction is not a prominent component of the disease.

CONTROL

Prevention of exposure to potential infectious, toxic, or environmental causes is prudent.

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EXERCISE-INDUCED PULMONARY HEMORRHAGE OF HORSES (BLEEDERS)

SYNOPSIS

Etiology Pulmonary hemorrhage during exercise.

Epidemiology Present in most (>80%) Thoroughbred and Standardbred racehorses, although clinical signs are less common. Occurs worldwide in any horse that performs strenuous exercise. Case-fatality rate is low, although because of the high incidence of the disease, deaths occur frequently during racing.

Pathogenesis Probably associated with rupture of pulmonary capillaries by the high pulmonary vascular pressures generated during exercise. There does not appear to be a contributory role for preexisting inflammation and obstruction of small airways, although this is unclear. There is tissue damage caused by large and rapid changes in intrathoracic pressure.

Clinical signs Epistaxis is an uncommon but very specific sign of EIPH in horses that have just exercised. Endoscopic examination of the trachea and bronchi reveals blood.

Clinical pathology Presence of hemosiderin-laden macrophages in tracheal aspirates or bronchial lavage fluid.

Lesions Fibrosis and discoloration of the caudodorsal regions of the lungs. Fibrosis, accumulation of hemosiderin-laden macrophages in interstitial tissue, inflammation and bronchial artery angiogenesis. Horses dying acutely have blood-filled airways and heavy, wet lungs.

Diagnostic confirmation Demonstration of blood in the trachea or bronchi by endoscopic examination, or cytologic examination of tracheal aspirates or bronchoalveolar lavage fluid.

Treatment Furosemide is effective in decreasing the frequency and severity of the disease.

Control There are no specific control measures; however, prevention of environmental and infectious respiratory disease might reduce the incidence of the disease.

ETIOLOGY

Exercise-induced pulmonary hemorrhage of horses (EIPH) is a disease that occurs in horses during strenuous exercise.[1] There is evidence of a genetic component to epistaxis in Thoroughbred racehorses ($h^2 = 0.27$-0.5),[2] but there are no reports of heritability or genetic factors contributing to EIPH.

EPIDEMIOLOGY

EIPH is primarily a disease of horses, although it occurs in racing camels and Greyhounds.[3] EIPH occurs in horses worldwide, and there does not appear to be any geographic distribution. It is a disorder of horses that run at high speed, such as Thoroughbred or Standardbred racehorses. The disorder is uncommon in endurance horses and is rare in draft breeds, although it does occur in horses used for these activities.[4] There is increasing recognition of its importance in sport horses (3-day event, show jumping, but not in dressage).[5]

The prevalence of EIPH varies with the method used to detect it and the frequency with which horses are examined, as discussed later in this section. Epistaxis associated with exercise is almost always attributable to pulmonary hemorrhage and occurs only in a small proportion of racehorses. Epistaxis occurs in only 3% of horses that have blood detected in the trachea by endoscopic examination performed within 2 hours of racing. The prevalence of epistaxis

in racehorses varies between 0.1% and 9.0%, with the frequency depending on the breed, age and sex of horses selected for study, the type of racing, and the timing and frequency of observation of horses after racing. Epistaxis is more common in older horses. There are conflicting reports of a sex predisposition, although epistaxis may be more common in female Thoroughbreds. Epistaxis is more common after races of less than 1600 m than in longer races, although not all sources agree on this point. However, horses in steeplechase races, which are typically longer than 2000 m, are at greater risk of epistaxis than are horses in flat races. Incidence of epistaxis in steeplechase horses in the United Kingdom is 5.3 per 1000 starts and 3.6 per 1000 starts in hurdle racing.[6] Risk factors for horses in jumps races (steeplechase) include previous epistaxis (odds ratio [OR] 6.1 [4.4–8.3]), racing in a claiming race (OR 5.9 [1.4–25]), greater than 9 starts in previous 4 to 6 months (OR 10 [2–47]), and racing on firmer ground.[6] *Epistaxis is relatively uncommon, and most horses with EIPH do not have epistaxis.*

There are a variety of other methods of detecting EIPH, including endoscopic examination of the airways and microscopic examination of tracheal aspirates or bronchoalveolar lavage fluid.

Almost all Thoroughbred racehorses in active training have hemosiderophages in bronchoalveolar lavage fluid, indicating that all have some degree of EIPH. The prevalence of EIPH decreases when diagnosis is based on endoscopic examination of horses after exercise or racing.

EIPH is very common in Thoroughbred racehorses, with estimates of prevalence, based on a single endoscopic examination of the trachea and bronchi, of 43% to 75%.[7,8] The prevalence increases with the frequency of examination, with over 80% of horses having evidence of EIPH on at least one occasion after examination after each of three consecutive races.[9] There can be considerable variability in severity of EIPH within an individual horse on repeated examination over a racing season.[8] The prevalence of EIPH in Standardbred racehorses is assumed to be lower, with 26% to 34% of horses reported to have blood in the trachea after racing. However, these studies were based on a single examination and one only reported as positive those horses with blood covering more than one half the tracheobronchial tree. When examined after each of three races, 87% of Standardbred racehorses have evidence of EIPH on at least one occasion, suggesting that EIPH is as common in Standardbred racehorses as it is in Thoroughbred racehorses.

Exercise-induced pulmonary hemorrhage occurs in approximately 62% of racing Quarter Horses and has been observed in Quarter Horses used for barrel racing. The disorder occurs in racing Appaloosa horses. Approximately 11% of polo ponies are affected with EIPH. The disease occurs in draft horses but is not well documented.

Age is considered a risk factor for EIPH, with the prevalence of the disorder being higher in older horses, but the risk factor is the amount of racing that a horse has completed, not its age.[10,11] There is no consistent association of sex with prevalence of EIPH. Among Thoroughbred racehorses there is an unclear relationship between the speed of racing and the risk of EIPH.[10,12] Lesions of EIPH are not detected in young Thoroughbred racehorses that have trained at speeds of less than 7 m/s.

The risk of EIPH increases with racing at lower ambient temperatures[10,12] and with the wearing of bar shoes during racing.[12] There is no association between risk of EIPH and track hardness.[10,12]

PATHOGENESIS

The cause of EIPH is rupture of alveolar capillary membranes with subsequent extravasation of blood into interstitial and alveolar spaces. The source of blood in such instances is the pulmonary circulation. Bleeding from bronchial circulation during exercise has been suggested, based on histologic evidence of bronchial angiogenesis in horses that have experienced previous episodes of EIPH, but contribution of the bronchial circulation to EIPH has not been demonstrated. Regardless of the contribution of bronchial circulation to blood in the airways, the likely initial lesion is in capillaries associated with the pulmonary circulation. There is increasing evidence that the primary lesion is arteriovenous remodeling of pulmonary veins.[13-16] Remodeling of pulmonary veins results in loss of distensibility and partial occlusion to blood flow with subsequent presumed increases in pulmonary alveolar capillary pressure.[13,17] Hemorrhage into the interstitial space and alveoli, with subsequent rostral movement of blood in the airways, results in blood in the trachea and bronchi.

Rupture of alveolar capillaries occurs secondary to an exercise-induced increase in transmural pressure (pressure difference between the inside of the capillary and the alveolar lumen). If the transmural stress exceeds the tensile strength of the capillary wall, the capillary ruptures. The proximate cause of alveolar capillary rupture is the high transmural pressure generated by positive intracapillary pressures, which are largely attributable to capillary blood pressure, and the lower intraalveolar pressure generated by the negative pleural pressures associated with inspiration.

During exercise, the absolute magnitudes of both pulmonary capillary pressure and alveolar pressure increase, with a consequent increase in transmural pressure. Strenuous exercise is associated with marked increases in pulmonary artery pressure in horses. Values for mean pulmonary arterial pressure at rest of 20 to 25 mm Hg increase to more than 90 mm Hg during intense exercise because of the large cardiac output achieved by exercising horses. The increases in pulmonary artery pressure, combined with an increase in left atrial pressure during exercise, probably result in an increase in pulmonary capillary pressure. Combined with the increase in pulmonary capillary pressure is a marked decrease (more negative) in pleural, and therefore alveolar, pressure during exercise. The pleural pressure of normal horses during inspiration decreases from approximately –0.7 kPa (–5.3 mm Hg) at rest to as low as –8.5 kPa (64 mm Hg) during strenuous exercise. Together, the increase in pulmonary capillary pressure and decrease (more negative) in intrapleural (alveolar) pressure contribute to a marked increase in stress in the alveolar wall. Although the alveolar wall and pulmonary capillaries of horses are stronger than those of other species, rupture may occur because the wall stress in the alveolus exceeds the mechanical strength of the capillary.

Other theories of the pathogenesis of EIPH include: small-airway disease, upper airway obstruction, hemostatic abnormalities, changes in blood viscosity and erythrocyte shape, intrathoracic sheer forces associated with gait, and bronchial artery angiogenesis. It is likely that the pathogenesis of EIPH involves several processes, including pulmonary hypertension, lower alveolar pressure, and changes in lung structure, that summate to induce stress failure of pulmonary capillaries.

Obstruction of either the upper or lower airways has been proposed as a cause of EIPH. Inspiratory airway obstruction results in more negative intrapleural, and therefore alveolar, pressures. This effect is exacerbated by exercise, with the result that alveolar transmural pressure is greater in horses with airway obstruction. The higher transmural pressure in such horses may increase the severity of EIPH, although this has not been demonstrated. Moreover, although inspiratory airway obstruction may predispose to EIPH, the prevalence of this condition is much less than that of EIPH, indicating that it is not the sole factor inducing EIPH in most horses.

Horses with moderate to severe EIPH have histologic evidence of inflammation of the small airways, and there is a clear association between the presence of EIPH and inflammatory changes in bronchoalveolar or tracheal aspirate fluid. However, instillation of autologous blood into the airways does not induce a marked inflammatory response in normal horses, and it is therefore unclear whether inflammation alone induces or predisposes to EIPH.[18,19] Theoretically, small-airway inflammation and bronchoconstriction have the potential to produce intrathoracic airway obstruction

and, therefore, a more negative alveolar pressure. Given that small-airway disease is common in horses, there is the potential for an important effect of factors such as viral infections, air pollution, and allergic airway disease to contribute to the initiation or propagation of EIPH.

The characteristic location of lesions of EIPH in the caudodorsal lung fields has led to the proposal that hemorrhage is a result of tissue damage occurring when waves of stress, generated by forelimb foot strike, are focused and amplified into the narrowing cross-sectional area of the caudal lung lobes. According to the theory, the locomotor impact of the forelimbs results in transmission of forces through the scapula to the body wall, from where they pass into the lungs and caudally and dorsally. As the wave of pressure passes into the narrower caudodorsal regions of the lungs it generates progressively greater shearing forces that disrupt tissue and cause EIPH. However, studies of intrapleural pressures have not demonstrated the presence of a systemic pressure wave passing through the lung and do not provide support for this hypothesis.

Horses with EIPH have been suspected of having defects in either hemostasis or fibrinolysis. However, although exercise induces substantial changes in blood coagulation and fibrinolysis, these is no evidence that horses with EIPH have defective coagulation or increased fibrinolysis.

Regardless of the cause, rupture of pulmonary capillaries and subsequent hemorrhage into airways and interstitium causes inflammation of both airways and interstitium with subsequent development of fibrosis and alteration of tissue compliance. Heterogeneity of compliance within the lungs, and particularly at the junction of normal and diseased tissue, results in the development of abnormal shear stress with subsequent tissue damage. These changes are exacerbated by inflammation and obstruction of small airways, with resulting uneven inflation of the lungs. The structural abnormalities, combined with pulmonary hypertension and the large intrathoracic forces associated with respiration during strenuous exercise, cause repetitive damage at the boundary of normal and diseased tissue with further hemorrhage and inflammation. The process, once started, is lifelong and continues for as long as the horse continues to perform strenuous exercise.

CLINICAL FINDINGS

Poor athletic performance or epistaxis are the most common presenting complaints for horses with EIPH. Although poor performance may be attributable to any of a large number of causes, epistaxis associated with exercise is almost always secondary to EIPH.

Epistaxis as a result of EIPH occurs during or shortly after exercise and is usually first noticed at the end of a race, particularly when the horse is returned to the paddock or winner's circle and is allowed to lower its head. It is usually bilateral and resolves within hours of the end of the race. Epistaxis may occur on more than one occasion, especially when horses are raced or exercised at high speed soon after an initial episode.

Exercise-Induced Pulmonary Hemorrhage and Performance

Failure of racehorses to perform to the expected standard (poor performance) is often, accurately or not, attributed to EIPH.[7] Many horses with poor performance have cytologic evidence of EIPH on microscopic examination of tracheobronchial aspirates or bronchoalveolar lavage fluid or have blood evident on endoscopic examination of the tracheobronchial tree performed 30 to 90 minutes after strenuous exercise or racing. However, it is important to recognize that EIPH is very common in racehorses, and it should be considered the cause of poor performance only after other causes have been eliminated. Severe EIPH undoubtedly results in poor performance and, on rare occasions, death of Thoroughbred racehorses.[1,7,20] Thoroughbred horses with EIPH have impaired performance compared with unaffected horses.[7] Affected horses have a lower likelihood of finishing in the first three places, are less likely to be elite money earners, and finish further behind the winner than do unaffected horses.

Results of studies in Standardbred racehorses indicate either a lack of effect of EIPH on performance or an association between EIPH and superior performance. There was no relationship between presence of EIPH and finishing position in 29 Standardbred racehorses with intermittent EIPH examined on at least two occasions, nor in 92 Standardbred racehorses examined on one occasion. However, of 965 Standardbred racehorses examined after racing, those finishing first or second were 1.4 times more likely (95% confidence interval 0.9-2.2) to have evidence of EIPH on tracheobronchoscopic examination than were horses that finished in seventh or eighth position.

Physical Examination

Apart from epistaxis in a small proportion of affected horses (Fig. 12-26), there are few abnormalities detectable on routine physical examination of horses with EIPH. Rectal temperature and heart and breathing rates may be elevated as a consequence of exercise in horses examined soon after exercise, but values of these variables in horses with EIPH at rest are not noticeably different from those of horses with no evidence of EIPH. Affected horses may swallow more frequently during recovery from exercise than do unaffected horses, probably as a result of blood in the larynx and pharynx. Coughing is common in horses recovering from strenuous exercise and after recovery from exercise; horses with EIPH are no more likely to cough than are unaffected horses.[1] Other clinical signs related to respiratory abnormalities are uncommon in horses with EIPH. Respiratory distress is rare in horses with EIPH and, when present, indicates severe hemorrhage or other serious lung disease such as pneumonia, pneumothorax or rupture of a pulmonary abscess. Lung sounds are abnormal in a small number of EIPH-affected horses and, when present, are characterized by increased intensity of normal breath sounds during rebreathing examination. Tracheal rales may be present in horses with EIPH but are also heard in unaffected horses.

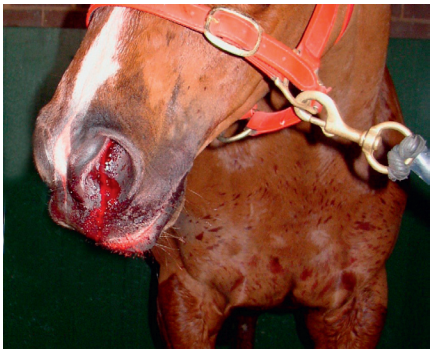

Fig. 12-26 Thoroughbred racehorse with epistaxis secondary to exercise-induced pulmonary hemorrhage during racing.

Tracheobronchoscopy

Observation of blood in the trachea or large bronchi of horses 30 to 120 minutes after racing or strenuous exercise provides a definitive diagnosis of EIPH. The amount of blood in the large airways varies from a few small specks on the airway walls to a stream of blood occupying the ventral one-third of the trachea. Blood may also be present in the larynx and nasopharynx. If there is a strong suspicion of EIPH and blood is not present on a single examination conducted soon after exercise, the examination should be repeated in 60 to 90 minutes. Some horses with EIPH do not have blood present in the rostral airways immediately after exercise, but do so when examined 1 to 2 hours later. Blood is detectable by tracheobronchoscopic examination for 1 to 3 days in most horses, with some horses having blood detectable for up to 7 days.

Bronchoscopic examination can be used to estimate the severity of EIPH through the use of a grading system. The interobserver repeatability of tracheobronchoscopic assessment of severity of EIPH using a grading scale of 0 to 4 is excellent, and this scoring system has been widely adopted for use (Fig. 12-27).[7,8,12,21]

- **Grade 0**: No blood detected in the pharynx, larynx, trachea, or mainstem bronchi.
- **Grade 1:** Presence of one or more flecks of blood or two or more short

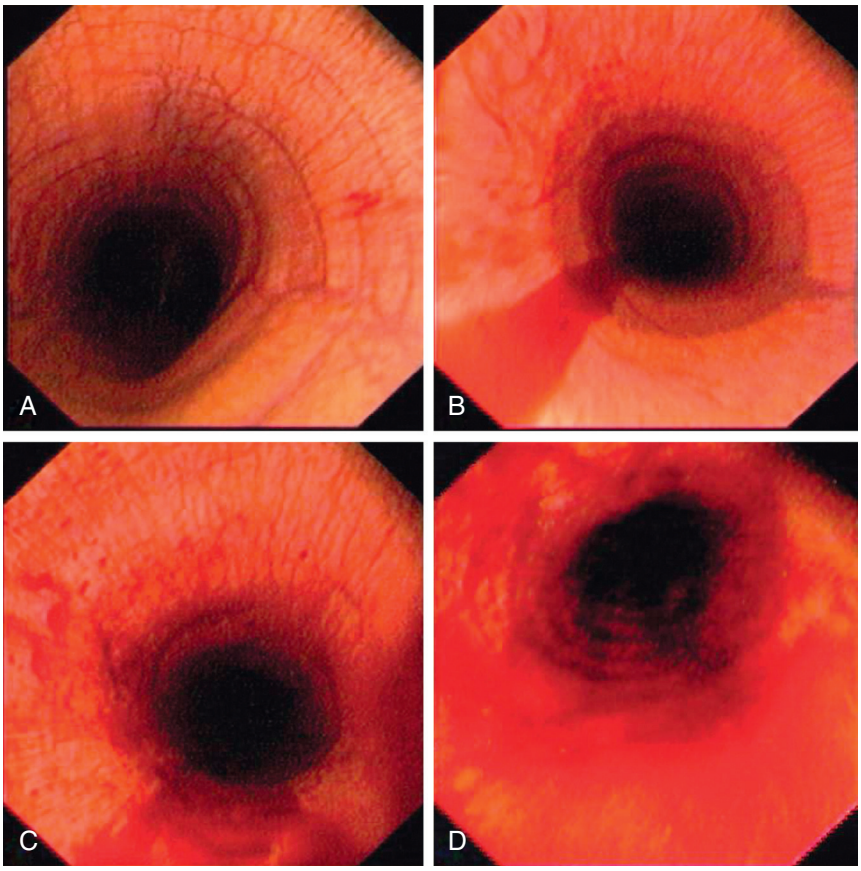

Fig. 12-27 Grading of EIPH in Thoroughbred racehorses—Grades 1 (A), 2 (B), 3 (C), and 4 (D). (Reproduced with permission Hinchcliff et al. 2005.[21])

(< one-quarter of the length of the trachea) narrow (<10% of the tracheal surface area) streams of blood in the trachea or mainstem bronchi visible from the tracheal bifurcation.

- **Grade 2:** One long stream of blood (> half the length of the trachea) or greater than 2 short streams occupying less than one-third of the tracheal circumference.
- **Grade 3:** Multiple, distinct streams of blood covering more than one-third of the tracheal circumference. No blood pooling at the thoracic inlet.
- **Grade 4:** Multiple, coalescing streams of blood covering greater than 90% of the tracheal surface with pooling of blood at the thoracic inlet.

It is assumed that a higher score represents more severe hemorrhage, but although the repeatability of this scoring system has been established, the relationship between the amount of blood in the large airways and the actual amount of hemorrhage has not been established.

Radiography

Thoracic radiography is of limited use in detecting horses with EIPH. Radiographs can demonstrate the presence of densities in the caudodorsal lung fields of some horses, but many affected horses have minimal to undetectable radiographic abnormalities.[1] Examination of thoracic radiographs of horses with EIPH can be useful in ruling out the presence of another disease process, such as a pulmonary abscess, contributing to the horse's pulmonary hemorrhage or poor athletic performance.

Prognosis

Horses that have experienced one episode of epistaxis are more likely to have a second episode. For this reason most racing jurisdictions do not permit horses with epistaxis to race for a period of weeks to months after the initial instance, with more prolonged enforced rest after a subsequent episode of epistaxis and retirement from racing after a third bout. The recurrence rate after one episode of epistaxis in Thoroughbred horses is approximately 13.5% despite affected horses not being permitted to race for 1 month after the initial episode. This high rate of recurrence suggests that the inciting pulmonary lesions have not healed.

Long-term examination of performance of horses with EIPH indicates the horses with grade 4 EIPH have shorter racing careers, but it is not clear if this is a function of the biology of the disease or a management decision by owners and trainers.[11] There is no association between measures of long-term performance measured over 10 years and grades 1, 2, or 3 of EIPH in Thoroughbred racehorses.[11]

Clinical Pathology

Examination of Airway Secretions or Lavage Fluid

The presence of red cells or macrophages containing either effete red cells or the breakdown products of hemoglobin (hemosiderophages) in tracheal or bronchoalveolar lavage fluid provides evidence of EIPH. Detection of red cells or hemosiderophages in tracheal aspirates or bronchoalveolar lavage fluid is believed to be both sensitive and specific in the diagnosis of EIPH. Examination of airway fluids indicates the presence of EIPH in a greater proportion of horses than does tracheobronchoscopic examination after strenuous exercise or racing. The greater sensitivity of examination of airway fluid is probably attributable to the ability of this examination to detect the presence of small amounts of blood or its residual products and the longevity of these products in the airways. Although endoscopic examination may detect blood in occasional horses up to 7 days after an episode of EIPH, cellular evidence of pulmonary hemorrhage persists for weeks after a single episode. Red blood cells and macrophages containing red cells are present in bronchoalveolar lavage fluid or tracheal aspirates for at least 1 week after strenuous exercise or instillation of autologous blood into airways, and hemosiderophages are present for at least 21 days and possibly longer.

Recent studies have reported on the use of red cell numbers in bronchoalveolar lavage fluid as a quantitative indicator of EIPH. However, this indicator of EIPH severity has not been validated nor demonstrated to be more reliable or repeatable than tracheobronchoscopic examination and visual scoring. Furthermore, considerable concern exists over the suitability of red cell counts in bronchoalveolar lavage fluid for assessment of severity of EIPH given that an unknown area, although presumably small, of the lung is examined by lavage and that there is a risk that this area of lung may not be representative of the lung as a whole, similar to the situation of examination of bronchoalveolar lavage fluid of horses with pneumonia. Bronchoalveolar lavage of sections of both lungs, achieved using an endoscope, may obviate some of these concerns.

Tracheal aspirates may be obtained any time after exercise by aspiration either during tracheobronchoscopic examination or through a percutaneous intratracheal needle. Aspirates obtained through an endoscope may not be sterile, depending on the collection technique. Bronchoalveolar lavage fluid

can be obtained through either an endoscope wedged in the distal airway or a cuffed tube inserted blindly into a distal airway. Collection of fluid through an endoscope has the advantage of permitting examination of the distal airways and selection of the area of lung to be lavaged. However, it does require the use of an endoscope that is longer (2 m) than those readily available in most equine practices. Use of a commercial bronchoalveolar lavage catheter does not require use of an endoscope, and this procedure can be readily performed in field situations.

DIFFERENTIAL DIAGNOSIS

Epistaxis and hemorrhage into airways can occur as a result of a number of diseases (Table 12-12).

Necropsy

Exercise-induced pulmonary hemorrhage is a rare cause of death of racehorses, but among racehorses that die during racing for reasons other than musculoskeletal injuries, EIPH is common.[20] Necropsy examination of horses is usually incidental to examination for another cause of death. Pertinent abnormalities in horses with EIPH are restricted to the respiratory tract. Grossly, horses examined within hours of strenuous exercise, such as horses examined because of catastrophic musculoskeletal injuries incurred during racing, may have severe petechiation in the caudodorsal lung fields. Horses with chronic disease have blue/gray or blue/brown discoloration of the visceral pleural surfaces of the caudodorsal lung fields that is often sharply demarcated, especially on the diaphragmatic surface. The discoloration affects both lungs equally, with 30% to 50% of the lung fields being discolored in severe cases. Affected areas do not collapse to the same extent as unaffected areas and, in the deflated lung, have a spleen-like consistency. On cut surface, the discolored areas of lung are predominantly contiguous with the dorsal pleural surface and extend ventrally into the lung parenchyma. Areas of affected lung may be separated by normal lung. There is proliferation of bronchial vessels, predominantly arteries and arterioles, in affected areas. Histologically, affected areas exhibit bronchiolitis, hemosiderophages in the alveolar lumen and interstitial spaces, and fibrosis of interlobular septa, pleura, and around vessels and bronchioles.

Treatment

Prevention of EIPH is contentious because it can involve the administration of medications on the day of racing. The efficacy of various interventions and medications has recently been evaluated in two systematic reviews, both of which concluded that there was moderately strong to strong evidence

Table 12-12 Causes of epistaxis in horses

Disease	Epidemiology	Clinical signs and diagnosis	Treatment and control
Hemorrhage into trachea or bronchi, sometimes with epistaxis			
Exercise-induced pulmonary hemorrhage (EIPH)	Horses after strenuous exercise. Most common in Thoroughbred and Standardbred racehorses.	Epistaxis is a rare but very specific sign of EIPH. Only occurs after exercise.	Efficacy of various drugs used for treatment and control is debated.
		Endoscopic examination of the airways is diagnostic,	Furosemide is used extensively before racing.
Trauma	Sporadic. Associated with trauma to head, neck, or chest.	Physical examination reveals site and nature of the trauma. Can require endoscopic examination of upper airways.	Symptomatic treatment.
Pneumonia	Recent transport or respiratory disease. Can occur as outbreaks though usually individual animals.	Fever, tachypnea, abnormal lung sounds, leukocytosis; radiography demonstrates lung lesions. Cytologic and microbiological examination of tracheal aspirate.	Antimicrobials, NSAIDs, oxygen. Control by vaccination and prevention of respiratory disease.
Lung abscess	Sporadic. Hemorrhage can occur after exercise.	Sometimes no premonitory signs. Fever, depression, anorexia, cough. Hemogram demonstrates leukocytosis. Hyperfibrinogenemia. Ultrasonography or radiography demonstrates lesion. Tracheal aspirates.	Antibiotics.
Intrabronchial foreign body	Sporadic.	Cough, hemoptysis, fever. Endoscopy or radiography reveals foreign body.	Removal of foreign body—often not readily achieved.
Pulmonary neoplasia	Sporadic. Often older horse, but not always. Hemangiosarcoma.	Cough, hemoptysis. Demonstrate mass on ultrasonographic or radiographic examination.	None.
Epistaxis (in addition to the previously listed diseases)			
Guttural pouch mycosis	Sporadic. Acute-onset epistaxis.	Severe, life-threatening epistaxis. Tachycardia, anemia, hemorrhagic shock.	Surgical ligation or occlusion of arteries in the guttural pouch.
Ethmoidal hematoma	Sporadic.	Epistaxis not associated with exercise. Usually unilateral.	Surgery or injection of mass with formaldehyde.
Thrombocytopenia	Sporadic.	Epistaxis, mild, intermittent. Petechiation and ecchymotic hemorrhages. Thrombocytopenia.	Glucocorticoids.
Neoplasia	Sporadic.	Neoplasia of upper airways.	None.
Trauma	Sporadic.	Injury to head or pharynx.	Symptomatic.
Sinusitis	Sporadic.	Endoscopic or radiographic examination of sinus.	Drainage. Antimicrobials.

that administration of furosemide before racing reduces the frequency and severity of EIPH in Thoroughbred racehorses.[1,22] There was either weak evidence or no evidence of efficacy of other interventions. There is a recommendation for use of furosemide, but because of the regulatory issues related to its use, this is only a weak recommendation.[1]

Therapy of EIPH is usually a combination of attempts to reduce the severity of subsequent hemorrhage and efforts to minimize the effect of recent hemorrhage. Treatment of EIPH is problematic for a number of reasons. First, the pathogenesis of EIPH has not been determined although the available evidence supports a role for stress failure of pulmonary capillaries secondary to exercise-induced pulmonary hypertension. Second, there is a lack of information using large numbers of horses under field conditions that demonstrates an effect of any medication or management practice (with the exception of bedding) on EIPH. There are numerous studies of small numbers of horses (< 40) under experimental conditions but these studies often lacked the statistical power to detect treatment effects and, furthermore, the relevance of studies conducted on a treadmill to horses racing competitively is questionable.[1] Treatments for EIPH are usually intended to address a specific aspect of the pathogenesis of the disease and will be discussed in that context but should be considered in the context of the amount and strength of evidence, which for most treatments is scant and weak.

Prevention of Stress Failure of the Pulmonary Capillaries

There is interest in reducing the pressure difference across the pulmonary capillary membrane in an effort to reduce EIPH. Theoretically, this can be achieved by reducing the pressure within the capillary or increasing (making less negative) the pressure within the intrathoracic airways and alveolus.

Reducing Pulmonary Capillary Pressure

Furosemide administration as prophylaxis of EIPH is permitted in a number of racing jurisdictions worldwide, most notably Canada, the United States, Mexico, and most of the South American countries. Within the United States and Canada, almost all Thoroughbred, Standardbred, and Quarter Horse racing jurisdictions permit administration of furosemide before racing.

The efficacy of furosemide in treatment of EIPH is now well documented.[9,22] The mechanism by which furosemide reduces the severity of EIPH is unknown, although it is speculated that furosemide, by attenuating the exercise-induced increase in pulmonary artery and pulmonary capillary pressure of horses, reduces the frequency or severity of pulmonary capillary rupture.

Furosemide is associated with superior performance in both Thoroughbred and Standardbred racehorses, which further complicates assessment of its efficacy in treating EIPH.

An increase in pulmonary capillary pressure secondary to altered rheostatic properties of blood during exercise has been suggested as a possible contributing factor for EIPH.

Increasing Alveolar Inspiratory Pressure

Airway obstruction, either intrathoracic or extrathoracic, increases airway resistance and results in a more negative intrathoracic (pleural) pressure during inspiration to maintain tidal volume and alveolar ventilation. Causes of extrathoracic airway obstruction include laryngeal hemiplegia and other abnormalities of the upper airway, whereas intrathoracic obstruction is usually a result of bronchoconstriction and inflammatory airway disease. Horses with partial extrathoracic inspiratory obstruction or bronchoconstriction and airway inflammation associated with recurrent airway obstructive disease (heaves) have pleural (and hence alveolar) pressures that are lower (more negative) than those in unaffected horses or in horses after effective treatment. Hypothetical relationships between the horse's bit, airway obstruction and EIPH are not supported to date by empirical evidence.[23,24]

Partial inspiratory obstruction, such as produced by laryngeal hemiplegia, exacerbates the exercise-induced decrease in intrapleural pressures with a consequent increase in transmural capillary pressures. These changes may exacerbate the severity of EIPH, although an association between upper airway obstructive disease and EIPH has not been demonstrated. Surgical correction of airway obstruction is expected to resolve the more negative intrapleural pressure, but its effect on EIPH is unknown.

Recently the role of the nares in contributing to upper airway resistance, and hence lowering inspiratory intrapleural pressure during intense exercise, has attracted the attention of some investigators. Application of nasal dilator bands (Flair strips) reduces nasal resistance by dilating the nasal valve and reduces red cell count of bronchoalveolar lavage fluid collected from horses after intense exercise on a treadmill. Furthermore, application of the nasal dilator strips to horses in simulated races reduces red cell count in bronchoalveolar lavage fluid of some, but not all, horses.

The role of small-airway inflammation and bronchoconstriction in the pathogenesis of EIPH is unclear. However, horses with EIPH are often treated with drugs intended to decrease lower airway inflammation and relieve bronchoconstriction. Beta-adrenergic bronchodilatory drugs such as clenbuterol and albuterol (salbutamol) are effective in inducing bronchodilation in horses with bronchoconstriction, but their efficacy in preventing EIPH is either unknown or, in very small studies, is not evident. Corticosteroids, including dexamethasone, fluticasone, and beclomethasone, administered by inhalation, parenterally, or enterally, reduce airway inflammation and obstruction but have no demonstrated efficacy in preventing EIPH. Cromolyn sodium (sodium cromoglycate) has no efficacy in preventing EIPH.

Water vapor treatment (inhalation of water-saturated air) has been proposed as a treatment for EIPH because of its putative effect on small-airway disease. However, water vapor treatment has no effect on EIPH.

The use of bedding of low allergenic potential (shredded paper) to prevent EIPH has no apparent effect on prevalence of the condition. Although it is suggested that preventing or minimizing small-airway disease may reduce the severity of EIPH, studies to demonstrate such an effect have not been reported. However, optimizing the air quality in barns and stables and preventing infectious respiratory disease appear to be sensible precautions.

Interstitial Inflammation and Bronchial Angiogenesis

Hemorrhage into interstitial tissues induces inflammation with subsequent development of fibrosis and bronchial artery angiogenesis. The role of these changes in perpetuating EIPH in horses is unclear but is probably of some importance. Treatments to reduce inflammation and promote healing with minimal fibrosis have been proposed. Rest is an obvious recommendation, and many racing jurisdictions have rules regarding enforced rest for horses with epistaxis. Although the recommendation for rest is intuitive, there is no information that rest reduces the severity or incidence of EIPH in horses with prior evidence of this disorder.

Similarly, corticosteroids are often administered, either by inhalation, enterally or parenterally, in an attempt to reduce pulmonary inflammation and minimize fibrosis. Again, the efficacy of this intervention in preventing or minimizing the severity of EIPH has not been documented.

Excessive Bleeding

Coagulopathy and Fibrinolysis

Exercise induces substantial changes in blood coagulation and fibrinolysis. However, there is no evidence that horses with EIPH have defective coagulation or increased fibrinolysis. Regardless, aminocaproic acid, a potent inhibitor of fibrin degradation, has been administered to horses to prevent EIPH. The efficacy of aminocaproic acid in preventing EIPH has not been demonstrated.[1] Similarly, estrogens are given to horses with the expectation of improving hemostasis, although the effect of estrogens on coagulation in any species is unclear.

There is no evidence that estrogens prevent EIPH in horses.

Vitamin K is administered to horses with EIPH, presumably in the expectation that it will decrease coagulation times. However, because EIPH is not associated with prolonged bleeding times, it is unlikely that this intervention will affect the prevalence or severity of EIPH.

Platelet Function

Aspirin inhibits platelet aggregation in horses and increases bleeding time. Seemingly paradoxically, aspirin is sometimes administered to horses with EIPH because of concerns that increased platelet aggregation contributes to EIPH. There is no evidence that aspirin either exacerbates or prevents EIPH.

Capillary Integrity

Capillary fragility increases the risk of hemorrhage in many species. Various bioflavonoids have been suggested to increase capillary integrity and prevent bleeding. However, hesperidin and citrus bioflavonoids have no efficacy in prevention of EIPH in horses. Similarly, vitamin C is administered to horses with EIPH without scientific evidence of any beneficial effect.

Summary of Treatment Options

Selection of therapy for horses with EIPH is problematic. Given that most horses have some degree of pulmonary hemorrhage during most bouts of intense exercise, the decision must be made not only as to the type of treatment and its timing but also which horses to treat. Moreover, the apparently progressive nature of the disease with continued work highlights the importance of early and effective prophylaxis and emphasizes the need for studies of factors such as air quality and respiratory infections in inciting the disorder.

The currently favored treatment for EIPH is administration of furosemide before intense exercise. Its use is permitted in racehorses in a number of countries but is contentious in many. A frequent practice is to administer furosemide before high-speed training, and not on the day of racing, in jurisdictions that do not permit race day administration of medications. There is increasing interest in the effect of furosemide administered 24 hours before racing; its efficacy in this situation remains to be determined. The association between furosemide administration and superior performance in Standardbred and Thoroughbred racehorses should be borne in mind when recommending use of this drug.

Prevention and Control

There are no documented preventive strategies. Rest is an obvious recommendation for horses with EIPH, but the hemorrhage is likely to recur when the horse is next strenuously exercised. The duration of rest and the optimal exercise program to return horses to racing after EIPH is unknown, although some jurisdictions require exercise no more intense than trotting for 2 months. Firm recommendations cannot be made on duration of rest because of a lack of objective information.

Although a role for lower airway disease (either infectious or allergic) in the genesis of EIPH has not been demonstrated, control of infectious diseases, and minimization of noninfectious lower airway inflammation appears prudent.

Concern about the role of impact waves in the genesis of EIPH has led to discussion of "low-stress" training protocols, but these have not been adequately evaluated.

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RECURRENT AIRWAY OBSTRUCTION (HEAVES)

SYNOPSIS

Etiology Combined genetic predisposition with environmental challenge of inhaled barn and feed dust containing inciting agents that can include particles of molds, endotoxin, mites, plant debris, and inorganic material.

Epidemiology Predominantly a disease of horses stabled in poorly ventilated barns and fed quality hay. Occurs worldwide but more commonly in the northern hemisphere. Increased prevalence in older horses. No breed or sex predilection.

Clinical signs Range of severity of clinical signs. Chronic cough, mucopurulent nasal discharge, poor athletic performance, increased respiratory rate, increased expiratory effort, wheezes on thoracic auscultation, and abundant mucopurulent material in the trachea on endoscopic examination.

Clinical pathology Neutrophilia in tracheal aspirate and bronchoalveolar lavage fluid.

Lesions Bronchiolitis with mononuclear cell infiltration, epithelial, and goblet cell hyperplasia, neutrophil accumulation in airway lumens, and alveolar hyperinflation.

Diagnostic confirmation Clinical signs, examination of bronchoalveolar lavage fluid, and the response to treatment.

Treatment Remove the inciting cause by providing a dust-free environment, and administer corticosteroids. Bronchodilators are useful for treatment of acute bronchoconstriction.

Control Prevent exposure to inciting cause. Ensure optimal air quality in stables or maintain horses at pasture.

Recurrent airway obstruction (RAO; heaves) is a recurrent disease of stabled adult horses characterized by neutrophilic airway inflammation and airway obstruction manifest clinically by the presence of coughing, excess mucus accumulation in airways, neutrophilic bronchoalveolar lavage fluid or tracheal aspirate, bronchospasm, tachypnea and increased respiratory effort, and exercise intolerance. Clinical severity ranges from mild coughing with minimal exercise intolerance during infrequent recurrences of the disease through to severe and persistent coughing, airway obstruction, and markedly increased work of breathing and abnormal breathing pattern. Removal of exposure to hay and straw and keeping the horse at pasture results in remission of the disease.

The disease should be differentiated from the usually transient inflammatory airway disease of young adult horses in which there is no clinically significant impairment of pulmonary function. The disease is classically considered to have an allergic component.

ETIOLOGY

Genetics

There is a genetic component to the disease, although the precise pattern of inheritance and gene association is yet to be determined.[1] There is a familial pattern to the disease in some breeds, and for Warmbloods and Lipizzan, affected parents increase the likelihood of off-spring developing recurrent airway obstruction (RAO) by fivefold.[2] Segregation analysis indicates a mixed mode of

inheritance in Warmbloods.[3] It appears likely that multiple genes are involved in the predisposition to RAO and the genes involved might differ with breed of horse or other animal or environmental factors. Evidence for a multigene cause for predisposition to RAO includes the finding of quantitative trait loci (QTL) located on two different chromosomes (ECA 13 and ECA 15) in different families of affected Warmbloods[4,5] and failure to identify a monogenetic predisposition to RAO. Further studies in half-sibling horses and from unrelated horses indicates that at least one causative variant is a QTL region located on ECA 13 that is not associated with any coding variants, suggesting that the cause is a regulatory mutation.[6] The cause does not appear to be abnormalities in the gene encoding DNAH3 (dynein—a component of cilia), although it is located in this QTL and has 53 polymorphisms including 7 nonsynonymous variants.[7] Similarly, mutations in the integrin alpha X gene, which is related to allergy in humans and is located in the region of the QTL in ECA 13, are not associated with RAO.[4,8] However, expression of interleukin 4 receptor gene, which is also located in the region of the QTL, is greater in bronchoalveolar lavage fluid of RAO-affected half-siblings than in unaffected animals, suggesting a role for this cytokine, or a mutation in its gene, in RAO.[9]

Environmental Factors

RAO is caused by inhalation by susceptible horses of dust particles found in barns, bedding, and feed materials such as dusty hay. The inhaled particles include endotoxin, mites, plant debris, inorganic materials, and conidia and fragments of molds. *Faenia rectivirgula* (formerly known as *Micropolyspora faeni*), *Aspergillus fumigatus,* and *Thermoactinomyces vulgaris* are molds commonly associated with respiratory disease in susceptible horses, as evidenced by experimental studies involving inhalation of mold or mold fragments by horses. Molds contain a number of inflammatory substances, including various allergens, glucans, mycotoxins, and proteases, and it is not clear which of these agents are the inciting cause of RAO. Furthermore, dust containing mold also contains endotoxin. Inhalation of fungal spores, endotoxin and silica microspheres causes RAO in susceptible horses but not in healthy horses.[10] There was neutrophilic inflammation in both healthy and RAO horses but bronchospasm in only RAO horses.[10] Endotoxin contamination of molds contributes to the airway response to inhalation of preparations of molds used in experimental studies and inhalation of endotoxin alone produces airway inflammation and impaired respiratory function in horses in a dose-dependent manner, with RAO-susceptible horses having an exaggerated response at lower doses. Endotoxin concentrations in the breathing zone of horses are eight times higher in stables than at pasture.[11] However, the response to endotoxin is less than that of susceptible horses exposed to hay dust containing endotoxin, indicating that endotoxin alone is not sufficient to cause the clinical signs of RAO. Other compounds in hay dust are integral to the development of RAO.

It is emphasized that there is not one causative agent acting alone but rather a range of agents that, when inhaled in sufficient concentration by susceptible horses, induce airway disease. It is likely that RAO is associated with the potentiating interactions among several agents present in barn or hay dust and is not simply a response to one agent. The mechanisms underlying development of airway inflammation and respiratory dysfunction are provided under "Pathogenesis." Viral infections and 3-methylindole intoxication are not considered important causes of RAO.

EPIDEMIOLOGY

Occurrence

Although RAO is one of the more common diseases of horses and is a major cause of loss of performance and wastage in European horses, there are few reports of its epidemiologic characteristics. The disease is common in Europe and North America but is rare in Australia. The prevalence of RAO in the Great Britain, based on a random survey of owners who use veterinary surgeons, is 14% (95% confidence interval of 10.7%–17.4%).[12] The 7-day incidence (ie, new occurrence of the disease in an animal) of RAO in horses and ponies in Great Britain is 0.4% (0%–0.8%), and the prevalence of RAO was 5.8% (95% CI 4.2%–7.5%), as reported by owners.[13] In Germany, 83% of horses believed to be healthy at an auction were found to have clinical evidence of chronic pulmonary disease.

Inflammatory airway disease is very common in horses, with 96% of racehorses in Hong Kong examined at necropsy and 27% of healthy racehorses in training having an increased proportion (>20%) of neutrophils in tracheal aspirate, indicating inflammatory airway disease. Among stabled pleasure horses in Michigan, ~17% had cytologic or endoscopic evidence of airway inflammation,[11] and 12% of horses examined in an abattoir in the northern United States had histologic evidence of bronchitis. However, although airway inflammation is a component of RAO, the airway inflammation common in young athletic horses and stabled horses is not generally considered to be RAO or necessarily a prodrome of RAO.

The **case-fatality rate** for moderately to severely affected horses is approximately 20% over a 2- to 4-year period. Among horses greater than 15 years of age, presence of RAO is not significantly associated with death ($P = 0.73$, hazard ratio 1.19, 95% CI 0.4–3.2).[14] Most mildly to moderately affected horses respond well to treatment and continue to perform at a satisfactory level.

Risk Factors

Animal Risk Factors

The disease occurs in adult horses and ponies. A survey of horse and pony owners in Great Britain found that the median age of horses and ponies with the disease is 18.2 years versus 12.7 for unaffected animals.[13] Another owner survey found the median age of RAO affected horses as 13 years (interquartile range [IQR] of 9.5–20 years), whereas that of unaffected horses was 10 years (IQR 7–14.4 y).[12] The odds of a horse having RAO (as reported by the owner) increases with greater age (odds ratio of 5.1, 8.1, 11.4, 9.5, and 18.3 for horses 5–7, 7–9, 9–11, 11–15, and greater than15 years of age compared with horses < 5 years).[12] Examination of a convenience sample stratified random sample of 3000 horses of 1e to 40 years of age in the Netherlands revealed spontaneous coughing during a 10 minute observation period in 1% of horses, nasal discharge in 1.9%, and abnormal respiratory effort in 1%.[15] Of 200 horses and ponies greater than 15 years of age in the United Kingdom randomly chosen for examination by a veterinarian, 13.6% had marked abnormalities (expiratory wheeze, cough and/or increased abdominal effort) during rebreathing examination that are consistent with RAO.[16] A further 17.8% had moderately severe abnormalities. Those horses and ponies with abnormalities identified during rebreathing examination were significantly older (median 21.2 years) than were animals without abnormalities (18.0 years).[16] Similarly, 15% of horses greater than 30 years of age have marked clinical signs consistent with RAO, and a further 19% have moderate abnormalities.[17]

There is no apparent breed, sex, or height predisposition,[12] with the exception that Thoroughbreds are 3 times more likely to be examined for the disease than are ponies, although this could represent a sampling bias in that owners of Thoroughbreds might be more likely to seek veterinary attention than owners of ponies. The finding of increased likelihood of Thoroughbred horses having the disease is not consistent among studies. A survey of donkey owners in the United Kingdom did not elicit any reports of signs consistent with RAO in any of the ~1700 animals.[18] This might represent underreporting of the condition in donkeys or a low prevalence of the disease in donkeys.

There are horses that develop the disease and other horses, maintained in an identical situation, that do not.[12] Development of disease is dependent on the horse being susceptible to the inflammatory effect of inhaled dust but the reasons for this individual susceptibility are poorly understood. As noted earlier ("Etiology—Genes"), familial predisposition has been suggested based on the observation that Lipizaners and German and

Swiss Warmbloods are 3.2 times more likely to have RAO if one parent was affected and 4.6 times as likely if both parents had RAO. There is no association between major histocompatibility markers (equine leukocyte antigens) and occurrence of RAO.

Exposure to inciting agents is associated with a variety of environmental factors, including potentially outdoor concentrations of aeroallergens and climatic factors but most importantly housing and feeding practices.

Environmental Risk Factors

Season

Horses are approximately 2 times more likely to be examined by a veterinarian because of the disease in winter or spring compared with summer, suggesting a seasonality to the occurrence of the disease perhaps as a result of increased stabling during winter. Signs of respiratory disease in horses with RAO are ~2 times more likely to occur in winter months, with peak values of 45% to 50% of RAO-affected horses having clinical signs of the disease in January and February in Great Britain (Fig. 12-28).

Housing and Hay Feeding

There is a clear association between housing, feeding of hay, and development of the disease. Typically, susceptible horses are clinically normal when at pasture and develop signs of disease within hours to days of being housed in stables and fed dusty hay. Moving affected horses to pasture, or improving air quality by increasing ventilation and feeding processed feedstuffs, results in resolution of the disease.

Horses living in urbanized environments are approximately twice as likely to have the disease. Although the reason for this association has not been demonstrated, it is reasonable to assume that at least part of the increased risk is attributable to poorer air quality for horses in an urban environment.[12]

Management practices that might contribute to development or exacerbation of RAO vary widely around the world, with differing practices related to duration of stabling, type of bedding, air quality in stables and such. Within Great Britain, 4% of horses are stabled 24 hours per day year round and 9% stabled all day (24 hours) in winter.[19] 61% are stabled part of each day with pasture turn-out and 36% are turned for 24 hours each day.[19]

Development of disease is related to inhalation of **respirable particles** that gain access to the lower respiratory tract. Respirable particles are less than 5 µm diameter, the principal source of these particles in stalls is hay, and the majority of particles are fungal spores. The concentration of particles in air of the stable is determined by the rate of release of particles from hay, which is dependent in large part on the quality of the hay, concentration of fungal spores in the hay, and the rate of clearance of dust from the stable, a function of the ventilation rate. Concentrations of respirable dust particles in the breathing zone of stabled horses can be as high as 20 mg/m^3. The severity of increases in neutrophil count and proportion and decreases in pulmonary function in experimental models of RAO are related in a dose-dependent fashion to the amount of dust inhaled. The presence of dust particles, and not the soluble products in hay dust, is responsible for most of the airway neutrophilia induced by inhalation of hay dust.

Hay is the usual original source of spores in stable air. However, **decomposing wood shavings** are also a source of spores of fungi that multiply during degradation of plant-based materials, and housing horses in poorly ventilated stalls deeply bedded with wood shavings may be detrimental to their respiratory health. Spores from hay enter the bedding either directly or after dispersal through the air and multiply in the bedding if it is not removed regularly. **Diced paper** and **wood shavings**, when fresh, usually contain very few spores. Barley and wheat straw are usually free of any small spores such as *A. fumigatus* or *M. faeni.* Bedding horses on fresh wood shavings and feeding a nutritionally complete pelleted ration results in a respirable dust burden 3% of that of horses fed hay and bedded with straw. Dust burdens measured in the air of the stall underestimate the respirable particle challenge of horses because of the high concentration of particles in hay and bedding, areas from which the horse inhales while eating.

Respiratory health of horses is related to **stable design and ventilation**, with horses in poorly ventilated barns having more respiratory disease than horses in well-ventilated barns. See "Control" for recommendations regarding stable design.

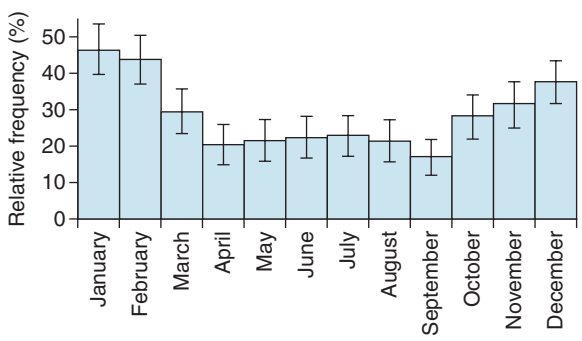

Fig. 12-28 Proportion of horses in Great Britain with recurrent airway obstruction (RAO) that display clinical signs of respiratory disease in each month. (Reproduced with permission.[12])

PATHOGENESIS

Susceptible horses, when exposed to adequate concentrations of respirable dust in the breathing zone, develop airway inflammation including neutrophilia in airway secretions, excess mucus accumulation, and bronchospasm within hours to days of exposure. Longer-term changes include bronchiolitis with peribronchial lymphocytic infiltration and increased thickness of submucosal smooth muscle and bronchial epithelium. Notably, eosinophils are not an important component of the inflammatory response in horses with RAO, either in bronchoalveolar lavage fluid or in peribronchial infiltrates.[20] These morphologic changes contribute to the reduction in airway diameter that underpins the physiologic effect of the disease. Emphysema and bronchiectasis develop as the severity of the disease worsens.

The mechanisms underlying these responses to inhalation of dust are not well defined but can be considered in the contexts of immune and inflammatory responses, mucus secretion, and pulmonary dysfunction.

Inflammatory and Immune Responses

The precise immunologic abnormalities and mechanisms causing airway and peribronchial inflammation in affected horses is unclear.[21] Inflammation is associated with excessive mucus production, airway swelling, and abnormal lung function. The inflammatory response in horses with RAO is neutrophilic, with lesser numbers of mast cells and rarely eosinophils.[20] The mechanisms underlying this inflammatory response have not been fully elucidated, but differing responses in RAO and healthy horses, and differing responses among families of RAO sensitive horses, to antigenic challenge supports an acquired immune-mediated process.[22] Furthermore, upregulated expression of IL1β, IL8, TLR4, TNFα, TGFβ1, and NFkβ transcripts in RAO-affected compared with healthy horses and the strong correlation with clinical variables indicative of disease severity provide support for an immune-mediated disease process.[23] There is currently no clear consensus on the immune mechanisms involved in development or perpetuation of RAO despite numerous studies examining cell types,[20,24-28] cytokines,[23,29-34] gene expression,[23,29,31,35] and antibody isotypes.[36]

The presence of allergen-specific IgE antibodies in bronchoalveolar lavage fluid is supportive of a hypersensitivity reaction (type 1

hypersensitivity response), as is the observation that reaginic antibodies (IgE and some classes of IgG) in serum of horses sensitive to *Aspergillus fumigatus* cause mast cell (rat origin) degranulation.[36] Others have proposed type 3 and type 4 immune reactions as the basis of the disease, and there are suggestions of polarized Th1 or Th2 responses. Detection of increased mRNA expression of **thymic stromal lymphopoietin**, a cytokine involved in lymphocyte development and driving a Th2 response, in bronchoalveolar lavage fluid (BALF) and peribronchial cells of horses with RAO is evidence of a contribution of a Th2 mechanism to RAO.[32] One proposed explanation is that RAO-susceptible horses exhibit a Th2-like immune response to inhalation of hay or barn dust characterized by increased expression of interleukins 4 and 5 and decreased expression of interferon-γ in cells obtained by bronchoalveolar lavage. Others have not detected a pure Th2-like cytokine profile, finding instead a mixed inflammatory response including increases in expression (mRNA) in cells obtained from bronchoalveolar lavage fluid of affected horses of interferon-γ, tumor necrosis factor-α, interleukins 1β and 4, and interleukins 8 and 17 (potent attractors of neutrophils) but not interleukins 2, 5, and 10. However, all the studies cited previously were performed on crude preparations of cells obtained by bronchoalveolar lavage, and the results could have been influenced by the varying proportions of types of cells in these preparations. A study examining just CD4 and CD8 lymphocytes in blood and bronchoalveolar lavage fluid of RAO-affected horses demonstrated a general down-regulation in expression of interferon-γ, and interleukins 4, 5, and 13 and no evidence of a cytokine profile consistent with either sole or predominate Th1- or Th2-like responses. The magnitude of the inflammatory response varies depending on the challenge (i.e., nature of the inhaled material) with responses to endotoxin characteristically being less than that of hay dust. Regardless of the underlying mechanism, it is clear that T cells are involved in mediating and likely modulating the response to exposure to inciting agents in susceptible horses[25] and this results in airway inflammation and interference with normal respiratory function. The role of the observed increased rate of T-cell apoptosis in resolution of clinical signs after removal of the inciting agents in RAO-affected horses is uncertain.[27]

Following inhalation of inciting agents there is recruitment of **neutrophils,** but not eosinophils or platelets, into the lungs in most horses that develop changes in lung function. Histologically, there is peribronchiolar accumulation of lymphocytes and luminal accumulations of neutrophils in affected horses. The neutrophilic response is airways is mediated at least in part by IL-8 and IL-17 and by MAPK and PI3K pathways in horses with heaves.[30] The entry of neutrophils into the airways is associated with activation of neutrophils and platelets. Neutrophil activation occurs in horses with RAO 10 and 24 hours after antigen challenge and is mediated by increased expression of CD13 on circulating neutrophils of susceptible horses exposed to inciting dust.[37] However, there is not increased expression of mRNA for TNF-alpha, IL-beta, IL-8, and MIP-2 in horses with RAO, suggesting that release of these cytokines is not necessary for the neutrophilic response characteristic of the disease.[38] The neutrophils of horses during episodes of RAO, but not when the horses are asymptomatic, have increased adherence in vitro to protein coated plastic suggesting a mechanism for the increased migration of neutrophils into airways of affected horses. However, neutrophils of asymptomatic RAO-affected horses (ie, those horses in remission of the disease) have an exaggerated serum concentration of TNF and mRNA expression after lipopolysaccharide exposure compared with cells of healthy horses.[26] A greater proportion of neutrophils in BALF of RAO-affected horses are viable and have a slower rate of apoptosis compared with those of unaffected horses, suggesting a role for increased neutrophil survival in airways in horses with RAO.[28]

Inhibition of neutrophil phosphodiesterase-4 activity does not alleviate clinical signs of RAO or decrease neutrophil numbers in bronchoalveolar lavage fluid in affected horses, suggesting that neutrophils are not primarily involved in the genesis of airway obstruction. The extent to which neutrophils in the airways are activated has not been determined, and their role in the development of respiratory dysfunction is unclear given that glucocorticoid administration attenuates the respiratory dysfunction but not airway neutrophilia in horses with RAO (see under "Treatment").

Airway inflammation is associated with increases in concentration of inflammatory mediators including leukotriene B_4, prostanoids including thromboxane, and proteases. Activity of matrix metalloproteinase-9 is higher in horses with RAO than in unaffected horses and is induced in a dose-dependent manner by inhalation of inciting substances including hay dust and endotoxin. MMP-9 is likely important in the inflammatory process associated with RAO through excessive gelatinolytic proteolysis that can contribute to lung injury and through a role in lung remodeling. Inflammation is also associated with increased oxidative stress in lungs of horses with RAO as indicated by elevated concentrations of epi-PGF2a and redox ratio of glutathione in pulmonary lavage fluid.

RAO is associated with platelet activation and increases in mean platelet volume, indicating consumption of platelets and bone marrow release of younger thrombocytes.[35,37] Platelets are consumed in part by formation of neutrophil-platelet aggregates.[37]

Mucus

Accumulation of excessive quantities of mucus in the large airways is characteristic of horses affected by RAO and can contribute to nonbronchospastic airway obstruction. Accumulation of mucus is attributable to decreased clearance and increased production. The mucus in horses with RAO differs in both composition and viscoelasticity from that of clinically normal horses and this might contribute to its decreased clearance. The viscosity of mucus can increase threefold in RAO susceptible horses stabled and exposed to hay dust. Increased production of mucus is associated with up-regulation of the equine MUC5AC gene, which is responsible for production of mucin, but not with IL-13 or epithelial gene (CLCA1, EGFR, Bcl-2 and MUC5AC expression,[39] in the small airways of horses with RAO.

Airway Function and Gas Exchange

Inhalation of inciting agents causes changes in lung function characterized by an increase in pulmonary resistance, lower dynamic compliance, altered distribution of ventilation, impaired gas exchange, increased functional residual capacity, and an altered breathing strategy. **Airway obstruction** is a result of bronchospasm, inflammatory thickening of airways, and accumulation of mucus and cells in the airways. Bronchospasm is largely relieved by administration of bronchodilator drugs or removal of the inciting cause, but residual effects on lung function remain and are attributable to inflammation and fibrosis and bronchoconstriction of small airways. **Bronchoconstriction** in both normal and affected horses is caused by parasympathetic activity and release of acetylcholine that reacts with muscarinic receptors on airway smooth muscle. However, the response is exaggerated in horses with RAO. Stimulation of airway sensory receptors results in an exaggerated bronchoconstrictive response, possibly because of the action of inflammatory mediators and/or byproducts. The exaggerated bronchoconstrictive response is not specific for allergens, and any substance that activates airway sensory receptors may incite bronchoconstriction once the sensitivity of the receptors is enhanced by inhalation of the inciting allergens. Exaggerated airway responsiveness to inhaled irritants persists for up to 3 days after a single exposure to the inciting agent and is likely important in the development of clinical signs of the disease. Bronchoconstriction increases work of breathing, but hypoventilation probably contributes little to the hypoxemia of affected horses, given that $PaCO_2$ is rarely increased.

Hypoxemia, which can be severe (<60 mm Hg, 8 kPa), is attributable to ventilation- perfusion mismatches and increased

dead-space ventilation. The increased minute ventilation of affected horses, a result of maintained tidal volume and increased respiratory rate, mainly supplies dead space and regions with high V/Q ratios. Pulmonary hypertension in affected horses is probably attributable to hypoxia and perhaps inflammatory mediators with vasoconstrictor activity.

The **elevated functional residual capacity** and characteristic breathing strategy of affected horses is attributable to airway obstruction. Airway obstruction causes trapping of air in alveoli and a higher end-inspiratory volume. The high end-inspiratory volume maximizes airway diameter and facilitates the high expiratory and inspiratory flow rates necessary for affected horses to maintain a normal tidal volume while increasing their respiratory rate.

Bronchiectasis (irreversible dilation and deformation of bronchi or bronchioles) occurs in some horses affected with RAO for a prolonged duration. Neutrophilic inflammation is essential for the development of bronchiectasis.

Surfactant of horses with RAO has a lower phospholipid concentration and lower percentage of phosphatidylglycerol that is not a result of leakage of plasma into BALF.[40] The clinical importance of this abnormality is unclear.

RAO causes pulmonary artery hypertension with consequent abnormal cardiac septal motion, decreased left ventricular diameter and stroke volume, and increased pulmonary artery diameter.[41]

CLINICAL FINDINGS

The degree to which horses are affected varies considerably and is quantifiable by consideration of a combination of clinical signs.[42] Minimally affected horses have airway inflammation evident on endoscopic or cytologic examination of the airways, but few other signs on physical examination, whereas severely affected horses have very obvious clinical signs. Owner-reported scoring systems are useful in staging the disease and predicting onset of clinical signs.[43,44]

The usual **history** is that of chronic cough in a stabled horse, and owner-reported rates of coughing correlate well with the clinical condition and bronchial sensitivity to histamine challenge of horse.[45] Typically, the disease is precipitated by exposure to hay and stabling, and disease remission occurs in most horses when pastured and removed from hay. There may be a history of reduced exercise tolerance.

Affected horses are usually bright and alert and have a normal appetite and rectal temperature. Severely affected horses appear anxious and have a greatly increased respiratory effort.

Coughing is common in horses with RAO, although it is neither particularly specific nor sensitive as an indicator of the disease. Coughing may consist of a single cough every few seconds to minutes, or there may be a paroxysm of coughing. The cough can also be elicited by digital massage of the larynx and proximal part of the trachea because horses with airway inflammation have increased sensitivity of the cough reflex. Stimulation of the larynx or proximal trachea by digital massage does not elicit coughing in normal horses. The cough becomes more pronounced and wheezing with exercise. It also occurs more frequently when the horse is exposed to cold air, physical activity, or excitement; when placed in a dusty environment; or if dusty feed is offered. The amount of coughing, which must be counted over at least 15 minutes and preferably 1 hour for accurate determination of its severity, correlates closely with the amount of mucus in airways, maximal change in pleural pressure (a measure of bronchoconstriction), and neutrophil count in bronchoalveolar lavage fluid. Coughing is more frequent in horses with RAO, and affected horses often have paroxysmal coughing, especially after barn cleaning and feeding.

An intermittent, bilateral, mucopurulent to serous **nasal discharge** is a common sign in affected horses.

The resting **respiratory rate** is increased from a normal of 12/min up to 24 to 36/min. There is a pronounced effort during expiration, and markedly affected horses have an obvious abdominal component to respiration. Normal horses have a biphasic pattern of airflow during inspiration and expiration, whereas affected horses lack the second phase of respiration—evident as lower thoracoabdominal asynchrony.[46] Longstanding cases develop a "heave line" in the flank as a result of hypertrophy of the abdominal oblique musculature. It is evident as a trough or furrow along the costal arch. In advanced cases the nostrils may be visibly dilated during inspiration, and the force of the expiratory effort causes the anus to protrude.

Heart rate is commonly within the normal range or only slightly increased. In horses with RAO, the heart rate is significantly higher during exercise than in healthy horses.

Abnormal lung sounds are one of the most frequent abnormalities detected on clinical examination and the sensitivity of this finding can be increased from 70% to almost 90% by auscultating the thorax while the horse breathes for 60 to 120 seconds with an airtight plastic bag over its nostrils. The bag should be large enough to enable the horse to breathe unhindered (10-15 L) and should not leak. Accumulation of carbon dioxide in the bag increases the horse's respiratory rate and tidal volume and accentuates lung sounds. Auscultation of the lungs in the early stages of the disease may reveal only a slight increase in the amplitude of normal breath sounds. Abnormal lung sounds become audible as the disease progresses. **Wheezing and crackling sounds** occur at the end of inspiration and the end of expiration. These abnormal sounds are audible over most of the lung but are usually easiest to detect over the upper one-half of both lung fields. **Auscultation of the trachea** usually reveals moist sounds characteristic of fluid in the trachea. Some affected horses have quieter than expected lung sounds.

Percussion of the thorax may reveal an increase in the area of resonance by as much as one to two intercostal spaces caudally. However, the area of resonance delineated by percussion is too labile and ill-defined to be of diagnostic value.

Endoscopic examination of the upper airways, trachea, and bronchi reveals an abundance of mucopurulent material in the trachea, which, in severe cases, is also present in the nasopharynx. Detection of signs of inflammation in the upper airway does not reflect the presence or severity of inflammation in the lower airway.[47] The amount of mucus can be graded on a scale from 0 to 5 (Fig. 12-29).[48]

- **Grade 0**—no visible mucus.
- **Grade 1**—small blobs of mucus that are not confluent.
- **Grade 2**—multiple blobs of mucus, some of which are confluent.
- **Grade 3**—mucus confluent in a stream in the ventral aspect of the trachea or multiple large blobs around the circumference of the lumen.
- **Grade 4**—large pool of mucus in the ventral aspect of the airway.
- **Grade 5**—Profuse amounts of mucus occupying more than 25% of the tracheal lumen.

Observation of tracheal mucus of grade 4 or 5 has a high specificity (92%) but low sensitivity (52%) for detection of RAO. Assessment of tracheal septum thickness is not useful in determining the severity of RAO.[49]

Radiographic examination of the thorax usually reveals evidence of bronchial disease with some evidence of interstitial disease. Radiography is more useful in ruling out other diseases, such as granulomatous or interstitial pneumonia, than in confirming RAO.

Sophisticated techniques for measuring pulmonary function, such as determination of tidal flow–volume loops, nitrogen washout, or forced expiratory flow–volume loops, may identify mildly or subclinically affected animals but have limited day-to-day clinical utility.

Measurement of **pleural pressure changes** by insertion of an esophageal balloon is relatively simple and may be useful in monitoring response to treatment. Affected horses have pleural pressure changes during respiration greater than 6 cm H_2O. Administration of atropine (0.02 mg/kg, IM or IV), isoproterenol (isoprenaline), or a β_2-adrenergic agonist such as terbutaline

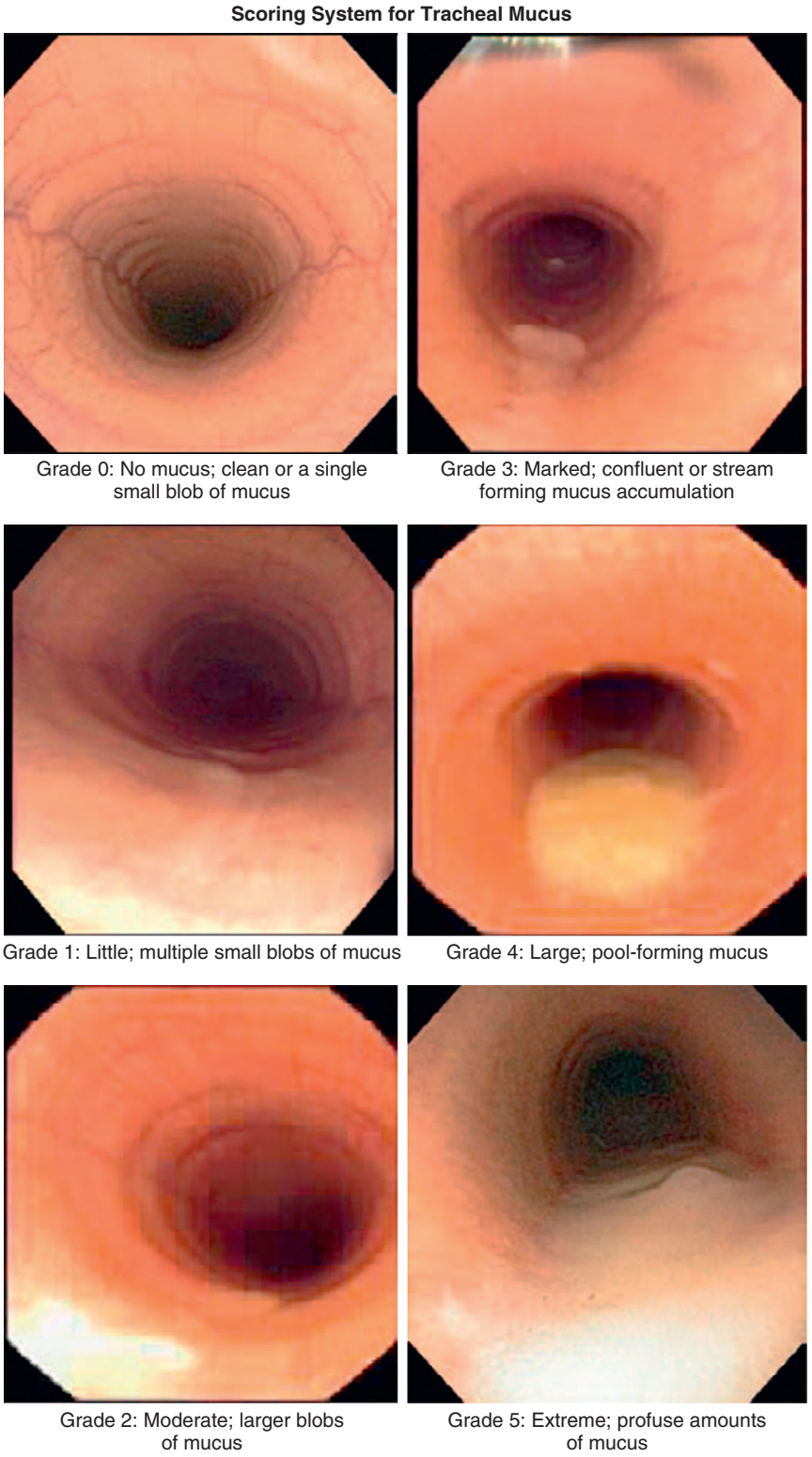

Fig. 12-29 Endoscopic grading of tracheal mucus in horses. (Modified from Gerber V, Straub R, Marti E et al. Endoscopic scoring of mucus quantity and quality: observer and horse variance and relationship to inflammation, mucus viscoelasticity and volume, Equine Vet J 2004,36: 576–582 with permission from the Equine Veterinary Journal.)

(0.002–0.005 mg/kg, IV) reduces the maximal change in pleural pressure of horses with RAO.

Abnormalities in left and right ventricular function are detectable in horses with RAO. These include reduced early diastolic filling velocities, elevated late diastolic filling velocities, thereby decreased E/A quotient, prolonged electromechanical coupling periods between electrocardiograph Q-wave and maximal velocities, and compensatory elevated systolic strain in the right heart[50] and abnormal cardiac septal motion, decreased left ventricular diameter and stroke volume, and increase pulmonary artery diameter.[41,51]

The **course of the disease** is dependent on the removal or continual presence of the precipitating cause. If the cause is removed in the early stages, complete recovery can occur. In the continual presence of the precipitating cause relapses occur commonly or the disease becomes progressive and affected horses become severely incapacitated. **Bronchiectasis**, evident on radiographic examination of the thorax, develops in horses with RAO of prolonged duration. With conscientious management and adequate housing, breeding animals and hunters or show jumpers with RAO can remain useful for many years.

Lung biopsy of horses with RAO is described,[52,53] but it is not a clinically relevant diagnostic test because of the risk of adverse events and clinicians' ability to detect and stage the disease based on less invasive clinical and clinicopathologic information.[42,43,45]

CLINICAL PATHOLOGY AND SPECIAL EXAMINATIONS

There are no significant changes in the hemogram or serum biochemistry of affected horses. The Pao_2 is below normal in moderately to severely affected horses and the $Paco_2$ is usually normal, although it may be increased in severely affected horses. Blood oxygen tension measurements should be corrected for the temperature of the animal and the altitude. At approximately sea level, Pao_2 values of normal horses are usually greater than 90 mm Hg (12 kPa), whereas affected horses have Pao_2 less than 82 mm Hg (10.9 kPa). With increases in altitude the values in both normal and affected horses decrease. The normal hypoxemia that occurs in horses during intense exercise is exacerbated by RAO.

Bronchoalveolar lavage fluid from affected horses during symptomatic episodes has a relative neutrophil count greater than 5% to 10%, and usually over 50%, of the absolute nucleated cell count. It is recommended that horses not be considered to have airway inflammation unless greater than15% of cells in bronchoalveolar lavage fluid are neutrophils. During periods of remission, the bronchoalveolar lavage fluid of previously affected horses is not different from that of normal horses. Absolute nucleated cell counts in bronchoalveolar lavage fluid of affected horses are reported, but the values depend on the collection technique used. The relative proportions of macrophages and lymphocytes in bronchoalveolar lavage fluid of affected horses are lower than those of normal horses. Eosinophil numbers in bronchoalveolar or tracheal aspirate fluid of affected horses may be mildly elevated (up

to 10%), but are usually low (<3%-5%). Higher values should raise the index of suspicion for *Dictyocaulus arnfieldi* or *Parascaris equorum* infestation. Bronchial collapse, observed endoscopically, during bronchoalveolar lavage is a sign of airway inflammation.[54]

Aspirates of **tracheal fluid** reveal a profound neutrophilia (>90%).

Measurement or identification of **precipitins** in serum of horses is not useful in identifying horses with RAO.

Intradermal testing using putative allergens has been investigated as a means of identifying horses with RAO or of identifying antigens with which to hyposensitize affected horses with variable or undetermined efficacy. Retrospective examination of records of horses with a history of RAO suggests that they are more likely to react, and react to a larger number, of intradermally injected allergens than horses without a history of RAO. However, reactions to individual allergens cannot be used to determine hypersensitivity to particular allergens, although it is suggested that overall patterns of reactivity, with a history of exposure of the horse to these allergens, might be useful in guiding management of affected horses. Contrary to these results, a prospective study demonstrated that horses with RAO did not have a greater rate of reaction to intradermal skin tests than did horses not affected by RAO. Intradermal testing did not distinguish clinically relevant reactions from those that were not clinically relevant. Horses with RAO have greater sensitivity to intradermal injection of histamine, which is commonly used as a positive control, than horses without RAO. Overall, intradermal skin testing is neither useful in detecting horses with RAO nor in determining hypersensitivity to particular allergens in individual horses. Results of such testing might be useful in management of horses, but this has not been demonstrated. The usefulness of intradermal skin testing and subsequent administration of preparations of antigens selected on the basis of intradermal testing, in an effort to hyposensitize horses with RAO, has not been determined. The apparent lack of efficacy of intradermal testing might be because the extent of reactivity to intradermal injection of mold preparations does not correlate with the severity of pulmonary dysfunction after inhalation of the same preparation in horses with RAO.

Lung biopsy demonstrates peribronchiolar lymphoplasmacytic inflammation, goblet cell metaplasia, alveolar fibrosis, and bronchial lumen exudate and neutrophils. The severity of bronchiolar neutrophil and mast cell infiltration correlates well with the severity of the clinical signs.

NECROPSY FINDINGS

The major findings are restricted to the lungs, which are pale and voluminous and do not collapse when the chest cavity is opened. The tissue damage is primarily centered on airways which are less than 2 mm in diameter. Microscopically, a variable degree of alveolar emphysema is accompanied by a chronic bronchiolitis featuring diffuse epithelial hyperplasia, goblet cell metaplasia, peribronchiolar fibrosis, and cellular infiltration by lymphocytes, plasma cells, mast cells, and sometimes eosinophils. Plugs of mucus with entrapped neutrophils often occlude bronchiolar lumina. Changes in airway collagen and elastic fiber content correlate with lung function in horses with RAO.[55]

Samples for Postmortem Confirmation of Diagnosis

- Formalin-fixed lung for light microscopic examination

DIAGNOSTIC CONFIRMATION

Confirmation of the disease is based on the presence of a history and clinical signs consistent with the disease, in particular the response to stabling and pasturing, and demonstration of reversible airway obstruction. Objective confirmation can be achieved by measuring the response of maximal changes in pleural pressure in response to bronchodilator drug (atropine or glycopyrrolate) administration.

DIFFERENTIAL DIAGNOSIS

Horses with respiratory distress may have the following conditions:

- Interstitial pneumonia
- Heart failure
- Bacterial pneumonia
- Pleuritis
- Pulmonary or mediastinal neoplasia, including leiomyosarcoma
- Parasitic pneumonia (*D. arnfieldi*)

Nasal discharge may be caused by the following:

- Guttural pouch diseases including empyema
- Dysphagia of any cause
- Esophageal obstruction
- Sinusitis
- Pneumonia

TREATMENT

The principles of treatment are as follows:

- Removal of the inciting cause
- Reduction of airway inflammation
- Bronchodilation
- Correction of hypoxemia

RAO is an inflammatory disease caused by inhalation exposure to inciting agents. Bronchoconstriction is secondary to inflammation. Control of the disease is based upon preventing inhalation of inciting agents and suppression of inflammation by administration of corticosteroids, practices supported by substantial evidence.[56] Relief of bronchoconstriction should be necessary only during acute exacerbations of the disease, and administration of bronchodilatory drugs for more than several days is not optimal treatment in most horses. Drugs used in the treatment of RAO are summarized in Table 12-13.

It is essential that the horse is **not exposed to the inciting agents** and irritant substances that could provoke or worsen the disease. Even relatively brief exposure of susceptible horses to the inciting agents, such as can occur if a horse is brought into a poorly ventilated barn to be fed, can result in airway hypersensitivity and the development or maintenance of clinical signs. Affected horses should be moved to a clean environment, **ideally pasture**, in which the concentration of airborne allergens is reduced to an absolute minimum. If the horse cannot be kept at pasture, then it should be housed in a well-ventilated barn (see "Control" for details), bedded with clean wood shavings or shredded paper and fed a complete pelleted ration. If affected horses are fed hay, it should be thoroughly wetted to minimize the release of spores. Remission of clinical signs can be expected in 4 to 21 days if the environmental changes are adequate. This may be all that is necessary to control the disease in many horses.

Antiinflammatory Drugs

The disease is essentially one of inflammation of the airways, and therefore one of the mainstays of treatment is administration of antiinflammatory drugs. Nonsteroidal antiinflammatory drugs such as phenylbutazone and flunixin meglumine are not effective. Corticosteroids including dexamethasone, prednisolone, triamcinolone, and betamethasone are effective in controlling the disease.[57] **Dexamethasone** (0.04-0.1 mg/kg, intravenously, intramuscularly or orally every 24-48 hours) can be given to control the acute signs of the disease, and then the dose reduced and eventually discontinued as environmental alterations have their effect. Similarly, **prednisolone** (1-2 mg/kg, orally once daily), but not prednisone (which is not absorbed after oral administration to horses), can be given initially, then the dose reduced by approximately one half every 5 to 10 days as the disease is controlled. Both prednisolone (2 mg/kg per day) and dexamethasone (0.05 mg/kg day) improve clinical signs of the disease in the face of continued exposure to inciting causes, and prednisolone improves lung function.[58] Often prednisolone or dexamethasone sodium phosphate is effective when administered every second day when the disease has been controlled. **Dexamethasone-21-isonicotinate** (0.04 mg/kg, intramuscularly) is effective when administered every 3 days, but not when administered only once. **Isoflupredone** (0.03 mg/kg, intramuscularly once

Table 12-13 Drugs used in the treatment of heaves in horses

	Drug	Dose and frequency	Route	Comments
Bronchodilators				
β_2-agonists	Clenbuterol	0.8–3.2 µg/kg q12 hourly	Oral or IV	Initial therapy with lowest dose. Gradual increments depending on response. For short-term therapy pending environmental control and corticosteroid administration.
	Albuterol	50 µg/kg	Oral	Unknown and doubtful efficacy.
	Albuterol	1–3 µg/kg q6–12 hours	Inhalation	Has short duration of action (1 hour). Can be combined with ipratropium to prolong duration of bronchodilation.
	Fenoterol	2–4 µg/kg as needed	Inhalation	Short duration of action.
	Pirbuterol	1–2 µg/kg as needed	Inhalation	Short duration of action.
	Salmeterol	0.5–1.0 µg/kg q6–12 hours	Inhalation	Longest acting β_2-agonist available for inhalation.
	Terbutaline	0.2 mg/kg as needed	Inhalation	Marked adverse effects including tachycardia. Not absorbed after oral administration.
	Terbutaline	0.005 mg/kg as needed	IV	Marked adverse effects, including sweating and tachycardia.
Parasympatholytics	Ipratropium	0.5–3.0 µg/kg q4–6 h	Inhalation	Usually combined with albuterol for rapidity of onset of bronchodilation. Duration of action is ~6 h.
	Glycopyrrolate	5 µg/kg as needed	IV or IM	Useful for short-term or emergency relief of bronchoconstriction.
	Atropine	0.01–0.02 mg/kg as needed	IV or IM	Useful for diagnosis of reversible airway obstruction and short-term relief of bronchoconstriction. Can cause colic.
Miscellaneous	Theophylline	5–10 mg/kg q8–12 hours	Oral	Antiquated therapy. Moderate bronchodilation, variable absorption, narrow therapeutic index, frequent adverse central nervous system effects. Not recommended.
	Pentoxifylline	10–15 mg/kg q12 hourly	Oral	Not used clinically. Experimental evidence of efficacy.
Antiinflammatory drugs				
Corticosteroids	Dexamethasone phosphate or in alcohol	0.02–0.1 mg/kg q24 hourly	IV, IM, or oral	Effective at reducing clinical signs within 3 days. Gradually reduce dose and frequency to lowest efficacious dose.
	Dexamethasone-21 isonicotinate	0.04–0.06 mg/kg q3 days	IM	Effective. Infrequent dosing.
	Prednisolone	1–2 mg/kg q24 hourly	Oral or IM	Effective at reducing clinical signs within 3 days. Gradually reduce dose and frequency to lowest efficacious dose.
	Prednisone	1–2 mg/kg q24 hourly	Oral	Variable efficacy and not efficacious in most horses. Do not use.
	Triamcinolone acetonide	0.011–0.022 mg/kg q2–4 weeks	IM, SC	Infrequent dosing and therefore lack of ability to taper dose. Should not be repeated at < 3-month intervals.
	Beclomethasone	1–9 µg/kg q12 hourly	Inhalation	Relief of bronchoconstriction within 3 days. Lowest dose does not cause adrenal suppression and is effective in relief of bronchoconstriction.
	Fluticasone	2–12 µg/kg q12 hourly	Inhalation	Potent and effective. Expensive.
Other	Cromolyn sodium	200 mg q12 hourly	Inhalation	Undetermined efficacy. Should be used before exposure to inciting agent.
	Montelukast	0.11 mg/kg q24 hourly	Oral	Leukotriene receptor antagonist. Not efficacious at this dose.

Data from: Couetil, L.L. (2014) In Hinchcliff KW, Kaneps AJ, and Geor RJ (eds): Equine Sports Medicine and Surgery: Basic and clinical sciences of the equine athlete, ed. 2. Elsevier Health Sciences. p 614.

daily) is as effective as dexamethasone in alcohol in control of exacerbations of RAO, although it does cause hypokalemia. **Triamcinolone** (0.09 mg/kg, intramuscularly) administered once provides long-term (weeks) relief of signs in some horses.

Inhaled corticosteroids, such as betamethasone, beclomethasone, or fluticasone, are useful in controlling the disease. Both inhaled and parenterally administered corticosteroids suppress adrenal function of horses but 500 µg of beclomethasone propionate inhaled twice daily effectively alleviated signs of RAO and causes less adrenal suppression than doses of 1000 µg or 1500 µg. Inhaled administration of fluticasone suppressed serum cortisol concentrations of horses for 8 to 24 hours after administration, and this effect persists with continued administration over 1 year.[59] The clinical importance of this long-term suppression is unclear, although it does not impair innate or acquired immunity, and adverse clinical signs are not detected.[60] It is important to reiterate that the use of glucocorticoids should only be as an adjunct to control of the horse's environment and reduction in the inhaled particle burden.

Clenbuterol decreases the production of inflammatory cytokines by cells obtained from bronchoalveolar lavage fluid of horses with RAO, suggesting that clenbuterol can have antiinflammatory effects in such horses. The clinical applicability of this finding remains to be determined.

In summary, a number of different corticosteroid preparations are useful in the control of RAO. Drugs administered by inhalation appear to have a reduced

potential for adverse effects including adrenal suppression, but they are more difficult to administer and require more frequent administration than drugs administered orally, intravenously, or intramuscularly. Improvements in respiratory effort and clinical signs are evident in approximately 3 days and persist for the duration of treatment. Cell counts and the neutrophilia in bronchoalveolar lavage fluid are not reliably reduced by administration of corticosteroids. Affected horses, after institution of appropriate measures to control inhalation of hay and barn dust, should be treated with the lowest dose that controls the disease and only for as long as necessary. The dose of corticosteroid can be reduced gradually and the frequency of administration decreased from once daily to once every second or third day (or greater, depending on the preparation) to achieve this end. Administration of the lowest effective dose is important because of the effects of corticosteroids in suppressing immune and adrenal function. It is suggested that dexamethasone and triamcinolone increase the likelihood of horses developing laminitis, but this relationship has not been conclusively demonstrated.

Bronchodilator Drugs

Bronchodilator drugs might be needed to provide acute relief of airway obstruction but should not be used as maintenance therapy. **Atropine** (0.02-0.04 mg/kg, intramuscularly) can be used to provide short-term relief of bronchoconstriction, but its use is associated with gastrointestinal side effects, including colic, that preclude its long-term use. Use of **N-butylscopolammonium bromide** causes fewer adverse effects than does atropine.[61] **Glycopyrrolate** (0.005 mg/kg intramuscularly every 8-12 hours) is a potent bronchodilator with minimal gastrointestinal effects. **Ipratropium bromide**, a parasympatholytic drug with minimal extrapulmonary effects when given by inhalation, is very effective in relieving airway obstruction in severely affected horses. Similarly, revatropate (1 mg, inhaled), a selective M-1 and M-3 muscarinic antagonist, is as effective as ipratropium (0.3 mg) in relieving clinical signs and improving lung function in horses with RAO.[62]

β_2-adrenergic agonists are potent bronchodilators frequently used in the management of horses with RAO. They can be administered orally or by inhalation, with the latter being preferred. **Clenbuterol hydrochloride** is used as maintenance therapy at a dose of 0.8 to 3.2 µg/kg, orally every 12 hours, and is effective in controlling signs in 75% of affected horses. The lower dose should be used initially and then increased in 0.8-µg/kg increments until the desired effect is achieved or side effects of tachycardia, muscle fasciculation, and sweating are apparent. Gradual, incremental increases in dose lessen the frequency and severity of side effects. **Terbutaline** is not absorbed after oral administration to horses. Terbutaline and clenbuterol can also be given intravenously, at a dose one-tenth of that given orally, to severely affected horses in which the need for bronchodilation is urgent. Adverse effects of β_2-agonist administration include tachycardia, sweating, and apprehension. Prolonged administration of clenbuterol is associated with potentially adverse effects on cardiac structure, alterations in body composition, and an impaired response to training. Delayed parturition may occur in mares treated in late pregnancy. β_2-Adrenergic agonists may transiently exacerbate hypoxemia in severely affected horses. Intratracheal administration does not produce detectable bronchodilation.

Bronchodilators administered by inhalation to horses include the β_2-agonists **albuterol, salbutamol, and salmeterol**, and the parasympatholytic ipratropium. The efficacy and duration of action of each of these drugs varies somewhat, but all are effective in producing bronchodilation in affected horses. Salmeterol produces bronchodilation in horses with RAO that persists for up to 6 hours, although onset of action requires 30 to 60 minutes. Similarly, ipratropium reduces pleural pressure changes and attenuates clinical signs of airway obstruction in horses with RAO. Efficacy of drugs is influenced by the mode of delivery, with handheld devices available for the delivery of some compounds.[63]

Theophylline (aminophylline) is a nonadrenergic bronchodilator given at a dose of 5–10 mg/kg PO every 8–12 hours. Signs of toxicity include tachycardia, excitement, and convulsions. Theophylline is not a drug of first choice for the treatment of RAO and is now used infrequently because of the availability of efficacious antiinflammatory drugs and other bronchodilators.

Other Drugs

Sodium cromoglycate is useful for the prophylaxis of RAO, but it has no direct bronchodilatory activity. Its mechanism of action is unclear, but it may act to prevent the degranulation of mast cells. It can be given at a dose of 80 to 200 mg per 425-kg horse by inhalation once daily for 4 days and then repeated in 1 to 2 weeks.

Pentoxifylline at high doses improves respiratory function, but not bronchoalveolar lavage fluid cytology, of horses with RAO. However, bioavailability after oral administration is quite variable, contributing to variations in the responses of horses to the drug.

Drugs that reduce **leukotriene** production or activity do not appear to be useful in the treatment of RAO. An experimental leukotriene D4 receptor antagonist was not effective in relieving signs of RAO. Similarly, montelukast did not improve respiratory function in 5 horses with RAO.

Mucolytics are often used but their efficacy is not established and is doubtful. **Cough suppressants** should not be used because they may impair clearance of mucopurulent material from the airways.

Antibiotics are often given to affected horses but are probably not necessary in the vast majority of cases.

Acupuncture is not effective in the treatment of RAO.

Administration of large quantities of **isotonic electrolyte solution** intravenously is associated with a decrement in respiratory function in both normal and heavy horses and is not recommended as a treatment for RAO.

An acoustic device ("sound therapy") did not improve clinical signs or lung function in horses with RAO.[64]

Integrated Therapy

Initial treatment of affected horses usually involves changes to the horse's environment and feed in combination with administration of corticosteroids. Corticosteroids and β_2-adrenergic agonists can be given as combined therapy to severely affected horses until the disease is controlled, at which time therapy should consist of environmental control and, if needed, administration of the lowest effective dose of corticosteroids. Bronchodilators are sometimes used as sole therapy, but their use without correction of the housing and feeding factors, and attempts to control inflammation, is not rational. Long-term administration of bronchodilators is not optimal therapy and, given the documented adverse effects, is not recommended. Long-term control of RAO is achieved by environmental management and administration of corticosteroids.

CONTROL

Control of RAO centers upon minimizing the exposure of horses to inciting agents. These agents are present in air of stables and barns and, when present in sufficient concentration or combination, induce the disease in susceptible horses. There is considerable evidence supporting the practice of minimizing inhalation by horses of poor-quality air in the control of RAO.[56]

Housing horses in stalls with good air quality is essential in reducing the occurrence of the disease.[65,66] **Adequate ventilation** is critical in maintaining good air quality in stalls. Few horse housing units have adequate ventilation although a well-designed individual box stall can meet the needs of the horse both for air hygiene and thermal comfort. Many horse barns have inadequate open space for ventilation in still air conditions when the doors are closed at both ends of the building. When the release rate of spores is low, ventilation rates of 4 air changes per hour are satisfactory.

However, suggested minima are 8 to 10 air changes per hour, airspace of 44 m/head, and floor space of 9.2 m/head. In practical terms, if the upper half of the stable door is open, and faces open air and not into a barn, the natural ventilation should exceed the minimum specifications. Hay and dusty feed materials should not be stored above stalls or in the same airspace as horses. Bedding should be changed frequently, preferably daily. Use of cardboard as bedding material is effective as part of an overall regimen to improve air quality.

A portable slit sampler is an accurate, quick, and simple semiquantitative method of assessing the mold contamination of source materials such as hay, straw, and other feeds and bedding collected from stables. Newer technology, such as real-time continuous particle monitors, is useful in evaluating the effects of interventions to reduce airborne dust concentrations in the breathing zone of horses.[65] Real-time continuous monitoring also provides information on peak particle counts, which might be more closely related to disease severity than average counts.

The greatest contributor to airborne dust concentrations in stables is hay with straw bedding being an important other source.[65] Dust and presence of molds in hay can be reduced by use of agricultural practices during hay making including early harvest, adequate drying (and lack of rain), and preparation as haylage.[67] The health hazard posed by any moldy source material depends on the types of organisms present and their abundance. The size of the respirable challenge from heated hays and straws arises from the prolificacy of the species involved and their small spore size. The highest respirable challenges are from the presence of thermotolerant and thermophilic mold species. The most critical factors in determining the microbial development in plant-based materials are water content and thermal environment. Hay baled at 15% to 20% moisture heats little; it is virtually dust-free and contains few spores. Baling hay with 20% to 30% moisture leads to temperatures of up to 35° to 45°C (95 to 113 F). At these temperatures, hazardous contamination may develop with the appearance of thermotolerant fungi and actinomycetes. The heaviest contamination of hay and straw occurs with baling at 35% to 50% moisture, when spontaneous heating up to 50° to 60°C (122 to 140 F) may occur. Microscopically, these hays show large numbers of fungal spores in the 2- to 5-µm size range.

Dust particle concentrations in the breathing zone can be reduced by feeding of soaked hay.[66]

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PASTURE-ASSOCIATED HEAVES (PASTURE-ASSOCIATED RECURRENT AIRWAY OBSTRUCTION OF HORSES)

Summer pasture-associated heaves occurs in horses in the southeastern region of the United States and in Great Britain. It appears to be a disease of adult horses that are on pasture most of the time in the summer. It occurs most commonly in the warm, humid summer months of June to September.[1] Affected horses gradually recover during the cooler months of winter and early spring, and the disease can recur in the same horse each successive summer. Most severe signs occurred during late spring and early summer during times of high airborne pollen counts. Allergy to pollens and fungal spores appears to be a factor and is associated with conditions conducive to production of fungal spores and grass pollens.[1] Affected horses have increased expression of interleukin-4 and interferon-γ in cells of bronchoalveolar lavage fluid and peripheral blood mononuclear cells but not increased concentrations of IgE in bronchoalveolar lavage fluid. Endothelin concentrations are higher in the BALF and serum of affected than unaffected horses.[2]

Affected horses have clinical findings typical of heaves including nasal discharge, coughing, tachypnea, labored expiratory effort, and crackles and wheezes on auscultation. There is moderate-to-severe accumulation of mucus in the large airways evident on endoscopic examination. Lung function testing is consistent with bronchoconstriction. Bronchoalveolar lavage fluid contains large numbers of nondegenerate neutrophils and lesser numbers of lymphocytes and mast cells. Necropsy reveals overinflated lungs that do not collapse when the chest is opened and that retain the impressions made by the ribs. The predominant histologic finding is accumulation of mucus in small airways. Inflammation is not severe and most inflammatory cells present are neutrophils and lymphocytes in peribronchial tissues. Treatment includes stabling and administration of corticosteroids and bronchodilators, as discussed for recurrent airway obstruction (see Table 12-13).

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SYNCHRONOUS DIAPHRAGMATIC FLUTTER IN HORSES (THUMPS)

Synchronous diaphragmatic flutter in horses is caused by an abrupt and powerful contraction of the diaphragm synchronous with the heartbeat. Contraction of the diaphragm occurs because of stimulation of the phrenic nerve as it passes over the atria of the heart. Thumps is often associated with electrolyte or acid-base abnormalities in horses. The disease occurs commonly in horses used for strenuous exercise, and in particular horses used for endurance racing, in which approximately 10% of horses eliminated from competing have thumps.[1] The disease occurs in Standardbred and Thoroughbred racehorses, and individual animals can be affected repeatedly. This disease also occurs sporadically in adult horses and ponies that have not exercised, and peripartum mares (as part of lactation tetany).

The syndrome is characterized by a violent hiccough occurring synchronously with every heartbeat. The lateral aspect of the thorax and cranial abdomen appear to jump or "thump" regularly in affected horses. It is often unilateral, with the contraction being felt very much more strongly on one side than the other. The horse is distressed because the hiccough interferes with eating, and to an extent with respiration. In some cases, there are additional signs suggestive of hypocalcemia. These include muscular rigidity and fasciculation, and a high-stepping gait. There is often hypocalcemia, hemoconcentration, alkalosis or mixed acid-base abnormalities, and hypokalemia, hypochloremia, and elevation of creatinine phosphokinase levels in affected horses. Hypocalcemia can be profound. The disease is reported as a consequence of hypocalcemia secondary to primary hypoparathyroidism in two Thoroughbred horses.

The principles of treatment are correction of abnormalities in blood electrolyte concentration, acid-base status, and hydration. Treatment with calcium borogluconate administered slowly intravenously rapidly resolves the condition in some horses that do not have severe abnormalities of electrolyte concentration or acid-base status. Horses might require administration of balanced isotonic polyionic electrolyte solutions intravenously (e.g., Ringer's solution or 0.9% sodium chloride), and consideration should be given to supplementing these fluids with calcium (e.g., 50 mL of 23% calcium borogluconate per liter of fluid).

The **pathogenesis** is thought to be related to hyperirritability of the phrenic nerve caused by metabolic disturbances, including hypocalcemia, and the phrenic nerve being stimulated by each atrial depolarization to fire with each heartbeat. The stimulation occurs because of the close physical proximity of the heart to the nerve in the horse. Dietary supplementation with calcium and other electrolytes during a ride is recommended, but excessive calcium feeding beforehand may reduce the activity of calcium homeostatic mechanisms and is to be avoided.

Regular veterinary inspection of all horses at the mandatory stops of endurance rides will reveal those animals with "thumps," and these horses should not be allowed to proceed in the event.

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RHODOCOCCUS EQUI PNEUMONIA OF FOALS

SYNOPSIS

Etiology Virulent strains of *Rhodococcus equi* (*Rhodococcus hoagii/Prescottella equi*)

Epidemiology Sporadic disease of 1- to 5-month-old foals that is endemic on some farms. Foals are infected likely by inhalation during first weeks of life.

Clinical signs Pneumonia, fever, respiratory distress, cough, lack of nasal discharge, failure to thrive, multiple distended joints and uveitis. Occasionally diarrhea or septic osteomyelitis.

Clinical pathology Leukocytosis, hyperfibrinogenemia, inflammatory cells in tracheal aspirate.

Necropsy lesions Pulmonary consolidation and abscessation. Nonseptic polyarthritis.

Diagnostic confirmation Culture or polymerase chain reaction (PCR) detection of *R. equi* from tracheal aspirate.

Treatment Administration of a macrolide in combination with rifampin, for example, azithromycin (10 mg/kg PO q24 h) and rifampin (5 mg/kg PO q12h or 10 mg/kg PO q24h).

Control Ensure adequate transfer of passive immunity. Decrease stocking density. Decrease environmental contamination by virulent strains of *R. equi*. Do not use mass medication of foals with subclinical disease.

Disease in foals caused by *Rhodococcus equi* is important because of its high incidence in valuable livestock, widespread distribution, insidious onset, high case-fatality rate in the more severely affected foals, cost of treatment, lack of a protective vaccine, and cost of implementation of control measures.

ETIOLOGY

Rhodococcus equi (*Rhodococcus hoagii/Prescottella equi*) is a gram-positive, pleomorphic rod that is a soil-dwelling actinomycete.[1,2] The most important manifestation of *R. equi* infection is pneumonia in foals. It also causes pleuropneumonia, pneumonia, osteomyelitis, and abortion in immunocompromised and normal adult horses; abscesses that must be differentiated from tuberculosis in pigs and ruminants; pneumonia in immunosuppressed humans; and lymph node infection in wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), and roe deer (*Capreolus capreolus*).[2,3] There is species tropism for ruminants, pigs, and horses, determined by host-adapted plasmids, whereas infection in humans appears to be zoonotic.[2] The organism is a natural inhabitant of soil, grows well at temperatures ranging from 10° to 40° C, and is readily isolated from the feces of herbivores and their environment. However, isolates of *R. equi* vary in virulence, with many isolates obtained from feces or soil not being pathogenic.

There are a large number of virulent strains of *R. equi,* based on pulsed-field gel electrophoresis of chromosomal DNA, and foals can be infected with multiple strains at the same time, with identical strains located in multiple sites in the body.[4] Although there is evidence of clustering of strains on farms and on most farms one or two strains predominate, there is little evidence of marked regional variations in prevalence of strains of virulent *R. equi*. Only rarely will it be possible to link infections to a given site or region on the basis of analysis of chromosomal DNA.

Virulence of *R. equi* is dependent on the ability of the organism to enter, survive in, and replicate in macrophages. It is a facultative intracellular parasite of macrophages, in which it survives by virtue of virulence factors that act to prevent phagosome-lysosome fusion and bacterial death.[2] Virulence is associated in part with the presence of highly immunogenic **virulence associated proteins** (Vap, coded by plasmid gene pVAP), of which pVAPA is apparently the most important, although the precise role of the other Vap proteins has not been determined. The presence of VapA is necessary but not sufficient for pathogenicity, which requires the presence of other virulence factors, including a number of microbial enzymes.[2] Virulence plasmids can be lost by *R. equi* strains and reacquired by conjugation with virulent strains.[5] Genomic analysis has identified additional virulence factors that are chromosomal rather than plasmid based.[2]

Vap A is a surface-expressed, lipid-modified protein that elicits an intense humoral response by foals. Expression of Vap A, C, D, and E is upregulated by incubation at 37° C (98.6 F), consistent with their role as virulence factors. Other genes probably involved in virulence are also upregulated by conditions that mimic those in vivo. The presence of the virulence proteins is associated with enhanced ability of virulent *R. equi* to survive and replicate within macrophages,

whereas avirulent strains replicate poorly or not at all. Virulence is associated with the presence of the plasmid, and loss of the plasmid by a strain of *R. equi* results in loss of virulence. A recent review provides a detailed discussion of the virulence factors of *R. equi*.[2]

EPIDEMIOLOGY

Occurrence

R. equi pneumonia in foals has a worldwide distribution. Clinical disease is often sporadic but on farms where the disease is endemic, annual **subclinical morbidity** can be high (up to 92%) and can vary widely from year to year. The median percentage of foals that developed clinical disease caused by *R. equi* at farms on which the disease was endemic was 6.6%, with 38% of farms having more than 10% of foals affected. Increased use of ultrasonographic examination of the thorax of all foals on a farm demonstrates the subclinical disease can affect many, if not the vast majority, of foals on farms in which the disease is endemic. For example, of 444 foals born on one stud farm during a breeding season, 426 (92%) had lesions consistent with *R. equi* infection. Eighteen of the foals had lung lesions less than 1cm (considered unaffected), 128 had lesion scores greater than 1 cm and less than 10 cm, and 280 had lesions greater than 10 cm.[6] The detection of *R. equi* pneumonia in one foal on a farm should prompt an examination of all other foals on that farm.

Case-fatality rates for foals on farms, as opposed to those treated at veterinary teaching hospitals, are 29% to 42% (for 113 and 19 affected foals, respectively). The median case-fatality rate for 32 farms in Texas was 25%, and the case-fatality rate was more than 50% for 22% of farms. The case-fatality rate among foals treated at veterinary teaching hospitals is approximately 28%.

Current evidence supports the hypothesis that foals are exposed and infected within the first 2 weeks of life.[7] The **age at onset** of clinical signs of disease associated with *R. equi* varies between 2 weeks and 6 months, but the peak prevalence for pneumonic disease is between 1 and 3 months. The disease is rare in adult horses. Risk factors in foals for development of *R. equi* pneumonia have not been determined, although a large number of factors have been examined. The month in which the foal was born, gestational age, dam's parity, antimicrobial administration during the first week of life, exposure to pasture at less than 2 weeks of age, need for treatment to correct inadequate transfer of passive immunity, and size of mare/foal groups were not associated with risk of disease on farms in Texas.

The prevalence of virulent *R. equi* in isolates from the environment does not appear to be greater on farms where the disease is endemic. Morbidity varies widely among geographic areas and individual farms, probably because of environmental factors that affect the number of virulent *R. equi* and the ease of infection. Because aerosol infection by virulent *R. equi* in dust is thought to be the most important route of infection of foals, factors that favor the accumulation and persistence of *R. equi* in soil and its ability to become aerosolized most probably increase the risk of infection. Such factors might include the following:

- Hot and dry weather, favoring formation of dust
- Soil pH and moisture
- Crowding of pastures with young horses
- Poor pasture hygiene, allowing accumulation of feces
- Dusty pastures

However, empirical demonstration of the importance of these risk factors has not been reported, with several exceptions. **Soil** pH, salinity, and concentrations of various elements including iron, zinc, and copper are not associated with the risk of foals developing *R. equi* pneumonia on farms in Texas. These soil-associated risk factors were examined because *R. equi* is a normal inhabitant of the soil and of the intestine of ruminants, horses, and pigs. It is not highly resistant, but it has been found to survive in moist soil for periods of longer than 12 months. The infection is considered to be soil associated and to be maintained through a soil–horse cycle. The number of organisms in the soil and stable areas on horse farms increases with the time that the farms have housed horses, although there is not a strong correlation between *R. equi* concentration in soil and prevalence of pneumonia in foals.

Farms of larger size, with more resident mares, greater numbers of foals (≥15), and greater foal density per acre,[8] and the presence of mares brought on to the farm for breeding, are all associated with greater risk of foals developing *R. equi* pneumonia. *R. equi* pneumonia does not appear to be associated with poor farm management or lack of preventative health practices such as vaccination, deworming, or administration of hyperimmune plasma. The practice of testing for failure of transfer of passive immunity is associated with an increased likelihood of the disease on a farm. However, this association probably reflects the facts that the disease is more likely on larger farms, which are more likely to perform this test, and that farms that have had the disease are more likely to institute preventive care procedures.

Transmission

Most foals are exposed to infection, as demonstrated by seroconversion or ultrasonographic examination of the thorax, but only a few develop severe disease, although many have subclinical infection and lesions. The organism colonizes the intestine of the normal foal during the first 2 months of life and has been detected in the feces as early as 5 days. Inhalation of the organism in dust is probably the most important route of transmission for pneumonic disease.[7,9] Intestinal disease, which may be clinically inapparent, usually occurs with pulmonary disease, but the source of the infection is unclear, although it may be ingestion of contaminated material or swallowing of infected respiratory secretions. Foals over 5 weeks of age have generally been resistant to experimental challenge.

Zoonotic Implications

R. equi is an occasional pathogen of humans.[2] Infection is more common in immunocompromised people but is only infrequently associated with strains of *R. equi* that are virulent in foals.

PATHOGENESIS

Exposure of foals to *R. equi* is common, based on rate of seroconversion, yet the development of clinical disease is much less common, although subclinical disease on farms with endemic disease can be as high as 95% (426 of 444 foals born on one farm in one breeding season).[6] The reason for this is not fully understood, although development of the disease probably depends on exposure to an infectious dose of organism and the susceptibility of the foal. Foals subject to experimental challenge exposure are much more susceptible to infection, and by lower doses, during the first week of life.[10] Higher doses are required to induce disease in 3-week and 6-week-old foals.[10] Lower challenge doses are associated with milder and more slowly progressive disease and spontaneous resolution of infection. For spontaneous disease, exposure presumably occurs within the first few days of life, before waning of maternally derived passive immunity. Infection results in increases in serum concentration of VapA-specific IgG(T) in foals.[11]

In adult horses, in which the disease is rare, protective immunity is associated with both cellular and humoral immune responses characterized by enhanced immunoproliferative responses of CD4 and CD8 cells and presence of IgGa and IgGb antibodies to Vap A. Opsonizing antibody to *R. equi* is an important defense mechanism in experimentally infected foals and administration of *R. equi* hyperimmune plasma or plasma rich in anti-Vap A and C antibodies protects experimentally infected foals from developing pneumonia. Overall, these results suggest that foals that develop *R. equi* pneumonia have a T-helper-cell (Th)2-like response to infection, rather than a Th1-like response. Th1-like responses, which are associated with enhanced CD4 and CD8 responses, are believed to be important in resistance to the disease. Whether the switch to a Th2-like response to infection is a function of virulent *R. equi* or an attribute of susceptible foals has not been determined.

Experimental and clinical studies indicate that the foal is infected several weeks or

months before clinical signs are observed. **Virulent strains** of *R. equi* are facultative intracellular parasites of macrophages, which they ultimately destroy. Neutrophils are bactericidal for *R. equi* but the organism can survive by inclusion in macrophages. Opsonization of *R. equi* by specific antibodies results in enhanced lysosome–phagosome fusion and greater killing of *R. equi* by equine macrophages and monocytes, whereas entry of *R. equi* into macrophages by nonimmune phagocytosis is not associated with enhanced killing. Its survival in the macrophage is associated with absence of phagosome–lysosome fusion. Nonvirulent strains do not proliferate in macrophages and monocytes. The combined action of humoral and cellular immune systems is important in preventing development of the disease after inhalation of bacteria. Without opsonization, the capacity of the pulmonary macrophage of foals to kill *R. equi* is impaired and the organism can persist in the pulmonary macrophage of infected foals. The inability of the pulmonary macrophages to destroy *R. equi* leads to persistent infection in the lung and a chronic bronchopneumonia with extensive abscessation and an associated suppurative lymphadenitis.

Intestinal infection is common in foals with *R. equi* pneumonia, although clinical manifestations of the intestinal infection, such as diarrhea, are uncommon. Gastrointestinal tract infection is characterized by ulcerative lesions of the mucosa of the large intestine and cecum. In rare cases bacteremia and subsequent **suppurative foci** may develop in many organs, including bones and joints, liver, kidneys, and subcutis.

CLINICAL FINDINGS

The most common manifestation of the disease is subclinical infection and pulmonary abscessation detected by ultrasonographic examination of the thorax of apparently healthy foals.[6,12]

R. equi pneumonia

***R. equi* pneumonia** of foals presents as an acute onset of inappetence, fever, depression, and tachypnea or as a more chronic disease characterized by cough and failure to thrive. Apparent acute onset of the clinical disease is preceded by a **long incubation period** during which clinical signs are minimal. Severe clinical disease is evident as respiratory distress, and the foal is reluctant to move and to suckle. Cyanosis can be present in severe cases. **Auscultation** of the chest can reveal crackles and wheezes, but abnormal lung sounds are often much less apparent than the severity of the respiratory disease suggests they should be. Foals with *R. equi* **abscesses** often do not have abnormal lung sounds, and there is usually minimal nasal discharge. It must be emphasized that the classical severe disease represents one end of the spectrum of consequences of infection of foals by *R. equi*, and much more common is subclinical disease that resolves spontaneously and without treatment. However, clinical disease is important because it is these foals that are at risk of death and that require prolonged treatment.

Table 12-14 Frequency and prevalence of extrapulmonary manifestations of infection by *R. equi* in 150 foals. (Reproduced with permission.[15])

EPD	No. of affected foals (%)	No. of foals with antemortem diagnosis	No. of foals with postmortem diagnosis
Diarrhea	50 (330)	50	0
Immune-mediated polysynovitis	37 (25)	36	1
Ulcerative enterotyphlocolitis	31 (21)	0	31
Intraabdominal abscesses	25 (17)	12	13
Abdominal lymphadenitis	25 (17)	5	20
Uveitis	16 (11)	16	0
Pyogranulomatous hepatitis	16 (11)	0	16
Septic synovitis	14 (9)	12	2
Mediastinal lymphadenitis	12 (8)	7	5
Peritonitis	11 (7)	10	1
Peripheral lymphadenopathy	11 (7)	10	1
R. equi bacteremia	11 (7)	11	0
Subcutaneous abscesses	8 (5)	8	0
Pyogranulomatous nephritis	7 (5)	0	7
Hyperthermia	6 (4)	6	0
Pericarditis	6 (4)	2	4
Osteomyelitis	5 (3)	5	0
Pleural effusion	5 (3)	2	3
Granulomatous meningitis	5 (3)	0	5
Vertebral body osteomyelitis	3 (2)	3	0
Paravertebral abscess	3 (2)	3	0
Cellulitis/lymphangitis	2 (1)	2	0
Immune-mediated hemolytic anemia	2 (1)	2	0

One (1/150 [0.7%]) foal each had an antemortem diagnosis of the following: sinusitis, immune-mediated thrombocytopenia, hyperlipemia, telogen effluvium, granulomatous dermatitis, myositis, lymphoid hyperplasia, omphalitis, bone marrow erythroid hypoplasia, seizures, and right ventricular double apex secondary to pulmonary hypertension. One (1/150 [0.7%]) foal each had a postmortem diagnosis of the following: pyometra, pyogranulomatous stomatitis, pyogranulomatous splenitis, pneumothorax, valvular endocarditis, and myelophthisis.

Ultrasonographic examination of the chest reveals the presence of pulmonary consolidation before clinical signs are apparent and is a useful means of detecting subclinical disease and triaging foals for treatment. **Radiographic examination** of affected animals shows evidence of consolidation of lung tissue, lymphadenopathy, and cavitating lesions in the lungs. Odds of survival are inversely related to severity of alveolar pattern and presence and number of cavitated lesions evident on thoracic radiographs.[13] Ultrasonographic examination of foals is more sensitive for detection of lesions than is radiographic examination, with radiography enabling detection of lesions in 20 of 42 affected foals in which disease was confirmed by ultrasonographic examination.[14]

Extrapulmonary manifestations of *R. equi* infection

Extrapulmonary manifestations of *R. equi* pneumonia are common in foals, and many are associated with an increased chance of death (Table 12-14).[15] Of 150 foals with *R. equi* pneumonia examined at a referral hospital, 111 (74%) had at least one extrapulmonary manifestation of the disease detected ante or postmortem. Of foals examined postmortem, 76% had an extrapulmonary manifestation of the disease that was not detected, or detectable, antemortem. Common abnormalities include diarrhea (50% of foals), immune-mediated polysynovitis (37%), ulcerative enterotyphlocolitis (31%), intraabdominal abscesses (25%), and abdominal lymphadenitis (25%).[15] *R. equi* infection of other structures includes septic synovitis, peritonitis, bacteremia, subcutaneous abscesses, osteomyelitis of the abaxial skeleton, and vertebral body osteomyelitis, in addition to other sites. Noninfectious abnormalities include immune-mediated hemolytic anemia,[16] uveitis, and hyperthermia.

Intraabdominal abscesses are associated with ill-thrift, weight loss, variable abdominal distension, fever, depression, and, in some cases, colic. Ultrasonographic examination can reveal the abscess.[17]

PROGNOSIS

The morbidity and case-fatality rates are provided under "Epidemiology." Presence of an

extrapulmonary manifestation of *R. equi* infection is associated with a case-fatality rate of 57% compared with 18% in foals with only *R. equi* pneumonia. A larger number of extrapulmonary manifestation was associated with an increased chance of death. Presence of uveitis, septic synovitis (but not immune-mediated polysynovitis), abdominal lymphadenitis and intraabdominal abscessation, pleural effusion, *R. equi* bacteremia and pyogranulomatous hepatitis were all independently associated with increased risk of death.

R. equi infection in Thoroughbred and Standardbred foals is associated with a reduced chance of racing as an adult compared with the overall population of foals, but affected foals that survive have a similar racing performance as adults to horses that did not have *R. equi* pneumonia.

CLINICAL PATHOLOGY

Hematologic evaluation usually reveals leukocytosis with neutrophilia and monocytosis, and elevation in the concentrations of acute-phase proteins, including plasma fibrinogen and serum amyloid A—changes characteristic, but not diagnostic, of *R. equi* infection. Monitoring of blood white cell concentration and plasma fibrinogen concentration is useful in foals from farms on which the disease is endemic. **White blood cell concentrations** above 13.0 × 10/L (13,000 cells/μL) have a sensitivity and specificity of 95% and 61%, respectively, for *R. equi* pneumonia. The high sensitivity means that few foals with the disease will be missed, whereas the moderate specificity means that a number of foals will be incorrectly suspected as having the disease. Because a high white cell count can be caused by a number of diseases other than *R. equi* pneumonia, foals with high white cell counts from farms on which the disease is endemic should be further examined for evidence of disease, including detailed clinical examination possibly including ultrasonographic examination, culture or PCR of tracheal aspirates, or thoracic radiography. Measurement of **plasma fibrinogen concentration** is less useful for detecting foals with *R. equi* pneumonia. Plasma fibrinogen concentrations of 400 mg/dL (0.4 g/L) have sensitivity and specificity of 91% and 51%, respectively, whereas concentrations of 600 mg/dL (0.6 g/L) have sensitivity and specificity of 38% and 96%, respectively. The positive and negative predictive values of the tests depend on the prevalence of the disease among the group of foals examined, being low for farms on which the disease is rare and increasing as the prevalence of the disease increases. Serial measurement of **serum amyloid A** concentrations is not useful for detecting foals with clinically inapparent *R. equi* pneumonia,[18] nor do foals with pneumonia reliably have higher serum amyloid A concentrations than normal foals.

Differentiation of pneumonia caused by *R. equi* from that caused by other infectious causes in foals can be challenging. Using microbiological culture as the "gold standard," identification of gram-positive coccobacilli in tracheal aspirates was highly specific (91%) but poorly sensitive (35%) for *R. equi* infection.[19] White cell counts greater than 20,000 cells/μL (86% specificity), fibrinogen concentrations greater than 700 mg/dL (92% specificity), radiologic evidence of thoracic abscessation (85% specificity), and the presence of gram-positive coccobacilli in tracheal aspirates (91% specificity) in pneumonic foals are highly suggestive of *R. equi* infection.[19]

Transtracheal aspirates from affected foals reveal a neutrophilic leukocytosis. Intracellular, gram-positive pleomorphic rods characteristic of *R. equi* may be present in tracheal aspirates, but the sensitivity of this observation has not been determined, and all tracheal aspirates should be cultured.

Although numerous **serologic tests** have been developed, including agar gel immunodiffusion, synergistic hemolysis inhibition, radial immunodiffusion, and various ELISAs, none has demonstrated value in the diagnosis of the disease in individual animals.[20] Currently available serologic tests, either as single or paired samples, are not reliable in confirming or excluding the presence of *R. equi* pneumonia in foals.

Culture of tracheal aspirates is the gold standard for antemortem diagnosis of the disease, although sensitivity of culture is less than that of PCR examination of tracheal aspirates. Culture of tracheal aspirates has a sensitivity of approximately 86%, based on diagnosis of *R. equi* pneumonia at necropsy. A **PCR** test for the rapid detection of *R. equi* in tracheal aspirates has a sensitivity of 100% and a specificity of 91% in foals with a clinical diagnosis of *R. equi* pneumonia. PCR examination of nasal swabs for presence of *R. equi* has a sensitivity of 15%, which is too low to be clinically useful. More recent quantitative real-time PCR assays permit the rapid detection and quantification of virulent (*VapA*-gene-positive) strains of *R. equi* in tracheobronchial aspirates. This assay detects *R. equi* at concentrations as low as 20 cfu/mL of tracheobronchial fluid, providing a specific and highly sensitive test for the presence of this organism. A **multiplex PCR** test simultaneously detects *R. equi* and the presence of virulence factors, thereby permitting rapid differentiation of pathogenic from nonpathogenic strains of *R. equi* in biological samples.

Collection and culturing of breath from foals is not useful in diagnosing *R. equi* pneumonia or in predicting onset of the disease.[21]

NECROPSY FINDINGS

The predominant lesions are a **pyogranulomatous pneumonia** plus **lymphadenitis** of the bronchial lymph nodes. Grossly, the firm, raised lung nodules may reach several centimeters in diameter and can be located anywhere in the lung field, especially in the cranioventral quadrant. If several nodules coalesce, the lesion may be misinterpreted as a suppurative bronchopneumonia. Histologically, organisms are easily demonstrated within the macrophages and giant cells comprising these lesions. Many cases also have ulcerative enterocolitis, with abscessation of mesenteric or cecocolic lymph nodes. Although necropsy may reveal widespread infection, many cases are subclinical.

Samples for Postmortem Confirmation of Diagnosis

- Bacteriology—chilled lung, affected lymph nodes, and swabs from atypical sites (CULT)
- Histology—formalin-fixed lung, lymph node, and colonic lesions

DIAGNOSTIC CONFIRMATION

Antemortem diagnosis is by culture of *R. equi* from aspirates of tracheal fluid. Currently available serologic tests do not provide confirmation of disease in individual animals.

DIFFERENTIAL DIAGNOSIS

The pneumonic form of the disease may be confused with other causes of pneumonia in foals (Table 12-15). Other causes of diarrhea in this age group include parasitism as a result of cyathostomes, infection by *Salmonella* sp., and antibiotic-induced diarrhea.

The aseptic synovitis and joint effusion that frequently accompanies *R. equi* pneumonia should be differentiated from septic arthritis as a result of *S. zooepidemicus, Salmonella* spp., *R. equi,* or other bacteria.

TREATMENT

The principles of treatment are cure of *R. equi* infection, relief of respiratory distress, and correction of associated immune-mediated diseases.

Elimination of infection requires the administration of antimicrobial agents that are both effective against the organism and able to penetrate infected macrophages to gain access to the organism. Customary **in vitro antibiotic sensitivity testing**, using Kirby-Bauer or dilution methodology, has not been demonstrated to be useful in predicting the clinical efficacy of treatment, and *ex vivo* testing of antimicrobial efficacy using macrophage culture systems more closely predicts in vivo efficacy.[22] *R. equi* isolates from ill foals are frequently sensitive in vitro to a variety of antibiotics, including the aminoglycosides gentamicin and neomycin, tetracycline, sulfonamides, and chloramphenicol, whereas most are resistant to cephalosporins and penicillin. However,

Table 12-15 Differential diagnosis of respiratory diseases of older (not newborn) foals

Disease	Epidemiology	Clinical findings	Clinical pathology	Necropsy findings	Treatment and response
Rhodococcus equi infection	Enzootic to a farm. Foals up to 6 months. Infection by inhalation. Case-fatality rate ≈30%.	Pneumonia in 1- to 6-month-olds. Occasional diarrhea. Aseptic synovitis and uveitis in affected foal. Septic osteomyelitis.	Inflammatory cells in tracheal aspirate. Culture or PCR detection of *R. equi* from tracheal fluid. Serum tests not useful in individual animals.	Suppurative bronchopneumonia. May be mesenteric and other lymph node abscess. Rarely septicemia.	Erythromycin estolate, or clarithromycin, plus rifampicin. Advanced cases may be refractory.
Interstitial pneumonia	Sporadic occurrence in foals to 6 months of age. Cause not identified.	Respiratory distress with minimal cough, slight nasal discharge and low grade to non-existent fever. Lungs sounds not remarkable.	None diagnostic. Rule out other diseases. Radiography useful.	Interstitial pneumonia.	Corticosteroids. Broad-spectrum antibiotics (e.g., penicillin and gentamicin). Supportive care.
Viral respiratory disease (see Table 12-16)	Foals usually over 2 months. Rhinitis virus, herpesvirus, and influenza virus infection.	Fever, cough, nasal discharge.	Viral isolation. Serology.	Usually survive although fatal influenza infection reported.	Supportive. Antibiotics for secondary bacterial (*Streptococcus zooepidemicus*) infection
Combined immunodeficiency of Arabian foals	Inherited as autosomal recessive trait. Affected animals are homozygous.	Poor condition, tire easily, cough, ocular and nasal discharge, diarrhea in some.	Severe lymphopenia. Hypogammaglobulinemic as passive immunity declines.	Lymphocytes absent from lymphoid tissue. Adenoviral pneumonia.	None.
Respiratory tract infection with *S. zooepidemicus*	Outbreaks in foals up to weaning. Likely secondary to viral infection.	Fever, nasal discharge, cough, inappetence. Minimal lymphadenopathy.	*S. zooepidemicus* in tracheal aspirates.	Usually survive.	Penicillin. Good recovery rate
Parasitic pneumonia	Migrating stages of *Parascaris equorum*. Foals > 6 weeks old.	Cough, slight nasal discharge. Rarely fever.	Eosinophils in tracheal aspirate.	Death rare.	Anthelmintics, e.g., fenbendazole
Pneumocystis jirovici (formerly *P. carinii*) pneumonia	Immunodeficient foals or foals administered corticosteroids.	Cough, mucopurulent nasal discharge, fever, lethargy, tachypnea.	Neutrophils and macrophages and *P. jirovici* cysts in tracheal aspirate or bronchoalveolar lavage fluid.	Pneumonia, diffuse with neutrophilic or lymphocytic/plasmacytic infiltration and alveolar edema. *P. jirovici* evident in silver-stained lung sections.	Sulfonamide/trimethoprim 30 mg/kg q12 h recommended but often not effective.

PCR, polymerase chain reaction.

treatment with antibiotics other than a macrolide (erythromycin, azithromycin, clarithromycin, gamithromycin) and rifampin is associated with a lower recovery rate. Treatment with **penicillin**, with or without **gentamicin**, chloramphenicol, or tetracycline, is not effective. **Trimethoprim–sulfadiazine** combinations might be effective in some foals but are not the preferred treatment. Neomycin has been recommended for treatment of *R. equi* pneumonia, but the risk of nephrotoxicosis, need for parenteral administration, and lack of demonstration of clinical efficacy do not support its use at this time.

The **treatment of *R. equi* pneumonia** in foals is achieved by administration of macrolide antibiotics in combination with rifampin. Conventional treatment is administration of the combination of an acid-stable **erythromycin** (preferably estolate) at a dose of 25 mg/kg orally every 12 hours and **rifampin** at a dose of either 5 mg/kg every 12 hours or 10 mg/kg every 24 hours. Other esters or preparations of erythromycin are less well absorbed or have shorter elimination half-lives than the estolate ester and must be administered more frequently. Erythromycin ethylsuccinate does not provide optimal therapy for *R. equi* pneumonia in foals because of poor absorption after oral administration. The macrolide antibiotics **azithromycin** and **clarithromycin** have also been used to treat foals with *R. equi* pneumonia. Treatment of foals with a combination of clarithromycin (7.5 mg/kg orally every 12 hours) and rifampin results in improved survival over foals treated with azithromycin (10 mg/kg orally q24h) and rifampin or erythromycin and rifampin in a veterinary teaching hospital. Azithromycin is typically administered with rifampin at a dose rate of 10 mg/kg q24h for every 24 hours for 5 to 7 days and then once every 48 hours. Gamithromycin (6 mg/kg intramuscularly [IM] or intravenously [IV] once every 7 days, with or without administration of rifampin) is currently not recommended for routine use pending results of studies demonstrating its equivalence to or superiority over other treatments. Administration of gamithromycin (6 mg/kg IM or IV once every 7 days, with foals administered IV gamithromycin also administered rifampin) was associated with resolution of lesions detected by ultrasonographic examination in 95% of foals with *R. equi* pneumonia.[23] IM was associated with marked lameness in 35% of foals and colic that required administration of analgesics in 45% of foals. Tulathromycin was not as effective as the combination of azithromycin–rifampin in treatment of *R. equi* abscesses in foals in a large prospective field study.[24] Tilmicosin is poorly active against *R. equi*.[25]

Ultrasonographic examination of the thorax of foals may permit identification of

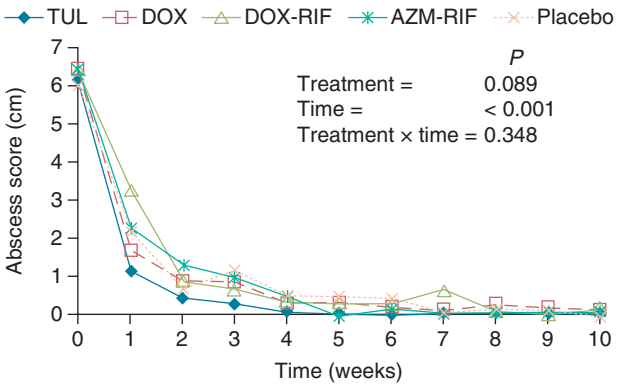

Fig. 12-30 Abscess score based on ultrasonographic examination of foals on a farm with endemic *R. equi* infection administered a placebo, tulathromycin, doxycycline, doxycycline and rifampin, or azithromycin and rifampin. There was no effect of antimicrobial treatment on resolution of the abscess score. (Modified from Venner M, Astheimer K, et al.: Efficacy of Mass Antimicrobial Treatment of Foals with Subclinical Pulmonary Abscesses Associated with Rhodococcus equi, J Vet Int Med 27:171, 2013.)

foals with clinically inapparent pulmonary abscesses, providing the opportunity for early intervention in the disease. However, many (~90%) lesions less than 10 cm in diameter will resolve without treatment, and treatment with antimicrobials confers no clear benefit over watchful waiting (Fig. 12-30).[12] Another study demonstrated resolution of lesions in 44% of foals with lesions greater than 1.0 cm administered a placebo—a resolution rate not statistically different from that in foals administered tulathromycin (IM), azithromycin alone, or azithromycin and rifampin.[6]

It is unclear if there is an advantage to combined therapy of rifampin and a macrolide compared with treatment with a macrolide alone. The current recommendation is to use the combination of drugs.

Gallium maltoate has been investigated for treatment of foals with *R. equi* pneumonia. The pharmacokinetics in foals have been determined, and it appears to be safe for administration to foals and was not inferior to administration of clarithromycin and rifampin in treatment of foals with pulmonary lesions consistent with *R. equi* infection.[26-30] However, the study did not include an untreated or placebo treated group, and given the high rate of spontaneous resolution of lesions without treatment in such foals,[6,12,31] it cannot be concluded that either treatment was superior to no treatment.

Therapy should be continued until the foal is clinically normal **and** has a normal plasma fibrinogen concentration and white blood cell count, which can require treatment for at least 1 month and often longer. Radiographic or ultrasonographic demonstration of resolution of the pulmonary consolidation and abscessation is useful in the decision to stop therapy. The case-fatality rate is approximately 30% (see "Epidemiology") even with appropriate treatment.

Adverse effects of macrolide–rifampin therapy include the development of **diarrhea** in some foals and their dams. Administration of erythromycin to foals is associated with an eightfold increase in the risk of diarrhea. Antibiotic therapy should be temporarily discontinued in foals that develop diarrhea.

During hot weather, some foals treated with erythromycin become **hyperthermic** (40–41°C [104–105.5°F]) and **tachypneic,** and occasional deaths result from this syndrome. The basis for this hyperthermic event, which may occur in healthy foals administered erythromycin, is unknown. Affected foals should be treated urgently with antipyretics, cold water bathing, and housing in a cooler environment.

The emergence of *R. equi* isolates **resistant to rifampin** and one or more macrolides has been documented and underscores the need for monitoring of *R. equi* sensitivity to these antimicrobials. Case-fatality rate is higher (75%) in foals with *R. equi* resistant to one or more of rifampin and a macrolide compared with that in foals infected with susceptible bacteria (30%).[32] The development of resistance during monotherapy with rifampin is a recognized contraindication to the use of this drug alone.

Ancillary therapy with NSAIDs, bronchodilators, and mucolytics might be of value. Foals in severe respiratory distress require intranasal or intratracheal administration of oxygen.

CONTROL

Control measures are designed to maximize the resistance of the foal to infection and to reduce the infection pressure on the foal by decreasing contamination of the foal's environment with virulent *R. equi*. Ensuring adequate transfer of **colostral immunoglobulins** in all foals through routine monitoring of serum immunoglobulin concentrations in 1-day-old foals is an essential part of any control program. To **decrease environmental contamination** with virulent *R. equi,* efforts should be made to reduce fecal contamination of pastures and to reduce or eliminate dusty or sandy areas. These efforts should include grassing or paving of bare areas, removal and composting of fecal material on a regular basis, reduction of stocking density, and reduction in the size of mare/foal bands.

On farms with endemic disease, regular physical examination, including ultrasonographic examination of the thorax of foals and once-daily monitoring of rectal temperature, can permit early identification of affected foals. These foals can then be monitored for resolution or progression of the disease, with animals in the latter group administered antimicrobials. Comments noted earlier about the effectiveness of mass medication of all foals with lung lesions should be noted.[6,12,31] Measurement of blood white cell count, as detailed previously, can be useful in early identification of affected foals. Identification of one foal affected with *R. equi* pneumonia on a farm should prompt an examination of all other foals on the farm.

The administration to foals of a hyperimmune serum, obtained from mares vaccinated with an autogenous vaccine, limits the severity of disease produced by experimental challenge but has not been consistently useful in preventing or decreasing the prevalence of naturally occurring disease. This unpredictable efficacy could be attributable to variable concentrations of *R. equi* anti-Vap-A IgG in batches of plasma.[33]

There are no vaccines effective in prevention of *R. equi* pneumonia in foals.[34,35]

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STREPTOCOCCUS ZOOEPIDEMICUS INFECTION

Streptococcus equi var. *zooepidemicus (S. zooepidemicus)* is one of the bacteria most commonly isolated from the upper respiratory tract of both clinically normal horses and horses with respiratory disease and from the female genital tract, wounds, and guttural pouch.[1] Almost all horses harbor a number of antigenic types of *S. zooepidemicus* in their tonsils, and this may be the source of opportunistic infections of other body systems, including the lungs and genital tract. Currently, over 300 variants of *S. zooepidemicus* are recognized by multilocus sequence typing, and disease is associated with specific variants.[2,3] *S. zooepidemicus* is the most common beta-hemolytic streptococcus isolated from horses at necropsy examination, representing 72% of isolates.[4] Most isolates are from placenta, fetal tissues, and genital tract of mares, but this likely represents the population of animals examined and would not include clinically normal horses in which *S. zooepidemicus* is commensal in the upper respiratory tract.

Outbreaks of upper or lower respiratory disease are associated with particular variants of *S. zooepidemicus* (eg, ST-24 and ST-307),[2,5] and endometritis is caused by a specific variant genetically distinct to those causing respiratory disease.[6] Pathogenicity of *S. zooepidemicus* in horses is related to the presence of superantigens (szeN and szeP, but not szeF).[7]

S. zooepidemicus can cause disease in humans, cats, dogs, and poultry.[8-12] Infection and disease of humans working with horses by *S. zooepidemicus* identical to or closely related to that isolated from horses with which the human cases had contact highlights the zoonotic potential of the organism.[12] An outbreak of disease in chickens was associated with a strain of *S. zooepidemicus* isolated from horses on the same farm,[9] and infection of three dogs, with disease in two, housed on horse stud farms.[8] The disease in dogs is usually a highly contagious often fatal pneumonia.[11] The organism also causes acute, severe pneumonia and systemic illness in cats usually as an outbreak of disease in catteries.[10]

S. zooepidemicus is frequently isolated from horses with pleuropneumonia, endometritis, neonatal septicemia, abortion, and mastitis, suggesting a role for this organism in the pathogenesis of these diseases.[4] *S. zooepidemicus* is likely important in the development of respiratory disease in foals and adult horses. *S. zooepidemicus* was isolated from 88% of foals with clinical evidence of lower respiratory tract disease, and isolation of the organism was associated with an increased proportion of neutrophils in bronchoalveolar lavage fluid, suggesting a causal role for this organism. Similarly, the number of *S. zooepidemicus* isolated from tracheal aspirates of adult horses is directly proportional to the number of neutrophils in the aspirate and the probability that they have a cough. The association of *S. zooepidemicus* and inflammatory airway disease in racehorses is independent of previous viral infection, suggesting a role for *S. zooepidemicus* as a primary pathogen. Presence and number of colony forming units (cfu) of *S. zooepidemicus* in tracheal aspirates of horses is significantly associated with the risk of the horse having inflammatory airway disease. Adult horses dying of pneumonia associated with transportation often yield *S. zooepidemicus* on culture of lung lesions, and the disease can be reproduced experimentally. *S. zooepidemicus* with *Chlamydophila caviae* causes conjunctivitis and rhinitis in adult horses.[13] These results clearly demonstrate a role for *S. zooepidemicus* in the pathogenesis of respiratory disease of horses. However, it is unclear whether *S. zooepidemicus* is a primary cause of disease, a secondary contaminant, or an invader of airways compromised by viral infection or other agents.

Clinical signs of *S. zooepidemicus* infection of the lower respiratory tract of foals and horses include coughing, mild fever, mucopurulent nasal discharge, and increased respiratory rate. Endoscopic examination of the trachea and bronchi reveals erythema and presence of mucopurulent exudate. Tracheal aspirates or bronchoalveolar lavage fluid of affected horses or foals have an increased (>10%) proportion of neutrophils. *S. zooepidemicus* is a frequent isolate from the cornea of horses with ulcerative keratitis.

Treatment consists of the administration of antimicrobials, including penicillin (procaine penicillin, 20,000 IU/kg IM every 12 hours) or the combination of a sulfonamide and trimethoprim (15-30 mg/kg orally every 12 hours). *S. zooepidemicus* isolates from horses in southern England demonstrate increasing resistance to tetracycline but not the combination of trimethoprim and sulfonamide (TMS).[14] Most *S. zooepidemicus* isolates (70%) are resistant to gentamicin, whereas 95% are sensitive to penicillin and 55% sensitive to TMS. Forty-five percent of isolates are resistant to enrofloxacin—a recent phenomenon.[14] Different sensitivity patterns are reported for *S. zooepidemicus* isolates from Western Canada, although the high proportion of isolates sensitive to penicillin (95%) and ceftiofur (99%) is consistent with that in England.[1] A higher proportion of Canadian isolates are sensitive to gentamicin (85%) or enrofloxacin (91%).

Control consists of isolation to prevent spread of infectious respiratory disease and vaccination to prevent viral respiratory disease.

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STRANGLES

SYNOPSIS

Etiology *Streptococcus equi* subsp. *equi*.

Epidemiology Highly contagious disease that affects horses of all ages but is most common in young animals. Prolonged carrier state in asymptomatic animals. *S. equi* causes disease in only equids.

Clinical signs Acute onset of fever, anorexia, depression, submandibular and pharyngeal lymphadenopathy with abscessation and rupture, and copious purulent nasal discharge. Metastatic infection in other organ systems.

Clinical pathology Culture of *S. equi* from nasal and abscess discharges. Polymerase chain reaction (PCR) of nasal, pharyngeal or guttural pouch swabs. High serum antibody titer to SeM.

Lesions Caseous lymphadenopathy with rhinitis and pharyngitis, pneumonia, and metastatic infection in severe cases.

Diagnostic confirmation Culture of *S. equi* or PCR.

Treatment Systemic administration of penicillin. Local treatment of abscesses.

Control Isolation and quarantine of cases. Serologic testing followed by PCR and culture of nasopharyngeal swabs or guttural pouch lavage of serologically positive horses enabling detection of carrier status. Vaccination might reduce the case attack rate and severity of disease but confounds identification of carrier horses.

ETIOLOGY

Streptococcus equi subsp. *equi* (*S. equi*) is a gram-positive coccobacillus that produces a beta-hemolysin, evident as a zone of clear hemolysis surrounding colonies growing on blood agar. There is evidence that *S. equi* is a biovar or genovar of *S. zooepidemicus. S. equi* is highly host-adapted to Equidae. Genetic analysis, particularly of the variable region of the SeM gene, demonstrates the existence of clones that vary geographically.[1-5] For instance, 21 SeM alleles were detected among 145 *S. equi* strains isolated in the United Kingdom,[1] and two SeM alleles detected in horses in New Zealand had distinct geographic distributions.[5] Similar analysis reveals the presence of two major *S. equi* clades in Ireland, with both being also common in the United Kingdom.[4] Individual outbreaks can be caused by *S. equi* of the same SeM type and be restricted geographically or by use of horse.[6] Analysis of SeM mutations in real time allows differentiation or linkage of strangles outbreaks and enables risk assessment of equine events where there is incursion of the disease. It is unclear whether changes in the SeM protein associated with these strains are associated with differing pathogenicity.[1] There is variation in virulence related to the amount of M protein and hyaluronic capsule produced. An atypical milder form of the disease is associated with a capsule-deficient variant of *S. equi,* and an intranasal vaccine is based on a live, attenuated, nonencapsulated SeM-2 strain, although this strain can cause disease.[7]

EPIDEMIOLOGY

Occurrence

Strangles occurs in horses, ponies, donkeys, and mules worldwide, with the exception of Iceland. Outbreaks are seen relatively frequently on breeding farms and in polo and racing stables, when the infection is introduced by new arrivals that are often asymptomatic, and in horses taken to fairs and riding schools. An **incidence** of 35% over a 3-year period is reported for horse studs in Australia, and there were approximately 600 recorded outbreaks in the United Kingdom in 2010.[2]

Strangles can affect horses of any age, although the **morbidity rate** is usually greater in younger horses such as foals and weanlings. Age-specific attack rates of strangles of 18% for brood mares, 48% for 1-year-old horses, and 38% for foals during an outbreak on a breeding farm are reported, although higher morbidity rates (100%) can occur, especially in young horses. The risk of occurrence of an outbreak of strangles increases with the size of the group of horses: farms with 100 or more horses have a 26 times greater risk of experiencing an outbreak than farms with fewer than 15 horses.

The **case-fatality rate** without treatment is about 9%, but with adequate early treatment it may be as low as 1% to 2%. Deaths are usually attributable to pneumonia.

Source of Infection and Transmission

S. equi is an obligate parasite of horses and all infections are attributable to transmission from infected horses, either directly or by fomites. **Nasal and abscess discharge** from infected animals that contaminates pasture, tack, stalls, feed and water troughs, grooming equipment, and hands and clothes of grooms and veterinarians is often the source of infection for susceptible horses. *S. equi* survives in the environment for less than 3 days, and although fomite transfer is important in transmission of infection, prolonged quarantine of facilities is not warranted.[8] Direct transmission from infected animals to susceptible animals occurs through contact.

Approximately 10% to 40% of horses that recover from the clinical disease have **persistent infection** of *S. equi* in the pharynx and guttural pouches for many months and are an important source of infection. Horses with **clinically inapparent disease**, such as some cases of guttural pouch empyema, can shed the organism for over 3 years. The period of infectivity is important in terms of the length of quarantine that needs to be imposed on horses that have apparently recovered from the disease. Because nasal shedding of *S. equi* can be intermittent, repeated culture of nasopharyngeal swabs or use of PCR examination of guttural pouch washings is necessary to document the carrier status of individual horses.

The clinically inapparent nature of the infection makes detection of carriers problematic, especially when considering introduction of horses into a previously closed herd in which strangles is not endemic. Endoscopic or radiographic examination of clinically inapparent shedders can demonstrate lesions in the guttural pouches, paranasal sinuses, or pharynx, but because some persistent carriers of *S. equi* do not have detectable abnormalities of the nasopharynx, the most reliable approach to detecting carriers is PCR examination of nasal swabs or guttural pouch lavage fluid (see "Control").[9]

Animal Risk Factors

Strangles is more common in young or naive horses, although the disease can occur in horses of any age. Animals that have previously had the disease are less likely than naive animals to develop the disease on subsequent exposure. A proportion (approximately 25%) of horses that recover from the disease do not develop a protective immune response and are susceptible to reinfection and a second bout of strangles. Resistance to the disease is associated with the production of **serum and mucosal IgG antibodies** to the streptococcal **M protein**. The presence in the nasopharynx of antibodies to streptococcal M protein is thought to be important in conferring resistance to the disease. Serum IgGb antibodies specific for SeM protein, which is important in the antiphagocytic activities of *S. equi,* are produced by most but not all horses during convalescence. Similarly, IgA and IgGb against SeM protein are detectable on nasal and pharyngeal mucosa after *S. equi* infection but not after intramuscular administration of vaccines containing M protein. Serum bactericidal activity alone is not considered to be a good indicator of resistance to the disease, especially if it is induced by administration of a vaccine. Antibodies similar to those found in the nasopharynx after infection with *S. equi* are present in colostrum and milk of mares that have recovered from the disease, are passed to foals via the colostrum, and are secreted into the foal's nasopharyngeal mucosa. These acquired antibodies are important in mediating the resistance of young foals to the disease.

Although **strong immunity** occurs after an attack, this immunity wanes.

Importance

Strangles is one of the most important diseases of horses in developed countries, accounting for up to 30% of reported infectious disease episodes. The disease is important not only because of the deaths that it causes but more importantly because of the disruption of the management of commercial horse establishments, the time necessary to treat affected horses, and the esthetic unpleasantness of the running noses and draining abscesses.

PATHOGENESIS

Virulence of *S. equi* is attributable to the presence of **M proteins** on the surface of the bacteria, a hyaluronic acid capsule and the production of a leukocidal toxin. M proteins are associated with *S. equi* adhesion to oral, nasal, and pharyngeal tissues; invasion of pharyngeal tonsils and associated lymphoid structures; and evasion of the innate host immune response. *S. equi* produces two M proteins—SeM and SzPSe. SeM is unique to *S. equi* and plays a dominant role in resistance of the organism to phagocytosis. Variations in structure of M protein are associated with decreased virulence. The M proteins interfere with the deposition of complement component 3b on the surface of the bacteria and bind fibrinogen, both of which reduce the susceptibility of the bacteria to phagocytosis by neutrophils. The antiphagocytic activity of *S. equi* reduces the efficacy of neutrophils in engulfing and destroying the bacteria.

The capsule of *S. equi* is associated with resistance to nonimmune phagocytosis and pathogenicity. Strains of *S. equi* that do not produce a capsule do not induce disease, although they are able to infect guttural

pouches and cause seroconversion in experimental studies.

Following exposure of the oral and nasopharyngeal mucosal surfaces to *S. equi,* bacteria lodge in the **pharyngeal and tonsillar lymphoid tissues,** where they multiply rapidly. There is no evidence of colonization of mucosal surfaces and streptococci can be detected in pharyngeal tonsils within hours of exposure.[10] The binding of *S. equi* to pharyngeal cells is caused by fibrinogen binding proteins associated with M protein. The resistance of *S. equi* to nonimmune phagocytosis results in accumulation of large numbers of organisms surrounded by degenerating neutrophils. Release of streptolysin S and streptokinase may contribute to tissue damage by directly injuring cell membranes and indirectly through activation of plasminogen. Bacteremia may occur. Migration of neutrophils into the lymph nodes causes swelling and abscessation within 48 hours of infection,[10] with associated disruption of lymph drainage and development of edema in tissues drained by the affected nodes. Swelling of retropharyngeal lymph nodes may interfere with deglutition and respiration. Most abscesses eventually rupture and drain, and the infection resolves with the development of an effective immune response. Nasal shedding of *S. equi* usually begins 4 to 7 days after infection, or 2 days after onset of fever, and persists for 2 to 3 weeks in most horses but up to years in exceptional cases. Cessation of shedding accompanies development of an effective serum and mucosal immune response.

Death is usually attributable to pneumonia caused by aspiration of infected material, although other causes of death include asphyxiation secondary to upper airway swelling and impairment of organ function by metastatic infection. Rare deaths also occur as a result of infarctive purpura hemorrhagica in horses infected with *S. equi.*

Metastatic infection of the heart valves, brain, eyes, joints, and tendon sheaths or other vital organs can occur and cause a chronic illness and eventual death. Metastatic infection may occur because of bacteremia or extension of infection along chains of lymph nodes. Purpura hemorrhagica can occur as a sequela to *S. equi* infection and is associated with high serum antibody titers to SeM.

CLINICAL FINDINGS

The disease manifests as an acute disease of varying severity, chronic infection of retropharyngeal lymph nodes and guttural pouches, and as chronic disease associated with metastatic infection of organs distant to the upper respiratory tract.[11,12] The severity of the acute disease varies with the age and immune status of the animal, the size of the inoculum, and the duration of exposure to infection. The term *strangles* derives from the enlarged retropharyngeal lymph nodes and guttural pouches causing respiratory distress in severely affected equids.

Acute Disease

The acute disease is characterized by mucopurulent nasal discharge and abscessation of submandibular and retropharyngeal lymph nodes. After an **incubation period** of 1 to 3 weeks the disease develops suddenly, with complete anorexia, depression, fever (39.5–40.5°C [103–105°F]), a serous nasal discharge that rapidly becomes copious and purulent, and a severe pharyngitis and laryngitis. Rarely there is a mild conjunctivitis.

Lymphadenopathy becomes apparent as the submandibular lymph nodes enlarge and palpation elicits a painful response. The pharyngitis may be so severe that the animal is unable to swallow, and there is a soft, moist cough. The head may be extended.

The febrile reaction commonly subsides in 2 to 3 days but returns as the characteristic abscesses develop in the lymph nodes of the throat region. The affected nodes become hot, swollen, and painful. **Swelling of the retropharyngeal lymph nodes** can cause obstruction of the oro- and nasopharynx with subsequent respiratory distress and dysphagia. Death by asphyxiation can occur at this time in severe cases. Obvious swelling of the nodes can take 3 to 4 days to develop; the glands begin to exude serum through the overlying skin at about 10 days and rupture to discharge thick, cream-yellow pus soon afterward. Average cases run a course of 3 weeks; severe cases can last as long as 3 months.

Retropharyngeal abscesses can rupture into the guttural pouches, resulting in guttural pouch empyema and ultimately in prolonged infection and formation of chondroids. Retropharyngeal lymph node abscessation might not be apparent on external evaluation and can often only be detected by radiographic or endoscopic examination of the pharynx. Infection of retropharyngeal lymph nodes and guttural pouches is important in persistent infection and carrier status of some horses.

If the infection is particularly severe, many other lymph nodes, including the pharyngeal, submaxillary, parotid, and retrobulbar nodes, can abscess at the same time. Local abscesses also occur at any point on the body surface, particularly on the face and limbs, and the infection can spread to local lymphatic vessels causing obstructive edema. This occurs most frequently in the lower limbs, where edema may cause severe swelling. Abscess formation in other organs probably occurs at this time.

An atypical form of the disease can occur and is characterized by widespread subclinical infection within a stable or yard and a mild disease. Affected horses have a transient fever for 24 to 48 hours and a profuse nasal discharge, and are anorexic. A moderate enlargement of the mandibular lymph nodes occurs in only about one-half of the affected horses.

Strangles in burros is a slowly developing debilitating disease. At postmortem examination the characteristic lesions consist of caseation and calcification of abdominal lymph nodes.

Complications

Complications occur in about 20% of cases. The most common fatal complication is the development of **suppurative necrotic bronchopneumonia**, which probably occurs secondary to the aspiration of pus from ruptured abscesses in the upper airway, or metastatic infection of the lungs.

Extension of the infection into the **guttural pouches**, usually as a result of rupture of retropharyngeal lymph nodes into the medial compartment, causes empyema, which can lead to the formation of accretions of inspissated pus (chondroids). Involvement of the guttural pouches is evident clinically as distension and, after resolution of other signs, unilateral or bilateral nasal discharge. Guttural pouches of affected horses should be examined endoscopically for evidence of retropharyngeal abscessation or guttural pouch empyema or chondroid formation.

Retropharyngeal lymphadenopathy can impair the function of the **recurrent laryngeal nerves,** with subsequent unilateral or bilateral laryngeal paresis and consequent respiratory distress.

Metastatic infection ("bastard strangles") results in the formation of abscesses in any organ or body site but most commonly in the lungs, mesenteric lymph nodes,[11,13] liver, spleen, kidneys, and brain. Clinical signs depend on the organ affected and the severity of the infection, but intermittent fever, chronic weight loss, and sudden death as a result of rupture of abscesses into a body cavity are common manifestations of metastatic infection. Rectal examination or percutaneous ultrasonographic examination can reveal intra-abdominal abscesses in some horses with metastatic abscesses in the abdomen. Peritoneal fluid from these horses is often abnormal.

Metastatic infections can occur in the **central nervous system**. Extension of infection to the meninges results in suppurative meningitis characterized clinically by excitation, hyperesthesia, rigidity of the neck, and terminal paralysis. Abscesses in the brain cause a variety of clinical signs, depending on location of the abscess, including severe depression, head pressing, abnormal gait, circling, and seizures. Metastatic infections of the ocular and extraocular structures, heart valves and myocardium, joints, bones, tendon sheaths, and veins may occur.

Purpura hemorrhagica can occur as a sequela to *S. equi* infection.

Two myopathic syndromes occur with *S. equi* infection in horses. **Muscle infarction**,

which may be extensive, is assumed to result from immune-mediated vasculitis associated with purpura hemorrhagica. Often the muscle lesions in these horses are associated with other lesions consistent with severe purpura hemorrhagica, including infarctions in the gastrointestinal tract, skin, and lungs. **Rhabdomyolysis and subsequent muscle atrophy** results in signs of muscle disease, including stilted gait and elevated serum activity of creatine kinase and other muscle-derived enzymes, and is assumed to be attributable to cross-reactivity of anti-SeM antibodies with myosin.

Myocarditis and glomerulonephritis have been suggested as sequelae to *S. equi* infection but have not been conclusively demonstrated to occur.

CLINICAL PATHOLOGY

Hematologic abnormalities during the acute phase of the disease include leukocytosis, with a neutrophilia reaching a peak as the lymph nodes abscess. **Hyperfibrinogenemia** is characteristic of both the acute and chronic disease. Hematologic and biochemical abnormalities associated with metastatic infection depend on the site of the infection and its severity. Leukocytosis with a **hyperproteinemia** attributable to a polyclonal agammaglobulinemia is characteristic of metastatic and chronic abscessation. **Hypoalbuminemia** may be present. Serum biochemical profile can reveal evidence of specific organ dysfunction. There can be an anemia, which is likely attributable to the hemolytic effect of streptolysin O, immune-mediated hemolysis, or anemia of chronic disease.

A number of serologic tests to measure antibodies to SeM have been developed. An early commercial test that measured the **serum IgG antibody titer to SeM** was used to determine response to vaccination, suitability for vaccination and presence of metastatic infection. This ELISA has a sensitivity and specificity of 90% and 77%, respectively.[14] The test is not useful in diagnosis of the acute disease. Serum antibody titers to SeM are very high (>1 : 12800) in horses with metastatic infection or purpura hemorrhagica. Further tests have been developed with the aim of detecting horses that have been exposed to *S. equi,* with the intent of enabling quarantine and control measures.[14] ELISA assays for antibodies to SeM that combined analysis of two antigens restricted to *S. equi* provides sensitivity and specificity of 93% and 99%, respectively.[14] Use of this assay allows detection of horses that have been exposed to *S. equi* and therefore might be carriers of the organism. These horses can then be examined using PCR of nasopharyngeal swabs (3 over 3 weeks) or guttural pouch lavage fluid (once).[9,15,16] The high sensitivity of the test means that horses that test negative are unlikely to have been exposed or to be carriers.[14]

PCR testing is useful to detect shedding of *S. equi* DNA and has a greater sensitivity than routine culture.[9,15,16] PCR testing of nasopharyngeal swabs or guttural pouch lavage fluid has a sensitivity of 90% to 95% and specificity of 86% to 97% with turnaround time of approximately 2 hours.[15,16] The test is reported to be more specific than culture for detection of *S. equi* shedding. The PCR does not differentiate between live and dead *S. equi,* and false-negative results occur in the presence of large numbers of *S. equi.*

Culture of nasal, pharyngeal, guttural pouch, or abscess discharge will usually yield *S. equi* in 30% to 40% of horses with active disease or in carriers.[9] Abscesses can rapidly become contaminated with *S. zooepidemicus,* which can impede isolation of *S. equi,* although the two can be differentiated by culture or PCR analysis.[17]

NECROPSY FINDINGS

In the rare fatalities that occur, necropsy examination usually reveals suppuration in internal organs, especially the liver, spleen, lungs, pleura, and peritoneum. When the last is involved, it is usually as a result of extension from abscesses in the mesenteric lymph nodes. The microscopic changes of abscessation and suppurative lymphadenitis are uncomplicated. The widespread ecchymotic hemorrhages of purpura hemorrhagica are not specific to this infection, but *S. equi* should always be investigated as a potential cause of such lesions.

Samples for Confirmation of Diagnosis

- Bacteriology—swab of abscess wall, enlarged lymph node (CULT), or PCR

DIAGNOSTIC CONFIRMATION

Confirmation of strangles depends on the detection of *S. equi* from nasopharyngeal swabs, discharges from abscesses, or guttural pouch lavage by PCR or culture. As discussed previously, PCR has greater utility at detecting presence of the organism. Shedding of *S. equi* in nasal discharges begins 1 to 4 days after the onset of fever, and ruptured abscesses often become contaminated with *Streptococcus zooepidemicus* and *S. equisimilis.*

History and clinical findings are usually highly suggestive of the disease, and classical cases of the strangles do not represent a diagnostic challenge. However, outbreaks of milder form of the disease are more challenging to diagnose, and confirmation is based on identification of the organism or demonstration of seroconversion. In acute disease, nasopharyngeal swabs or pus aspirates from abscesses can confirm *S. equi* infection. Because false negative culture results occur in 30% to 40% of cases, and qPCR has a sensitivity 94% and specificity 96%, and combining qPCR with culture will detect more than 90% of infected horses.[9]

Carriers are defined as horses shedding bacteria more than 6 weeks after clinical recovery. These horses will have serologic evidence of infection and can be detected by a series of at least 3 nasopharyngeal swabs at weekly intervals, or a single guttural pouch lavage ideally combined with a single nasopharyngeal swab, submitted for qPCR combined with culture. This will detect greater than 90% of carriers.[18]

Infection by *Actinomyces denticolens* caused submandibular abscessation in horses that can appear clinically similar to strangles. Diagnosis is based on bacterial culture.[19,20]

TREATMENT

The **specific treatment** of choice for *S. equi* infection of horses is **penicillin**, either as procaine penicillin G (22,000 IU/kg intramuscularly every 12 hours) or potassium or sodium penicillin G (22,000 IU/kg intravenously every 6 hours). Tetracycline (6.6 mg/kg intravenously every 12-24 hours) and sulfonamide–trimethoprim combinations (15-30 mg/kg orally or intravenously every 12 hours) can be efficacious but should only be used if penicillin cannot be administered. Aminoglycosides, such as gentamicin or amikacin, and the fluoroquinolones are not effective. Proportions of a small number (10) *S. equi* isolates from horses in southern England during 2007 to 2012 resistant to various antimicrobials were as follows: enrofloxacin 40%, gentamicin 80%, penicillin or ceftiofur 0%, trimethoprim–sulfonamide combination 20%, doxycycline 10%, oxytetracycline 0%, and resistant to three or more antimicrobials 20%.[21] Similar sensitivities are reported for 22 isolates from Western Canada, with all isolates sensitive to ampicillin, ceftiofur, cephalothin, penicillin, erythromycin, amoxicillin-clavulanic acid, and no isolates sensitive to amikacin or neomycin. Approximately 80% of isolates were sensitive to TMS or tetracycline.[22] Use of ceftiofur, a third-generation cephalosporin, in horses is discouraged on public health grounds.[21]

DIFFERENTIAL DIAGNOSIS

See Table 12-16 for a list of differential diagnoses of infectious upper respiratory tract disease of horses. Pneumonia should be differentiated from pleuropneumonia associated with transport or other stress. Chronic weight loss as a result of metastatic infection should be differentiated from equine infectious anemia, parasitism, inadequate nutrition and neoplasia, especially gastric squamous-cell carcinoma, alimentary lymphosarcoma, and granulomatous enteritis.

Table 12-16 Differential diagnosis of diseases of the upper respiratory tract of horses

Disease	Epidemiology	Clinical signs: Respiratory tract	Clinical signs: Other	Diagnosis and clinical pathology
Strangles (*Streptococcus equi* infection)	Incubation period 4–8 days. Course 10–21 days. Spreads by inhalation or ingestion. Mostly young horses in recently commingled groups. Long period (many months) of inapparent infection in some horses.	Copious, purulent nasal discharge. Cranial lymphadenitis and rupture. Moist cough. Obstruction of pharynx can cause dyspnea.	Severe illness with suppuration, fever. Atypical cases show involvement of other organs. Serious sequelae include pneumonia, metastatic spread of infection, mesenteric abscess or purpura hemorrhagica.	*S. equi* in nasal, pharyngeal or guttural pouch swabs, oropharyngeal pus. or lymph node abscess pus. PCR of nasal, pharyngeal or guttural pouch swabs. Serology to detect exposed horses. Leukocytosis. Hyperfibrinogenemia.
Equine viral arteritis (EVA)	Incubation period 1–6 days. Course 3–8 days. Some deaths.	Serous/purulent nasal discharge. Slight cranial lymphadenitis, cough. Conjunctivitis, purulent with edema or petechiae. Dyspnea.	Severe disease. Anasarca. Ventral edema, prepuce, legs, scrotum. May be diarrhea, jaundice. Up to 50% of mares abort.	Virus in blood at fever peak. Serology. Leukopenia.
Equine viral rhinopneumonitis (EHV-1)	Incubation period 2–10 days. Course 2–5 days. Cough may last as long as 3 weeks.	Serous/purulent nasal discharge. Slight cranial lymphadenitis, coughing, conjunctivitis. Mild respiratory disease; in young.	Abortion in mares. Virus may cause myelopathy.	Virus in nasal discharge or peripheral blood buffy coat. PCR of nasal discharge or blood. Tissue culture and serologic tests. Leukopenia. Virus in intranuclear hepatic inclusions of fetus.
Equine viral rhinopneumonitis (EHV-4)	Incubation period 2–10 days. Course 2–5 days. Cough may last as long as 3 weeks.	Serous/purulent discharge. Slight cranial lymphadenitis, coughing, conjunctivitis.	Mild respiratory disease; in young horses.	Virus in nasal discharge. Tissue culture and serologic tests. Leukopenia.
Equine influenza (H3N8 rarely H7N1)	Incubation period 2–3 days. Course 7 days. Cough may persist 3–4 weeks. Enzootic, worldwide (not Australia). Explosive outbreaks; 80%–100% morbidity in young.	Nasal discharge slight, serous only. Slight cranial lymphadenitis. Severe cough. No conjunctivitis and no respiratory distress.	Minimal extrarespiratory signs. Temperature 39–41°C (102–105°F).	Virus in nasal discharge. Good serologic tests available. Rapid ELISA test for viral antigen in nasal secretions. PCR of nasal secretions.
Equine rhinitis virus	Incubation period 3–8 days. Rapid spread, high morbidity (70%). Solid immunity after natural infection. Excreted in urine.	Pharyngitis, pharyngeal lymphadenitis, nasal discharge serous to mucopurulent. Cough persists 2–3 weeks.	Mild disease. Emphasis on coughing. Fever to 39.5°C (103°F).	Equine rhinitis virus on tissue culture. Serologic tests available.
Equine adenovirus	Many inapparent infections. High proportion of population serologically positive.	Mild respiratory signs in adults. Fatal pneumonia in Arabian foals with combined immunodeficiency.	Transient softness of feces. In mares can cause abortion without clinical illness.	Adenovirus in oropharyngeal swabs. Serologic tests available.

ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction.

There is considerable debate about the treatment of horses with strangles. Folklore and anecdotal reports suggest that antibiotic treatment of horses with strangles is contraindicated because it promotes the development of metastatic infection. There is no experimental or empirical evidence to support this contention, and horses with strangles should be treated with therapeutic doses of an appropriate antibiotic, such as procaine penicillin, for a period of time sufficient to effect a cure, as appropriate.

Treatment for *S. equi* infection depends on the stage of the disease, as follows:

- Horses with **early clinical signs** including fever, anorexia, depression, and purulent nasal discharge should be isolated and treated with therapeutic doses of penicillin for at least 5 days. The purpose of treatment is to prevent further development of the disease in the affected animal and to minimize environmental contamination with *S. equi* and transmission to other horses. Treatment should start as soon as clinical signs are observed, and the full course of treatment should be completed to minimize the chances of recrudescence of the infection. Treatment at this stage causes rapid resolution of fever, anorexia, nasal discharge, and lymphadenopathy in individual horses and may abort an incipient outbreak of the disease in a stable or yard. However, treated horses may not develop a protective immune response and consequently may be at risk of reinfection if exposed to *S. equi* after completion of the course of treatment, leading one authority to

recommend that only severely affected animals be treated.

- Horses with submandibular **lymph node abscessation** but without other clinical abnormalities probably do not require antibiotic treatment. Such horses should be isolated and efforts made to aid maturation and rupture of affected lymph nodes.
- Systemic antibiotic therapy with penicillin is indicated in horses with **advanced signs of strangles**, including prolonged fever, depression, anorexia, or dyspnea resulting from retropharyngeal lymphadenopathy. Retropharyngeal abscessation frequently responds to antimicrobial therapy, although surgical drainage may be required in some instances.
- Horses with **metastatic infection** require systemic penicillin therapy in combination with specific therapy for the complication. Pulmonary and mesenteric abscesses are problematic because they are usually not amenable to surgical drainage, and prolonged antimicrobial therapy is required to attempt to effect a cure.
- **Guttural pouch empyema** requires either surgical drainage or repeated flushing of the affected pouch through the pharyngeal openings. Removal of pus and inspissated material in the guttural pouches can be achieved under endoscopic guidance. Alternatively, rigid or flexible indwelling catheters can be inserted for repeated flushing of the pouches with sterile isotonic electrolyte solutions (such as 0.9% NaCl) and topical medications. Substances and solutions that are irritating or injurious to mucus membranes, such as iodine,[23] hydrogen peroxide, and similar irritant compounds, should not be infused into the guttural pouches. Combined topical and systemic administration of potassium benzyl penicillin may be beneficial. Chondroids can often be removed using wire snares. Horses with metastatic or guttural pouch infections are likely infectious and should be isolated.
- Treatment of **purpura hemorrhagica** is dealt with elsewhere.
- Management of horses that have been **exposed to** horses with strangles is controversial. Some authorities recommend treatment of such in-contact horses with penicillin until affected horses are isolated and no longer are a source of infection. However, close examination of exposed animals, including monitoring rectal temperature, and treatment of horses at the first sign of illness is probably a more reasonable approach.

Ancillary treatment consists of administration of nonsteroidal antiinflammatory drugs (NSAIDs) to reduce swelling and provide pain relief, application of hot poultices to encourage rupture of abscesses, provision of intravenous hydration in animals unable to drink, and wound care, including cleaning of ruptured abscesses and application of petroleum ointment to surrounding skin to prevent scalding. Horses with severe upper airway obstruction may require placement of a short-term tracheotomy.

CONTROL

All establishments that house multiple horses, and at which horses both enter and leave, should have biosecurity plans detailing the measures to be taken before new horses enter the facility. The principles of control measures include the prevention of transmission of *S. equi* from infected horses (cases or carriers) to susceptible animals and enhancement of resistance to infection and disease.

There are two basic approaches to strangles prevention: eradication or control.[24] The eradication approach aims to create and maintain a guaranteed disease-free state within the group and is most suited for closed herds. The control approach aims to reduce the frequency and severity of outbreaks but accepts that disease will occur from time to time. In many facilities with large numbers or frequent turnover of horses, such as large training yards or stud farms, a control approach may be more achievable than eradication.[24]

The approach to managing horses entering a facility in which *S. equi* infection is not present involves serologic testing of all horses before entry using an ELISA of known high sensitivity and specificity. Horses that are seropositive on arrival have prima facie evidence of exposure and are considered to be potential carriers of *S. equi* until demonstrated by PCR and culture to be negative for the organism. These horses are then screened for *S equi* carriage by guttural pouch lavage combined with a nasopharyngeal swab tested by qPCR and culture.[9,15] Horses with negative or equivocal serology on arrival should not be admitted to the facility and should be retested 10 to 14 days later to establish serologic status and then either admitted to the facility if they are seronegative or screened for *S equi* carriage if they are seropositive.[24] Any horses testing positive for carriage need to be treated (see previous discussion) and cured of infection before entry into the yard.

Vaccination provides a useful adjunct to management changes, especially in groups of horses with open management systems, and may be more appropriate for yards aiming for control rather than eradication.[24] However, vaccination complicates interpretation of serologic screening of new arrivals because it is not possible at this time to differentiate between serologic responses to vaccination and infection.

Prevention of Transmission

Methods to control transmission of *S. equi* on affected premises are detailed in Table 12-17 and are as follows:

- Infected animals should be isolated immediately.
- All potential sources of fomites—including pails, brooms, grooming brushes, and blankets—should be thoroughly cleaned and disinfected and the bedding burned. Disinfection with phenolic compounds is preferred because they retain their activity in the presence of some organic matter, whereas bleach and quaternary ammonium compounds are inactivated by organic material.
- Emergency prophylactic treatment, using injections of benzathine penicillin every 48 hours in foals and yearlings that are most susceptible, has been used but most treated animals develop strangles when the treatment is discontinued. This method of prophylaxis is not recommended.
- People who care for affected horses should, ideally, avoid contact with susceptible animals. If this is not practical, then strict isolation protocols, including the wearing of protective boots and clothes that are changed between affected and normal horses, should be implemented.
- Horses with elevated temperatures should have nasopharyngeal or guttural pouch swabs cultured.
- As detailed previously, horses should be examined by nasopharyngeal swab or guttural pouch lavage to detect carriers. Carriers should be treated and demonstrated to be no longer carriers before being allowed access to potentially susceptible horses.

Enhanced Resistance

The majority of horses develop solid immunity to strangles after recovery from the spontaneous disease. This immunity lasts for up to 5 years in approximately three-quarters of recovered horses. Maximum resistance to disease probably requires both systemic and mucosal immunity to a variety of *S. equi* factors including, but not limited to, M protein. As noted previously, vaccination will result in positive results of serologic testing for exposure to *S. equi*. It is not possible at this time to differentiate between responses

Table 12-17 Aims and associated measures used to control transmission of *Streptococcus equi* in affected premises and herds

Aim	Measure
Prevent spread of *S. equi* infection to horses on other premises and to new arrivals on the affected premises	Stop all movement of horses on and off affected premises immediately and until the outbreak is controlled. Horses with strangles and their contacts should be maintained in well-demarcated quarantine areas. Clustering of cases in groups allow parts of the premises to be allocated as contaminated or clean.
Establish whether clinically recovered horses are carriers.	At least three nasopharyngeal swabs or washings taken at weekly intervals from all recovered cases and their contacts and examined by culture and PCR. Horses that are consistently negative are returned to the clean area.
Investigate apparently healthy horses from which *S. equi* is recovered.	Serology to determine exposure, with positive horses subject to nasopharyngeal swabbing (three times) or guttural pouch lavage and PCR and culture. Serologically negative horses should be retested in 10–14 days.
Eliminate *S. equi* from guttural pouches.	Treatment of guttural pouches, as detailed under "Treatment."
Prevent infection of uninfected horses by *S. equi* from infected horses.	Personnel should have dedicated protective clothing when dealing with infected horses. Personnel should not deal with infected and uninfected horses. If this is not possible, then infected horses should be dealt with after uninfected horses. Strict hygiene should be implemented, including provision of disinfection facilities for personnel and diligent and thorough cleaning of stables and barns. If practicable, equipment should be destroyed after use with infected horses. Organic material should be removed from stables and then appropriate phenolic disinfectants or steam should be applied. This cleaning should be repeated. Feces and waste from infected animals should be composted in an isolated location. Uninfected horses should not be introduced to pastures used to house infected horses for 4 weeks. Water troughs should be disinfected daily. Horse vans should be thoroughly cleaned and disinfected after each use.

PCR, polymerase chain reaction.
Source: modified from Sweeney CR et al. J Vet Intern Med *2005; 19:123–134.*

to vaccination and responses to natural infection. This ambiguity confounds use of serologic tests in control of the disease. The benefits of potential increases in resistance to the disease induced by vaccination should be weighed against the restrictions this imposes on use of serologic testing in control programs.

The efficacy of **vaccination** of adult horses with *S. equi* bacterins or M protein extracts of *S. equi* administered intramuscularly is controversial. Administration of M protein vaccines elicits an increase in the concentration of serum opsonizing antibodies but does not confer a high degree of resistance to natural exposure. However, in a controlled field trial, vaccination with an M protein commercial vaccine three times at 2-week intervals reduced the clinical attack rate by 50% in a population of young horses in which the disease was endemic. Horses vaccinated only once were not protected against strangles. A modified live vaccine induced a strong antibody response but caused substantial morbidity and some deaths among young ponies, highlighting the challenges with use of attenuated vaccines.[25] Administration of a live, attenuated submucosal vaccine to mares appears to be safe.[26]

This result suggests that, in the face of an outbreak, vaccination might reduce the number of horses that develop strangles but will not prevent strangles in all vaccinated horses. A common vaccination protocol involves the administration of an M protein vaccine intramuscularly for an initial course of three injections at 2-week intervals, with further administration of the vaccine every 6 months in animals at increased risk of contracting the disease. On breeding farms, vaccination of mares during the last 4 to 6 weeks of gestation and of the foals at 2 to 3 months of age might reduce the incidence of the disease.

The vaccines are administered by the intramuscular route and frequently cause swelling and pain at the injection site. **Injection site reactions** are usually less severe with the M protein vaccines. Injection into the cervical muscles may cause the horse to be unable to lower its head to eat or drink for several days—injection into the pectoral muscles is preferred for this reason. There are reports of **purpura hemorrhagica**, the onset of which was temporally associated with administration of a *S. equi* vaccine. Owners should be clearly warned of the limited efficacy and potential adverse effects of vaccination. The effect of vaccination in confounding interpretation of results of serologic testing used in control of the disease should be considered before horses are vaccinated.

Foals that receive adequate high-quality colostrum from exposed or vaccinated mares have serum and nasopharyngeal mucosal immunoglobulins (IgGb) that provide them with resistance to *S. equi* infection. This passive immunity wanes at approximately 4 months of age. Vaccination of brood mares 1 month before foaling increases colostral IgG antibodies to M protein, and presumably serum and mucosal immunoglobulin concentrations in their foals, but the efficacy of this approach in preventing strangles in foals is not reported.

An **intranasal vaccine** of an avirulent live strain of *S. equi* has recently been developed and appears useful. Use of the intranasal modified live vaccine can result in strangles caused by the vaccine strain.[7] The vaccine is composed of a live variant (strain 707-27) that does not possess a capsule and is therefore avirulent when administered intranasally. Anecdotal reports suggest that recent manipulation of the genome by deletion of genes HasA and HasB, associated with formation of the capsule, has increased the genetic stability of the vaccine strain. The live attenuated vaccine should only be administered intranasally to healthy horses. The efficacy of the vaccine in field situations, safety in the face of an outbreak and in pregnant mares, incidence of adverse effects, and risk of reversion to virulence have not been reported. It should not be used in potentially exposed horses during an outbreak of the disease. Intramuscular injection of the vaccine results in the formation of abscesses. The vaccine should not be administered to horses concurrently with intramuscular administration of other vaccines because of the risk of contamination of needles and syringes with *S. equi* vaccinal strain and subsequent development of abscesses at injection sites.

An experimental modified live vaccine administered intramuscularly to ponies conferred protection to experimental challenge.[27]

Vaccination by **submucosal** injection of a modified live vaccine is reported to provide short-lived (90-day) immunity to disease. The commercial form of the vaccine is administered into the submucosal tissues of the upper lip and is recommended for use in horses at moderate to high risk of developing strangles. At present there is no evidence of reversion of the vaccinal strain to virulence, and horses developing strangles subsequent to vaccination have all been infected with virulent strains of *S. equi,* apparently before

development of immunity as a result of vaccination. The vaccine appears to be safe for use in pregnant mares.[26]

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GLANDERS

SYNOPSIS

Etiology *Burkholderia mallei*

Epidemiology Contagious disease of solipeds (equids) and possibly camels. Important potential zoonosis.

Clinical findings Acute or chronic form, and characterized by pneumonia and nodules or ulcers in the respiratory tract and on the skin. The disease is highly fatal.

Clinical pathology Complement fixation test, mallein test, isolation of organism

Necropsy findings Extensive bronchopneumonia in acute cases. Miliary nodules in internal organs and ulcerated nodules in skin and respiratory tract.

Treatment and control Control is by slaughter of clinically affected and carrier animals detected by serologic or mallein tests. Rarely are affected animals treated, and if so it is by prolonged administration of antimicrobials.

ETIOLOGY

Burkholderia mallei, a gram-negative bacterium, is the causative organism of glanders. It has close genetic and antigenic relatedness to *Burkholderia pseudomallei.* Isolates of *B. mallei* recovered from three continents over a period of 30 years have identical allelic profiles, but phylogenetic determination of strains can be achieved using molecular diagnostic techniques (for example, next-generation whole-genome sequencing and multiple-locus variable-number tandem repeats).[1-3] Determination of phylogenetic relationships is a powerful tool for determining the source, and epidemiologic characteristics, of outbreaks of the disease.

The only natural hosts of the organism are equids, with infection in other species being a result of transmission from infected equids. Humans in close contact with affected equids can be infected and develop an often fatal disease. Infection in humans is also caused through inadvertent exposure in laboratories. The organism is considered a category B biothreat (biologic warfare agent) by the Centers for Disease Control in the United States.[4,5]

EPIDEMIOLOGY

Geographic Occurrence

Glanders is restricted geographically to South America, eastern Europe, Asia Minor, Asia, and North Africa. Recent cases in Western Europe (Germany) are reported in horse imported from Brazil,[6] where the disease is present,[3] and in another horse, some years later, that was born in Germany.[7] These cases highlight the need for vigilance in detection of glanders.[6] Occurrence of outbreaks of the disease since 1986 is cataloged and available.[8] The disease has reemerged, or at least been detected, recently in India and Pakistan.[2,9,10] An outbreak in Bahrain was attributed to multiple introductions of infection, rather than simply one source.[1]

The disease was more widespread but has been **eradicated** from most countries. Glanders was an important disease when there were large concentrations of horses in cities and armies, but now has sporadic occurrence, or occurs in localized outbreaks, even in infected areas.

Host Occurrence

Horses, mules, and donkeys are the species usually affected. The disease can occur naturally in camels, although the number of reported cases is low, suggesting that camels are not particularly susceptible to infection.[11]

Humans are susceptible and the infection is often fatal. Carnivores, including lions can be infected by eating infected meat and infections have been observed in sheep and goats.

Source of Infection and Transmission

B. mallei is an obligate parasite and is readily destroyed by light, heat, and the usual disinfectants and is unlikely to survive in a contaminated environment for more than 6 weeks.

Infected animals or **carriers** that have made an **apparent recovery** from the disease are the important sources of infection. Carriers can be clinically normal and shed the organism for years. **Chronic nodular lung lesions**, which have ruptured into the bronchi, infect upper airway passages and nasal or oral secretions. Spread to other animals occurs mostly by **ingestion**, the infection spreading on fodder and utensils, particularly **communal watering troughs**, contaminated by nasal discharge or sputum. Rarely the cutaneous form appears to arise through contamination of skin abrasions by direct contact or from harness or grooming tools. Spread by inhalation can also occur, but this mode of infection is probably rare under natural conditions.

Experimental Reproduction

An experimental model for disease has been reproduced by intratracheal inoculation of horses with cultures of *B. mallei.* Horses showed fever within 24 to 48 hours of challenge followed by the progressive development of signs of respiratory distress with epistaxis and purulent nasal and ocular discharge. On postmortem there was lymphadenopathy, ulcerative lesions in the nasal septa, and pneumonia.

Host and Pathogen Risk Factors

Horses tend to develop the chronic form, **mules and donkeys** the acute form, but all types of equid and all ages are susceptible. The disease is more likely when animals are in a **stressed state** from heavy work, and animals that are poorly fed and kept in a poor environment are more susceptible.

The stress associated with movement of a large number of horses can precipitate an outbreak with high mortality rates. In the few animals that recover, there is a long convalescence with the frequent development of the **"carrier"** state. Animals rarely make a complete recovery.

Economic Importance

The disease has little current economic importance, although the threat of horse movement reintroducing glanders into countries that have eradicated it is a concern.

Zoonotic Implications

Although humans are not highly susceptible, the infection can gain access through skin abrasions to produce granulomatous disease and pyemia. Infection can also occur from inhalation of infectious material. The case fatality is high. Horse handlers in general are at risk, and veterinarians conducting postmortem examinations without proper precautions are at particular risk. The organism is identified as a possible agent of bioterrorism.

PATHOGENESIS

Invasion occurs mostly through the intestinal wall and a septicemia (acute form) or bacteremia (chronic form) is set up. Localization always occurs in the lungs but the skin and nasal mucosa are also common sites. Other viscera can become the site of the typical nodules. Terminal signs are in the main those of bronchopneumonia or, in acute disease, chronic wasting.

CLINICAL FINDINGS

Acute Disease

Acute disease presents with high fever, cough, and nasal discharge, with rapidly spreading ulcers appearing on the nasal mucosa and nodules appearing on the skin of the lower limbs or abdomen. Death as a result of septicemia occurs in a few days.

Chronic Disease

The disease is evident as fever, inappetence, weight loss, enlargement of submandibular lymph nodes, and exercise intolerance in almost all affected horses. Cough, dyspnea, and nasal discharge occur in approximately two-thirds of cases, and greater than 70% of cases have ulcers on the nasal septum or nodules and ulcers in the skin, usually of the legs.[12]

Three major manifestations are described, although one or more of all three can occur in the same animal:

1. Pulmonary
2. Skin
3. Nasal, although the chronic nasal and skin forms commonly occur together.

Pulmonary Form of Disease

The **pulmonary** form manifests as a chronic pneumonia with cough, frequent epistaxis, and labored respiration.

Nasal Form of Disease

In the **nasal form**, lesions appear on the lower parts of the **turbinates** and the cartilaginous **nasal septum**. They commence as nodules (1 cm in diameter), which ulcerate and may become confluent. In the early stages there is a serous nasal discharge that may be unilateral and that later becomes purulent and blood stained. Enlargement of the submaxillary lymph nodes is a common accompaniment. On healing, the ulcers are replaced by a characteristic **stellate scar**.

Skin Form of Disease ("Farcy")

The **skin** form is characterized by the appearance of subcutaneous nodules (1-2 cm in diameter), which soon **ulcerate** and discharge pus of the color and consistency of dark honey. In some cases the lesions are more deeply situated and discharge through fistulous tracts. Thickened **fibrous lymph vessels** radiate from the lesions and connect one to the other. Lymph nodes draining the area become involved and may discharge to the exterior. The predilection site for cutaneous lesions is the medial aspect of the hock, but they can occur on any part of the body.

Animals affected with the chronic form are usually ill for **several months**, frequently showing improvement but eventually either dying or making an apparent recovery to persist as occult cases.

CLINICAL PATHOLOGY

Chronic disease caused anemia and a moderate leukocytosis and neutrophilia.[12]

The principal tests used in the diagnosis of glanders are demonstration of presence of the organism by culture or detection of specific DNA (such as by PCR testing),[13] the mallein test, or one of various serologic tests—complement fixation test,[14,15] C-ELISA, immunoblot,[16] Rose Bengal test, indirect hemagglutination, agar-gel immunodiffusion, indirect fluorescent antibody testing, counterimmune electrophoresis, and dot-ELISA.[8] Details of test procedures are available in the *OIE Manual* on diagnostic tests and vaccines.[17]

All serologic tests are dependent on the host mounting an immune response to infection. Detectable immune responses might require a period of up to 2 weeks after infection to develop to the stage where they are detectable. The precise time depends on host factors and the characteristics of the particular serologic test.

The intent of testing affects the test chosen for use. Tests intended to screen horses for international travel must have a high sensitivity, to avoid false negative results, but also high specificity, to ensure that there are few false-positive results. From the point of international movement of horses, tests should first have a high sensitivity to ensure that there are few false-negative results—with the potential for consequent transportation of infected animals—whereas detection of diseased animals in populations of horses in which the disease is rare demands tests with high specificity. The solution is often to first screen with tests of high sensitivity, such as the complement fixation test, followed by a test with much higher specificity (but often lower sensitivity), such as immunoblotting.[16] The outcome of such serial testing is a high sensitivity and specificity. The diagnostic performance of various tests has improved with use of refined reagents (including use of recombinant or purified bacterial proteins or lipopolysaccharide,[16] or antibodies) and optimized tests conditions,[18] such as the temperature at which complement fixation tests are incubated.[15] All serologic tests can be inaccurate for periods up to 6 weeks following performance of the mallein test.

Molecular diagnostic techniques must discriminate between *B. mallei* and the closely related *B. pseudomallei.* Whereas older molecular diagnostic tests did not do so because of the close genetic relationship between these organisms, more modern tests do discriminate at a level that is clinically useful.[13,19]

Discussion of all the currently available tests is beyond the scope of this text, and readers are referred to recent publications.[5,8]

Mallein Test

The test is not generally recommended because of animal welfare concerns; however, it can be useful in remote endemic areas where sample transport or proper cooling of samples is not possible.[17] The mallein test involves the intradermal injection of mallein, a purified or semipurified protein of *B. mallei,*[20] into the subcutaneous tissues of the eyelid or lateral side of the neck. Mallein (0.1 mL of a 1.0 mg/mL concentration of mallein) is injected intradermally with a tuberculin syringe. Ideally, the thickness of the skin is measured using calipers before injection of mallein and 48 hours following injection. Some infected animals exhibit a general hypersensitivity reaction after inoculation. The mallein test can be negative in recently infected animals, in those with acute disease, and in advanced cases in horses.[17]

The mallein test has poorer sensitivity (~75%) than does serologic testing (Rose Bengal—90%, complement fixation—97%, and others).[21]

NECROPSY FINDINGS

In the **acute** form there are multiple petechial hemorrhages throughout the body and a severe catarrhal bronchopneumonia with enlargement of the bronchial lymph nodes.

In the more common **chronic** form, the lesions in the lungs take the form of **miliary nodules**, similar to those of miliary tuberculosis, scattered throughout the lung tissue. **Ulcers** are present on the mucosa of the **upper respiratory tract**, especially the nasal mucosa and to a lesser extent that of the larynx, trachea, and bronchi. Nodules and ulcers may be present in the **skin and subcutis** of the limbs, which may be greatly enlarged. Local lymph nodes receiving drainage from affected parts usually contain foci of pus and the lymphatic vessels have similar lesions. Necrotic foci may also be present in other internal organs. *B. mallei,* and sometimes *Arcanobacterium pyogenes,* are isolated from infected tissues, and this is the main means of confirmation of diagnosis at necropsy.

DIAGNOSTIC CONFIRMATION

In live animals that could be carriers, the complement fixation test is used as the official test in most countries. The mallein test is used in those horses whose sera is anticomplementary.

DIFFERENTIAL DIAGNOSIS

- Epizootic lymphangitis
- Ulcerative lymphangitis
- Sporotrichosis
- Melioidosis
  - Strangles
  - *Rhodococcus equi* infection
  - Equine pleuropneumonia
- Other causes of pneumonia

TREATMENT

There is little information on treatment because control of the disease requires death of affected equids to prevent further spread of infection, and the granulomatous nature of the disease likely requires prolonged administration of antimicrobials capable of penetrating abscesses. Antimicrobial sensitivity of *B. mallei* isolates is reported.[22]

However, in instances in which high-value animals are treated, a treatment protocol of enrofloxacin (8 mg/kg IV q24 h) and trimethoprim–sulfadiazine (32 mg/kg IV q24h) for 7 days, followed by enrofloxacin (4 mg/kg IV q24h) and trimethoprim–sulfadiazine (16 mg/kg IV q24h) for 2 weeks, and then 6 mg/kg doxycycline PO q12h for 9 weeks has been used. Treated horses responded within 1 week to treatment, with reduction of pyrexia and improved appetite. Nodules on the legs had resolved by week 3 of treatment. All 23 treated horses recovered and did not have evidence of disease recrudescence or a carrier status 1 year after the cessation of treatment.[12]

CONTROL

Control of glanders involves measures to reduce spread of the disease among equids in areas where the disease is endemic and eradication of the disease when desired or when the disease occurs as an emergency disease outbreak in areas where the disease is not endemic.

Control of glanders is based on identification of infected animals by either serologic testing, intradermal mallein testing, or detection of the organism (culture or PCR) (see previous discussion). The mallein test and complement fixation test are the OIE-approved tests for glanders for the purposes of international movement of horses—noting the comments given previously about the characteristics of these tests. When attempting to identify infected animals, the delay in seroconversion or development of a positive mallein test after infection should be considered. Mallein testing can influence the sensitivity of subsequent serologic testing.

If glanders is detected, or suspected, in area free of the disease, then the affected horse and contact animals should be promptly quarantined until their disease status has been established. Eradication of the disease involves identification of infected animals with subsequent euthanasia and controlled disposal of these equids. Equids that could have been infected but that are negative on serologic or bacteriologic testing should have serologic tests repeated in 2 to 3 weeks. During this time, they should be quarantined.

Complete quarantine of affected premises is necessary. A vigorous disinfection program for food and water troughs and premises generally should be instituted to prevent spread while eradication is being carried out. Carcasses of infected animals and contaminated or potentially contaminated bedding, feed, and tack that cannot be disinfected should be burned or deeply buried, consistent with local culture and laws. *B. mallei* is susceptible to most common disinfectants, including benzalkonium chloride, 1% sodium hypochlorite, 70% alcohol, and others.[5] *B. mallei* does not persist in soil and is destroyed by exposure to sunlight or heating (>55°C >131 F for at least 10 minutes).[5]

B. mallei is a potential zoonosis that can cause severe illness and death in people. Barrier precautions, including the wearing of surgical masks, face shields, gloves, and gowns, are strongly recommended for people dealing with infected or suspect equids.[5]

There is currently no vaccine for glanders in animals or people.[17]

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VIRAL INFECTIONS OF THE RESPIRATORY TRACT OF HORSES

Viral respiratory tract disease is considered by veterinarians in the United States to be second only to colic among medical diseases in importance to the health and welfare of horses. The situation is likely similar in most developed countries and especially those in which equine influenza is endemic. Episodes of upper respiratory tract disease characterized by fever, nasal discharge, and cough are common in horses, especially young animals and horses housed in groups in stables and barns. An estimated 17% of equine operations in the United States have one or more horses affected by upper respiratory disease each year, and 1.5% of horses develop the disease every 3 months.[1] Upper respiratory disease is most common in spring and least common in winter. Strangles was an uncommon cause of disease, occurring in only three horses per 1000 per 3 months. Viral respiratory disease is approximately three times more common in horses less than 5 years of age.

With the exception of *Streptococcus equi* and possibly *Mycoplasma* spp., all the other known or suspected causes of nonparasitic infectious upper respiratory disease of horses are viral and include the following: equine herpesvirus types 1, 2, 3 (rarely), and 4; equine influenza virus; arguably, equine rhinitis virus types A-1 and B-1, 2, and 3; equine adenovirus; equine viral arteritis; and, historically, equine parainfluenza type 3 virus. Equine Hendra virus and African horse sickness cause signs of severe respiratory disease. There is minimal evidence that equine coronavirus causes respiratory disease in horses[2,3] and there is evidence that the Middle Eastern respiratory syndrome (MERS) coronavirus, a disease of humans and camelids, does not cause disease in horses.[4] Both s. equi and equine arteritis virus infection can be mild and lack outstanding clinical signs, thus closely resembling disease associated with some viral causes of upper respiratory tract disease. Therefore differentiation among diseases associated with these agents based on clinical signs and epidemiologic characteristics is difficult, and definitive diagnosis is only achieved through serologic or microbiological examination of blood or nasal discharge.

Isolation and identification of a causative organism from nasopharyngeal swabs or airway washings of acutely affected horses provides a definitive diagnosis, although on occasion more than one potential pathogen may be isolated. Demonstration of **seroconversion** or a three- to fourfold increase in titer from serum samples collected during the acute and convalescent (usually 3 weeks after onset of clinical signs) phases of disease is persuasive evidence of infection. Immunofluorescence,

enzyme-linked immunosorbent assay (**ELISA**) and polymerase chain reaction (**PCR**) tests may provide rapid diagnosis through detection of viral particles in nasal swabs and tissue specimens. The ability to determine the cause of an outbreak of upper respiratory disease in horses is enhanced by the use of multiple diagnostic tests and obtaining samples from more than one horse in an outbreak. However, definitive diagnosis of the cause of nasal discharge, cough, and fever is often not achieved.

All the agents known to cause upper respiratory disease in horses are relatively sensitive to environmental influences, and spread of the agent is dependent on **transmission from infected horses**, either directly or on fomites. Introduction of an infected horse into a susceptible population of horses may result in an explosive outbreak of upper respiratory tract disease. Such events are common on stud farms and in racing stables, where relatively closed bands of horses are maintained for much of the year. The movement of horses over long distances may facilitate the introduction of pathogens to which the local population of horses is naive.

The opposite situation occurs when young horses are introduced into larger bands of mixed aged animals, such as happens in racing stables or barns of pleasure horses. The younger, possibly naive, horse is then exposed to endemic pathogens to which the resident horses have developed resistance.

Young horses are at particular risk of developing infectious disease of the upper respiratory tract. The diseases are usually a problem only in yearlings and 2-year-olds; young foals acquire a passive immunity from the dam and adults have acquired a permanent immunity through exposure or vaccination. In a horse population it is the average age and the mix of ages that largely determine its herd resistance, and when 30% to 40% of that population has not previously been exposed to infection then major outbreaks are likely. All of the diseases are transmitted by droplet infection, and over long distances, so that limitation of their spread is possible only by rigid isolation and intensive sanitary precautions, and even the best protected studs are likely to be infected from time to time.

Parainfluenza-3 Virus

Upper respiratory tract disease associated with equine parainfluenza-3 (PI-3) is characterized by a mild self-limiting disease that is not clinically distinguishable from the others in the group. The epidemiology and economic importance of disease associated with this agent is unknown.[1]

Equine Adenovirus Infection

Two antigenic types of equine adenovirus, EAdV-1 and EAdV-2, are recognized that have been associated with **respiratory disease** in foals and adult horses and **diarrhea** in foals, respectively.[1] The virus causes fatal pneumonia in Arabian foals, and likely Fell pony foals, with severe combined immunodeficiency and has been isolated from otherwise apparently healthy foals with severe pneumonia, but its importance in clinical respiratory disease of immunocompetent foals is uncertain. EAdV-1 can be isolated or detected by PCR from healthy adult horses, although at a very low rate,[5,6] and from 1% to 3% horses with signs of upper respiratory disease.[7,8] Genomic analysis of EAdV-2 indicates markedly different lineage to that of EAdV-1.[9] The virus is readily isolated from, or detected by PCR, in nasal swabs of approximately 50% of sick or healthy foals.[6] Postparturient mares can shed the virus.[6] Infection with EAdV-1 and EAdV-2 is worldwide. Serologic surveys differ in the proportion of seropositive horses, likely at least partially a result of the testing methodology, with serum neutralization tests yielding higher seropositive rates than ELISA tests.[5] Approximately 80% of horses in New South Wales, Australia are positive by serum neutralization assay for either or both of EAvV-1 or EAdV-2.[5] EAdV is considered to cause a mild respiratory disease with fever, coughing, nasal discharge, and conjunctivitis. Foals are assumed to acquire the infection from their dams, which secrete the environmentally stable virus in nasal discharge, urine, and feces. The virus is not associated with inflammatory airway disease in racehorses in England, but it has been associated with small outbreak of upper respiratory tract disease.

Diagnosis can be made on cell smears taken from conjunctiva or nasal mucosa that reveal characteristic adenoviral intranuclear inclusion bodies. **Serologic methods** include serum neutralization, hemagglutination inhibition, complement fixation, ELISA, or precipitating antibody tests. The serum neutralization test is most accurate, but the hemagglutination inhibition test is most suitable for a screening test. Virus genetic material can be detected by specific PCR testing.[5,7] No specific **control measures** are indicated for normal foals.

Reovirus

A reovirus, or a series of serotypes, cause mild upper respiratory tract disease of horses. Infection with these agents appears to be of little clinical or economic importance.

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EQUINE INFLUENZA

SYNOPSIS

Etiology Influenza virus H3N8 (previously A/equine 2) of two lineages (Eurasian and American) and numerous, evolving, strains. Currently circulating viruses are of the American lineage, Florida clades 1 and 2. H7N7 has not been identified as a cause of disease for decades.

Epidemiology Short incubation period and highly contagious nature of the virus result in explosive outbreaks of disease. Viral shedding by subclinically affected horses is important for introduction of infection to populations. Prolonged carrier state is not recognized.

Clinical signs Upper respiratory disease complicated by pneumonia. Abortion is not a feature of the disease.

Clinical pathology None characteristic.

Lesions Rhinitis, pneumonitis. Rarely causes death.

Diagnostic confirmation Demonstration of virus in nasopharyngeal swab either by culture, enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), or membrane-bound immunoassay.

Treatment Supportive care. There is no specific treatment.

Control Quarantine to prevent introduction of the virus. Hygiene and disinfection to prevent fomite spread. Vaccination in enzootic areas with vaccine containing strains or antigens protective against currently circulating strains (Florida clade 1 and clade 2), to prevent clinical disease.

ETIOLOGY

Equine influenza is associated with infection by influenza A virus—either equine **influenza A/H7N7** or **equine influenza A/H3N8** virus, members of the influenza virus A genus of the family Orthomyxoviridae. Influenza A viruses are typed according to the surface proteins—hemagglutinin (HA) and neuraminidase (NA) of which there are 18 HA subtypes (H1-H18) and 11 NA subtypes (N1-N11).[1] Influenza virus is an RNA virus that has eight segments to its genome that encode 10 proteins. The hemagglutinin and neuraminidase proteins are used for antigenic characterization of virus strains. Mutations in these genes or poor-fidelity RNA copying results in changes in amino acid composition of viral proteins that can be detected by serologic tests (see "Clinical Pathology") and that have important consequences for infectivity and pathogenicity of the virus.

Of the two serologically distinct subtypes of equine influenza virus, all reported outbreaks in the past three decades have been associated with strains of EIV-A/H3N8. There are no reports of disease associated with EIV-A/H7N7 in the past 35 years, and reports of seroconversion might be related to use of vaccines containing EIV-A/H7N7 antigen. There are no reports of other influenza viruses, such as the H1N1 avian virus, causing disease in horses, although the avian-like influenza A/I/Jilin89 (H3N8) caused severe disease and high mortality among horses in China in 1989 and there is a single report of avian H5N1 being isolated from sick horses, which also had serologic evidence of exposure to the virus, in Egypt during an outbreak of the disease in birds.[2]

Equine influenza H3N8 virus can infect dogs and cause serious disease and death.[3,4] Canine influenza virus infection, which originated in horses, is now endemic in dogs populations in much of the world.[3] Dogs are also susceptible to infection with equine influenza virus (Florida clade 1) when in close contact with horses infected with, and clinically ill from, the virus.[5] Equine influenza virus H3N8 was isolated from one of ~400 healthy Bactrian camels sampled in Mongolia.[6] The H3N8 virus can infect pigs but is not associated with disease;[7] seals, in which it can cause a fatal respiratory disease; and birds.[8,9] Experimental infection of cats with equine N3H8 influenza virus causes respiratory disease, and the infection can spread to in-contact cats.[10]

Canine influenza, N3N8, which is of equine origin, does not appear to pose a zoonotic risk.[10] At this time, equine H3N8 virus does not appear to be an important zoonotic threat.

Equine influenza H3N8 virus was first detected as a cause of respiratory disease in horses in 1963 in the United States. It subsequently became widely distributed, appearing in the United Kingdom in 1965, and evolved into multiple lineages and sublineages. There are two major lineages of EIV-H3N8 that circulate in horse populations—a Eurasian lineage and an American lineage (the names of which do not reflect the current geographic distribution of the viruses). This divergence in the virus occurred in the early 1980s, and there has been subsequent evolution of the American lineage into Kentucky, Argentinian, and Florida sublineages, with the Florida sublineage composed of two clades—clade 1 and clade 2 (Fig. 12-31).[11] For purposes of vaccine production, clade 1 is represented by A/eq/South Africa/04/2003-like or A/eq/Ohio/2003-like viruses, and clade 2 is represented by A/eq/Richmond/1/2007-like viruses.[12,13]

The predominating virus lineage or strain varies from year to year and from region to region. Both of Florida clades currently cocirculate and coevolve worldwide. Viruses of the Eurasian lineage have not been detected since 2005 (2015 OIE data).[12,14] The important point is that there is continual change in the viral lineage or strain in some populations of horses and that constant monitoring of viral strains is vital for appropriate composition of vaccines and for molecular epidemiology. For instance, the majority of viruses from Europe (France, Italy and the United Kingdom) and North America characterized antigenically and/or genetically between January 2003 and April 2004 were of the American lineage. This continues to be the case with American lineage, Florida sublineage, clade 2 being the virus detected in Europe,[15] Northern Africa,[16] Asia (India, China, Mongolia),[17-19] Ireland (before 2009), and the United Kingdom.[12,16] Within clade 2, there are at least two identified virus subpopulations with amino acid substitutions in HA1 at either position 144 or position 179. The majority of 2014 viruses characterized had a valine at position 144 and an isoleucine at position 179.[13] Equine influenza viruses detected in recent outbreaks in the United States of America have been Florida sublineage clade 1,[12] although clade 2 was isolated from a horse in California that had been imported from Europe.[20] Although clade 1 viruses predominate in America and clade 2 in Europe, clade 1 viruses have caused outbreaks in Europe (France),[15,21] Australia, Africa, and Asia, and the virus circulating in Ireland in 2014/15 is from clade 1 (although different from the virus that caused outbreaks in Australia and Japan in 2007).[22]

Viral Evolution

Identification of the lineage and sublineage of the virus is based on nucleotide sequencing of the hemagglutinin gene to detect mutations in the gene resulting in amino acid substitutions in the HA1 domain. These amino acid substitutions alter the charge, acquisition of glycosylation sites, and/or receptor binding avidity of the virus and hence its biologic activity including infectivity, immunogenicity, and virulence.[11] Hemagglutination inhibition assay (HI) has been used to type viruses, but this is now being complemented by genetic testing and determination of amino acid composition of major antigens (HA and NA). For instance, the amino acid composition of clade 1 and clade 2 viruses differs by at least seven amino acids in the HA1 domain of hemagglutinin.[23] Information about EIV strains changes constantly and is available at the equiflunet or OIE websites.[12,13]

The existence of lineages and strains of virus is important in the epidemiology of the disease because the antigenic differences among strains can be sufficient to prevent cross-protection provided by natural infection or vaccination. Cross-protection refers to the ability of one antigen (virus strain) to produce immunity in the horse against infection with another type of antigen (virus strain). Infection or challenge with the same type of antigen is referred to as homologous challenge, whereas that with a different antigenic type is referred to as heterologous challenge. Strains of influenza virus circulate between and among populations of horses, with more than one strain of virus circulating at any one time in some horse populations, although individual disease outbreaks are associated with a single viral strain. Many, but not all, of these virus strains are constantly evolving, and evolution of the viruses is necessary for perpetuation of cycles of infection through the emergence, or reemergence by cycling, of heterologous strains. Evolutionary stasis, the continued circulation of older strains of virus, occurs and has importance for vaccine composition for many diseases, but not, apparently, for equine influenza virus (EIV), where emergence of new strains is common and of great importance for control of the disease. Evolution of strains of equine H3N8 virus occurs through antigenic drift. **Antigenic drift**, the accumulation of point mutations in the gene coding for the major surface protein hemagglutinin, occurs continuously in virus circulating in horse populations. Antigenic drift occurs most rapidly in hemagglutinin protein but also occurs in M and NS genes. Antigenic drift, by producing heterologous viral strains, contributes to the continuing susceptibility of horses to infection and the reduced efficacy of some vaccines.[11] For example, the 2007 outbreak of equine influenza in Japan in a population of vaccinated horses, was associated with the Florida clade 1 virus, whereas the vaccines in use at that time included viruses of Eurasian and American (Argentinian) strains.[24]

Antigenic shift is an event in which there is a dramatic alteration in the viral genome occurring by reassortment of viral genes during coinfection of a cell by two different types of virus (for example infection of a pig by both avian and human influenza viruses). Antigenic shift, which has not been documented for influenza viruses infecting horses, has the potential to produce new viruses with markedly different host infectivity and pathogenicity to either parent virus.

RNA viruses, such as equine influenza virus, are genetically labile, and during an outbreak there is considerable genetic variation of the viruses infecting a single animal, with dominant and one or more less dominant variants of a strain proliferating in the horse and being transmitted to other horses.[25,26] Furthermore, the dominant form of the virus within a horse can change over the course of the infection. This pattern of multiple variants infecting one horse and being transmitted to other horses results in a relatively large number of variants of the virus in a group of horses during an outbreak, constituting a loose bottleneck to viral evolution.[25,26]

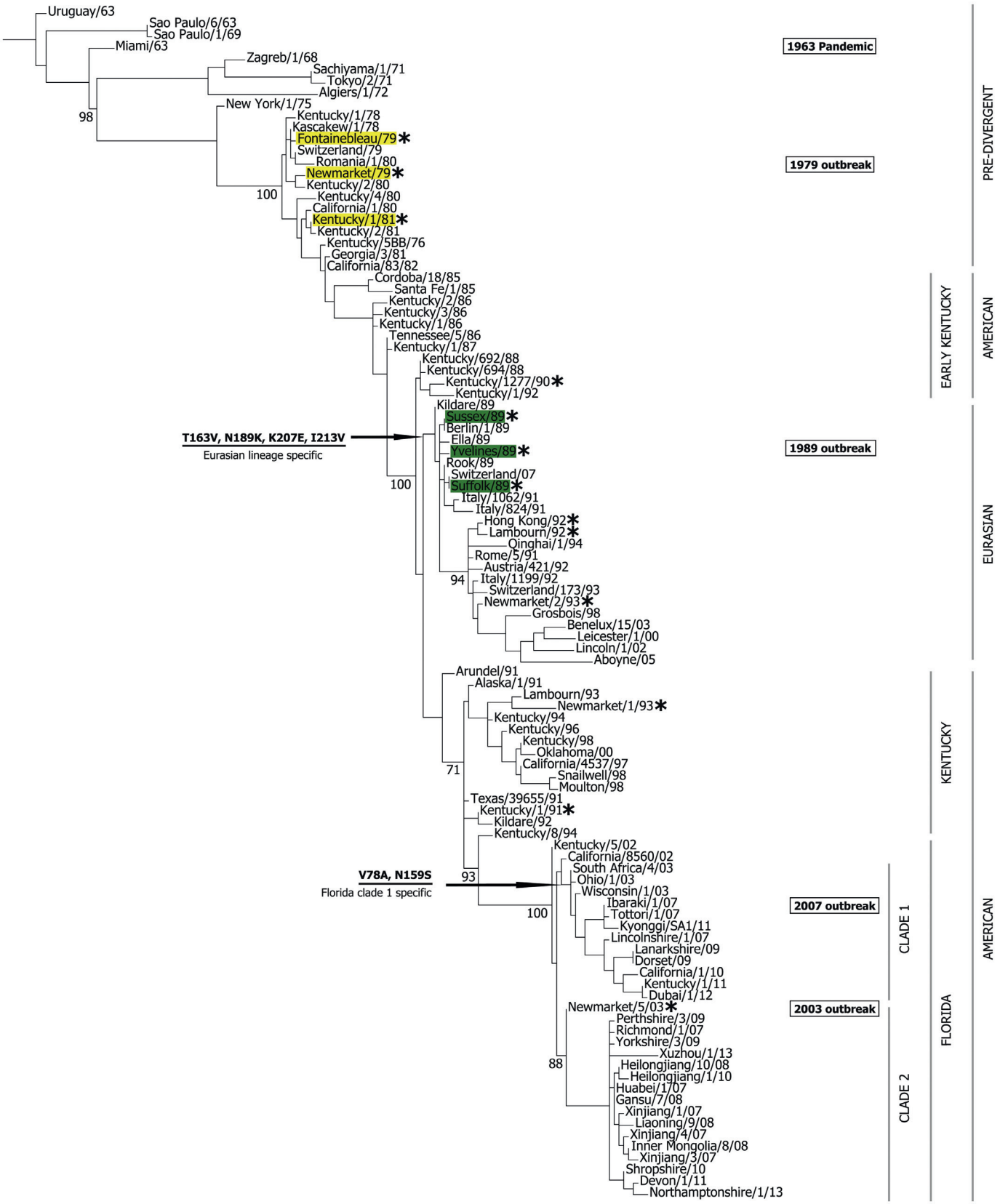

Fig. 12-31 **Phylogenetic tree illustrating evolving nature of equine influenza virus N3H8 from the virus originally detected in 1963. Note that there are two lineages (Eurasian and American) that diverged in the early 1980s; that the American lineage further evolved into Kentucky, Argentinian, and Florida sublineages (clades 1 and 2); and that these continue to evolve. Strains causing substantial outbreaks are highlighted in green, dates of large outbreaks are identified in the boxes, and vaccine strains are identified in yellow.** (Reproduced with permission.[11])

Persistence in the Environment

According to the Ausvet Plan, equine influenza virus is inactivated by exposure to ultraviolet light for 30 minutes, by heating at 50° C (122 F) for 30 minutes, and by ether and acid (pH 3) treatment.[27] Exposure to sunlight for 15 minutes at 15° C (59 F) also inactivates the virus. The virus persists in canal water (pH 6.9) for up to 18 days at 22° C (72 F) and 14 days at 37° C (98.6 F); in tap water (pH 7.0) for 14 days at 4° C (42 F) and up to 2 days at 37° C (98.6 F); in horse blood for 18 hours at 37° C (98.6 F); in horse urine (pH 8.0) for 5 to 6 days at 4° C (42 F), 15° C (59 F), and 37° C (98.6 F); in soil under dark storage at 18° C (65 F) for 24 hours; and in soil exposed to sunlight for 8 hours at 15° C (59 F).[27] The capacity for the virus to persist in carcasses is unknown.[27]

EPIDEMIOLOGY

Occurrence

Worldwide, the only large horse populations in which **influenza virus infection** does not occur are in Australia and New Zealand, although Australia experienced its first outbreak of equine influenza in 2007 and subsequently was declared free of the virus.[28,29] Widespread use of aircraft to move horses between countries in short periods has increased the spread of equine influenza viruses, as exemplified by the 2007 outbreak in Australia allegedly associated with importation of horses and failure to contain the infection in the quarantine facility;[30] the 2003 outbreak in South Africa associated with a virus from North America;, and an earlier outbreak in Hong Kong. In all cases virus was introduced by imported horses.

Epidemics of equine influenza have occurred in Europe or North America in 1956 (H7N7), 1963 (H3N8), 1969, 1979, and 1989, although this does not represent a comprehensive listing of large-scale outbreaks. Epidemics affecting more than 1 million horses occurred in China in 1989 (associated with the novel H3N8 Jilin virus) and 1993/1994 (associated with a conventional H3N8 virus closely related to 1991 European isolates). Epidemics in Japan, Europe, and North America have been associated with introduction of a novel virus (for example, the 1963 appearance of H3N8 virus in Miami) or antigenic drift of existing viruses and resultant inefficacy of extant vaccines.[14,31,32] The epidemics in Australia (2007) and South Africa (2003) were associated with introduction of the virus into a naïve and unvaccinated population of horses.[30]

Localized outbreaks of disease in stables or race courses occur almost annually in countries in which the disease is endemic, likely related to the movement of horses into the training and racing populations, with subsequent introduction of virus and development of disease in at risk horses (see "Animal Risk Factors"). Disease associated with equine influenza virus usually occurs as outbreaks associated with the introduction of virus into a population of susceptible horses. Virus may be introduced by clinically affected horses or, more commonly, by horses that are not noted to be clinically ill. Vaccinated horses can become infected and shed influenza virus while not becoming ill,[33] especially if vaccinated with heterologous strains, and this is likely a common method of introduction of virus into susceptible populations.

Outbreaks of influenza virus infection can cause clinical disease in nearly all (98%) horses in a susceptible population, although in populations of horses of mixed age and with varying serum titers to equine influenza the morbidity rate can be much lower (16%-28%). The incidence of disease in one race track population was approximately 130 cases per 1000 horses at risk per month, although this rate likely varies widely among outbreaks. The mortality rate is usually very low (<1%) with most deaths associated with secondary bacterial infections. However, an outbreak of disease in China associated with a novel H3N8 (Jilin virus) strain was associated with a morbidity rate of 80% and mortality rate of 20% to 35%.

The disease in populations of vaccinated or previously exposed horses is associated with a lower morbidity and mortality and slower spread, as a result of the milder disease induced by influenza virus infection of immune or partially immune horses. In an outbreak among vaccinated racehorses in Hong Kong, 75% of horses had serologic evidence of infection, 37% had clinical signs of infection, and 0.2% died. Horses imported from Australia and New Zealand, where the disease does not occur, had a morbidity rate of 52%, whereas horses from the northern hemisphere had a morbidity rate of 20%, likely reflecting the effect of previous exposure to influenza virus or repeated vaccination. The attack rate of infection (proportion of at risk horses infected by the virus) during an outbreak in a large population of naïve horses in Australia was ~98% (mean) varying from 10% to 100%, with farms with lower numbers of horses generally having a higher attack rate.[34] The mortality rate attributable to equine influenza was 0.6% and case-fatality rate 6%, based on a questionnaire of more than 1200 horse owners in Australia during the influenza outbreak (46% return rate) that involved self-reporting and was therefore at risk of sampling bias.[35] The mortality rate appeared to be highest among foals (68% of reported deaths).[35]

The profile of an epidemic can vary from explosive with a large proportion of a small group of susceptible horses housed in close proximity, such as a small band, developing clinical disease within 24 to 48 hours, to much more prolonged outbreaks lasting several weeks in larger groups of horses of varying susceptibility housed in multiple barns. During larger outbreaks among horses of varying susceptibility there is a characteristic three phase pattern. The first stage is associated with the first cases of disease and slow spread over 10 to 14 days. This stage is followed by one of rapid spread of the disease to horses clustered in stalls around horses affected during the first phase of the outbreak. The third phase is characterized by declining numbers of cases.

Origin of Infection and Transmission

Equine influenza virus is relatively susceptible to environmental conditions, and during an outbreak infection must originate from an infected horse, although the proximate source of virus can be contaminated equipment or other fomites, including people. **Transmission** of equine influenza virus occurs by direct contact, inhalation of aerosols of infected material, and on fomites. Survival of the virus on clothing and surfaces, including vehicles used to transport horses shedding the virus, can result in transmission of infection in the absence of horse to horse contact. **Fomite transfer** on veterinary clothing, equipment, or vehicles was likely responsible for the spread of infection from quarantined horses in both South African outbreaks. However, in most instances, horses are infected by other horses that are in close proximity or have physical contact, for instance, exercise ponies (horses or ponies used to accompany racehorses from the stable to the track in preparation for racing or training gallops) or stable mates. Aerosol spread occurs over distances of 35 m, possibly further (see "Meteorologic factors" on this page), and is enhanced by the frequent coughing characteristic of the disease. Equine influenza virus in aerosols survives longer (24–36 hours) than human or porcine strains (15 hours).

Clinically affected horses excrete more virus than do horses in which the infection is inapparent. The duration of infectivity of clinically affected horses is 3 to 8 days and with the short incubation period of 2 to 3 days combine to produce the potential for a very rapid new infection rate and a characteristic explosive outbreak.

Risk Factors

Animal Factors

All age groups of horses, including newborn foals, are susceptible. The greatest risk appears to be between the ages of 2 and 6 months, serum levels of passively acquired antibodies being lost by foals at 2 months of age. A recent survey of more than 8000 horses in the United States revealed that only 20.2% of horses aged 6 to 17 months had a detectable influenza antibody titer (HI), compared with 89.0% of horses aged 20 years or more. The percentage of horses that had a high equine influenza antibody titer increased as the horse's age increased such that 45% to 51% of horses older than 5 years had high titers. This observation is consistent

with most cases of the disease occurring in 2-year-old or younger horses, probably because older horses are immune through either natural exposure or vaccination. Thoroughbred racehorses 2 years of age or older were 5 to 8 times more likely to develop influenza than were horses 5 years of age in a well-characterized series of outbreaks. Seronegativity to a H3N8 virus (Saskatoon/90) was associated with a 13- to 38-fold increase in likelihood of developing influenza, independent of the effect of age. It is probable that outbreaks occur as a result of a natural accumulation of young animals that have not been previously exposed, the comingling of these susceptible animals with older infected ones at race and show meetings, and the significant level of antigenic "drift." This capacity of the virus to change slightly and continuously in antigenic composition leads to the frequent appearance of new strains that are likely to breach existing natural and induced immunologic barriers.

Outbreaks can occur at any time of year, and their timing probably depends on husbandry and management practices, such as yearling sales, transport of horses for racing and sale, and movement of show and breeding animals. These events often provide the combination of a population of susceptible animals housed in crowded, poorly ventilated barns that facilitate transmission of the virus.

Immunity depends on the means of exposure (vaccination or natural infection), the strain of the virus, and the time since exposure. After infection, protective immunity to homologous strains of the virus is present and persists for 1 year, possibly up to 2 years. Field studies of disease outbreaks indicated that the concentration of antibodies in serum that provide some resistance to disease might be less than that suggested from experimental studies. Protective immunity induced by natural infection is characterized by production of IgA in nasal secretions and IgGa and IgGb in serum, whereas administration of an inactivated, alum-adjuvanted commercial vaccine induces only a serum IgG(T) response that is not protective against challenge. Immunity after vaccination lasts for a much shorter period of time, 3 to 4 months, and is specific for the subtypes, and their strains, of virus included in the vaccine. Immunity following infection or vaccination is less protective against infection by a heterologous strain. Similarly, vaccination exposure to a heterologous virus may induce only a poor anamnestic immune response. These observations are consistent with the concurrent circulation of multiple viral strains influenza virus in horse populations and the cycling of virus strains causing disease in consecutive years.

Management Factors

Housing of large numbers of horses in close contact, or in enclosed environments such as large barns or stables, provides optimum conditions for facilitating contact and aerosol spread of the virus. Shed barns, which characteristically have poorer ventilation and greater stocking density than pole barns, are associated with a fourfold increase in risk of influenza.

Presence of small numbers of horses with access to large numbers of at risk horses might affect the course of an epidemic. Track ponies, which have close contact with large numbers of horses on a daily basis, are important in spread of influenza in racing barns.

Meteorologic Factors

The influence of weather and wind on spread of equine influenza has not been extensively investigated. During the outbreak in Australia, the hazard of equine influenza infection was higher when relative humidity was less than 60% and lowest on days when daily maximum air temperature was 20° to 25° C (68 to 77 F).[36] The increased risk of spread at lower relative humidity is mediated by both virion and aerosol droplet nuclei stability.[36] In cool, dry conditions, droplets are desiccated and remain small, which may stabilize influenza aerosols and facilitate longer range transmission, whereas at high relative humidity, the droplets absorb water and settle, thereby decreasing the time aloft available for dispersion by wind.[36]

There was a relationship between the direction of **prevailing winds** and spread of equine influenza infection during the 2007 outbreak in Australia. There was a clear trend for appearance of newly infected premises to occur to the west of previously infected premises, consistent with predominant wind patterns.[37] It is likely that wind carriage of the virus facilitated dispersal of infection. In a cluster of 437 infected premises, 81% were not contiguous to a previously infected premise and the mean distance from newly infected premises to the closest previous infected premises was 0.85 +/– 1.50 km, with a range of 0.01 to 12.94 km.[37] Wind speeds greater than 30 km per hour from the direction of nearby infected premises were associated with increased hazard of infection.[36] At wind speeds of greater than 30 km per hour, an aerosol of influenza droplet nuclei would only need to be stable for minutes to be able to infect horses on nearby premises.[36] This is consistent with spread of equine influenza virus over 1 to 2 km, or possibly up to 13 km, via wind-borne aerosol.[37,38]

Economic Importance

Influenza causes minimal loss through death of horses, but it causes much inconvenience in racing stables because it occurs in explosive outbreaks and affected horses have to break training. Such outbreaks have the capacity to close down the racing industry in a country for a period of months. An additional cost is incurred because of restrictions on international movement of horses and associated quarantine periods.

Zoonotic Potential

There is evidence that humans can be infected by equine influenza virus H3N8, especially among individuals working with horses, although such seroconversion appears to be uncommon.[39-41]

AUSTRALIAN OUTBREAK (2007)

Australia experienced its first ever outbreak of equine influenza in 2007 as a result of equine influenza in horses in a quarantine station and a breach in the quarantine of these infected imported horses.[42,43] Equine influenza virus H3N8, American subtype, Florida clade 1 lineage, with an HA sequence identical to that of a virus isolated from a contemporaneous outbreak in Japan,[44] was introduced into a population of horses that were naïve to the infection and unvaccinated for the disease. Over the course of 4 months, nearly 70,000 horses were infected on over 9,000 premises in New South Wales and Queensland, with a mean attack rate of 98%.[34] In the first 10 days of the equine influenza outbreak in Australia, horses on 197 premises were infected.[38] Timely and complete implementation of a horse movement ban ("standstill") is widely credited as the most effective of the control measures that facilitated the rapid eradication of this disease from the Australian horse population. The effectiveness of this ban was impressive: of 1052 horse movements in a contact-tracing data set, 978 occurred during the first 10 days of the epidemic.[38] Vaccination was introduced in an attempt to control spread of the disease, but this began 6 weeks into the outbreak, well after the peak of reported daily infections.[45] Modeling indicates that vaccination could have contributed to abbreviating the duration and reducing the geographic size of the outbreak by 8% to 9%.[45]

A comprehensive series of articles describing aspects of this epizootic is available.[5,28-30,34,35,37,38,43-98]

PATHOGENESIS

The disease is principally one of inflammation of the upper respiratory tract, although pulmonary lesions are common in adult horses, and the disease can cause severe, fatal pneumonia in foals. The virus is inhaled and attaches to respiratory epithelial cells with its hemagglutinin spikes, fuses with the cell, and is released into the cytoplasm, where it replicates. New virions are released from the cell surface and infect other cells or are expelled into the environment. Initial viral infection and replication occurs mainly in the nasopharyngeal mucosa, but by 3 to 7 days after infection, virus can be recovered from cells throughout the respiratory tract. Infection of the respiratory mucosa results in death of epithelial cells, inflammation, edema, and loss of the protective mucociliary clearance.

Death of cells is a result of influenza virus-induced apoptosis of respiratory epithelial cells and local and systemic increases in interferon and interleukin-6. Proliferation by opportunistic bacteria, commonly *Streptococcus zooepidemicus,* occurs because of the disruption of normal clearance mechanisms and can exacerbate the inflammation and cause bronchopneumonia. Viremia, if it occurs, is mild and brief, although it may be related to some of the systemic signs of the disease. Some speculate that myocarditis, myositis, and encephalitis occur occasionally in response to influenza virus infection, but definitive proof is lacking and was not evident in horses in the Australian epizootic[97] or in ponies experimentally infected with equine influenza virus.[99] Influenza virus has not been isolated from tissues other than those of the respiratory tract. Enteritis was reported in horses in the 1989 Chinese outbreak (Jilin/89), but is not reported for disease associated with conventional virus strains.

CLINICAL FINDINGS

Outbreaks of equine influenza are characterized by a sudden onset and rapid spread of disease. Typically, in a large group of susceptible horses the incidence of the disease peaks about 1 week after the first case is noticed, and new cases do not develop after 21 to 28 days. The disease may have an attenuated clinical course in a population of vaccinated or previously exposed horses, and there is evidence that disease severity varies widely among even naïve horses, with some horses having severe signs of disease and others having no clinical evidence of infection.[71,78,90,92]

The mild disease in immune animals may be clinically indistinguishable from upper respiratory diseases associated with other common agents such as EHV-4, equine rhinitis virus, and arteritis virus.

Clinically, the disease starts with a fever (38.5–41°C [101–106°F]) after an incubation period of 24 to 72 hours. Horses may be depressed, refuse feed, and be reluctant to move. The dominant sign is cough, which is dry and hacking in the beginning and moist later, and that commences soon after the temperature rise and lasts for 1 to 3 weeks. It is easily stimulated by manual compression of the upper trachea. During the early stages of the disease, nasal discharge is not a prominent sign and, if it occurs, is watery. There is no marked swelling of the submaxillary lymph nodes but they may be painful on palpation in the early stages of the disease, especially in younger horses. Limb edema or swelling is unusual in horses with influenza. Abnormal lung sounds, characterized by crackles, wheezes, and increased intensity of normal breath sounds, may be apparent in both uncomplicated disease and in horses with secondary bacterial pneumonia. Ultrasonographic examination of lungs of horses with influenza, even clinically mild disease, reveals pulmonary consolidation, fluid bronchograms, and peripheral irregularities. Tracheal aspirates are neutrophilic, yield heavy growth of *S. zooepidemicus,* and are consistent with bronchitis and pneumonia. Horses, unwisely, forced to exercise have reduced endurance. Horses that are protected against environmental stress pursue an uncomplicated course, with most horses have complete recovery in 7 to 14 days, although a mild cough can persist for weeks.

The previous paragraphs provide a description of the classical disease. However, in outbreaks there is a range of disease severity. Mucopurulent nasal discharge is observed in 75% to 90% of horses, cough in approximately 60%, fever in 20% to 50%, inappetence in 20% to 30%, and signs of depression in 20% to 40%. Undoubtedly, the proportion of horses showing each of these signs will vary from outbreak to outbreak depending on the age and susceptibility of horses in the population, among other factors.

Late term mares and young foals were the groups of horses that appeared to have large numbers or proportion of severely affected individuals during the Australian epizootic. Late term mares appeared to have a greater frequency of severe, paroxysmal coughing and a higher-than-expected incidence of dystocia, although these reports are largely anecdotal.[83,90,92] Young foals had the highest case-fatality rate (see below) and death was often attributable to interstitial pneumonia.[100,101]

Complications and a more severe disease occurs in a small number of horses. Horses that are worked, transported, or exposed to adverse climatic conditions can experience a worsening of the cough, and severe bronchitis, pneumonia, and edema of the legs may develop. Complications are usually associated with secondary bacterial infection, usually *Strep. zooepidemicus,* that results in a mucopurulent nasal discharge, persistent fever, and markedly abnormal lung sounds. Icterus, encephalitic signs, incoordination, and myoglobinuria are reported as rare complications. Electrocardiographic abnormalities have been reported in horses with influenza and were attributed to myocarditis. However, there is no objective evidence of myocarditis secondary to influenza infection of horses,[97,99] nor is there a clear association between influenza infection and electrocardiographic abnormalities.

A more severe form of the disease, associated with an antigenically distinct strain of equine influenza 2, is reported from China. The mortality rate is 35%, and death is attributable to pneumonia and enteritis.

A severe form of the disease is also reported in young foals.[100,101] Foals develop fever, severe respiratory distress, and acute interstitial pneumonia that is commonly fatal. The disease is not invariably associated with failure of transfer of passive immunity.

CLINICAL PATHOLOGY

There are no characteristic changes on hematologic or serum biochemical examination of horses clinically affected by equine influenza virus infection.

Confirmation of the diagnosis of infection by equine influenza virus is achieved through virus isolation, indirect demonstration of virus in nasopharyngeal swabs by detection of viral genome (RT-PCR or variations) or proteins (ELISA), and/or serology.[102]

Serology

Measurement of antibody concentrations against the viral hemagglutinin antigen is important in determining susceptibility to infection, vaccine efficacy, and exposure—factors important in implementing control measures (see following discussion). Documentation of seroconversion, a three- to fourfold increase in **hemagglutination inhibition** (HI) antibody titer, or a doubling in antibody titer measured by the **single radial hemolysis test**, in paired sera collection 14 to 21 days apart provides retrospective confirmation of the diagnosis. The single radial hemolysis test is more reproducible than the hemagglutination inhibition test, is the preferred test for determining concentrations of antibody against the hemagglutinin antigen, and better correlates with susceptibility to infection.[102] For the single radial hemolysis test, the virus is coupled to red blood cells that are then included in agarose. Wells are punched in the agar plate filled with test sera. Influenza antibodies then cause lysis of red cells, with the diameter of the zone of hemolysis proportional to the concentration of the strain specific antibody in the serum. Antibodies against the nonstructural protein (nucleocapsid protein, NS1) are detectable in horses after natural infection, but not after vaccination with an inactivated virus, thereby permitting differentiation of immunologic responses to infection and vaccination by canary pox–vectored vaccines,[74] but not by other subunit vaccines.[103]

ELISA tests for detection of antibodies to equine influenza virus are available and have been characterized. A blocking ELISA for influenza A was accurate (area under curve = 0.993 +/– 0.003 standard error), informative ($z = -32.0$; $p < 0.0001$) and had sensitivity and specificity at cut-point percentage inhibition greater than or equal to 50 of 0.99 (95% CI: 0.98–0.99) and 0.97 (95% CI: 0.96–0.98), respectively, and detected seroconversion as early as day 3 after onset of clinical signs and in 50% of horses by day 5.[104,105] Other commercially available ELISA tests have similar diagnostic test characteristics.[106]

Rapid Detection of Virus

Rapid identification of the cause of the outbreak is important when instituting control measures. Timely demonstration of virus in

nasopharyngeal swabs can be achieved by use of tests that detect viral antigen (ELISA or similar tests) or viral genome (real-time reverse-transcription polymerase chain reaction [rtRT-PCR or qRT-PCR]) or a reverse-transcription loop-mediated isothermal amplification assay. Such tests can be invaluable in confirming an outbreak or occurrence of disease, in monitoring infection rates, and in achieving control.[54,62,65,74,76,77,87,107] Detection of viral antigen by ELISA or similar methods and of viral genome by RT-PCR is not necessarily associated with shedding of live (infectious) virus (a false-positive test result). Both methodologies can detect nonviable remnants of viruses, often for long periods of time, and detection of low levels of viral RNA or antigen should be considered in the context of available clinical and epidemiologic data. Viral RNA has been detected in nasal swabs of horses for up to 34 days after natural infection, with RNA detected in all of 36 horses tested for the first 10 days after onset of clinical signs.[105]

Virus can be detected rapidly in clinical specimens by a reverse-transcription PCR (RT-PCR) test for nucleoprotein gene, hemagglutinin gene of H3N8 viruses and hemagglutinin gene of H7N7 virus. The rtRT-PCR is widely available, is the most sensitive of the currently available tests, and is suited for throughput of large numbers of samples when rapid decision making is required, such as during outbreaks of the disease.[87,108-110] rtRT-PCR can detect virus as early as 1 day after experimental infection of horses, at the same time as virus isolation detected the virus, and before onset of clinical signs.[111] RT-PCR is highly sensitive and can result in detection of even very small quantities of viral RNA, such as can occur when nasal swabs are contaminated with inactivated intramuscular vaccine.[94]

Recent development of **field tests** (on-site tests) to detect viral genome will further enhance diagnosis and control of the disease. The insulated isothermal RT-PCR (iiRT-PCR) method on the POCKIT™, a field-deployable device, is about 100-fold more sensitive than the rRT-PCR assay targeting the NP gene of EIV subtype H3N8 (Miami 1/63/H3N8).[112] The iiRT-PCR assay identified accurately 15 EIV H3N8 strains and two canine influenza virus (CIV) H3N8 strains, and it did not cross-react with H6N2, H7N7, and H1N1 subtypes or any other equine respiratory viral pathogens. There was 100% agreement between the iiRT-PCR assay and the universal influenza virus type A rRT-PCR assay in detecting the EIV A/equine/Kentucky/7/07 strain in 56 nasal swab samples collected from experimentally inoculated horses.[112] The utility of this test in field situations remains to be demonstrated.

The **Directigen Flu A** test (Becton Dickinson) is a rapid test designed for use with humans that identifies influenza viral nucleoprotein (NP, which is highly conserved among influenza A viruses) by a membrane-bound enzyme immunoassay. It has been validated for use in horses and is effective because of the conserved nature of the target antigen across influenza A strains. Results are available in as little as 15 minutes. The test had sensitivity of 68% to 83% and specificity of 78% to 95%, compared with RT-PCR or virus culture.[113] Sensitivity was 54%, but specificity and positive predictive value were 100% compared with serologic diagnosis. The low sensitivity compared with serology was ascribed to inadequate collection of nasopharyngeal swabs, or collection of samples when horses were not excreting virus. The high specificity and positive predictive value of the test mean that a positive result confirms the diagnosis of influenza infection. The relatively low specificity means that samples should be collected from a number of horses in various stages of the disease. Nasopharyngeal swabs should be collected by inserting a cotton gauze swab approximately 30 cm (12 in.) into the nostril or, preferably, nasopharynx of an adult horse and leaving it in place for 60 seconds. The swab should then be transferred to specialized transport media and shipped to the laboratory.

Other rapid diagnostic tests include the **Flu OIA (Biostar)** assay for influenza A and B viral antigen. The test cross reacts with equine herpesvirus 2 and is therefore not useful for diagnosis of upper respiratory disease of horses. Other ELISA diagnostic assays, including an antigen capture ELISA, are available.[114]

Use of rapid tests is not a substitute for viral isolation, which is important for typing of the isolate and subsequent epidemiologic studies and vaccinal applications. Isolation of the virus provides a definitive diagnosis and is best achieved when samples are collected during the first 48 hours after onset of clinical signs. Material for viral culture should be inoculated into the transport medium quickly. The transport medium should contain phosphate buffered saline (PBS) containing either 40% glycerol or 2% tryptose phosphate broth with 2% antibiotic solution (penicillin [10,000 units], streptomycin [10,000 units] in sterile distilled water [100 mL]), and 2% fungizone (250 mg/mL stock).[115] If the samples are to be inoculated within 1 to 2 days they may be held at 4° C (39 F), but, if kept for longer, they should be stored at –70° C (–94 F) or below. Samples should be kept cool during transport to the laboratory.

NECROPSY FINDINGS

Necropsy material is rarely available for adult horses, and the lesions in these fatalities are usually complicated by other pathogens. Histologically, a necrotizing bronchiolitis accompanies widespread pulmonary edema. Foals dying of acute respiratory distress associated with influenza infection have severe diffuse interstitial pneumonia that is characterized histologically by necrotizing bronchitis and bronchiolitis and multifocal interstitial pneumonia.[97]

Samples for Postmortem Confirmation of Diagnosis

- **Nasal swabs** in viral transport media, and sections of lung and trachea should be submitted for virus isolation or demonstration by fluorescent antibody or PCR testing.
- **Formalin-fixed nasopharynx, trachea, and lung** should be submitted for light microscopic examination.

DIFFERENTIAL DIAGNOSIS

See Table 12-16.

TREATMENT

Currently, there is no specific treatment of influenza virus infection of horses.

Amantadine is used in humans for prophylaxis and treatment of influenza infection in high-risk populations, and it has been investigated for use in horses. Amantadine administered intravenously caused transient neurologic abnormalities in experimental horses. **Rimantidine** (30 mg/kg PO q12 hour) administered 12 hours before experimental inoculation of horses with equine influenza KY/91 mitigated signs of disease but did not eliminate viral shedding. Administration of **peramivir** (~8 mg/kg IV once) to horses experimentally infected with equine influenza virus attenuated the severity of clinical signs.[116,117] The safety and efficacy of permavir, amantadine, and rimantidine in horses with naturally occurring disease have not been demonstrated at this time. Until these issues are resolved, and because the infection has such a low case-fatality rate, the use of these drugs in horses cannot be recommended.

Antibiotic treatment of uncomplicated cases is probably not warranted, but horses that develop prolonged fever (longer than 5 days), signs of pneumonia, or a profuse mucopurulent nasal discharge should be treated with broad-spectrum antibiotics, such as potentiated sulfonamides (15-30 mg/kg, PO, IM, or IV, every 12 h), ceftiofur (2.2 mg/kg, IM, every 12 hours), or procaine penicillin (20,000 IU per kg, IM, every 12 hours) with or without gentamicin (6.6 mg/kg, IM, every 24 hours). The usual cause of secondary bacterial infection is *S. zooepidemicus,* which is susceptible to penicillin.

Supportive treatment includes rest, provision of a dust-free environment and, on occasion, administration of nonsteroidal antiinflammatory drugs (NSAIDs). However, NSAIDs should be used judiciously, as their analgesic properties may mask signs of

complications, such as pleuritis. Corticosteroids are contraindicated in the treatment of this disease. Cough suppressants are also contraindicated because coughing is a normal protective mechanism that aids in the clearance of material from the airway. Mucolytics can be administered but their efficacy is unknown. Clenbuterol administration does not alter the course of the disease and is not recommended.

CONTROL

The fundamental aims of a control program are the following:

- Increase the immunity of both individual animals and the population to infection.
- Reduce the opportunities for spread of infection between horses.
- Prevent the introduction of infection or of novel strains of the virus into a population.

These aims are achieved by vaccination, hygiene, and quarantine. It is important to note that effective quarantine that includes isolation of horses for 4 weeks before introduction into a new population of horses prevents introduction of the disease.[115]

Immunity and Vaccination

The aim of vaccination in enzootic areas is to prevent clinical disease caused by infection with equine influenza virus. This aim includes two components—induction of herd immunity by widespread vaccination of almost all horses in a population and induction of protective immunity in individual horses.[102,118]

The first aim is actually more difficult to achieve because of the perception by horse owners in enzootic areas that influenza is not an important disease or that their horse(s) are not at risk and the inability of regulators to mandate vaccination on a country-wide scale.[118] The result is that an insufficient proportion of horses in a population are vaccinated to confer herd immunity on the population as a whole, with consequential localized outbreaks or epizootics of the disease.[102,118]

Induction of protective immunity in individual horses through vaccination is achievable with certain limitations. Immunity induced by vaccination, especially vaccination using inactivated virus, is not as durable as that conferred by natural infection, nor does it induce the same type of immune response,[1] although this might not apply for modified live vaccines.[119] Furthermore, many factors influence the onset, duration, spectrum, and efficacy of vaccination programs, including the product administered, frequency of administration, frequency with which different equine influenza vaccines are administered to horses, age, and sex.[120-128] That said, the responses of most horses to vaccination are predictable, and with increasing knowledge of the immune responses to vaccination, vaccination programs can be expected to confer immunity to clinical disease when properly applied.[1,33,102,119,127-129] Reports of vaccine failure might in actuality be failures of vaccination programs.[118]

Vaccination does not induce sterile immunity to equine influenza virus, and this is important in understanding of the epidemiology and control of the clinical disease.[27,33,115] The extent of cross-protection against infection and/or disease by each of two influenza virus strains depends on the antigenic distance between the strains (see Fig. 12-31).[1] Infection by equine influenza virus or vaccination using an effective vaccine can induce immunity and resistance to infection and development of disease as a result of a homologous virus. For example, vaccination with an inactivated Florida clade 2 virus, or vaccine containing the relevant HA antigen, confers considerable resistance to infection by a Florida clade 2 strain of the virus, but limited immunity, or immunity only at peak antibody titers, to infection by a Eurasian lineage virus or a Florida clade 1 virus.[102] Therefore, the level of protection provided by vaccines is often critically dependent on how closely the vaccine strain matches the virus encountered by the horse. This phenomenon is the basis for the recommendation that contemporary vaccines contain both Florida clade 1 (A/equi2/South Africa/4/03 or Ohio/03) and a clade 2 (Richmond/1/07) virus strains or HA antigens.[12,13,118]

A large number of vaccines to prevent disease as a result of infection by equine influenza virus and recommendations for vaccination programs are available.[12,13,118,130,131] These include vaccines that include whole inactivated virus or viruses,[110,126] virus subunits (e.g., HA protein) either as the purified protein or in a live vector (canary pox),[122,123,129] and vaccines containing various adjuvants and immune stimulating complexes,[132] or live attenuated virus for intranasal administration.[119,133] There are numerous reports of the efficacy of one or more of these vaccines.[33,103,110,119-127,129,132-134] A comparison of each vaccine is beyond the scope of this text, but several principles should be considered when selecting a vaccine: the vaccine should induce a measurable immune response and demonstrable protection against disease (natural or experimental), it should contain pertinent viral strains, it should be safe, and it should be practical (i.e., readily administered). Ideally, it should be possible to differentiate naturally infected horses from those that are seropositive as a result of vaccination.

Immunity to influenza through administration of inactivated vaccines can be assessed by measurement of serum antibody concentrations against hemagglutinin, using the single radial hemolysis test, whereas immunity gained through natural infection is independent of serum antibody concentration and appears to be mediated largely by cell. However, serum antibody concentration is currently used as an indicator of susceptibility of individual horses to infection, and as a guide in the development and application of vaccination protocols, including monitoring of need for vaccination in individual horses. Serum antibody concentrations to hemagglutinin measured by single radial hemolysis are specific for the strain of virus and are strongly predictive for resistance to disease associated with that virus in both experimental and field challenge. Failure of a commercial inactivated virus multivalent vaccine to induce detectable increases in antibody concentration in Thoroughbred racehorses was associated with lack of protection against natural infection by a heterologous influenza virus. It is important to reiterate that resistance to disease after vaccination or natural infection is greatest for homologous virus and less for challenge by heterologous virus. Thus horses with antibody concentrations protective to disease associated with homologous virus can be susceptible to disease associated with heterologous virus.

Vaccination against equine influenza is now in general use in countries where the disease occurs, and use of efficacious vaccines is effective in limiting the severity of clinical illness and morbidity during an outbreak.[12,13] Administration of a subunit, canary pox–vectored vaccine, is partially credited with abbreviating the equine influenza epizootic in Australia and for allowing continued commercial racing.[63,81,98] Vaccine efficacy is limited by the short duration of immunity induced by vaccination, the presence in horse populations of multiple viral strains and of antigenic drift in these strains, and the poor immunity induced by vaccines (and natural infection) to challenge by heterologous virus. Furthermore, the immune responses induced by administration of inactivated virus or subunit vaccines, which are primarily an increase in serum IgG(T) antibody titer, differ markedly from the immune responses to natural infection, which are production of IgA in nasal secretions and IgGa and IgGb in serum.

Multiple factors are important in determining the efficacy of a vaccine in protecting against disease. Factors include efficacy of the vaccine in stimulating an immune response, viral strains included in the vaccine amount of antigen in a dose of vaccine adjuvant, and timing and frequency of administration of the vaccine.

Vaccines

A complete listing of current commercially available vaccines, the viral stains or antigens included, and the adjuvant used is available at the equiflunet website.[13] This site should be consulted for up-to-date information on equine influenza vaccines as this is a rapidly developing field. At time of writing, there was only one vaccine that included both Florida clade 1 and Florida clade 2 virus

antigens (clade 1 is represented by A/eq/South Africa/04/2003-like or A/eq/Ohio/2003-like viruses, and Clade 2 is represented by A/eq/Richmond/1/2007-like viruses[12,13]), consistent with the most recent OIE recommendations.[13] The OIE recommends that H7N7 and H3N8 (Eurasian lineage) virus no longer be included in vaccines.

Most vaccines are comprised of inactivated or subunits of virus combined with an adjuvant. Inclusion of an adjuvant is important in maximizing the immune response to vaccination. The important factor in vaccine composition is the inclusion of adequate amounts of antigen of pertinent strains of virus. H7N7 virus is no longer a cause of clinical disease, and it should not be included in contemporary vaccines. Inclusion of antigen from both American and Eurasian lineages of H3N8 virus is essential, and vaccine composition should be regularly updated to reflect those viruses currently circulating in the horse population. The vaccine must include an adequate amount of antigen, measured by the single radial diffusion assay, preferably, the single radial hemolysis assay, because there is a clear relationship between dose of antigen and magnitude and duration of antibody response. There is increasing concern, and some evidence, that inclusion of multiple antigens in vaccines (for example, tetanus toxoid, equine herpesvirus, encephalomyelitis virus) reduces the efficacy of influenza vaccines. Although this concern has yet to be proved conclusively, it should be borne in mind when formulating vaccine programs for horses at high risk of influenza.

A modified live-virus vaccine is available in North America and has proven to be effective in experimental studies in preventing disease against heterologous virus challenge (both American and Eurasian lineages).[122] Furthermore, the duration of protection is at least 6 months after completion of a course of vaccination and there is a strong anamnestic response.[122] Vaccinated ponies had had significantly lower clinical scores, had smaller increases in rectal temperature, and shed less virus over fewer days than did the unvaccinated controls in response to challenge 6 months after vaccination. After challenge at 12 months, vaccinates had rectal temperatures and duration and concentration of virus shed significantly reduced compared with those in unvaccinated animals.

A live recombinant canary pox–vectored vaccine has been used in Europe, America, South Africa, and Australia.[70,129] The vaccine uses the viral vector to introduce influenza hemagglutinin genes into host cells. The recombinant virus expresses the hemagglutinin gene of both Florida clade 1 H3N8-Ohio/03 and Florida clade 2 H3N8-Richmond/1/07.[13] The canary pox infection of the host cell is abortive, with no virus produced, but influenza viral gene is expressed and presented through MHC class 1 by the host cell, with subsequent induction of an immune response. A form of this vaccine, including both European and American lineages, was used to aid in the control of the 2003 influenza outbreak in South Africa and the 2007 epizootic in Australia.[63] An advantage of use of this vaccine is the ability to differentiate vaccinated horses from horses that seroconvert as a result of natural infection. This is achieved by detection of antibodies to both HA and internal viral proteins (NP) in naturally infected horses and only antibodies to HA in vaccinated horses.[54,74] There are variations of this testing, based on the same principle of differential induction of antibodies, that can differentiate infected horses from those receiving an inactivated virus vaccine.

Other novel vaccine strategies include use of DNA or vector vaccines.[135] Although effective in inducing a protective response, technological issues currently limit the widespread use of DNA vaccines.

Objective

The objective of a vaccination program is to ensure that horses have maximal immunity at the times of greatest risk of exposure to influenza virus. Therefore young horses should be adequately vaccinated before being introduced into larger populations of horses. Older horses should receive frequent booster vaccinations before, and during, the racing or show season. Mares should be revaccinated before being shipped to breeding farms. It is important in any control program that all horses in a herd be vaccinated so that the population immunity to infection is maximal.

The objective of vaccination during the epizootic in Australia was to first control and then eliminate infection from the continent.[63] As such, the program of vaccination involved prophylactic vaccination, implemented before a disease outbreak, and reactive vaccination, implemented as part of a response to a disease outbreak, in conjunction with quarantine and movement control measures, as set out in the Ausvet Plan.[27] Reactive strategies include ring vaccination around identified sources of infection to limit further spread by producing an immune buffer, blanket (mass) vaccination and predictive vaccination whereby selective groups of horses are vaccinated because they are identified as having the potential to contribute most to future spatial transmission of infection. Ring vaccination was implemented through the creation of vaccination buffer zones 10-km wide or wider around foci of infection where lateral spread of infection was occurring. Predictive vaccination was used where there were large accumulations of horses and/or movement of personnel or fomites (vehicles) in contact with horses. Groups of horses suitable for predictive vaccination include racehorses, breeding horses, police horses, and other essential groups. Blanket vaccination refers to vaccination of all horses in an area, thereby permitting limited movement and use of horses in that area.[27,63]

Timing

Foals

Timing of vaccination of foals depends on the immune status of the mare and consequent acquisition of passive immunity by the foal. The presence of even small amounts of maternally derived antibody interferes with the immune response of foals to vaccination. Furthermore, vaccination of foals while they continue to have passive immunity can result in impaired responses to subsequent vaccinations. The practical significance of this latter observation is unknown but because of its potential importance should be considered when developing vaccination protocols for foals. Therefore vaccination of foals born to mares vaccinated more than once yearly should be delayed until the foals are at least 24 weeks of age when the immunity resulting from the vaccination is much better; this might leave some foals unprotected because passively acquired immunity is short lived, and some foals of recently vaccinated dams are seronegative by 4 weeks of age. Foals of unvaccinated mares can be vaccinated at less than 1 month of age. Vaccinations are carried out at 6- to 12-week intervals for at least two injections. Subsequently, booster injections are given at least once a year, although more frequent vaccination confers a greater immunity.

Racehorses and Show Horses

Yearlings and young horses are at increased risk of disease, and careful attention to their vaccination status is important in reducing the incidence of disease in this group. Yearlings and 2 year olds in racing stables in Great Britain typically have antibody concentrations against influenza before vaccination on arrival at the stable that are not protective. Vaccination increases antibody titer such that approximately three quarters of yearlings and 2 year olds have protective titers. For yearlings entering training the important predictors of antibody titer before vaccination on arrival at the stable were the time since a previous vaccination, total number of previous vaccinations, and the age at first vaccination. This study demonstrates the need for appropriate vaccination of young horses before they enter larger populations of horses, both to protect the young horse from disease and also to confer herd immunity on the population that they are entering.

Vaccination of racehorses and show horses and other horses at increased risk of exposure should be frequent. Booster vaccines should be timed to maximize immunity at the time of greatest exposure, such as introduction to a new stable or at the beginning of the show season. For maximal protection subsequent booster injections should

be administered at intervals of 6, or even 4, months. Measurement of antibody concentrations by single radial hemolysis can be useful in determining the need for booster vaccination. Previously, vaccination during the racing season was disliked by trainers because of transitory swellings at injection sites and an infrequent mild systemic reaction; however, administration of contemporary vaccines is rarely associated with these adverse effects. In general, vaccination appears to have no adverse effect on performance.

Schedule

Various schedules have been proposed for influenza vaccinations of horses, with different regulatory bodies having specific recommendations. The FEI requires all horses competing in FEI competition to provide evidence of sufficient vaccination against equine influenza.[136] This involves regular six monthly booster vaccinations following a primary vaccination course. All horses and ponies for which an FEI Passport or a National Passport approved by the FEI has been issued must have the vaccination section completed and endorsed by a veterinarian, stating that it has received two injections for primary vaccination against equine influenza, given between 1 and 3 months apart. In addition, a booster vaccination must be administered within each succeeding 6 months (± 21 days) following the second vaccination of the primary course. None of these injections must have been given within the preceding 7 days, including the day of the competition or of entry into the competition stables.

The British Horse Racing Authority has strict vaccination requirements that must be complied with to enter horses in their competitions or onto its premises.[130] The program includes a first equine influenza vaccination to be followed by a second vaccination 21 to 92 days later, with a third vaccination 150 to 215 days from the second vaccination. Thereafter vaccinations should be annually, with the last permissible day being the same date as the previous year's vaccination.

A schedule proposed for control of influenza in a large area includes the following rules:

- Mandatory vaccination for all horses entering racing premises
- Horses not to race in the 10-day period following vaccination
- Horses coming from international locations must be vaccinated before departure.
- All horse events, including shows, sales, and gymkhanas, should apply the same restrictions.
- The recommended vaccination program using inactivated or subunit vaccine is as follows:
  - Mares should be vaccinated during the final 4 to 6 weeks of gestation to ensure adequate passage of passive immunity to the foal.
  - Vaccination of foals at 6 months of age
  - Two vaccinations initially at 21 days, and not more than 92 days apart
  - A booster vaccination 5 to 7 months later
  - Annual boosters or, in the face of increased infection pressure or when the risk of infection is high, boosters should be at 6-month or even 4-month intervals.
  - When vaccination schedules break down and a horse goes longer than 12 months without a booster, recommence with a two-vaccination schedule.
  - Yearlings and 2-year-olds may require an additional vaccination between the second vaccination of the primary series and the booster at 6 months.

Control Measures

Spread of equine influenza virus is by infected horses, fomites (humans, equipment, and vehicles), and by dispersion of infected aerosols by wind or, more proximately, by coughing and sneezing. Control is achieved by preventing movement of infected or potentially infect horses and their contact with uninfected horses (i.e., quarantine), disinfection of fomites or personnel or prevention of contact of potentially contaminated fomites or personnel with susceptible horses, and reduction of ambient contamination of the virus.

Hygienic precautions can be of value in limiting the spread of the disease, as was documented during the epizootic in Australia.[57,64,72,82,86,89,98] Vehicles used for the transport of horses are thought to play a large part in transmission and should be thoroughly disinfected between shipments.

The surfactant action of soaps and detergents is an effective decontaminant for EI virus because of the susceptibility of the virus's outer lipid envelope. Soap and water or alcohol-based hand rubs, applied for at least 20 seconds are satisfactory for personal disinfection of human influenza virus and likely equine influenza virus.[137,138] Virkon® and quaternary ammonium compounds are suitable for decontaminating surfaces and equipment and for foot dips. Virkon® is not approved for use on skin and is unsuitable for disinfecting vehicles because it is corrosive.

A comprehensive description of means of decontaminating personnel, vehicles, and equipment is provided in a number of references,[27,115] and evidence of efficacy in preventing spread of infection is documented in some instances.[72,86]

Quarantine is imperative to prevent introduction of virus by animals in the incubation period of the disease or subclinically infected horses. The most common introduction of infection, especially internationally, is through importation of subclinically infected horses. Also, because vaccinated animals can be infected and be shedding virus but not have signs of infection, isolation of introduced animals is an essential precaution, especially when an outbreak is in progress. The period of isolation should be at least 21 days and ideally 28 days.[115] The degree of isolation required cannot be specified because of lack of basic information, but it is suggested that droplet infection can occur over a distance of 32 m and windborne spread of up to 13 km.[38] There should be maximum biosecurity with regard to clothing, utensils, and personnel because this is effective in preventing spread of infection.[86,89]

It is important to recognize that abrupt imposition of quarantine and "standstill" of horses during an epizootic of the disease is associated with considerable psychological, emotional, logistic, and economic impact to owners, trainers, and horse-related businesses.[50-52,57,60,66,80,93]

Control measures during an outbreak are intended to eliminate sources of infection, reduce transmission of virus, enhance the resistance of at-risk horses, and decrease the number of horses at risk. Infected horses (identified by stall-side or rapid laboratory tests), and clinically affected horses should be removed from the group and isolated for 3 to 4 weeks. Ventilation of shed rows and barns should be optimal to minimize aerosol spread of the virus. No horses should be introduced or allowed to leave until the outbreak is over, probably about 4 weeks after the first case is identified. Movement of horses between barns or paddocks should be avoided. Training and racing should be suspended. The opportunity for fomite transfer on clothing, tack, feed utensils, or vehicles should be minimized by strict hygiene. Vaccination of clinically normal horses in the face of an outbreak can enhance the immunity of at-risk horses and is probably safe.

A comprehensive plan for management of incursion of equine influenza into an area free of the infection is provided in Austvet Plan for equine influenza and in the special issue of the *Australian Veterinary Journal* (2011, Volume 89, Supplement) describing responses to the 2007 incursion of equine influenza into Australia and its prompt eradication.[27]

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EQUINE RHINITIS (EQUINE RHINITIS VIRUS)

The equine rhinitis viruses are ubiquitous in populations of horses. Infection has been associated with mild respiratory disease, although their role in the common infectious respiratory diseases of horses is uncertain.

The equine rhinitis viruses are of four types—ERAV-1 and ERBV-1, 2, and 3. Equine rhinitis A virus (formerly equine rhinovirus-1) is a species within the genus Aphthovirus containing a single serotype, equine rhinitis A virus-1 (ERAV-1). There is little genetic variation in this virus.[1] The genus includes foot-and-mouth disease virus and bovine rhinitis virus, of the Picornaviridae. Equine rhinovirus 2 and equine rhinovirus 3 are now classified in the genus *Erbovirus* as equine rhinitis B virus (ERBV) types 1 and 2, respectively.[2,3] A third equine rhinitis B virus has been identified—ERBV-3.[4]

The viruses are widely distributed in horse populations globally and infect substantial proportions of horses worldwide. For example, antibodies to ERBV were detected in 86% of 50 weanling Thoroughbred from three stud farms in Australia, with 48%, 10%, and 62% of yearlings being seropositive for ERBV-1, ERBV-2, and ERBV-3, respectively.[5] Fifty-six percent of the horses had evidence of infection by two serotypes, and 12% had evidence of infection by all three serotypes.[5] Others find similar seroprevalence for ERBV-1 and ERBV-2 in Australia.[6] Virus genome is detected by PCR in nasal swabs in a much smaller proportion of horses (0%-16%), suggesting differences in sensitivity of the assay, minimal or intermittent shedding of the virus or differences in the populations of horses sampled.[7-9]

Serologic evidence of infection with ERAV is present in 50% to 100% of horses, although the prevalence of seropositive animals varies from almost zero in yearlings, to 8% in horses newly introduced to a training yard, to 61% in the same horses 7 months later.[6] Twenty-eight percent of 113 horses in

Ontario with signs of respiratory disease were seropositive for ERAV.[10]

ERAV is present in nasal discharge, feces, and/or urine of experimentally infected clinically normal horses and can be shed in the urine for at least 37 days.[11,12] Virus was detected in 23% of 215 urine samples collected from Thoroughbred racehorses as part of postrace drug monitoring.[11] These horses were therefore presumed to not have signs of infectious respiratory disease, and this represents a shedding rate in clinically normal horses.[11] The importance of urine shedding of the virus in transmission of infection is unclear, although the inhalation of aerosols of infected urine might transmit the virus. The virus has been detected in semen of a stallion.[13]

The role of ERAV or ERBV in genesis of respiratory disease of horses is unclear. The disease can be reproduced experimentally in seronegative ponies,[12] and there are a small number of reports of ERV association with respiratory disease, but clear causal association with common naturally occurring respiratory disease is lacking. High titers to ERAV are detected in horses with disease caused by equine influenza virus in Ontario, and it is suggested that this could be important in the development of respiratory disease, but without conclusive evidence.[10] The high prevalence of ERV infection in horses means that detection of the virus in horses with signs of respiratory disease could occur by chance in a proportion of individuals with ERV not being causative. None of 52 horses with signs of infectious respiratory disease in New Zealand had ERAV or ERBV virus (by tissue culture) or viral DNA (by PCR) detected in nasal swabs.[9] Similarly, ERAV was not detected in nasal swabs of any of 336 horses with respiratory disease (or in any of the 39 healthy controls) in the western United States, and ERBV was detected in 9 of 336 samples.[14] Although there is some evidence to indicate a causative role for ERAV and ERBV in common infectious respiratory disease of horses, the importance of the virus remains to be determined.

The **disease** thought to be associated with ERAV is characterized by an incubation period of 3 to 8 days, fever, pharyngitis, pharyngeal and submandibular lymphadenitis, and a copious nasal discharge that is serous early and becomes mucopurulent later. A cough persists for 2 to 3 weeks. The uncomplicated disease is mild and self-limiting. Among a group of susceptible horses, there is rapid spread of infection and disease. Studies in England have not identified the virus as an important cause of inflammatory airway disease in racehorses.

The virus has been associated with abortion in dromedary camels.[15]

Virus neutralizing antibody develops within 7 to 14 days of infection and persists for long periods.[11] Immunity after natural infection is said to be solid and long-lasting.

Diagnosis is based on serologic testing, detection of viral DNA, or tissue culture of the virus, which is environmentally resistant.

There is no specific treatment, and a commercial vaccine is not available. Planned exposure of young horses to infection has been recommended, but this should be reconsidered in light of current knowledge of the prolonged shedding of the virus in urine and feces. The virus appears to have minimal zoonotic potential.

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EQUINE VIRAL RHINOPNEUMONITIS (EQUINE HERPESVIRUS-1 AND -4 INFECTIONS)

SYNOPSIS

Etiology EHV-4, and EHV-1, alpha-herpesvirus.

Epidemiology Transmission between horses and by mediate contagion. Lifelong latency of infection with putative periodic reactivation of virus shedding. Respiratory disease occurs as sporadic disease and as outbreaks. Younger animals more commonly affected by disease. Immunity following vaccination or infection is apparently short lived.

Clinical signs Upper respiratory disease; rarely, abortion or myeloencephalopathy.

Clinical pathology Seroconversion or increase in titer detected by enzyme-linked immunosorbent assay (ELISA) able to differentiate EHV-1 from EHV-4.

Diagnostic confirmation Virus isolation from, or polymerase chain reaction test on, blood, nasopharyngeal swabs, or tissue. Seroconversion or increase in titer detected by ELISA able to differentiate EHV-1 from EHV-4.

Treatment There is no specific treatment.

Control Vaccination (of minimal efficacy). Quarantine. Hygiene.

ETIOLOGY

Upper respiratory disease of foals and adults is associated with equid herpesvirus-4 (EHV-4) or, less commonly, EHV-1, an alpha-herpesvirus. The DNA sequence of EHV-4 has been determined. There appear to be strains of EHV-4 that vary in virulence, based on severity of clinical disease, but at present it is not possible to differentiate between strains of low and high virulence by laboratory methods.

EPIDEMIOLOGY

Occurrence

Infection with EHV-4 is endemic in horse populations worldwide.[1-8] The serologic surveys of prevalence of serologic evidence of infection are of limited value because earlier studies used techniques that were unable to differentiate between antibodies to EHV-1 and EHV-4. Recent serologic surveys using ELISA tests capable of differentiating between antibodies to EHV-1 and EHV-4 demonstrate that almost all horses and foals greater than 60 days of age have evidence of infection by EHV-4. Young foals can be seropositive as a result of transfer of immunoglobulins from seropositive dams, making determination of the time of first infection, and active seroconversion, difficult. Furthermore, serologic tests are also unable to differentiate between responses to natural infection and to vaccination.

EHV-4 can be isolated from both clinically normal foals and those with signs of upper respiratory disease with similar frequency. Shedding of virus is more likely in foals with nasal discharge. There is a marked seasonal distribution to the pattern of shedding, with the most frequent detection of shedding being in early autumn (March).

Upper respiratory tract disease attributable to EHV-4 is very common and probably affects almost all horses during the first 2 years of life. EHV-4 rarely causes abortion in mares, septicemia in newborn foals, or myeloencephalopathy in adult horses. The frequency of respiratory disease attributable to EHV-1 is unclear but is apparently much less common than that for EHV-4.[1,2,5,7] Seroprevalence of EHV-1 specific antibodies is 9% to 28% in adult Thoroughbred horses, 26% of Thoroughbred brood mares, 11% of Thoroughbred foals, and 46% to 68% of 1- and 2-year-old Thoroughbred racehorses in Australia. Sixty-one percent of 82 normal horses and horses with upper respiratory tract disease had antibodies to EHV-1 in New Zealand. Prevalence of serum antibodies to EHV-4 and EHV-1 were 93% and 1%, respectively, in young Thoroughbreds presented for sale as yearlings in South Africa.[5] EHV-4 DNA was detected in 14% of animals, whereas EHV-1 DNA was not detected in any horses at sale.[5] Similarly, in England, EHV-1 was not associated with clinical respiratory disease in Thoroughbred racehorses.

Method of Transmission

EHV-4 is highly infectious, and transmission probably occurs by the inhalation of infected droplets or by the ingestion of material contaminated by nasal discharge. Foals infected with EHV-4 have prolonged and profuse shedding of virus in nasal secretions. Mediate infection may occur, the virus surviving for 14 to 45 days outside the animal.

Infections always arise from other horses, both by direct contact and via fomites. Horses and foals are infectious during the active stage of disease and, because horses become latently infected, presumably during subsequent periods of viral reactivation and shedding. The duration of latency is unknown but is assumed to be lifelong. EHV-4 establishes latency in the trigeminal ganglion, which is the origin of the maxillary branch of the trigeminal (5th cranial) nerve that provides sensory innervation to the nasal mucosae. It is assumed that reactivation of the virus and subsequent virus shedding poses a risk to in-contact, susceptible animals, but this has not been definitively demonstrated in field situations. If this were the case, then clinically normal animals harbor latent virus that during periods of reactivation can infect susceptible animals. If true, this feature of the disease has obvious importance in the prevention, control, and management of outbreaks of disease.

Risk Factors

Immunity

Immunity resulting from natural infection of the respiratory tract is of short duration despite the persistence of serum virus-neutralizing (VN) antibodies. If similar to EHV-1, immunity to EHV-4 is likely associated with cytotoxic T-cell responses because of the importance of cell-associated virus in dissemination of infection throughout the horse. Because of the short duration of immunity an animal can become clinically affected a number of times during its life, although subsequent disease tends to be milder. Foals born to mares with serum antibodies to the virus acquire a protective passive immunity that persists for up to 180 days, provided that they ingest sufficient high-quality colostrum. Unfortunately, VN antibodies are not necessarily an indication of resistance to infection.

Age

Foals are infected by EHV-4, presumably from the dam or other mares in the band of mares and foals, early in life and excrete large quantities of virus in nasal secretions. Horses are infected repeatedly throughout life, with episodes of disease being less frequent and milder with increasing age. EHV-4 is isolated more frequently from younger than from older horses, suggesting an age-associated decrease in susceptibility to disease.

Economic Importance

Disease associated with EHV-4 is apparently of considerable economic importance because of the loss of training time and opportunities to perform during convalescence and quarantine. Although the upper respiratory disease is a mild inflammation of the respiratory tract of horses, characterized by coughing and nasal discharge, the importance of the disease is the large numbers of animals affected in an outbreak. Fatalities in uncomplicated cases of rhinopneumonitis are rare.

PATHOGENESIS

The pathogenesis of EHV-4 infection and disease is assumed to be similar to that of EHV-1, with the exception that the virus does not commonly cause abortion, neonatal septicemia, or myeloencephalopathy. The virus is inhaled and binds to epithelium of the upper respiratory tract, enters epithelial cells, and reproduces. The infection then spreads throughout the respiratory tract, including trachea and bronchioles, and to lymphoid tissues associated with the respiratory tract. There is a viremia, although this may be of short duration. There is cell death and development of intranuclear inclusion bodies in the respiratory tract and associated lymphoid tissues. The EHV-4 virus then becomes latent, as evidenced by isolation of virus from lymph nodes associated with the respiratory tract and detection of viral genome in trigeminal ganglia, although this has not been a consistent finding. The factors causing viral recrudescence from these latent sites have not been determined. It should be noted that definitive evidence of viral recrudescence of EHV-4 as a cause of outbreaks of disease is lacking, and experimental induction of recrudescence is achieved only by administration of large doses of corticosteroids.

CLINICAL FINDINGS

The classical respiratory tract form of the disease (rhinopneumonitis) is virtually indistinguishable on the basis of clinical signs from the other respiratory tract diseases of horses. There is an incubation period of 2 to 20 days. Fever, conjunctivitis, coughing, and mild inflammation of the upper respiratory tract are the cardinal manifestation of the disease, but inapparent infection is common. The temperature varies from 39° to 40.5° C (102.5–105.5° F). There is enlargement, but not abscessation, of the submandibular lymph nodes, especially in foals and yearlings. These signs are more likely to occur in young horses or when horses are assembled in sale barns. Edema of the limbs and diarrhea occur rarely. The length of the illness is usually 2 to 5 days, although the nasal discharge and cough may persist for 1 to 3 weeks. Secondary bacterial invasion, usually *Streptococcus equi* subsp. *zooepidemicus,* may exacerbate the clinically inapparent viral pneumonia. Young foals can develop primary viral pneumonia.

EHV-4 only rarely causes abortion or neurologic disease.

CLINICAL PATHOLOGY

Results of hematologic and serum biochemical examinations are neither specific nor diagnostic. In adult horses with rhinopneumonitis there may be pronounced leukopenia, largely attributable to depression of neutrophils.

Serologic tests are of critical importance in diagnosis and control of equine herpesvirus infections. Serum antibody levels to EHV-1/4 may be determined by ELISA, virus neutralization (VN), or complement fixation (CF) tests. The CF and VN tests are not able to differentiate between seroconversion associated with EHV-1 and EHV-4, whereas an ELISA using recombinant antigens specific for EHV-1 and EHV-4 is able to differentiate infection by each of these types of equine herpesvirus. Many, if not all, adult horses have serum antibodies to EHV-4 as a result of previous infection or vaccination. Thus the demonstration of antibodies is not in itself sufficient to confirm a diagnosis of the disease. **Complement-fixing antibody** appears on the 10th to 12th day after experimental infection but persists for only a few months. Demonstration of a three- to four-fold increase in the serum concentration of specific complement-fixing antibodies in acute and convalescent serum samples provides persuasive evidence of recent infection, albeit by either EHV-1 or EHV-4. Complement-fixing antibodies persist for only a short time (several months), whereas VN antibodies persist for over a year, and testing for them is therefore a more reliable means of determining that previous infection with the virus has occurred. Until recently, serologic differentiation of antibodies to EHV-1 and EHV-4 was not possible. However, highly specific **ELISA** tests based on the variable region of the C terminus of glycoprotein G, at least one of which is commercially available, have been developed that can differentiate between antibodies to EHV-1 and EHV-4 in horse serum. The ELISA is reported to be more sensitive, easier to perform, more rapid, and more reproducible than the virus neutralization test. Importantly, the ELISA test is able to differentiate between infections associated with EHV-1 and EHV-4.

Identification of the virus in nasal swabs or blood buffy coat by culture or a PCR test provides confirmation of infection.[9] The use of seminested or multiplex PCR provides rapid identification of EHV-4 viral genome in pharyngeal swabs. The test is at least as sensitive as viral isolation in identifying presence of virus. However, the use of rapid and innovative diagnostic techniques based on ELISA, PCR, immunohistochemical staining with peroxidase, or nucleic acid hybridization probes is often restricted to specialized

reference laboratories. Therefore the method of choice for diagnosis of rhinopneumonitis by diagnostic virology laboratories handling many routine samples continues to be the traditional methodology of cell culture isolation followed by sero-identification of the isolated viruses. The virus can be isolated in tissue culture, chick embryos, and hamsters, from either nasal washings or aborted fetuses.

Samples of nasopharyngeal exudate for virus isolation are best obtained from horses during the very early, febrile stages of the respiratory disease, and they are collected via the nares by swabbing the nasopharyngeal area with a 5 × 5 cm gauze sponge attached to the end of a 50-cm length of flexible, stainless steel wire encased in latex rubber tubing. A guarded uterine swab device can also be used. After collection, the swab should be removed from the wire and transported promptly to the virology laboratory in 3 mL of cold (not frozen) fluid transport medium (serum-free minimal essential medium [MEM] with antibiotics). Virus infectivity can be prolonged by the addition of bovine serum albumin or gelatine to 0.1% (w/v).

NECROPSY FINDINGS

Fatalities are extremely rare in the respiratory forms of EHV-4 infection or EHV-1 disease restricted to the respiratory tract.

Samples for Confirmation of Diagnosis

- **Virus isolation or identification** by fluorescent antibody testing or PCR of nasal swabs or blood

DIFFERENTIAL DIAGNOSIS

The upper respiratory diseases of horses are listed in Table 12-16. There is no specific treatment, although antibiotics are often administered to horses with respiratory tract disease to prevent or treat secondary bacterial infection. There is, however, no evidence that antibiotic treatment shortens the duration of the disease or prevents complications.

CONTROL

Principles of a control program include the following:

- Enhancing the immunity of individual horses by vaccination
- Minimizing the risk of introducing EHV-4 infection to the farm or stable
- Hygiene to prevent spread of virus on fomites such as clothes and tack
- Rapid isolation of any horse with disease that could be attributable to EHV-4

Vaccination

Vaccines for protection against rhinopneumonitis contain both inactivated EHV-1 and EHV-4 virus, presumably because both viruses cause respiratory disease in horses. None of the currently available vaccines consistently prevents infection of vaccinated horses or provide complete protection against disease associated with EHV-4, although a combined EHV-1/EHV-4 inactivated virus vaccine attenuated the clinical signs of disease in experimentally infected foals. The development of modified live-virus vaccines administered intranasally holds promise for effective control of both EHV-1 and EHV-4 in foals and adults.

Hygiene

Standard hygienic procedures should be adopted to avoid spread of the disease, with particular attention being given to the isolation of introduced horses.

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EQUINE MULTINODULAR PULMONARY FIBROSIS

Equine multinodular pulmonary fibrosis (EMPF) is a recently described progressive fibrosing lung disease of adult horses.[1]

ETIOLOGY

EMPF is strongly associated with infection by equine herpesvirus-5 (EHV-5), a gammaherpesvirus.[1-10] Most cases of EMPF have detectable EHV-5 in the lung lesions or in bronchoalveolar lavage fluid, the virus is most abundant in lesions compared with unaffected areas or lung from unaffected horses, and inoculation of healthy horses with EHV-5 derived from affected horses results in nodular pulmonary fibrosis.[6]

EHV-5 antigen can be detected in lungs of unaffected horses, and the virus is widely distributed in horses worldwide, with both healthy horses and horses with evidence of non-EMPF respiratory disease having detectable virus in respiratory secretions.[11-16] The ubiquity of EHV-5 infection in horses raises the possibility that infection with EHV-5 in horses with EMPF is coincidental and not causative or contributory to the disease. However, the almost universal detection of EHV-5 in horses with EMPF and the similarity of distribution of EHV-5 antigen in lungs and lesions of horses inoculated with the virus and naturally occurring cases of EMPF suggests a role for EHV-5 in the genesis of the lesions. It is plausible, but increasingly unlikely, that EHV-5 infects the abnormal tissues of lungs of horses with EMPF after development of the lesions and is not a contributory agent to the disease. Although Koch's postulates have not been fulfilled for EHV-5 and EMPF, the weight of evidence supports a role for EHV-5 in the initiation, development, or proliferation of nodular pulmonary fibrosis in horses.

Both asinine gammaherpesvirus-5 and equine gammaherpesvirus-5 were isolated from two horses with EMPF.[4,17] The importance of this finding is unclear and might just represent the high frequency of infection of horses by asinine gammaherpesvirus-5.

EHV-5 is also associated with skin and ocular disease and lymphoma in horses, and both EMPF and lymphoma can occur in the one animal.[18-21]

EPIDEMIOLOGY

The epidemiology of EMPF is not well established, and current knowledge is based on individual reports of small numbers of cases with the resultant potential for reporting and case-selection bias. The disease has been reported from Australia, New Zealand, Brazil, Europe, the United Kingdom and North America and appears to have a worldwide distribution.[1,2,4,5,7,8,17,19,22-25] Horses most commonly reported as affected are middle-aged to older light breeds (Thoroughbreds) and Warmbloods, although the disease is reported in horses as young as 2 years of age.[5,7,17,26,27] There are no readily apparent risk factors.

The case-fatality rate is high (>50%) in untreated and treated horses. There are no estimates of cause-specific mortality or morbidity rates.

PATHOGENESIS

The molecular pathogenesis of EMPF is unknown, although parallels are drawn between Epstein-Barr virus associated interstitial (fibrosing) pneumonia in people and the disease in horses.[6] The lesion in horses progresses through proliferation of type 2 pneumocytes to alveolar fibrosis and focal obliteration of normal lung architecture. There is systemic evidence of inflammation, and the fever, weight loss, lethargy, and exercise intolerance of affected horses demonstrate a systemic response to the disease. Exercise tolerance could be attributable to diminished gas exchange in damaged lungs, the systemic inflammatory effects of the disease, or, more likely, a combination of both.

CLINICAL SIGNS

Horses with EMPF have various combinations of weight loss, recurrent cough, depression, anorexia, fever, tachycardia, tachypnea, or respiratory distress.[5,7,8,22,27] Signs of respiratory disease might not be apparent at

initial examination, but as the disease progresses respiratory distress develops in most, but not all, cases. The usual history is of a gradual onset of increased respiratory effort, although some horses have a sudden onset of respiratory distress. Heart and respiratory rates are often elevated. Pyrexia is not a constant finding and can be intermittent in affected horses. There can be a nasal discharge but this is not invariable or characteristic. Thoracic auscultation might reveal only increased intensity of normal breath sounds or the presence of occasional crackles and wheezes. Typically, there is tachypnea with an increased respiratory effort.

Thoracic radiography reveals pulmonary disease, usually apparent as severe, diffuse interstitial disease with nodular opacities.[7] The interstitial opacity can be diffuse or nodular with multiple well-defined opacities against an overall background of increased interstitial density. Ultrasonographic examination often reveals the presence of multiple nodules in the lung parenchyma confluent with the pleural surface.[7] There is no excess pleural fluid.

Lymphoma in horses with EMPF and lymphoma associated with EHV-5 infection and treated with acyclovir have been reported, raising the possibility of a common etiology of the two diseases.[19,20]

CLINICAL PATHOLOGY

Hematologic examination usually reveals a neutrophilic leukocytosis, mild anemia, lymphopenia, and hyperfibrinogenemia.[2,7,8,22] Pancytopenia occurs in a small proportion of cases.[2] Hypoproteinemia and hypoalbuminemia are common. Arterial oxygen tension is not invariably abnormal but declines as the disease progresses.

Examination of a tracheal aspirate reveals neutrophil inflammation. Macrophages contain occasional intranuclear inclusions.[7,8,28]

Serologic testing for antibodies to fungi including *Blastomyces, Coccidioides, Histoplasma, Aspergillus,* and *Cryptococcus* spp. assists with ruling out diseases caused by these organisms. EHV-5 can be detected by PCR examination of bronchoalveolar lavage fluid in most affected horses.[7]

NECROPSY FINDINGS

Gross lesions are restricted to the lungs and occur in two distinct forms, the more common form being numerous coalescing nodules of fibrosis with little unaffected lung present (diffuse nodular form).[1] Individual nodules are up to 5 cm in diameter, pale tan to white, and moderately firm. The less common lesion consists of multiple discrete nodules up to 10 cm in diameter and separated by grossly normal lung (discrete nodular form). The nodules are otherwise similar in appearance and texture to those of the diffuse form. Bronchial lymph nodes may be markedly enlarged.

Histopathologic findings are restricted to the lungs and bronchial lymph nodes, and the lesions are similar regardless of the gross pathology. Nodules are sharply demarcated from unaffected lung tissue, and they consist of marked interstitial expansion of alveolar parenchyma by well-organized mature collagen.[1,7] In most cases, the alveolar architecture is preserved, but in rare cases, fibrosis is arranged in broad interlacing bundles without preserving the alveolar structure. Affected alveoli are lined by cuboidal cells, and the lumen contains inflammatory cells, primarily neutrophils and macrophages, the latter occasionally containing intranuclear inclusion bodies consistent with a herpesvirus infection. Changes in bronchial lymph nodes consist of marked lymphoid hyperplasia, often with nonspecific sinus histiocytosis.

Specimen for Laboratory Diagnosis

Specimens for diagnosis include lung nodules for histopathology, in situ hybridization, and PCR.

DIFFERENTIAL DIAGNOSIS

The differential diagnoses include the following: lung abscess, chronic pleuropneumonia, silicosis, lipid pneumonia, eosinophilic pneumonia, fungal pneumonia, pulmonary neoplasia (either primary—granular cell tumor—or secondary such as metastatic squamous-cell carcinoma), congestive heart failure, or chronic kidney disease.

DIAGNOSTIC CONFIRMATION

Diagnosis is confirmed by demonstration of compatible lesions in lungs at necropsy or on biopsy.

TREATMENT

There are no treatments with established efficacy, and management of the disease is based on first principles and empirical treatment with antiviral drugs, antiinflammatory drugs, and antimicrobials.

Reduction of inflammation and relief of pain is achieved by administration of nonsteroidal antiinflammatory drugs (phenylbutazone, fluniximе meglumine, ketoprofen) or corticosteroids (dexamethasone or prednisolone). Antimicrobials are administered to treat secondary bacterial infection and include penicillin, penicillin in combination with an aminoglycoside, or tetracycline or doxycycline.

Antiviral drugs have been administered to horses with EMPF, and some of these treated horses have survived.[7,28] Acyclovir and valacyclovir (a metabolite of acyclovir) are both active in vitro against gammaherpesviruses. Acyclovir is administered orally (20 mg/kg PO q8h) but has variable absorption compared with valacyclovir, and one cannot be confident that adequate concentrations in the blood are achieved in all horses.[29-31] The preferred drug, based on pharmacokinetic properties, is valacyclovir (30-40 mg/kg PO q8h).[30,31] A 2-week course of treatment with valacyclovir was associated with resolution of the disease in one horse.[28]

CONTROL

There are no known control measures.

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EQUINE HENDRA VIRUS INFECTION

ETIOLOGY

An acute disease of horses transmissible to humans and characterized in horses by fever and respiratory distress, but with capacity for pleiotropic clinical expression, occurs in northeastern Australia. The disease is associated with infection by equine Hendra virus (henipavirus, HeV, in the family Paramyxoviridae), which is closely related to Nipah virus (classified as the same genus).[1,2] There is very little genomic variation in HeV.[3] Infection by HeV, or Nipah virus, causes meningoencephalitis or, less frequently, respiratory disease in humans in contact with infected horses.[4]

A disease syndrome in horses and humans in the Philippines in 2014 was

associated with infection by a henipavirus closely related to Nipah virus.[5] The disease caused encephalitic signs and death in both horses and humans, there was horse–human and human–human spread, and the source of infection appeared to be fruit bats. The case-fatality rate in humans was approximately 50%, with a higher fatality rate among those with an acute encephalitic disease. At least 10 horses died, although this number likely underrepresents the actual number of horse deaths. Infection in some humans was associated with butchering or eating horses.[5]

EPIDEMIOLOGY

The disease in horses is uncommon, in that the morbidity or mortality rate within the population of at risk horses is low, with approximately two to four outbreaks reported each year involving a small number of horses. Between 1994 and 2013 there were 48 outbreaks of disease in horses, of which approximately 6 involved human disease.[2] The case-fatality rate was high in early outbreaks, and because of control measures that involve test and slaughter of infected horses, all horses infected in outbreaks are destroyed.

The disease is important because of the zoonotic nature of the infection and the high case-fatality rate in infected humans.

Transmission

The source of the virus is a wildlife host, the frugiverous pteropoid bats (fruitbats and flying foxes, *Pteropus* spp.). Approximately 25% of pteropid bats, including representatives of all four main species in eastern Australia (the grey-headed flying-fox, *Pteropus poliocephalus;* the black flying-fox, *Pteropus alecto;* the little red flying-fox, *Pteropus scapulatus;* and the spectacled flying-fox, *Pteropus conspicillatus*), were identified as being seropositive for HeV. The bats are seropositive for antibodies to the virus, the only seropositive mammals of 34 wildlife species sampled, and the virus can be isolated from pteropoid postpartum uterine fluid and fetal tissue. Mechanism of spread from bats to horses is uncertain, but it is speculated that ingestion by horses of infected bat fetal fluids and tissues might transfer infection from bats to horses. Fruit bats are consistently present when the disease occurs in horses.[6]

The disease spreads from bats to horses, and there is considerable interest in determining risk factors associated with transmission.[7-11] Infection of horses likely involves contact with virus soon after (hours) it is excreted from bats.[9] This is consistent with the 40× increase in risk of disease for horses in postal codes where fruit bats roost.[8] Serologic evidence indicates waxing and waning infection on a seasonal basis, and epidemiologic evidence and modeling favors an effect of anthropogenic changes in bat habitat favoring urbanization of bat colonies and reduced migration of bats. Urbanization increases the risk of spread of infection to horses, and reduced migration of bats reduces herd immunity in flocks, resulting in outbreaks of virus shedding and spread to horses.[12,13]

Dissemination of infection between horses by mechanical spread of infected nasal discharge likely occurred in the largest outbreak, and this could have been the route of infection of the human fatality. The virus is present in nasal discharges and urine of infected horses, and spread from horse to horse might also occur through inhalation of infected urine. Horse-to-horse transmission of infection is uncommon,[6] likely because the virus does not persist in the environment but can occur. Human-to-human transmission of infection has not been reported.

Disease occurs in horses, humans, cats, and guinea pigs, although in the latter two species the disease was a result of experimental infection. Dogs can become infected, but they do not appear to be at high risk of developing the disease, if they are at any risk at all, and there is no evidence that they propagate infection. Fruit bats do not develop clinical disease when experimentally infected.

Zoonotic Potential

The disease has important zoonotic implications; there have been four human deaths (~60% case-fatality rate) as a result of meningoencephalitis or pneumonitis and respiratory failure. Deaths all occurred in people who had close contact with infected horses, and the high risk associated with treating infected horses, or performing postmortem examinations on horses that have died of the disease, has prompted some veterinarians in endemic areas to exit from equine practice.[14] The reasons are concern about personal safety or legal liability for the safety of coworkers and owners of horses. However, the virus is not easily transmitted to humans, as evidenced by the observation that most people in contact with clinically affected horses do not develop antibodies to the virus.

CLINICAL SIGNS

The **incubation period** of the spontaneous disease is 8 to 11 days, but it is much shorter in experimentally induced disease. Death usually occurs within 24 to 48 hours of first onset of clinical signs, and affected horses housed in paddocks are often found dead.[6] **Clinical signs** of the disease in horses include lethargy, which is often marked, depression, loss of appetite, fever, ataxia, blindness, head pressing, aimless wandering, tachycardia, tachypnea, and copious frothy nasal discharge. Horses can show aimless pacing and can become entangled in fence—which can be mistaken for an accident rather than a consequence of neurologic disease associated with HeV infection.[6] There can also be hemorrhagic nasal discharge and swelling of the head. Some horses have muscle tremor. Death in acutely affected horses is sometimes associated with severe respiratory distress. Clinically inapparent infections of horses can occur.

An important understanding is that HeV can cause protean clinical signs, which might be interpreted as evidence of respiratory (dyspnea), neurologic (ataxia, blindness), muscular (muscle fasciculations), hepatic (head pressing) or gastrointestinal (terminal colic) disease.

CLINICAL PATHOLOGY

Characteristic changes in the hemogram or serum biochemical profile are not reported. If infected animals survive more than a few days after the onset of clinical signs, they develop serum-neutralizing antibodies. The recommended range of samples for HeV exclusion from the live horse are 10 mL of clotted, EDTA, and heparin blood; pooled nasal swabs from each nostril; swabs from other mucosal surfaces (e.g., oral cavity, rectum, or conjunctiva); or urine collected in, preferably, phosphate-buffered glycerol saline or isotonic sterile saline.[6] Antibodies are detectable by immunofluorescence microsphere immunoassays, or rapid immune plaque assay.[15] Viral genome can be detected by RT-PCR that is highly specific. Viral isolation in Vero cells or imaging using electron microscopy demonstrate presence of the virus. Details of diagnostic tests are available from the OIE.

NECROPSY

Necropsy examination reveals pulmonary edema with hemorrhage and froth in the airways. Histologic examination reveals an interstitial pneumonia characterized by extensive vascular damage and necrosis of alveolar macrophages. Pulmonary vascular changes include edema and hemorrhage within alveoli, plus necrosis and thrombosis of alveolar capillaries and small arterioles. The distinctive histologic feature is the presence of syncytial giant cells within blood vessels of the lungs and other organs. Retrospective diagnosis of the disease can be documented using an immunohistochemical technique or demonstration of viral nucleic acid in tissue by a test based on the PCR. Postmortem, 10 mL of blood can be collected from the jugular vein in addition to the submandibular lymph node and swabs as per a live horse. Field experience suggests that it is relatively easy to safely collect jugular blood from recently dead horses.[6]

TREATMENT AND CONTROL

There is **no specific treatment** for this disease. Ribavirin has been investigated for use in infected or exposed humans but is not used in horses, for which control measures are implemented.[2]

The **control measures** in the described outbreaks included slaughter of all infected horses, extensive serologic testing, and control of movement of horses within a defined disease control zone. The disease in index cases is likely attributable to contact of susceptible horses with infected fluids of pteropoid bats, and interventions that prevent or reduce the frequency of this occurrence are sensible, although the efficacy of this control technique has not been determined.

An effective vaccine is available, and its use is strongly advised in horses living or visiting areas where the disease is endemic.[16-18] In addition to preventing disease in horses, the vaccine provides veterinarians attending horses in endemic areas with some level of confidence that the horse is not infected with HeV.[17]

Strict biosecurity measures must be used by veterinarians examining potentially infected horses in areas where the disease is endemic, although this practice is often met with resistance.[18] Because of the protean nature of the disease, all sick horses should be considered as sources of infection. Biosecurity practices should be in place for examination of all horses, and the degree to which personal protective equipment is used can be adjusted based on the risk that the horse being examined is infected. Detailed guidelines for personal biosecurity are available.[19]

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PULMONARY AND SYSTEMIC ASPERGILLOSIS (*ASPERGILLUS* SPP.)

Diseases of horses and cattle associated with infection with *Aspergillus* spp. are characterized by either localized infections with slow progression or fulminant systemic or pulmonary disease. Localized infections are of the nasal cavities and paranasal sinuses;[1-3] eye; reproductive tract, including placenta;[4] mediastinum; or guttural pouch (see "Guttural Pouch Mycosis"). Systemic disease can affect any organ, including the brain, liver, and kidney,[5] but the most common manifestation is as acute pulmonary disease with or without infection of other tissues.[3,5-9]

ETIOLOGY

The causative organism is *Aspergillus* spp., usually *A. fumigatus* but occasionally one of *A. flavus, A. deflectus, A. nidulans, A. niger, A. clavulatus, A. nidulans,* or *A. sydowii.*[3,5,7,8,10,11] Aspergilli reproduce both sexually and asexually and hence are classified as dimorphic fungi. Asexual reproduction is by production of conidiophores and conidia. The organism is ubiquitous in organic material, and infections are opportunistic and associated with heavy contamination with the organism or decreased host defenses, although obvious risk factors are not always identified. Because its ubiquitous, the organism is often recovered from tracheal aspirates performed using contaminated equipment in horses with mild signs suggestive of noninfectious respiratory disease, such as heaves. In this instance recovery of the organism is of no clinical importance.

EPIDEMIOLOGY

Risk factors for development of aspergillosis include heavy environmental contamination with conidia and decreased host resistance, such as in horses with immune suppression associated with myeloproliferative disease (lymphoma), enterocolitis, or administration of immunosuppressive drugs such as corticosteroids. Specific risk factors for guttural pouch mycosis and infections of the nasal cavity or paranasal sinuses have not been identified, with the exception of an association between surgical resection of ethmoidal hematoma and subsequent nasal aspergillosis. Systemic or pulmonary aspergillosis is commonly associated with rumenitis, third-compartment ulceration in camelids,[7] enterocolitis, or administration of immunosuppressive drugs in adult horses. An outbreak pulmonary aspergillosis causing death of five albino Asinara donkey foals aged 20 to 30 days, but not of nonalbino herdmates, occurred without history of intercurrent disease or drug administration.[6]

CLINICAL FINDINGS

***Aspergillus* spp.** causes both localized and systemic disease in horses, cattle, camelids, and likely other species. Localized diseases include **guttural pouch mycosis**, which is discussed in detail elsewhere in this text. Fungal granulomas in the **paranasal sinuses** or **nasal passages** in any species of farm animal are caused by a number of organisms, including *Cryptococcus neoformans, Conidiobolus* spp., *Rhizomucor pusillus, Scedosporium apiospermum,* and, rarely, *Aspergillus* spp.[1-3,11-13] The disease is evident as nasal discharge that is usually unilateral, distortion of the contour of the head over the affected sinus, and lesions detectable on endoscopic examination of the nasal passages. Radiography can reveal the presence of a mass in the paranasal sinuses or nasal cavity associated with lysis and proliferation of bone. There is hyperfibrinogenemia and leukocytosis.

Systemic aspergillosis, including aspergillus **pneumonia**, is a severe disease usually evident as acute death without localizing signs in animals with other preexisting systemic disease, such as enterocolitis, neonates with inadequate passive immunity, or those receiving immunosuppressive drugs.[3,8,9] Horses with aspergillus pneumonia often have a very brief clinical course once signs of respiratory disease develop. Most commonly, horses with pulmonary aspergillosis die without signs of respiratory disease. Signs of pulmonary aspergillosis include fever, tachypnea, crackles and wheezes on thoracic auscultation, epistaxis, and frothy nasal discharge. Radiography reveals diffuse, miliary, nodule interstitial pneumonia (Fig. 12-32). Ultrasonographic examination demonstrates numerous small intrapulmonary masses adjacent to the pleural surface. Affected horses have hyperfibrinogenemia and leukocytosis at the time of development of the disease, but usually they have had neutropenia as a result of the enterocolitis. *Aspergillus* spp. can be isolated from tracheal aspirates of affected horses. The prognosis is very poor.

Aspergillus fumigatus can cause solitary, cavitated lesions in the lungs of foals.[8]

Disseminated aspergillosis has a variety of manifestations but is always a severe disease with a brief clinical course. Affected horses often have severe depression and can have signs of brain disease as a result of mycotic vasculitis and encephalitis.[5] The prognosis is very poor.

Aspergillus spp. is also associated with development of granulomas in the **mediastinum** of horses without apparent predisposing factors. Affected horses have progressively worsening respiratory distress, cough, fever, and occasional nasal discharge. Horner's syndrome can develop if the mass encroaches on the vagosympathetic trunk within the thorax. The mass is evident on radiographic examination of the thorax. Cultures of tracheal aspirates yields *Aspergillus* spp. Affected horses have neutrophilia, hyperfibrinogenemia, hyperglobulinemia, and mild anemia.

Keratomycosis attributable to *Aspergillus* spp. infection is infrequent in horses. The disease is characterized by blepharospasm, photophobia, epiphora, and corneal ulceration and opacity. *Aspergillus* spp. infections of the reproductive tract include mycotic **placentitis** and abortion and mycotic

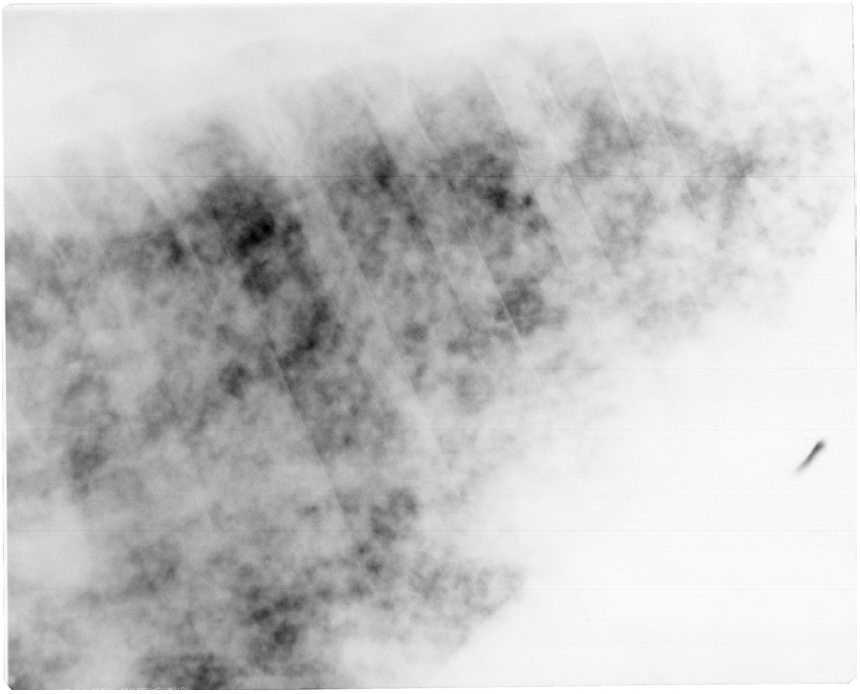

Fig. 12-32 **Radiograph of the caudal thorax of an adult horse with pulmonary aspergillosis secondary to acute enterocolitis. Note the military and interstitial densities.**

endometritis.[4] Fungal osteomyelitis of the proximal sesamoid occurs in horses that have received intraarticular administration of corticosteroids.[14]

CLINICAL PATHOLOGY

Definitive diagnosis of the disease is based on demonstration of organisms within lesions, either by histologic examination, culture, or use of PCR to demonstrate fungal DNA.[2] Antemortem demonstration of high concentrations of antibodies to *Aspergillus* spp. provides persuasive, but not definitive, evidence of infection. Both agar gel immunodiffusion assays and ELISA assays are available. These assays might not be useful in immunocompromised animals or in those with fulminant disease.

NECROPSY FINDINGS

Acute lesions are characterized by purulent, necrotizing inflammation. Chronic lesions are granulomas that contain macrophages, neutrophils, and giant cells. Pulmonary lesions are characterized by an acute necrohemorrhagic alveolitis. Organisms morphologically consistent with *Aspergillus* spp. are detected in the lesions as fungal hyphae, although these must be differentiated from *Pseudoallescheria boydii* or *Fusarium* spp. Reagents for immunofluorescent detection of *Aspergillus* spp. in lesions are available and useful in confirming the diagnosis.

TREATMENT AND CONTROL

Treatment of systemic or pulmonary disease is usually unrewarding, although surgical resection of a single large cavitating lesion in the lungs of a foal followed by administration of voriconazole (10 mg/kg PO q24h for 2–4 weeks) effected a cure.[8] A dose of voriconazole of 4 mg/kg orally q24h produces concentrations of drug greater than 0.5 µg/mL in body fluids. This concentration is greater than the concentration of voriconazole required to inhibit growth of filamentous fungi.[15]

Localized disease can be treated by surgical resection and administration of antifungal agents. Antifungal agents reported to be effective in treatment of localized disease in horses associated with *Aspergillus* spp. include itraconazole (3 mg/kg q12h, PO for 3–5 months) or enilconazole (0.2%–2.0% solution administered topically via an indwelling intranasal catheter q12h for 2–5 weeks). The lesions were debulked before treatment with enilconazole was started.[1] Seven of eight horses with nasal aspergillosis treated in this way recovered.[1] Topical treatment with enilconazole (10 mg/mL of solution) after surgical resection resulted in resolution of aspergillosis of the frontal sinus of a horse. Topical administration of natamycin (25 mg) was used for varying periods of time to treat mycotic rhinitis in three horses.

Amphotericin is likely effective against *Aspergillus* spp. and is cheaper than the azole class of drugs, but it is potentially nephrotoxic and must be administered intravenously. Fluconazole is not effective against the filamentous fungi, including *Aspergillus* spp.

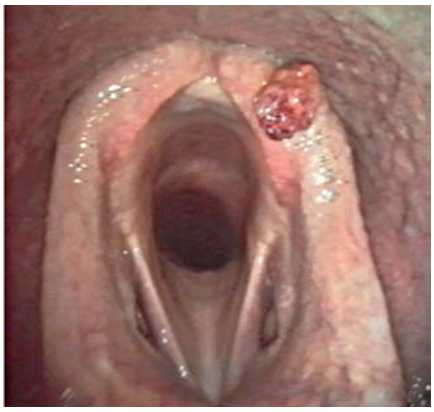

Fig. 12-33 **Granulomatous lesion caused by *R. seeberi* in a Belgian Warmblood horse.** (Reproduced with permission.[1])

There are no specific control measures or means of preventing disease associated with *Aspergillus* spp.

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RHINOSPORIDOSIS

Rhinosporidiosis is a chronic disease of the nasal mucosa in cattle and nasal mucosa, pharynx, and larynx in horses that causes formation of large polyps or granulomatous lesions (Fig. 12-33).[1,2] The causative agent, *Rhinosporidium seeberi,* is an aquatic protist that typically causes disease in amphibians. Its exact taxonomy is the subject of debate.[2,3]

Exposure to the organism is almost universal, based on serologic studies, in buffalo, cats, cattle, dogs, goats, horses, and in some areas with high prevalence of the disease, humans (such as Sri Lanka).[4,5] The disease is not endemic in western Canada or the United Kingdom, and affected horses in those areas were imported from Argentina.[2,6-8] The disease is reported in a Warmblood horse in Belgian that had never left the country.[1] Other cases in horses are reported from the Costa Rica, the southern United States, South Africa, and South America.[7,9]

The disease is evident as single or multiple, pedunculated or sessile, pink to red masses in the mucous membranes of the

nose and nasopharynx. The lesions can bleed and become evident as epistaxis.[7] No uniformly effective treatment is described, and surgical removal in three horses with pharyngeal or laryngeal lesions was not associated with cure—the disease progressing slowly over many months.[1,2,7] Excision of a single mass in the rostral nares of a mule was curative.[9] There is no effective pharmacotherapy.

Confirmation of disease is achieved by examination of biopsy material demonstrating moderate multifocal hyperplasia and ulceration of the mucosa, mild to moderate, multifocal, lymphoplasmacellular inflammatory infiltrate multiple and spherical to polygonal organisms of variable appearance, consistent with *R. seeberi,* in the lamina propria mucosae.[7] PCR analysis of affected tissue reveals presence of *R. seeberi* DNA.[2,7]

A related condition in cattle also thought to be caused by an unidentified fungus, similar to *Rhinosporidium* spp., is nasal granuloma, in which the lesions are small (0.5–2.0 cm diameter) mucosal nodules in the anterior third of the nasal cavity. Histologically, there is a marked eosinophilic reaction, and yeast-like bodies are present in cells or free in the tissue spaces. Clinical signs include severe dyspnea with loud stertor and a mucopurulent or blood-stained nasal discharge. A high incidence of the disease may occur on some farms and in particular areas.

Other diseases with similar clinical profiles include nasal obstruction associated with the blood fluke *Schistosoma nasalis* and chronic allergic rhinitis.[1]

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LUNGWORM IN HORSES

SYNOPSIS

Etiology The nematode parasite *Dictyocaulus arnfieldi.*

Epidemiology Infection is by ingestion of larvae on herbage; donkeys and foals shed most larvae, but adult horses can perpetuate life cycle.

Signs Chronic cough in adult horses.

Clinical pathology Eggs or larvae in feces (but often absent in affected adults); eosinophils in tracheal mucus.

Lesions Discrete areas of hyperinflation in lung tissue.

Diagnostic confirmation Response to treatment if no eggs/larvae in feces.

Treatment Eprinomectin, ivermectin, fenbendazole (elevated dose); mebendazole over 5 days for donkeys.

Control Avoid grazing donkeys and horses on same pasture.

ETIOLOGY

Lungworm disease in horses is associated with the nematode parasite *Dictyocaulus arnfieldi.*

LIFE CYCLE

The life cycle of *D. arnfieldi* is direct and is almost identical to that of *D. viviparus,* except that the eggs do not hatch until shortly after they are passed in the feces.

EPIDEMIOLOGY

Infestations with *D. arnfieldi* are recorded more commonly in donkeys than in horses, and the former are considered to be the more normal host. Patent infections may persist in donkeys throughout their lives but in horses are generally confined to foals. These animals therefore provide the most important sources of pasture contamination. Nevertheless, a small proportion of infected adult horses shed low numbers of eggs, and this may be sufficient to perpetuate the life cycle even in the absence of donkeys and foals. As with *D. viviparus,* larvae can cross field boundaries by fungal transfer.

PATHOGENESIS

Adult worms are found in the smaller bronchi, which they almost completely block. In adult horses however, few larvae reaching the lungs develop to this stage. Bronchioles in affected areas are surrounded by dense infiltrations of inflammatory cells, the epithelium becomes hyperplastic, and excessive mucus is produced. The consequent interference with airflow leads to patches of hyperinflation in the lung tissue.

CLINICAL FINDINGS

Lungworm disease in horses is characterized by a chronic cough. Experimental infections produce an afebrile condition with coughing, increased respiratory rates, and forced expiration being most intense during weeks 3 to 5 after infection. Thereafter the signs decrease in severity but coughing may persist for several months. Heavy infestations in donkeys do not cause clinical illness. Horse foals may also be symptomless, although some show clinical signs.

CLINICAL PATHOLOGY

Characteristic eggs may be found in the feces of a small proportion of cases. Eosinophils and sometimes eggs or larvae may be demonstrated in tracheal mucus.

NECROPSY FINDINGS

The most obvious lesions at necropsy are discrete patches of overinflation.

DIAGNOSTIC CONFIRMATION

D. arnfieldi eggs in fresh feces are oval, are thin shelled, and contain a larva. Because the eggs may have hatched before arrival at the laboratory, it is usual to harvest larvae with the Baermann technique. The larvae resemble those of *D. viviparus,* but the tail ends in a small spine. Because many clinical cases are nonpatent and because tracheal mucus is difficult to sample, confirmation of diagnosis is often dependent on response to treatment.

DIFFERENTIAL DIAGNOSIS

- Recurrent airway obstruction (heaves)
- Pulmonary abscessation and pneumonia
- Inflammatory airway disease

TREATMENT

TREATMENT
Eprinomectin (0.5 mg/kg, top.) (R2)
Ivermectin (0.2 mg/kg SQ) (R2)
Mebendazole (20 mg/kg, q1d for 5 days) (R3)

Eprinomectin, as a pour-on formulation (0.5 mg/kg), has 100% efficacy in eliminating fecal larvae in donkeys within 7 days after treatment.[1] Ivermectin at the standard equine dose is highly effective against immature and mature stages. For donkeys, mebendazole may be used at 15 to 20 mg/kg daily for 5 days, but this should not be attempted within the first 4 months of pregnancy.

CONTROL

Donkeys and horses should not be grazed on the same pasture. If this is impossible, the former should be treated regularly for lungworm. If there is a problem in a closed herd of adult horses, individuals with patent infection can be identified by fecal screening and treated.

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Diseases of the Swine Respiratory Tract

PROGRESSIVE ATROPHIC RHINITIS (CONCHAL ATROPHY OF SWINE)

Atrophic rhinitis is a disease affecting primarily young pigs but causing anatomic lesions that may persist for life. The term *nonprogressive atrophic rhinitis* is used for the

slight to severe rhinitis and usually transient atrophy of the conchal bones (formerly called the turbinates) in which no toxigenic *P. multocida* are found, when there are no clinical signs and no obvious growth retardation. This mild form is probably as a result of infection with *Bordetella bronchiseptica* (BB) or nontoxigenic *P. multocida* (PM).

The term *progressive atrophic rhinitis* is proposed for the infection with toxigenic *P. multocida* (PM) (capsular serotype D and A strains) characterized by shortening or distortion of the snout, sneezing, nasal discharge, and epistaxis. Progressive atrophic rhinitis is often accompanied by reduced growth rates in severe cases.

The organism is a zoonosis,[1] but this is rarely from the pig,[2] although pig farmers often have PM in their nasal cavities.[3]

SYNOPSIS

Etiology Toxigenic strains of *Bordetella bronchiseptica* and *Pasteurella multocida*

Epidemiology Young growing pigs. High percentage of pigs reared under intensive conditions may have some degree of atrophic rhinitis. Infection is widespread and transmitted by carrier sow to piglet. Housing and ventilation risk factors. Immunity develops in herd. Major economic importance because may affect growth rate and predispose to pneumonia.

Signs Initially sneezing when piglets 3 to 9 weeks of age. Nasal discharge. Deformity of face with nasal bones (twisted snout). Growth rate may be decreased.

Clinical pathology Culture organism from nasal swabs; polymerase chain reaction (PCR)

Lesions Varying degrees of severity of atrophic rhinitis.

Diagnostic confirmation Necropsy examinations of snouts.

Differential diagnosis list
- Inclusion-body rhinitis
- Necrotic rhinitis
- Inherited prognathia

Treatment Antimicrobials in early stages; nothing later.

Control Eliminating toxigenic strains of *P. multocida*. Depopulation and repopulation. Reduction of infection. Mass medication. Medicated early weaning. Vaccination.

ETIOLOGY

Infection of the nasal cavities with BB followed by toxigenic strains of PM—primarily capsular type D and occasionally type A—results in progressive turbinate atrophy. PM has four subspecies (*multocida, septica, gallicida,* and *tigris*), but multocida is usually isolated from pigs[4]. There are five capsular serotypes (A-F) of PM. PM type A strains were formerly thought to be associated entirely with lung infections but there is increasing evidence that some strains of PM type A are toxin producers and may be involved in atrophic rhinitis. Toxin production appears to be independent of serotype. The strains of PM isolated from the lungs are usually nontoxigenic and of capsular type A but a small proportion are toxigenic and are capsular type D. Serotype B is probably the most common one associated with septicemic pasteurellosis.

The majority of cases of progressive atrophic rhinitis were associated with toxA-containing capsular type D strains.

Somatic antigens reflecting differences in lipopolysaccharides have also been used, in addition to a variety of other techniques.[5] The poultry type of analysis[6] based on multilocus sequence typing will be adapted for use for the pig eventually. There may a limited genetic heterogeneity in both the healthy pig strains and the PAR strains.[7]

EPIDEMIOLOGY

Occurrence

Atrophic rhinitis occurs worldwide where pigs are reared under intensive conditions. It has, however, become much less important with the onset of vaccination, improvement in resistance by pig breeding companies, and general attention to the environment in the farrowing house.

Some surveys have shown that 50% of finished pigs and sows at slaughter have lesions of atrophic rhinitis. The incidence of clinical disease varies from 5% to 30%, which in part depends on the method of detection of the gross lesions. Abattoir surveys of the snouts of slaughtered pigs indicate that the incidence of gross lesions ranges from 14% to 50%. However, the incidence of gross lesions in abattoir surveys is biased by the source of the pigs; the incidence may be low in pigs from herds that have attempted to control the disease and high in some commercial herds with no control program. In pigs slaughtered from pig testing stations the incidence of lesions may be uniform over a long period. The published data on the incidence of gross lesions are also variable because of the lack of a uniform method of evaluating and quantifying the lesions.

The incidence and severity of the lesions may vary with the season and the type of facility in which pigs are reared. In a slaughter survey of the snouts and lungs of pigs from 21 pig herds over one winter and one summer, the lesions of atrophic rhinitis were more severe among pigs slaughtered in the summer, whereas lesions of pneumonia were more severe among pigs slaughtered in the winter. Lesions of atrophic rhinitis were also more severe in pigs farrowed in central, enclosed farrowing houses and finished in enclosed, mechanically ventilated buildings than in pigs farrowed individually in sow huts and finished on dirt lots. It is possible that the incidence and severity of the lesions at slaughter may be a reflection of the condition of the housing facilities when the animals were piglets several months previously, but many other factors could have been involved.

Prevalence of Infection

B. bronchiseptica readily colonizes the ciliated mucosa of the respiratory tract of pigs and infection of the nasal cavities of pigs is present in almost every pig herd, with the prevalence of infection in pigs in commercial herds varying from 25% to 50%. Serologic surveys of individual herds have found that up to 90% of the pigs are positive, which indicates that there is no reliable correlation between the frequency of isolation of the organism and the percentage of animals with antibody. The prevalence of infection is just as high in specific-pathogen-free herds as in nonspecific-pathogen-free herds.

The prevalence of infection of toxigenic PM type D is higher in herds with clinical disease. The organism can be present in 50% to 80% of weaned pigs in a herd with clinical disease in the finishing pigs. Toxigenic type D PM was first detected in New South Wales, Australia, in 1986; in all herds examined, the introduction of pigs from an infected herd in South Australia was associated with an increased risk of infection. Toxigenic PM type D has been isolated rarely from herds free of atrophic rhinitis.

Whereas BB is eliminated from the respiratory tract of most infected pigs, leaving only a few infected at slaughter, PM often persists.

Method of Transmission

Direct contact and droplet infection are presumed to be the most likely methods of transmission. The reservoir of infection is the infected sow, and litters of piglets become infected at an early age. Colonization of the tonsil by PM in conventionally reared pigs is common. In the Netherlands, it has been recognized that infection is usually by one of four possibilities. These are artificial insemination centers, laborers, neighborhood infection by direct aerosol or indirect local contact, and the presence of carrier animals and birds.

The infection is usually introduced into a herd by the purchase of infected pigs. Spread between piglets is probably enhanced after weaning when mixing of litters occurs, and 70% to 80% of a large weaned group may become infected. Infection persists for up to several weeks and months, followed by a gradual reduction in the intensity and rate of infection. In herds where BB is the initiating agent, up to 90% of pigs 4 to 10 weeks of age will have nasal infection, but this infection rate falls to approximately 15% by 12 months of age, and the proportion of carrier pigs within the breeding herd decreases with increasing age of sow. The

prevalence of infection is also much higher during the period from October to March than at other times of the year, and the prevalence of serologically positive animals is highest from July to December. This is most probably a result of the winter housing conditions, with few air changes per hour, fluctuating temperatures, and high humidity.

The epidemiology of toxigenic strains of PM as a causative agent of atrophic rhinitis is not as well understood. The organism colonizes the tonsils of clinically normal pigs. In contrast to BB, which is ubiquitous in pig herds, the toxigenic isolates of PM appear to be restricted to herds affected with progressive atrophic rhinitis. The organism is invariably present in herds with progressive atrophic rhinitis but may also be present in about 5% of the pigs in a herd with no clinical history of atrophic rhinitis. The main source of toxigenic isolates of PM for young pigs appears to be the pharyngeal tissues of the breeding stock. About 10% to 15% of sows in farrowing houses may be infected with toxigenic isolates, and piglets become infected within a week after birth. In contrast to BB, infection of piglets at 12 to 16 weeks of age with toxigenic PM will still result in varying degrees of severity of lesions.

It is possible for growing pigs to develop lesions of atrophic rhinitis well beyond the age of 3 weeks if they are exposed to pigs affected with disease and infected with PM and BB.

Risk Factors

Animal Risk Factors

The age at which piglets first become infected with BB has an important effect on the development of lesions. The most severe lesions occur in nonimmune animals infected during the first week of life. Animals infected at 4 weeks of age develop less severe lesions, whereas those infected at 10 weeks do not develop significant lesions.

Immune Mechanisms

The level of immunity in the young pigs will influence the level of infection and the incidence of clinical disease. Colostral immunity from sows serologically positive to BB is transferred to piglets and provides protection for 2 to 5 weeks. Clinical disease does not occur in piglets with high levels of passive antibody. Older pigs from 10 to 12 weeks of age may become infected but are less likely to develop severe turbinate atrophy and may develop inapparent infection and become carriers.

Vaccination of the sow before parturition to increase colostral immunity or vaccination of the young pig will increase the rate of clearance of the organism from the nasal cavity and reduce the incidence of clinical disease. In chronically affected herds a level of immunity develops with increasing age of the breeding herd.

Pathogen Factors

The virulence characteristics of BB and the toxigenic isolates of PM are important risk factors. Both organisms are required to produce lesions similar to the naturally occurring progressive disease. The virulence of BB is dependent on the ability to produce heavy, persistent colonization in the nasal cavity and the production of a heat-labile toxin. *Bordetella* spp. produce several virulence factors and toxins, which are regulated by a two-component sensory transduction system encoded by the bvg locus. These virulence factors include adhesins such as filamentous agglutinin, pertactin, and fimbriae; the adenylate cyclase–hemolysin toxin; and the dermonecrotic toxin. In cell cultures the dermonecrotic toxin stimulates DNA and protein synthesis and assembly of actin stress fibers while inhibiting cell division, resulting in polynucleation of cells. It mediates these through the modification and activation of the small guanosine 5′-triphosphate (GTP)-binding protein Rho.

There are both toxigenic and nontoxigenic strains of BB. Colonization of the nose was greater with the dermonecrotic-toxin-positive strains than with the dermonecrotic-toxin-negative mutant strains. This was maintained for the first, second, and third weeks postinoculation, but by the fourth week the position had changed to the opposite. All dermonecrotic-toxin-positive pigs had pneumonia but the dermonecrotic-toxin-negative animals were able to colonize the lung more freely. There is an outer membrane protein P68 perlactin (BB perlactin gene [prn]), an adhesin, that may play a part in the protective immunity and may be extremely variable. The most important experiment is one that shows that PM mutant strains without the capacity to produce PM type D toxin did not produce turbinate atrophy. Only certain porcine phase 1 cultures possess both properties. However, even the most virulent of 10 isolates of BB did not cause progressive turbinate atrophy or significant snout deformation in experimental infections. The severe lesions of atrophic rhinitis cannot be attributed to this organism alone. Experimental inoculation of specific-pathogen-free or gnotobiotic pigs with the organism results in a nonprogressive moderately severe turbinate atrophy 2 to 4 weeks after infection, followed frequently by regeneration of the turbinates. These virulence characteristics of BB are consistent with the observations that in herds where the organism is common it can provoke sneezing and coughing but no evidence of clinical turbinate atrophy. Examination of the turbinates within 2 weeks after the sneezing will reveal some mild lesions, but no lesions will be evident when the pigs are examined at slaughter. It may be that the adhesins left over in the nasal cavity from an infection of BB are subsequently available for the attachment of other bacteria.

Toxigenic isolates of PM colonize the nasal cavities, elaborate several toxins, and produce progressive lesions of the turbinate bones and snout. Toxigenic PM can colonize the upper respiratory tract of pigs, and the presence of the capsule is a virulence factor. The presence of BB can enhance the colonization of PM, particularly the toxigenic type D strains isolated from pigs. The cytotoxin of BB is required for optimum growth by toxigenic PM; other products of phase 1 BB growth assist colonization by PM, and the degree of atrophy of the turbinates in these mixed infections is related to the numbers of toxigenic PM in the nasal cavity. Severe turbinate damage and shortening of the snout can be reproduced in specific-pathogen-free and gnotobiotic pigs by combined infection with BB and certain strains of PM. Following experimental infection both organisms may persist in the nasal cavities for up to 64 days. The cell envelope proteins and lipopolysaccharides of PM strains associated with atrophic rhinitis have been characterized and compared. At least three protein patterns and six lipopolysaccharide patterns can be distinguished, which can be used to predict the pathogenic character of some of the strains. This will obviate the need to use the guinea-pig skin test to distinguish those strains that are associated with atrophic rhinitis and those that are not.

The gene for the osteolytic toxin of PM has been cloned and expressed in *E. coli*; the protein expressed has been shown to have the same properties as the native toxin. The toxin is the main colonization factor produced by toxigenic strains of the organism and antitoxin made from the toxin is protective experimentally and cross-protective between toxins from different capsule types. The toxin can produce turbinate atrophy when injected intranasally and also when given intramuscularly, intraperitoneally, intravenously, or intradermally. Fingerprinting techniques have been used to show that outbreaks of atrophic rhinitis since 1985 in Australia have been associated primarily with a single strain of toxigenic type D PM.

Environmental Factors

The effects of housing, population density, and adequacy of ventilation on the prevalence of infection of BB and toxigenic isolates of PM and on the incidence and severity of atrophic rhinitis have not been examined in detail. Atmospheric ammonia, dust, and microbial concentrations in the farrowing house and dust in weaner barns have a significant role in the severity of atrophic rhinitis. The mean daily gain of gilts with atrophic rhinitis exposed to ammonia may be smaller than that of those not affected. Undocumented field observations suggest that the disease is more common and severe when pigs are confined, overcrowded, and housed in poorly ventilated unsanitary

barns, all of which promote the spread of infection.

There is no effect of high levels of ammonia on the severity of turbinate atrophy. It has been shown that high levels of ammonia have no effect on the disease progression of atrophic rhinitis and pneumonia but do enhance the colonization of the nasal turbinates by toxigenic PM. A recent experiment has shown that higher numbers of PM bacteria were isolated from the tonsil than the nasal membranes per gram of tissue. Aerial pollutants contribute to the severity of lesions associated with atrophic rhinitis by facilitating colonization of the upper respiratory tract by PM.

Management factors such as confinement farrowing and the use of continual throughput farrowing houses and weaner houses are also considered to be important risk factors. Adverse climatic conditions (below thermoneutrality with drafty periods) can result in a lower amount of energy available for production because of increased maintenance requirements, which results in growth retardation associated with lowered feed intake.

Economic Importance

Historically, it was accepted as dogma that atrophic rhinitis was an important cause of economic loss in pig herds because of decreased growth rate, less-than-optimal feed efficiency, and the fact that it was a major risk factor in enzootic swine pneumonia. A number of field studies have found an association between atrophic rhinitis and reduced growth rate in some herds, whereas other observations were unable to show an association between the presence of the disease and growth rate. The lack of a standard system for evaluation of conchal lesions may be a factor in the variable results between observations.

Some field studies have failed to show that the disease has an effect on growth rate in finishing pigs or that there is a cause and effect relationship between atrophic rhinitis and pneumonia. The presence of pneumonia in pigs from a test station reduced mean daily weight gains by 33% for each 10% of affected lung, but atrophic rhinitis did not affect daily gain and there was no association between the development of atrophic rhinitis and the development of pneumonia. Pigs vaccinated against BB had turbinate atrophy scores or mean daily gains no different from those of unvaccinated pigs. In another study there was a low positive correlation between the herd mean turbinate atrophy score and the herd mean percentage pneumonia score. A recent report from Illinois indicates that the prevalence of clinical atrophic rhinitis in farrow-to-finish herds ranged from 0% to 20%, and in pigs from those herds examined at the abattoir the incidence of turbinate lesions ranged from 5 to 92%. In some of the herds the mean daily weight gain was 15% to 18% higher than in herds where pigs had severe turbinate lesions. In an Australian report there was no correlation between the severity of atrophic rhinitis and growth rate or back-fat thickness.

In one study of three commercial pig herds, the snouts and lungs of individual pigs were examined and scored at slaughter, and the results were correlated with growth indicators for each pig (average daily gain during the growing and finishing phases, and days to reach market). Scores for lung lesions were also correlated to scores for snout lesions. Contrary to findings in many other studies, pigs that reached market weight at the youngest age did not have the lowest score for lung lesions, nor the lowest grade for snout lesions, nor the least extensive or severe lesions. It was concluded that lung lesions and grades for snout lesions in pigs at slaughter are not valid indicators for determining the economic effect of either pneumonia or atrophic rhinitis on growth performance of pigs.

PATHOGENESIS

Following infection of the nasal cavity, BB becomes closely associated with the ciliated epithelium of the respiratory tract. It can bind to respiratory tract mucus. The organism produces a heat-labile toxin that results in a nonprogressive, moderately severe turbinate atrophy that is apparent within 2 to 4 weeks after infection, followed frequently by regeneration of the conchae. There is, initially, ciliary loss and ciliary stasis, followed by reduction in mucociliary clearance, followed by hyperplasia and metaplasia of the nasal epithelium, fibrosis in the lamina propria, and resorption and replacement fibrosis of the osseous core. Experimental infection with BB alone does not result in severe persistent conchal atrophy or twisting or shortening of the snout. The strains of BB that produce cytotoxin may predispose to the colonization of PM in the nasal cavities.

The preferred habitat of PM appears to be the tonsillar crypt, but following damage by BB, it can inhabit the epithelium of the URT.

Infection and colonization of the nasal cavities, particularly the mucus, with the toxigenic strains of PM results in the elaboration of a toxin that causes progressive conchal atrophy. The toxin is thermolabile and dermonecrotic and is called the dermonecrotic toxin of PM. It interferes with G-protein and Rho-dependent signaling pathways in the cells. It is encoded by the toxA gene. The inoculation of a toxin from a toxigenic strain of type D PM into the nasal cavities of gnotobiotic pigs results in severe bilateral atrophy of the conchae. Atrophy of the ventral conchae can be produced experimentally with pathogenic BB in piglets at 6 weeks of age and with toxigenic PM strains in piglets as old as 16 weeks of age.

The toxin enhances osteoclastic resorption and impairs osteoblastic synthesis of the conchal osseous core; irreversible changes can occur within a few days. The toxin is a one chain toxin of 1285 amino acids, and different domains of the toxin are involved in cell uptake and intracellular activities. The toxin is able to subvert cell cycle progression and cell–cell signaling systems in osteoblasts and osteoclasts. The toxin is the sole agent responsible for the conchal atrophy, and the effect appears to be related to the total exposure to the toxin; that is, it is dose dependent. The toxin PMT activates various heterotrimeric G proteins, which causes the deamidation of the alpha-subunits of the G proteins.[8-11] More important, this also appears to have an immunomodulatory effect. There is an inverse relationship between the number of PM and the total concentration of immunoglobulin. This may in part be one of the reasons that local changes in the nose produce such adverse growth effects, and they may be a result of the fact that the PM type D toxin has in fact changed the immune functions and that the PM may have predisposed to many other agents. These authors' conclusion is that PM significantly suppresses the antigen-specific IgG immune responses of pigs to parenteral antigen challenge. The epithelium and the submucosa undergo secondary atrophy, and the conchae may disappear almost completely within 10 to 14 days. These lesions can persist until the animal is 90 kg in body weight. The conchal atrophy is not accompanied by an inflammatory reaction. The effect of the PM toxin is restricted to the nasal cavity; this is supported by the intriguing observation that the parenteral injection of the toxin into gnotobiotic piglets results in turbinate lesions and shortening and twisting of the snout. The parenteral injection of the dermonecrotoxin of PM capsular type D into specific-pathogen-free adult pigs will result in moderate conchal atrophy. In piglets 7 days of age, the intramuscular injection of the purified dermonecrotoxin will result in severe atrophy of the conchae. The culture filtrate of a nonatrophic-rhinitis pathogenic PM will not cause lesions after intramuscular injection. The disappearance of the conchae and the involvement of the bones of the face lead to deformity of the facial bones with the appearance of dishing and bulging of the face and, if the lesion is unilateral, to lateral deviation of the snout.

The effect on growth rate, if any, may be attributable to the chronic irritation and interference with prehension. Experimentally, atrophic rhinitis suppressed the health of pigs, reducing their activity and feed intake. Experimentally, parenteral injections of the toxin decrease physeal area and reduce chondrocyte proliferation in long bones, in addition to conchal atrophy.

Reliable experimental models of atrophic rhinitis in gnotobiotic pigs are now available and are useful for studying the pathogenesis of the disease and testing vaccine strategies.

A sterile sonicate of a toxigenic strain of BB is instilled into the nasal cavities of piglets at 5 days of age followed by intranasal inoculation of toxigenic strains of PM at 7 days of age.

The toxin can also affect the liver and urinary tract and decrease the physeal area in the long bones.

CLINICAL FINDINGS

The clinical findings of atrophic rhinitis depend on the stage of the lesions. In acute cases in piglets 3 to 9 weeks of age, irritation of the nasal mucosa causes sneezing, some coughing, small amounts of serous or mucopurulent nasal discharge, and transient unilateral or bilateral epistaxis. The frequency of sneezing may be a measure of the incidence and severity of the disease. In piglets born from sows vaccinated with BB and PM vaccine before farrowing, followed by two vaccinations within 3 weeks of age, the frequency of sneezing at 3 to 9 weeks of age was much less than in piglets given only BB vaccine. There may be rubbing of the nose against objects or on the ground. A watery ocular discharge usually accompanies this and may result in the appearance of dried streaks of dirt below the medial canthus of the eyes. There may be a decrease in growth rate. In infection with BB these clinical signs will disappear spontaneously in a few weeks, when the pigs will appear normal. In severe cases, respiratory obstruction may increase to the point of dyspnea and cyanosis, and sucking pigs may have great difficulty in nursing. The nasal secretions become thicker and nasal bleeding may also occur.

In the more chronic stages, inspissated material may be expelled during paroxysms of sneezing. During this chronic stage, there is often pronounced deformity of the face as a result of arrested development of the bones, especially the conchae, and the accumulation of necrotic material in the nasal cavities. The nasal bones and premaxillae turn upward and interfere with approximation of the incisor and, to a lesser extent, the molar teeth. There are varying degrees of brachygnathia superior and protrusion of the lower incisor teeth. Prehension and mastication become difficult, with a resulting loss of body condition. Facial distortion in the final stages takes the form of severe "dishing" of the face with wrinkling of the overlying skin. If the condition is unilateral, the upper jaw may be twisted to one side. These visible facial deformities develop most commonly in pigs 8 to 10 weeks old within 3 to 4 weeks after infection, but they may occur in younger pigs.

The most serious effects of the advanced disease are depression of growth rate and unthriftiness. The appetite may be unaffected, but much feed is lost by spillage, and feed efficiency may be reduced in some instances.

CLINICAL PATHOLOGY

Culture and Detection of Bacteria

It is important to be able to detect infected animals in a herd, especially the carrier animal. Nasal swabs are used to detect the bacteria and to determine their drug sensitivity. The collection of the nasal swabs must be done carefully and requires a special transport medium to ensure a high recovery rate. A sampling technique and a special culture medium to facilitate the isolation and recognition of BB are described. The external nares are cleaned with alcohol, and a cotton-tipped flexible wire is pushed into the nasal cavity (of each side in turn) until it reaches a point midway between the nostril and the level of the medial canthus of the eye. On removal, the cotton tip is cut off into 0.5 mL of an ice-cold sterile transport medium comprising phosphate-buffered saline (PBS, pH 7.3) with fetal calf serum (5% v/v). The samples are then placed on special media, preferably within 4 hours. Normally the organism grows well on conventional culture media, especially when younger pigs are sampled. However, in the carrier pig the organism may be sparse, and the selective medium is recommended.

The nasal culturing procedure has been used as an aid in the control of atrophic rhinitis associated with BB. A series of three nasal swabs from each animal is considered to be about 77% efficient in detecting infected animals for possible culling and elimination from the herd. However, in some studies there may be no marked difference in the prevalence of BB or PM in pig herds with or without clinical atrophic rhinitis.

Toxigenic PM grow readily in the laboratory but are difficult to isolate from nasal swabs because they are frequently overgrown by commensal flora. Selective laboratory media containing antimicrobial agents have been developed to promote the isolation of PM from nasal swabs. Inoculation of cotton swabs to selective medium on the same day as the sampling provides the best isolation of toxigenic PM. Immersion of pigs at slaughter in the scalding tank can result in a marked reduction in the isolation of toxigenic PM.

A cell culture assay using embryonic bovine lung cell cultures is available and is a sensitive in vitro test for the differentiation of toxigenic from nontoxigenic isolates of PM. This test can replace the lethal tests in mice or the dermonecrotic tests in guinea pigs.

Serology

Agglutination tests and an ELISA test are available for the detection of pigs infected with BB, especially carrier animals. Serology is of value in the assessment of the response of pigs vaccinated with the BB vaccines. There are currently no reliable serologic tests for Pasteurellae.

Antigen Detection

A PCR method originally described in 1996 for the enhanced detection of toxigenic PM directly from nasal swabs has been described and upgraded. This was shown to be 10 times more sensitive than PM type D toxin (PMT) ELISA and 5 times more sensitive than clinical bacteriology with subsequent use of PMT ELISA. A nested PCR has also been described. Similarly, a PCR method for the detection of BB has been described that produces 78% more positives than culture, particularly with swabs with a high mixed bacterial load. Recently a nested-PCR has been described that was reported to be more specific and sensitive than the other PCR methods previously described. It does not require culture, it is less laborious, and the results can be provided within 24 hours. The authors concluded that this test was suitable for breeding company evaluations and for eradication schemes.

Radiography

Some aids to the clinical diagnosis have been examined but are not highly accurate. Radiography of the nose is not reliable in detecting the severity of conchal atrophy.

NECROPSY FINDINGS

The typical lesions of atrophic rhinitis are restricted to the nasal cavities, although concurrent diseases, especially virus pneumonia of pigs, may produce lesions elsewhere. In the early stages there is acute inflammation, sometimes with the accumulation of pus, but in the later stages, there is evidence only of atrophy of the mucosa and decalcification and atrophy of the conchae and ethmoid bones, which may have completely disappeared in severe cases. The inflammatory and atrophic processes may extend to involve the facial sinuses. There is no evidence of interference with the vascular supply to the affected bones. The changes in the nasal cavities are most readily seen if the head is split in the sagittal plane but for accurate diagnosis the degree of conchal symmetry, volume, and atrophy and medial septum deviation should be assessed by inspection of a vertical cross-section of the skull made at the level of the second premolar tooth.

The clinical diagnosis is confirmed and the severity of the lesions is assessed by the postmortem examination of a cross-section of the snout. The snout must be sectioned at the level of the second premolar tooth because the size of the conchal bone reduces anteriorly and may give a false-positive result if the section is taken too far forward. Quantification of the severity of the lesions has been of value for monitoring the incidence and severity of the disease in a herd. Several systems have been used for grading the severity of lesions of the snout. Most of them have used a subjective visual scoring system in which snouts are grade 0 (complete normality) to 5 (complete conchal atrophy).

Reasonable agreement among observers recording morphologic changes of nasal conchae is achievable with some training.

The standards for each grade are as follows:

- **Grade 0**: No deviation from absolute normality, with nasal septum straight and conchae symmetric and filling nasal cavities.
- **Grade 1**: Slight irregularity, asymmetry, or distortion of the nasal structures without atrophy.
- **Grade 2**: Marked distortion of nasal structure but without marked atrophy.
- **Grade 3**: Definite atrophy of the conchae with or without distortion.
- **Grade 4**: More severe atrophy with severe atrophy of one or more conchae.
- **Grade 5**: Very severe atrophy in which all conchae have virtually disappeared.

Such a discontinuous grading system does not provide a direct quantitative relationship. Regular examination of the snouts from heads of pigs sent to slaughter can be used to assess the level of conchal atrophy in the herd. Morphometric methods, using either point counting or semiautomated planimetry applied to photographic or impression prints of sections of the snout to measure the extent of conchal atrophy on a continuous scale as a morphometric index, are now available. Cross-sections of the snout are photographed or used to make impression prints, which are then measured. A morphometric index is determined, which is the ratio of free space to total cross-sectional area of the nasal cavity. The system correlates well with the visual grading system of 0 to 5 but is labor-intensive and relatively expensive. The conchal perimeter ratio may be a more reliable morphometric measure of atrophic rhinitis and also provides parametric data suitable for quantitative analysis. A morphometric analysis using conchal area ratio is the best method for quantifying gross morphologic turbinate changes. Descriptions of the methods for making snout impressions are available. Computed tomography has been described.

A major limitation of the grading system is that conchal atrophy occurs as a continuous spectrum, and it is difficult to decide, for example, if a pig with a grade 3 lesion represents the more severe manifestation of BB infection, which may not progress further, or an early manifestation of infection with toxigenic PM, which could develop into a severe herd problem.

Histologically, the lesions vary according to the stage of the disease; initially there is a neutrophilic infiltrate followed by more chronic mononuclear cell infiltration. The conchal bones are eroded by osteoclasts, and new bone formation is reduced with degeneration dystrophy and reparative processes.

Samples for Confirmation of Diagnosis

- Bacteriology—nasal swabs are not as good as tonsil swabs but are easier to obtain. The highest isolation rates are achieved with Knight medium or KPMD. Conventional biochemistry can then be used to identify.[12]
- Histology—formalin-fixed cross-section of snout at level of second premolar
- Antigen detection—nasal swabs. ELISAs based on the use of PMT-specific monoclonal AB are rapid, sensitive and specific. The *kmt1* gene has been used as a target for the loop-mediated isothermal amplification method.[13] Diagnostic tests have been reviewed.[14]

Computer tomography can be helpful.[15]

DIFFERENTIAL DIAGNOSIS

The occurrence of sneezing in the early stages and of facial deformity in the later stages are characteristic of this disease. Diagnosis depends on clinical signs, pathology, and demonstration of PM and its toxin.

Inclusion-body rhinitis as a result of a cytomegalovirus is a common infection in young piglets in which there is sneezing and conjunctivitis. However, by itself it does not progress to produce turbinate atrophy and facial distortion. Under good hygienic conditions the course of the disease is about 2 weeks, and the economic effects are minimal. In the early acute stages, atrophic rhinitis may be mistaken for swine influenza, which, however, usually occurs as an outbreak affecting older pigs and accompanied by a severe systemic reaction without subsequent involvement of facial bones.

Necrotic rhinitis is manifested by external lesions affecting the face, and virus pneumonia of pigs is characterized by coughing rather than sneezing.

The inherited prognathic jaw of some breeds of pigs has been mistaken for the chronic stage of atrophic rhinitis; protrusion of the lower jaw is quite common in adult intensively housed pigs and has been attributed to behavioral problems of pushing the snout against fixed equipment such as bars and nipple drinkers.

TREATMENT

Treatment early in the course of the disease will reduce the severity of its effects, but it is of little value in chronically affected pigs, and these pigs are best culled at an early age because of their persistent poor growth rate and high food conversion.

Tylosin at 20 mg/kg BW, oxytetracycline at 20 mg/kg BW, or trimethoprim–sulfadoxine (40 mg/200 mg/mL) at 0.1 mL/kg BW may be given parenterally, or the creep feed may be medicated with sulfamethazine and/or tylosin at 200 and 100 mg/kg of feed respectively. Parenteral injections need to be repeated every 3 to 7 days for at least three injections, and feed medication should be given for 3 to 5 weeks. The problem with early creep medication is in obtaining adequate intakes of the antibacterial. This is seldom achieved before 2 weeks of age, and parenteral antibiotics may be required if significant infection occurs before this stage.

The parenteral administration of antimicrobial agents to individual piglets at - to 7-day intervals beginning at 3 days of age for a total of three to five injections per piglet has been recommended for the treatment and control of atrophic rhinitis. However, in a large herd such a treatment regimen would be a major task, and until a cost–benefit analysis indicates a beneficial effect over other methods, we cannot recommend such a practice.

The treatment of experimental BB infection in young pigs has been successful with the use of trimethoprim–sulfadiazine in the drinking water at levels of 13.3 and 77.6 µg/mL respectively, for 3 weeks. This method would remove the necessity to inject pigs repeatedly.

Tilmicosin has proved useful; fed continuously over 6 weeks at concentrations of 200 g per ton of feed, it controlled transmission of atrophic rhinitis, weight gains were positively affected, and fewer nasal swabs were positive for PM at the end of the study period. A resistance to some antibiotics has recently been reported.[12,16]

CONTROL

Effective control depends on developing methods of eliminating or controlling the prevalence of toxigenic isolates of PM, which cause progressive atrophic rhinitis if they become established in the nasal cavity. Previous infection of the nasal cavity with BB may enhance the establishment of toxigenic PM and result in progressive atrophic rhinitis.

Although there is considerable information available on the ecology of BB and the methods by which it might be eliminated or controlled in a herd, there is little documented information available on methods that can be used for control of the toxigenic isolates of PM associated with atrophic rhinitis.

Control of atrophic rhinitis can be attempted in at least four ways:

- Total eradication
- Reduction of infection pressure
- Mass medication with antimicrobials to reduce the severity and adverse effects of infection
- Vaccination

Regardless of the method employed, any effective control program must have a system for monitoring the incidence of clinical disease in the herd and the incidence and severity of conchal lesions of the pigs sent to slaughter. Accurate and reliable methods for monitoring clinical disease are not available,

but the incidence of acute rhinitis and facial deformities could be recorded regularly. At slaughter, snouts can be examined for lesions of conchal atrophy and for assessing a mean snout score for each group of pigs slaughtered.

Eradication

Total eradication can only be achieved with confidence by complete depopulation for a 4-week period and repopulation with primary or purchased specific-pathogen-free stock. This approach has the added advantage of also eliminating enzootic pneumonia, which may be a significant contributing factor to the economic importance of this disease. However, this method of control is extremely costly, and the economic importance of the disease would need to be carefully evaluated in relation to this cost before this method was instituted. Other techniques of obtaining pigs free of atrophic rhinitis, such as the isolated farrowing of older and presumed noncarrier sows with subsequent clinical and postmortem examinations of a proportion of the litters, have had a significant failure rate in the field and are not recommended. Eradication by repopulation with cesarean-derived stock may be essential in breeding nucleus herds where a high generation turnover results in a low herd sow age and a low herd level of immunity. The breakdown rate of herds established by this method can be significant, presumably because the initiating organisms are not solely confined to pigs.

A pilot control scheme was initiated in Britain in which a herd had to meet the following conditions:

- It must be inspected by a veterinarian every 6 months over a period of 2 years, over which time there must be no clinical evidence of atrophic rhinitis.
- The herd owner must certify that atrophic rhinitis has not been suspected over the same time period.
- Cross-sections of snouts taken from at least 30% of marketed pigs must be examined regularly by a veterinarian, and over a 2-year probationary period the average six-month snout score must not exceed 0.5.
- There must be no vaccination or treatment for atrophic rhinitis.
- New breeding stock can be introduced only from other qualified herds or herds derived by hysterectomy, artificial insemination, or embryo transfer techniques.

Over a 5-year period 45 herds qualified at some stage, and 34 were still qualified at the end of 5 years. As of 1988, some herds had exceeded the snout score limit of 0.5, with their average scores increasing to 2.24. In these herds, there was no clinical, epidemiologic, or bacteriologic evidence that they were at risk of developing severe atrophic rhinitis. It is suggested that the higher scores were associated with a group of recurrent husbandry factors, especially overstocking and unsatisfactory conditions in the weaner barns. These increased scores suggested the possibility that the upper limit for the snout scores in qualifying herds could be raised and allow bacteriologic testing to be confined to more doubtful herds.

Eradication in the Netherlands was based on the fact that they thought that there were four main possibilities for the spread of toxigenic PM: artificial insemination centers, laborers, neighborhood infection either by aerosol or by local spread, and carrier animals or birds. They assumed that most herds were closed or buying certified stock and that the major source of infection was therefore the boar. In this study they tested boars; in herds with less than 50 boars they tested all, and in those with more than 50 they tested 50 as the minimum. They took nasal and tonsil samples, which were placed in cold transport medium and sent to the laboratory within 24 hours under cooled conditions for overnight culture followed by PCR.

Reduction of Infection

Reduction of infection pressure can be attempted. Infection of piglets occurs primarily either from carrier sows or from other infected piglets in the immediate environment and severe atrophic rhinitis generally results from infection of piglets under 3 weeks of age. If these factors can be minimized, the incidence and severity of the disease can be reduced. An all-in, all-out pig flow is one of the most effective methods of control of atrophic rhinitis. Changing to an all-in, all-out pig flow from continuous flow management can improve snout scores by 50%, lung scores by 55%, average daily gain by 0.14 lb, and days to market by 13 days.

Because severe lesions depend on infection of the piglet under 3 weeks of age, every attempt should be made to minimize the severity of the challenge to young piglets. It is a common observation that the effects of atrophic rhinitis are minimal under good systems of management and adequate ventilation, nondusty conditions, and good hygiene. The use of continual-throughput farrowing houses and weaner houses allows a buildup of infection with the presence of actively infected pigs that can provide a high infection pressure on piglets born into or introduced into these areas. The use of all-in, all-out systems of management in these areas is recommended, and young piglets should be kept in a separate area from older pigs.

Mass Medication

The prophylactic use of antimicrobials is frequently employed to reduce the incidence of the disease within the herd. Antimicrobials are used both within the breeding herd to reduce the prevalence of carriers and in young suckling and weaner pigs to reduce the severity of the infection. The medication is begun about 2 weeks before farrowing, continued throughout lactation, and incorporated in the creep feed for the sucking pigs and the starter feeds for the weaned pigs. In this way there is continuous medication of the sow and the piglets during the most susceptible period. For the breeding herd, sulfamethazine at levels of 450 to 1000 mg/kg feed, with the higher levels being given to dry sows on restricted feeding, has been recommended. Sulfonamide resistance has proved a problem in some countries but beneficial results may still be achieved with these levels. It is recommended that medication be continued for a 4- to 6-week period. Carbadox at a level of 55 ppm in combination with sulfamethazine at 110 ppm is reported to be effective in clearing experimentally induced BB infection, and when used alone improved growth rate and feed efficiency in pigs with naturally occurring atrophic rhinitis. In the starter period, carbadox fed alone or in combination with sulfamethazine improved average daily gain in piglets from herds with naturally occurring atrophic rhinitis. Use of the medication, however, did not result in a reduction of mean nasal lesion scores as a result of atrophic rhinitis. Sulfamethazine at 110 mg/kg of feed is more effective than sulfathiazole at the same concentration for the control of experimentally induced atrophic rhinitis attributable to BB. Sulfamethazine may also be incorporated in creep rations, and the use of tetracyclines (200 mg/kg), tylosin (50-100 mg/kg), and penicillin (200 mg/kg) has also been suggested.

Medicated early weaning is recommended to obtain pigs free from pathogens, including BB that are endemic in the herd of origin. The sows are fed medicated feed from 5 days before to 5 days after weaning, and the piglets are dosed from birth to 10 days of age.

Vaccination

There has been considerable interest in the development of vaccines for the control and prevention of atrophic rhinitis attributable to BB. Inactivated vaccines have been used to vaccinate the pregnant sow 4 to 6 weeks before farrowing; in some cases, this is followed by vaccination of the piglets at 7 and 28 days of age. In general, the use of the vaccine in pregnant sows in herds where the disease has been endemic has reduced the incidence of clinical atrophic rhinitis. However, the results from one study to another have been highly variable. Vaccination of the pregnant sow results in an increase in colostral antibody titer, which does improve the clearance rate of BB in the piglets. However, it has been difficult to evaluate the efficacy of the BB used alone because the conchal atrophy associated with infection of piglets with BB experimentally or naturally heals and regenerates completely

when they are reared to about 70 to 90 kg BW in good housing conditions.

Vaccination with both components (BB and PM) in a vaccine reduces lesions considerably compared with a placebo and a group with only PM type D toxin in the vaccine, but neither vaccine eliminated toxigenic PM from the upper respiratory tract.

Experimentally, piglets born from sows vaccinated with PM are protected from a challenge with atrophic rhinitis toxin. This indicates that artificial immunization for atrophic rhinitis should be possible. Vaccination of sows at least three times before farrowing for the first time and during each subsequent pregnancy with a vaccine containing BB and PM was highly successful in reducing the incidence of atrophic rhinitis in the pigs. The incidence in affected herds was reduced from 7.5% to about 2%. Experimentally, the vaccine provides good protection against challenge in piglets from vaccinated sows.

A recombinant PM toxin derivative vaccine given to gilts 4 to 5 weeks before farrowing and again 2 to 3 weeks later provided excellent protection in their piglets against experimental challenge with BB and toxigenic PM. This indicates the excellent immunoprotective properties of the nontoxic derivative of the PM toxin. In five field trials, a single-component vaccine containing a nontoxic but highly immunogenic protein, as the antigen, provided much better protection than the control vaccine containing killed PM and killed BB.

Experimental infection and vaccination of pregnant minimum-disease sows with BB resulted in much higher agglutinins in serum and colostrum than in sows only vaccinated or control animals, and the piglets were provided with protection against experimental disease. Vaccination of pregnant gilts with purified inactivated PM toxin resulted in a high degree of protection of their progeny against progressive atrophic rhinitis.

A new vaccine has been described using a truncated PM type D toxin that is immunogenic and nontoxic, a toxoid for BB, and an adjuvant. Sows were vaccinated at 8 to 6 weeks and 4 to 2 weeks before farrowing. The vaccinated animals had fewer organisms.

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FACIAL NECROSIS (FACIAL PYEMIA)

Facial necrosis (facial pyemia) was formerly called necrotic rhinitis or bullnose or paranasal abscessation and is often confused with atrophic rhinitis (AR). It occurs in growing pigs usually before 1 week of age and may occur in herds where AR is present and even in the same pig, but there appears to be no relationship between the two diseases. The diseases differ by the presence of oral and facial lesions. Necrotic ulcer in pigs may involve the mouth and face, but the lesions are erosive rather than necrotic.

There are a variety of other conditions of the face of the young pig that can be confused. The common occurrence of *Fusobacterium necrophorum* in the lesions suggests that any injury to the face or nasal or oral cavities may lead to bacterial invasion, especially if the environment is dirty and heavily contaminated. The disease is now rarer following a general improvement in hygiene in piggeries but possibly also as a result of the declining occurrence of AR following vaccination and eradication of *P. multocida* toxigenic type D and much greater care in teeth clipping of the young pig. It is also associated with fighting in piglets trying to reach a teat, especially when milk is in short supply.

The lesions develop as a necrotic cellulitis of the soft tissues of the nose and face but may spread to involve bone and produce osteomyelitis. Local swelling is obvious, and extensive lesions may interfere with respiration and mastication. The lesions may be ulcerated, crusty, and extensive. Depression of food intake and toxemia may result and poor growth, and some deaths result. Treatment by the local application of debridement, disinfection with substances such as chlorhexidine or iodophors and the use of antibiotic creams and parenteral antibacterial drugs, and the oral administration of sulphonamides is satisfactory in early cases. Oral dosing with sulphadimidine has been effective in young pigs. Improvement of sanitation, elimination of injuries, and disinfection of pens usually result in a reduction of incidence, and cross-fostering will reduce competition and fighting.

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BORDETELLA RHINITIS

Bordetella bronchiseptica (BB) is capable of causing two major disorders on its own. The first is *Bordetella* rhinitis, and the second *Bordetella* bronchitis. It is also capable of infecting man but the contribution of pig strains to human disease is unknown.

ETIOLOGY

It is a small, aerobic, gram-negative bacterium that produces a beta hemolytic 1- to 2-mm gray colony on some nutrient blood agars but is nonhemolytic on some enriched media. On MacConkey media it produces nonlactose fermenting colonies in 48 hours. Nearly all the strains express one of two antigenically distinct O-antigen serotypes (O1/O2) that are not cross-reactive.[1] Variation in virulence can result from strain variation[2,3] and may be related to different phylogenetic lineages.[4]

EPIDEMIOLOGY

The bacterium is often isolated from healthy animals.[5] Carrier animals usually introduce it to a herd. Strains from other animals (dogs, rodents, etc.) are not so likely to colonize the pig because only a few strains occur in the pig, and these tend to be different from other species. Spread is by aerosol from sneezing and through direct and indirect contact.

Infection usually occurs early in life and what happens then depends usually on the state of immunity. Maternal antibody usually lasts long enough to cover the establishment of infection and prevents pathology but does not lead to removal of the agent.

Cross-fostering; multiple ages in the same house; multisourcing to a nursery or finishing house; poor ventilation and environmental control and, in particular, lack of an all-in, all-out policy followed by effective cleaning, disinfection, and drying policy are conducive to the spread of the condition.

PATHOGENESIS

Bordetella is a complicated organism with several virulence factors. It exists in four colony phases. Expression of the virulence genes requires cooperation of the BvgAS (virulence genes system).[6]

Phase I colonies contain fully virulent organisms (Bvg +) expressing genes for flagellae (*fla*), the mannose-resistant filamentous hemagglutin,[7,8] and the outer membrane protein pertactin (PN), all of which are involved in adhesion. Other factors include a hemolysin that is adenylate cyclise, a cytotoxin, an osteocytic toxin, and the dermonecrotic toxin (*dnt*). The adenylate cyclise may modulate cytokine production in dendritic cells and alter immunomodulatory function.[9] The tracheal cytotoxin is likely to act on the cilia and cause ciliostasis. The Bvg + organisms also possess the *bfrZ* gene for the exogenous ferric siderophore receptor, which

is essential because BB has huge requirements for iron.

Phases II and III do not have all these. Reversion to phase I only takes place in vivo. The organisms also differ between strains in the presence of genes for flagellae and fimbriae. The organisms colonize the cilia of the URT and then proliferate, and then the cilia are lost as the organisms increase further in number. Pertactin may be required for this.[9] Toxic substances then diffuse from the BB into the epithelium and below and damage the osteoblasts. Mild turbinate atrophy may then begin but usually resolves by about 70 days postinfection.

In the lung, BB causes a pneumonia similar to *Mycoplasma hyopneumoniae* (EP), and the organism lives in large numbers in the main bronchi (formerly called bronchitis X), where it may cause a mucopurulent tracheitis and bronchitis.

The organism also enhances the ability of other organisms to colonize the respiratory tract, notably *P. multocida,*[10] *S. suis,* and *H. parasuis.* In turn, PRRSV predisposes to infection with BB. Coinfection of BB with PRCV and SIV leads to a longer outbreak of more severe pneumonia.[11]

CLINICAL SIGNS

Clinical signs may be severe in newly established herds, rapidly expanding herds, or in herds with poor immunity or where there are immunosuppressive disorders.

Normally, outbreaks of sneezing will occur in baby pigs. It may be paroxysmal or be accompanied by epistaxis. There may be tear staining. The signs of progressive atrophic rhinitis are rarely seen with just BB infection.

In the pneumonic form there may be fever to 40°C (104 F), anorexia and loss of condition, and possibly a high mortality. It may cause a reduction in growth rate that may reach 20% to 30%. Coinfection with other agents contributes to an increased severity of signs, and respiratory viruses may favor the colonization by BB.[12]

PATHOLOGY

In an uncomplicated infection there is a mild catarrhal rhinitis. There may be some degree of conchal (turbinate) atrophy with deviation of the nasal septum, and excess mucus production.

In the lung infection there may be consolidation of cranial and middle lobes of the lung. Histologically, the nasal epithelium is infiltrated with inflammatory cells, it sometimes shows mucous metaplasia, and there may be fibrosis that is almost pathognomonic for BB infection. In the lung there may be a catarrhal exudate with neutrophilic infiltration.

DIAGNOSIS

In early cases, severe sneezing and tear staining will be a good indication. Sneezing is the method of clearing the nasal cavity of irritation (infection, noxious gases such as ammonia or heavy burdens of dust) and is the clinical sign indicating the nasal cavity is stressed. In early cases of bronchial infection there may be a cough, which indicates that the trachea, main-stem bronchi, and the major part of the bronchial tree are clogged with exudate that needs to be physically removed because the normal mucociliary clearance mechanism is overcome.

In early infections BB can be isolated from the whole of the respiratory tract, but in chronic or recovered cases it may only be isolated from the nasal cavity (ethmoturbinates in particular). Nasal swabs using cotton tips can be collected, placed in transport media, and cultured on special media.

At postmortem the BB can be grown on blood agar plates with 48 hours of incubation.

PCR tests based on the dermonecrotic toxin have been used successfully[13] and in multiplex PCRs with *P. multocida.*

Antibody tests (agglutination and ELISAs) can also be used to assess the herd status.

IMMUNITY

There is an IgM immunity to the hemagglutinin within 7 days and IgG appears 4 to 5 weeks later. This immunity usually prevents turbinate atrophy and pneumonic damage. It is necessary for a good IgA response to clear the URT of infection,[14] but vaccine protection is not as good as natural infection protection.[15]

TREATMENT

Parenteral treatment with almost all antibiotics is possible for severe acute case because in vitro sensitivity to most antibiotics is high. Only after this should treatment via water and food should be considered. BB are, however, largely resistant to Ceftiofur, and there is evidence that they are becoming more resistant to trimethoprim–sulphonamide combinations

CONTROL

Medication can be used to control the onset of the problem.

Threatened pigs in a single airspace should all be given antibiotics in the feed after weaning (trimethoprim/sulphonamides at 30 mg/kg daily) or tetracyclines.

Strategic medication using the same antibiotics, given parenterally, at 3, 10, and 21 days of age will also reduce the clinical signs.

Medicated early weaning techniques and long-term treatment in the water for 28 days have also been used to eradicate the agent.

Vaccination using formalin killed alum adjuvenated vaccines usually combined with *P. multocida* toxoid have been successfully used for a long time.[16] They can be given to sows 6 and 2 weeks before farrowing to stimulate maternal antibody and to piglets at 7 and 28 days of age, but in this case they may be negated by maternal antibodies.

All-in, all-out management, with good ventilation reduces the level of infection. Purchasing clean stock with a period of isolation and quarantine will also remove the infection, as will treating incoming stock. It is sensitive to several on-farm disinfectants.[17]

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PLEUROPNEUMONIA OF PIGS ASSOCIATED WITH *ACTINOBACILLUS PLEUROPNEUMONIAE*

ETIOLOGY

Actinobacillus pleuropneumoniae (APP), formerly known as *Haemophilus pleuropneumoniae,* is the causative organism of pleuropneumonia in pigs. Some strains require V factor (NAD) for growth (biotype I), but some strains do not require this factor (type II). It forms small translucent mucoid beta-hemolytic colonies around staphylococcal streaks on sheep blood agar. It is a small gram-negative, encapsulated rod. The organism causes severe, rapidly fatal fibrohemorrhagic and necrotizing pleuropneumonia. The survivors often have bacteria-laden sequestra in the lungs that are poorly penetrated by antibiotics but do act as sources of the organism for later outbreaks. It does not affect humans and has no public health significance.

Recently a completely nonpathogenic species, *A. porcitonsillarum,* has been identified.

EPIDEMIOLOGY

Occurrence

It is widely distributed worldwide. The primary reservoir is domesticated pigs, but wild boar are also affected.[1]

The only natural host is the pig but it has been isolated from cattle, deer, lambs, and

some rodents can be infected experimentally. It is probably not carried by birds and rodents. The diversity of strains isolated from healthy pigs could be higher than that of strains recovered from diseased pigs.

It appears that few pigs are infected from their sows, and then the organism spreads after weaning as the maternal antibodies disappear. The disease occurs worldwide in growing pigs from 2 to 6 months of age, with rapid spread both within the initially affected group and subsequently to other older or younger pigs in a herd. There are probably large numbers in the nose of affected animals. Abattoir surveys have found that the lungs of pigs from about 50% of herds monitored for several months may have lesions attributable to APP. This chronic pleurisy is presumably associated with APP.[2,3] Sero-epidemiologic surveys have found that pigs in 70% of herds may have antibodies to one or more of several recognized serotypes of the organism. The prevalence of infection continues to increase—presumably as a result of confinement rearing, crowding, inadequate ventilation, close contact, and commingling of pigs of various age groups. The incidence of clinical disease is much less than the prevalence of infection. In most countries, the more dense the pig population, the more prevalent the APP is likely to be, and this has been documented in Belgium. In the United States it may not be as important as in Eurasia, and it was not reported to be of major importance at the Iowa clinic.[4] In Europe, it may have become more common in recent years.

Most herds have one or more types but these are usually avirulent. In some countries there may have been a shift from virulent to avirulent serotypes. In Canada, over 75% of the pigs were positive for APP in the upper respiratory tract.[5] There is a relative homogeneity within a particular serotype.[6]

A strain may be virulent in one country but not in another depending on the genetic makeup determining the presence of virulence factors.

Morbidity and Case Fatality

The morbidity rate can exceed 50%, and the mortality may vary from 1% to 10%.

Methods of Transmission

Transmission is usually by pig to pig contact or more correctly nose-to-nose contact. Aerosol droplets only carry over short distances before they are precipitated. In Denmark it was suggested that the paramount factor in the spread of APP was the aerosol spread from infected neighbors.[7] In this study, the trading of subclinically infected animals, the frequency of stock purchases, the use of multiple sources, and poor biosecurity were factors associated with the spread of APP in the Danish SPF herds. Most of the herds use AI and bring in pigs in sterile containers. The organism can be spread in the air for a distance of 1 m. Aerosol transmission of APP9 was possible over 2.5 m. An experiment with transferring air from a group of pigs with serotype 2 showed that if 10% of the air was transferred, then there was no transmission, but if 70% was transferred, then the APP did spread. Experimental aerosol exposure of pigs to serotype 9 results in infection and induces protection to subsequent challenge from the homologous strain.

Only a few organisms need to be carried in the tonsil and nasopharynx for a pig to become infectious during travel. Pigs may carry the APP in the nose, and the carriage occurs for both low- and high-virulence strains. The carrier state can be activated by stress or other pathogens. The subclinically infected carrier pig is the most common source of infection. It has been suggested that shedding only takes place at the time of active infection, not when the organism is just carried.

Transmission is by the respiratory route, principally via nose-to-nose contact. Overcrowding and inadequate ventilation may facilitate spread. Peak transmission may occur at around 11 weeks. Experimental intranasal challenge has been followed by death in a period as short as 24 hours. The mixing of infected pigs (seeder pigs) with normal susceptible pigs for 48 hours can mimic field infection, with the development of clinical disease, febrile responses, lung lesions, and mortality.

The subclinically infected carrier pig is the most common means by which the infection is transmitted between herds. Severe outbreaks may occur unexpectedly in susceptible breeding herds with no previous history of the disease or in intensive feeder pig operations in which pigs are introduced on a regular basis from a variety of sources. Herds that continuously introduce replacement stock are highly susceptible to an outbreak. Following the initial outbreak, general herd immunity develops, but the infection persists, and sporadic cases may continue to occur. The organism is not readily isolated from normal respiratory tissues, but persists in chronic lesions within the lungs of recovered and apparently clinically healthy pigs. These pigs provide a source of infection, especially in a finishing herd buying from diverse sources. The indirect transmission of infection has been proposed but may be rare. An on-farm study described five cases of being transmitted by aerosol or boots or clothes, but the other three cases could have been any combination of these three or even other indirect sources.

Risk Factors

Pathogen Factors

Biotypes

There are two biotypes. Biotype 1 requires NAD (NAD dependent) (13 serotypes), and biotype 2 does not (NAD independent; 2 serotypes). Biotype I should be differentiated from other *Actinobacillus* species. The isolation of biotype 2 may be increasing. It easily grows on blood agar plates, as does *A. suis* (see later discussion), which may also under some circumstances produce pleuropneumonia.[5] In addition, atypical biotype II strains belonging to APP serotypes 2, 4, 7, 9, and 11 have also been identified.[8] In Canada, two biotype I APP13 strains have been found (should be biotype II).[9]

Serotypes

In 1997, two new serotypes were proposed: APP14 and APP15. Serotypes APP1 to APP12 form biotype 1 together with APP15. Biotype 2 is composed of APP13 and 14. Within these categories there are variations because strains may acquire characteristics of other strains. Some of the serotypes are heterogeneous (they share antigenic determinants with other serotypes). Heterogeneity has been reported for APP3, 6, and 8; APP4 and 7; and APP1, 9, and 11. Restriction endonuclease fingerprinting analysis can be used for comparison of serotypes.

Serotype 5 is subdivided into subtypes A and B. Serotype 1 has also been divided into antigenic subtypes 1A and 1B. The prevalence of serotypes of APP varies considerably according to geographic location. APP1, 5, and 7 are common in North America; APP2 and 9 are common in continental Europe; and APP3 is common in England and Ireland. APP8 is also found in Ireland. In the British Isles, APP2 and 8 were most common, with 3, 6, and 7 also occurring frequently. APP5, 9, 10, and 12 occurred only rarely. APP1 and 4 were not isolated.

In Denmark they routinely find 9 strains of the 15. It is usually APP2 followed by 6, 5, and 12. APP1, 7, 8, and 14 are infrequent, and APP3, 4, 9, 11, 13, and 15 have not yet been found. APP2 is the dominant isolate in Sweden and Switzerland. APP 10 is common in France (also Brazil). APP4 is common in Spain as APP7 and many are nontypeable, but APP4 rarely appears elsewhere.[8] And in Spain, biotype II is also quite common.

APP1, 7, and 12 are common in Australian pigs, with APP1 being the most common, and APP 15 is also found there and in Japan.[10]

In North America, the most common serotypes, in order of frequency, are APP1, 5, and 7. APP1 is most common in eastern Canada, accounting for 66% to 83% of the isolates, and is the second most prevalent isolate in western Canada and the United States. APP2 is of low frequency in Canada. However, serotype 2 has now been reported as causing disease in growing and finishing pigs in the United States. Serotype 3 has a low incidence in Canada and the United States. Serotype 5 isolations are common in Canada and the United States. The most common serotypes isolated in Quebec were 1, 5, 2, and 7 in that order. Serotype 6 has not been reported in North America.

The serotyping of isolated strains is important in the epidemiologic and immunologic study of APP infection. It is also important when comparing or analyzing the effectiveness of different treatments to know the virulence of the strains and sensitivity to antimicrobials. An effective immunization program also depends on consideration of the multiplicity of immunogenic types that occur in a particular area or country.

It is important to realize that some strains share lipopolysaccharide O-chains and may therefore cross-react. The antigenic cross-reactions between APP3, 8, and 15 can also be explained by the presence of structural similarities.[11]

In one study it was suggested that the presence of APP9 may go clinically unnoticed as *M. hyopneumoniae* as this potentiates the APP infection.[12]

Virulence Factors

APP attaches to tonsillar epithelium. It also adheres to tracheal rings in vitro and alveolar epithelial cells. Genes that are involved in energy metabolism, nutrient uptake, and stress response are essential for the survival of APP in the pig host. These would include enzymes that are produced in vivo to ensure that there is oxygen. A metalloprotease has been found that can degrade porcine IgA and IgG.

Several other virulence attributes and their biological effects have been described. Multiple virulence factors are involved in the development of the disease, and lesions are likely caused by toxic factors associated with the organism.

The capsular components are antiphagocytic and inhibit bactericidal activity of the serum but do not cause any lesions themselves. Discovery of mutants without capsules that were no less able to adhere to respiratory tract tissues suggests that the outer membrane proteins were then unmasked (without the capsule), and these were able to adhere to epithelial cells. It is therefore an LPS independent adherence. The outer membrane proteins (OMPs) (60 kDa) adhere to fibers of type III collagen in the lung. The outer membrane proteins appear to be common to all serotypes. Some of the outer membrane proteins are also involved in iron uptake, which is essential for proliferation.

The lipopolysaccharides (LPS) of APP are serotype specific but will cross-react with one another. LPS of APP is an important adhesin. It also induces inflammation by stimulating TNF-α, IL-1, Il-6, and IL-8. However, the construction of antibodies to LPS blocked adherence to tracheal cells, so our understanding is not yet complete. The LPS causes an endotoxemia and reproduces certain typical lesions of the natural disease but not the hemolytic or necrotizing effects. Some LPS also help APP to stick to mucus, tracheal rings, and lung, but they do not seem to be involved in adherence to cultured porcine alveolar epithelial cells.

Porcine hemoglobin also binds to LPS with APP, and this is a property of the APP OMP.

Under iron-deficiency growth conditions, APP expresses 2 transferrin binding proteins. Recently a ferrichrome receptor in APP has been described.

There are adhesins (fimbriae) involved in attachment. They are particularly associated with serotype 1 but also 3 and 5 and are usually a feature of subculture 1 (56% of strains), but only 8% on subculture 2 and none on 3.

Apx Toxins

Not all the differences in virulence are explained by capsules, LPS, hemolysins, and Apx toxins. Certainly, all strains need ApxIV and two out of Apx II or III. There is no certain way to differentiate virulent from avirulent strains

Several exotoxins are produced including hemolysins. The hemolytic activity of this organism is characteristic of this species of bacteria. This range of exotoxins is part of the pore-forming RTX group known as the Apx toxins. The latest is Apx IVA, and the gene is present in all APP strains and is species specific and therefore can be used to confirm identification of the organism. The Apx IVA gene is found in all APP serotypes and is absent in the other related species in the *Pasteurellacae* and, therefore, is considered species specific for APP and is thus being used in a PCR to identify APP strains. It is secreted by a type I secretion system.

The Apx toxins are described in the following discussion: I through III can be produced in vitro, but Apx IVa is only produced in vivo and is specific to APP. All 90 strains investigated in one study had Apx IVa genes. Mutants without the capacity to produce Apx toxins do not cause disease. There are basically four different patterns. Both ApxI and II are essential for the production of lesions. Apx III specifically targets leukocytes by binding CD18[13] The Apx gene is present in all APP strains.

Major RTX toxins in APP are as follows:

- **ApxI** 110 kDa is strongly hemolytic and weakly cytotoxic.
- **ApxII** 102 kDa is weakly hemolytic and moderately cytotoxic.
- **ApxIII** 120 kDa is not hemolytic but strongly cytotoxic.
- **ApxIV** 202 kDa has largely unknown actions but is essential for full virulence of APP.[14]

Box 12-2 Relative virulence of strains of A. pleuropneumoniae in pigs

Very highly virulent	Highly virulent	Moderately virulent	Low virulence
1	2, 4, 6, 8, 15, 9, 11 (10 + 14)	2, 5, 9, 10, 11	3, 7, 12

Serotypes 1, 5a, 5b, 9, and 11 produce I and II; serotypes 2, 3, 4, 6, and 8 produce II and III; serotypes 7 and 12 produce II; serotype 10 only produces Apx I.

There are differences in opinion as to what constitutes virulence. Generally, the following is representative, but it does vary considerably from country to country and isolate to isolate (Box 12-2).

These are toxic to alveolar macrophages, neutrophils, and endothelial cells. In small doses they are stimulatory but in large doses lethal. The gene expression is controlled over the growth curve by a novel regulating pathway. Several genes have recently been identified that have helped in survival, including the knowledge that it can produce toxins under anaerobic conditions. The LPS of APP can also stimulate the release of nitric oxide from macrophages by virtue of the enzyme nitric oxide synthase that damages tissues and may disrupt vascular tone, neuronal signaling, and host defense mechanisms. Nitric oxide synthase 2 and cyclooxygenase 2 have been found in swine experimentally infected with APP. Urease activity may also be required for APP to establish infection in the respiratory tract.

The increase in antimicrobial drug resistance that has occurred is an indirect virulence factor and an important disease-promoting mechanism. The ability of the organism to resist complement killing in vitro may reflect a virulence mechanism in vivo that assists bacteria in avoiding the pulmonary defenses of swine and promotes bacterial invasion of the lung.

Differences in pathogenicity exist between serovar 1 and serovars 7, 3, and 2. The differences between serotypes 1, 2, and 7 are low. Serotype 3 seems less virulent than 1. The differences in capsular structure and biochemical composition between virulent and avirulent isolates may contribute to virulence. A smooth-type lipopolysaccharide and a rough-type lipopolysaccharide have been isolated and characterized from serotype 5. The intrabronchial infusion of the preparations into pigs induces lesions typical of those in pigs that die of acute pleuropneumonia.

APP may interact with *P. multocida* to produce a severe pneumonia, whereas *P. multocida* alone is relatively nonpathogenic. Experimentally, a combination of *P. multocida* and the crude toxin of APP resulted in moderate-to-severe pneumonic pasteurellosis.

Of increasing importance and recognition is the formation of biofilms at mucosal

surfaces. It is part of the extracytoplasmic stress response to the presence of APP.[15] Many strains of APP under appropriate growth conditions form biofilms.[16] Serovars 5b and 11 may exhibit biofilm formation, and a histone-like protein H-NS regulates biofilm formation and virulence of APP.[17]

Animal Risk Factors

The major animal risk factors are related to the immune mechanisms and the immune status of pigs of varying ages. A major animal risk factor is that clinically recovered pigs commonly serve as carriers of the organism and never fully recover from the infection. Normally, the APP is detected in mixed bacterial samples from the tonsils and/or nasal samples by PCR from the age of 4 weeks on, but it has been detected as early as 11 days in tonsil samples, so it is possible for the sow to infect the piglet. Isolations become more common from 4 to 12 weeks as maternal antibody wanes. The median length of tonsillar carriage may be 7 to 8 weeks. Colonization of the lungs can develop from around 12 to 16 weeks in some herds to as late as 23 weeks in others.

Factors associated with pleurisy in pigs in a case-control analysis of slaughter pigs in England and Wales[18] showed that risk factors included the following:

- No all-in/all-out policy
- Pigs with more than 1-month age difference in the same shed
- Repeated mixing
- Moving during the rearing phase

Decreased incidence was associated with the following:

- Grow to finish or wean to finish in a house filled with less than 3 sources
- With cleaning and disinfection of grower and finisher groups between groups and extended down time of grower or finisher units

Noninfectious factors in the occurrence of pleurisy have been investigated in France.[19] This study was in 143 farrow to finish herds, where management, husbandry, and housing conditions were recorded. An increased risk for extensive pleuritis occurred where there was a short temperature range for the ventilation control, lack of disinfection in the farrowing room, late surgical procedures on the piglets, a mean temperature below 23° C (75 F) in the finishing room, and a herd size above 200 sows.

Immune Mechanisms

Colostral immunity lasts from 2 weeks (usually 5) to 3 months. After an experimental or natural infection antibodies occur 10 to 14 days postinfection and reach their height at 4 to 6 weeks postinfection. In the animals that are subclinically affected there may be no antibodies produced to the toxins.

In most herds, high antibody levels in 4-week-old piglets can still be detected, and this maternal antibody (AB) continues to decrease until about 12 weeks, and then the AB starts to rise with the acquisition of a pathogenic burden. The presence and decay of acquired colostral antibodies between 2 weeks and 2 months determines the age at which APP infection is most likely to occur. The maternal antibody titers halve every 3 weeks and therefore may remain for 12 to 56 days.

Nasal colonization can occur as early as 4 weeks, and APP can be found in the lungs from 12 weeks; it is usually 12 to 23 weeks before there is any seroconversion to Apx toxins. In other words, nasal colonization does not always produce antibodies.

Active immunity to disease usually follows experimentally induced and naturally occurring infections, and infection with one serotype of APP confers a strong immunity to the same serotype and a partial protection against heterologous strains. Most recovered pigs have a strong humoral immunity but it does not necessarily stop them from becoming carriers and thence possible shedders of APP. Vaccination with killed bacteria produces partial protection against the homologous strain and none against heterologous strains. Second-generation vaccines with Apx toxins produce good protection against clinical disease caused by any serotype but do not prevent animals from becoming carriers through subclinical infections. However, vaccine immunity is serotype specific.

The antibody response to APP infections or vaccination is demonstrated by the complement fixation test or other serologic tests. There is a good correlation between a CF titer and resistance to infection, and the organism usually cannot be isolated from seropositive animals. Susceptibility to APP can be predicted by the absence of neutralizing antibodies to the organism, whereas protection can be predicted by the presence of these antibodies. An aerosol exposure of pigs to viable or inactivated serotype 9 induced antibodies in pulmonary fluids and serum, and protected against homologous challenge. However, the organism may persist in necrotic foci in the lungs or tonsils of pigs considered immune to the infection. Within 2 to 3 weeks of an acute disease outbreak, the morbidity decreases because of the development of immunity. Clinical disease is unlikely in adult immune animals, and immune sows confer passive immunity to their piglets that provides protection for the first weeks of life. However, acute disease may occur in piglets 3 to 8 weeks of age if colostral immunity is initially low and wanes to below protective levels. Also, severe cases can occur in nonimmune gilts and boars introduced into infected herds.

Pigs infected with hemolytic *Actinobacillus* spp. may become false-positive reactors for APP. Such pigs may also be less susceptible to pleuropneumonia caused by APP.

Environmental and Management Factors

Outbreaks of the disease appear to occur in pigs that lack immunity, are overcrowded, or have been subjected to recent stressors, such as marked changes in ambient temperature or a failure in the ventilation system. The organism survives better when conditions are wet or in mucus and may last days or even weeks. It survives in water for 30 days at 4° C (39° F) but has a very short survival under dry and warm conditions. Outbreaks may occur in breeding herds following transportation to and from livestock shows and sales. Presumably, the infection was contracted by commingling with clinically healthy but infected pigs. The hypothalamic–pituitary–adrenal axis is stimulated in response to a wide variety of stressors, and this may lead to activation of the organism from the tonsils. The highest risk is associated with the introduction of pigs from sales barns and the lowest risk from stock whose health status is known to the purchaser.

Economic Importance

The economic losses associated with the disease are considered to be attributable to peracute deaths, the costs of treatment of individually affected pigs and mass medication of the feed and water, and chronic disease that delays the marketing of finishing pigs. Field observations indicate that 5.64 additional days are required for pigs with subclinical infection to reach market weight of 113.6 kg compared with uninfected herdmates. However, other observations and investigations indicate that average daily gain is not significantly affected by infection with APP. Undoubtedly, there are major economic losses associated with the endemic nature of the disease, which is characterized by peracute deaths that recur sporadically, sometimes punctuated by outbreaks.

PATHOGENESIS

The interactions of APP with host epithelial cells seem to involve complex interactions resulting in the regulation of various bacterial genes, including some coding for putative adhesins.[20]

The natural route of infection is aerogenous. In growing pigs the disease appears to be a respiratory infection without septicemia, producing a fibrinous necrotizing hemorrhagic pleuropneumonia with pleuritis. Early after intranasal inoculation the bacteria were mainly associated with the stratified squamous epithelium and detached epithelial cells in the tonsil. If only a few organisms are inhaled, probably they are trapped in the tonsil and remain there until they are activated. If large numbers are inhaled or if spread from the tonsil reservoir occurs, then a bacteremia probably results. Vacuolation and desquamation of the tonsillar epithelium was observed and there were many migrating neutrophils and these distend the

tonsillar crypts. They do not bind to the tracheal (perhaps in the newborn) or to the bronchial epithelium, but they can stick to the alveolar wall.[20] The ApxI of APP10 induces apoptosis in porcine alveolar epithelial cells.[21] The adhesion of bacteria to cells appears to be essential and seems to be mediated by polysaccharides and proteins.[22] The role of the fimbriae is not clear. Discharge of vesicles containing proteases and Apx toxins from APP1 has been described. Later the bacteria are associated with the crypt walls and detached cells in the crypts. Experimental aerosol exposure of pigs to APP results in a severe fibrinous hemorrhagic necrotizing pleuropneumonia that simulates the natural disease. The organism expresses a number of factors that help to acquire iron and it can use a variety of compounds, including hemoglobin.[22] Normally, the APP are kept out of the alveoli by the mucociliary clearance mechanism but not if there are large numbers of APP or there is preexisting damage to the clearance such as occurs as in *M. hyopneumoniae* infection.[12] It is a very determined battle in the alveolus between the APP virulence factors and the host defense mechanisms. The cytokine production excites the defenses and increases the permeability of the alveolar capillary walls and allows access of antibodies and complement. The macrophages need opsonins to help phagocytosis as APP is resistant to the action of complement. The Apx 1 toxin induces apoptosis in the macrophages, which are then killed by leukotoxins, and these then release further amounts of proteases etc. The characteristics of the pathogenesis have been described.[23-25] Within a few hours following endobronchial inoculation of various doses of the organism into 12-week-old pigs, clinical evidence of dyspnea and fever are obvious. An aerosol infection with the organism results in pulmonary edema with multifocal petechial hemorrhages and a diffuse neutrophilic bronchiolitis and alveolitis within hours of infection. In the lung, the recruitment of neutrophils is directed toward the viable APP organisms, and possibly 30% of the lung neutrophils respond. This is further enhanced by IL-8 activity. The porcine mononuclear cell phagocytic populations during inflammation produced by APP have been described.[26] The lesion is particularly marked in the dorsocaudal regions of the lung. The ability of APP hemolysin to debilitate pulmonary macrophages may enhance the multiplication of the organism, but experimentally the hemolysin of serovar 2 is not an essential factor for the production of the lesions. In the acute stages there are marked vascular changes in the lungs. The lesions resemble infarcts because of the vasculitis, thrombosis, and hemorrhage. There are many necrotic foci that serve as reservoirs of the organism in pigs that recover. In the experimental disease, the leukogram is typical of acute inflammation; however, hypoxemia and alveolar hypoventilation are not features of the disease. The hematologic and physiologic findings indicate that the peracute disease resembles septic shock. Immediately after infection the levels of IL-1, IL-6, and TNF-α begin to rise. Moderate levels help in defense, but high levels make things worse. At the same time the IL-10 suppresses TNF-α and IL-1 production in macrophages and monocytes, which up-regulates the other inflammatory cytokines. Pretreatment of the pig with IL-10 reduces the severity of the pleuropneumonia. The prolonged survival of APP during the infections may be attributable to the effect the organism has in downgrading the protective responses of the host.

The distribution of porcine monocytes in different lymphoid tissues and the lungs during experimental. *A. pleuropneumoniae* infection and the role of chemokines has been described.[27] This study showed that monocyte counts in various organs changed during inflammation. The CD163 + monocyte counts were found in the lungs and TBLN from APP-infected pigs, suggesting that monocytes migrate just to these organs.

CLINICAL FINDINGS

The clinical signs vary with the immune status and environmental stress and customarily may be seen between 6 and 20 weeks of age. In all cases there is a reduced growth rate and reduced feed intake, therefore leading to reduced weight gain. There is no relationship between average daily gain and serologic response to APP. The illness may be peracute, acute, subacute, or chronic. In all stages there is very little exercise tolerance, with varying degrees of increase in respiratory rate. The onset is sudden. Several pigs that were not seen ill may be found dead, and others show severe respiratory distress. Affected pigs are disinclined to move and are anorexic. A fever of up to 41°C (105.8°F) is common, and labored respirations with an exaggerated abdominal component ('thumps'), cyanosis, and frequently a blood-stained frothy discharge from the nose and mouth are characteristic, particularly just before death. In peracute cases, the clinical course may be as short as a few hours, but in the majority of pigs it is 1 to 2 days. In many cases, the animals "dog-sit" with elbows abducted to relieve pressure on the lungs, and they show dyspnea. Chronic cases, which usually appear after the acute phase has disappeared, are febrile and anorexic initially, but respiratory distress is less severe, and a persistent cough may develop. If affected pigs are not treated, there will be a high case-fatality rate. Otitis media in a weaned pig caused by infection of the middle ear with the organism has been described. There may also be lesions in the joints with fluctuating swellings of the hocks and the synovial membranes replaced by granulation tissue.

The course of the disease in a herd may last for several weeks, during which time new acute cases develop and chronic cases become obvious by an unthrifty appearance and chronic coughing.

Abortions may occur and the disease may cause sudden deaths in adult pigs, particularly those that are kept outdoors during the summer months and exposed to very warm weather.

Computer tomography and radiography have been described as aids to diagnosis.[28]

Recently a very mild condition very similar to swine influenza, with just a slight increase in respiratory rate, has been described.[29]

CLINICAL PATHOLOGY

Plasma cortisol rises 24 hours postchallenge. Haptoglobin is increased. Within 48 hours IL-1a, IL-1β, and IL-8 were increased, and there was a 50% reduction in iron and zinc. Plasma IGF-1 concentrations were reduced in response to the APP challenge as they were with endotoxin challenge. The LPS of APP produces rises in inflammatory cytokines (TNF-α, IL-6, and IL-10). Band neutrophils are significantly increased in early infections from 18 to 48 hours, and the early changes have been described.[30]

Culture of Organism

In an outbreak, the diagnosis is preferably made by culture at necropsy. Carrier pigs can be identified by culturing the organism from the upper respiratory tract using nasal swabs from live pigs on the farm and samples from tonsils at slaughter. A selective medium for the culture of the organism from the airways of slaughtered pigs may increase the isolation rate because of the high degree of contamination. The culture of APP has recently been complicated by the identification of the nonpathogenic *A. porcitonsillarum.*

Serotype of Organism

Tests to determine the serotype include slide agglutination, immunodiffusion, ring precipitation, indirect hemagglutination, immunofluorescence, coagglutination, and counterimmunoelectrophoresis. The latter is quicker, more sensitive, and more easily performed than direct immunofluorescence and immunodiffusion procedures. The coagglutination test is simple and rapid, the immunodiffusion test is considered to be the most serotype-specific, and there is a good correlation between the rapid slide agglutination test and the indirect fluorescent antibody tests. The rapid slide agglutination test is the method of choice of some workers, but the coagglutination test is serotype-specific, sensitive, simple, rapid, reproducible, and easier to read and interpret than the rapid slide or tube agglutination tests. The International Pig Veterinary Society has recommended that the coagglutination test is currently the method of choice for routine

serotyping of field strains. This technique does not allow separation of the heterogeneous serovar 8 from serovars 3 and 6, the heterogeneous serovar 9 from serovar 1, or the heterogeneous serovar 7 from 4. The results are reported as group 9–1, group 8–3–6, and group 7–4, respectively. The final identification of heterogeneous serovars can only be achieved by the agar gel diffusion test and by indirect hemagglutination. Reference strains and the corresponding antisera are available to bring some uniformity into serotyping.

Detection of Antigen

The polymerase chain reaction (PCR) is a highly sensitive test for the detection of the organism from tissue samples. A PCR for type 4 has been developed. Some detect OMP; others detect Apx genes. Apx IVA based ELISAS can be used for evaluating APP status in commercial herds, but some appear limited by high carriage rates of low-virulence APP.[31] Immunomagnetic separation of APP1 and 2 has been described with greater sensitivity than possible with isolation or even PCR. A PCR-based RFLP analysis of the OM1A gene may also be of value in differentiating APP serotypes. A multiplex PCR has been developed. There is often disparity between immunologic and PCR-based serotyping.[32]

Serology

Serology is the best method for surveillance purposes and is the best way to detect subclinical infections but may give unexplained results.[33] In addition, some of the strains do not produce ApxIV do not produce antibodies.[34] Sometimes diagnostic interpretation is difficult.[22,33]

Tests for antibodies to toxins and/or capsular antigens have a low specificity and can also be positive for *A. suis* infection. Most commonly used are antigens using O-chain LPS.[35] They tend to be grouped together: (1, 9, 11), (2, 3, 6), (8, 4, 7), (10 and 12), (3 and 5), (15, 3, 6).[11]

For the serologic diagnosis of infection in live animals the complement fixation test is reliable, but an enzyme-linked immunosorbent assay (ELISA) test is highly specific and more sensitive than the complement fixation test.

The **complement fixation test** has been used routinely in the past in some countries and has a high degree of sensitivity and specificity. It is, however, a cumbersome test, and many laboratories find it difficult to perform, and so it is rarely used nowadays. Pigs being imported into China and Russia still require a CFT negative test.

The **ELISA** is a rapid and sensitive test and can be adapted to automation. The ELISA for serotypes 1, 2, 5, and 7 distinguishes exposed from unexposed pigs or herds. Because of cross-reactivity with other serotypes and *A. suis,* the serodiagnosis of serotype infections cannot be made with certainty. A blocking ELISA is available for detection of antibodies against serotype 2 and also 2, 6, 8, and 12, which is the dominating serotype in Danish swine herds, causing approximately 70% of diagnosed outbreaks of pleuropneumonia. A similar test is available for serotype 8. A mixed-antigen ELISA for serodiagnosis of serotypes 1, 5, and 7 has a sensitivity of 96% and specificity of 99.5% and can be used for herd health monitoring programs. The long-chain lipopolysaccharide of serotypes 4, 5, and 7 is a superior antigen to the crude extracts used as antigens in the ELISA for the serodiagnosis of pleuropneumonia.

There are now ELISAs for the detection of antibodies to the Apx toxins, and the one for type II Apx was described as sensitive, inexpensive, and highly discriminatory. A multiplex PCR for all toxins in one test is a reliable typing system. A new ELISA for the Apx IV produced by all 15 serotypes means that you can detect all APP with one test. It has a specificity of 100% and a higher sensitivity than culture (93.8%). This is important because you can find Apx I to III in pigs associated with *A. suis* and *A. rossii,* but Apx IV is only produced by APP in vivo. It will detect the toxin from 2 to 3 weeks postinfection.

An inhibition enzyme immunoassay for the serodiagnosis of serotypes 2 and 5 had a sensitivity and specificity of 100% and 98.9%, respectively. The detection of antibodies to APP is an essential feature in the epidemiologic study and control of pleuropneumonia in pigs. Serologic testing can be used to monitor the level of infection in a breeding herd over a period of time and as the piglets become older. A minimum of 30 serum samples from adult pigs is necessary to provide a reliable assessment of the herd's infection status. None of these serologic tests is completely reliable, and in certain situations a combination of two tests is needed for interpretation of low titers in some pigs. In most instances, serologic diagnosis is type-specific, and protection obtained by vaccination is type-specific and will protect only against the serotype contained in the vaccine. Thus it is important to determine the serotypes that are causing disease in the herd.

An important strategy of control of this disease is to detect infected pigs in a herd or to exclude infected pigs from being imported into a herd. Because there is no reliable method for the detection of every infected pig, the effectiveness of this barrier is reduced whenever pigs, such as breeding stock or weanlings, are allowed into a herd. There is a need for a highly sensitive and specific test for the identification of infected pigs. Although bacteriologic culture is specific it is not sensitive. The ELISA test may be a useful test for the antemortem diagnosis of infected herds.

NECROPSY FINDINGS

Characteristic lesions are confined to the thoracic cavity and consist of hemorrhagic and fibrinous pleuropneumonia with a tendency to sequestration in the chronic form. In peracute cases the lungs are swollen, firm, and dark red. In peracute cases the trachea and bronchi are full of frothy fluid. Fluid and blood ooze from the cut surface, and there may be marked edema of the interlobular septa, reflecting widespread thrombosis and alterations in capillary permeability. There may be hemorrhagic areas of necrosis that are very variable. In acute cases there are layers of fibrin on the pleural surface and pericardium. In pigs that die less acutely, focal black or red raised areas of pneumonia are present. Lesions may occur throughout the lung, including the diaphragmatic lobes. The quantitative morphology of peracute pulmonary lesions induced by the organism has been described. In chronic cases there is fibrosis of the fibrinous pleurisy and adhesions result between the visceral and parietal pleura, and on removal of the lungs from the thorax portions of lung may remain adherent to the thoracic cage.[36] A fibrinous pleuritis overlies the affected lung tissue, and a fibrinous pericarditis may also be present. The organism can be isolated from affected lung tissue, but generally not from other internal organs. Occasionally, otitis, endocarditis, pericarditis, and serous arthritis may follow, particularly when infection involves serotype 3. An osteomyelitis and arthritis caused by APP has been demonstrated using fluorescent in situ hybridization.

Histologically, vasculitis and widespread thrombosis is usually evident, in addition to an abundance of fibrin and neutrophils within alveoli. A fibrinous thrombosis with IHC demonstration of APP has been described. In situ hybridization can be used to detect IL-1, IL-6, and TNF-α in streaming degenerate alveolar leukocytes (oat cells) and the boundary zone of oxidative necrosis. A less intense signal was seen in the dense zone of degenerate cells in granulation tissue surrounding the necrotic areas. IL-1 was also seen in the scattered endothelial cells bordering zones of coagulative necrosis. IL-6 is the cytokine that is most elevated, and serum amyloid and haptoglobin are also elevated.

In a chronically infected herd, fibrous pleural adhesions may be present in a large proportion of the pigs at market as a result of infection several months earlier. Subacute to chronic lung lesions are encapsulated by fibrous tissue, and sequestra may be present. A high prevalence of fibrous or fibrinous pleuritic lesions on inspection at the abattoir is very suggestive of APP infection.[37,38]

DIAGNOSIS

The provisional diagnosis of pleuropneumonia associated with APP in the pig is usually based on history, clinical signs, and the postmortem picture. The acute cases then require

laboratory investigation to confirm, and chronic cases may prove antigen negative (the lesions are usually fibrous or fibrinous) but possibly antibody positive. A variety of samples need to be taken from acute cases and should be from lesions not from inflammatory exudates and particularly not from the lungs.

Samples for Confirmation of Diagnosis

The evolution of diagnostic tests has been described as follows:[39]

- Bacteriology—lung culture is relatively easy if the carcass is freshly dead. The culture is achieved on 55 sheep blood agar with a cross-streak of *Staphylococcus epidermidis* or *S. aureus*. The plates are incubated overnight with 5% CO_2, and a clear zone of complete hemolysis results. Typing will confirm the identity of APP1-15 and if atypical PCRs can be used. PCRs for 3, 6, and 8 were described;[40] 1, 7, and 12;[41] 15 and 7;[42] and also 1, 2, and 8. Sometimes serotypes cannot be differentiated. Toxin typing using a PCR can be used to determine which Apx toxin genes are carried by a certain isolate. They can also be isolated from pure and mixed bacterial cultures by immunomagnetic separation.
- Histology—formalin-fixed lung (LM). APP can be further identified by IHC, which is particularly useful in chronic cases and ISH.
- Serology—used to check the herd status. Coagglutination can be used first, with confirmation by agar gel diffusion and indirect hemagglutination.

DIFFERENTIAL DIAGNOSIS

The rapidity of onset and spread with fever, anorexia, severe dyspnea, and high mortality differentiates APP from the majority of respiratory diseases in pigs.

Enzootic pneumonia is more insidious in its occurrence and has distinctively different epidemiologic, clinical, and pathologic features.

Pasteurellosis is characterized by a necrotizing bronchopneumonia.

Swine influenza is characterized by an explosive outbreak of respiratory disease. However, this is not restricted to growing pigs and the mortality is low. There is a distinct difference in the respiratory lesion on necropsy examination.

Glasser's disease is characterized by serositis, arthritis, and meningitis, and occurs in younger pigs.

Mulberry heart disease may present with similar clinical findings, but there is no pneumonia on necropsy examination.

A. porcitonsillarum also produces and secretes ApxII by an operon that does not occur in APP

Actinobacillus suis shares cross reactions with APP 3, 6, and 8.

Actinobacillus lignieresii have some cross reactions with APP serotypes.

TREATMENT

Antimicrobial Therapy

The results of treatment are often disappointing because of the severity of acute disease and persistence of infection in recovered pigs. It is best to assume that APP cannot be eliminated using antibiotic therapy.[41] Although antimicrobials may reduce mortality and improve average daily gain, treated animals often continue to harbor the organism and are a source of infection to other animals. If animals are clinically ill, then injection of antimicrobials is necessary. Affected and in-contact pigs should be treated parenterally with antimicrobials. Tetracycline, spectinomycin, and penicillin have been effective and are recommended unless drug resistance has occurred. Penicillin may have inconsistent results.[43] Fluoroquinolones are distributed to bronchial secretions, bronchial mucosa, and alveolar macrophages. The pharmacokinetics of danofloxacin are favorable for APP treatment. In fact, elevated C-reactive protein, IL-6, and haptoglobin (all elevated rapidly after infection) all return to normal, as do the reduced plasma zinc, ascorbic acid, and alpha tocopherol rapidly after treatment. Ceftiofur and fluoroquinolones were the most active agents against APP. APP is only eliminated from the respiratory tract in animals medicated with enrofloxacin. Tilmicosin is useful for treating outbreaks.

In a large study in Switzerland of 83APP and 58 *A. porcitonsillarum* (PT) strains screened for susceptibility to 20 antimicrobial agents, it was found that there was resistance to sulphamethoxazole, sulphonamide–trimethoprim, tiamulin, tilmicosin, tetracycline, and ampicillin. A few of the PT strains showed increased susceptibility to enrofloxacin.[44] Both APP and PT remain susceptible to cephalosporins, fluoroquinolones, and phenicols, which are not be used except in special cases. In the last few years resistance to tetracyclines and trimethoprim-sulphonamide has increased.[45-47] There is no clear association between antimicrobial susceptibility and serotype.[44] There have been enrofloxacillin-resistant APP isolates found in Taiwan.[48]

In finishing units, where outbreaks of the disease have been confirmed, the twice-daily intramuscular injection of pigs early in the course of the disease with antimicrobials, based on drug sensitivity tests, daily until clinical recovery occurred, was superior to the mass medication of feed and water. A considerable amount of labor is required, but it is considered to be the most cost-effective method.

In a study of SPF pigs experimentally infected with APP2 and treated with enrofloxacin(E), tetracycline(T), or penicillin(P) at the onset of disease or left untreated, it was found that the animals treated with E and T recovered rapidly. All except the E group developed antibodies. They were later challenged with APP2 again, but here the E group developed serious disease. The implication is that the E was so successful initially in eradicating the APP that it did not allow an antibody response to develop to resist the rechallenge.[43]

Antimicrobial Sensitivities

The antimicrobial sensitivities of isolates of APP have been monitored, and there is some variation based on geographic location. The large expansion in the size of swine herds, and the introduction of breeding stock from many different sources, has led to an increase in the incidence of porcine pleuropneumonia and extensive use of parenteral antimicrobials. To ensure an optimal response to therapy, it is necessary to monitor antimicrobial sensitivity on a herd basis.

The antimicrobial sensitivity of the organism was determined in isolates from Europe, Japan, South Africa, and North America between 1989 and 1991. They were highly susceptible to danofloxacin and moderately susceptible to amoxicillin, ceftiofur, and trimethoprim–sulfamethoxazole. There was widespread resistance to other currently available antimicrobials. In another study, thiamphenicol and metronidazole had good activity, and the cephalosporins and fluoroquinolones were most active. A comparison of the minimum inhibitory concentrations (MICs) of several antimicrobials against several bacterial pathogens of swine, including APP, from the United States, Canada, and Denmark found that ceftiofur and enrofloxacin were the most active antimicrobials.

Plasmid-mediated antimicrobial resistance has been found in isolates of the organism that are resistant to certain antimicrobials.

Antimicrobials in Experimental Disease

In these experimental infections enrofloxacin and ceftiofur are particularly effective and also tulathromycin.[49]

The therapeutic efficiency of some commonly used antimicrobials has been evaluated for the treatment of experimentally induced pleuropneumonia using serotype 1 APP. Florfenicol in the feed at 50 ppm prevented pneumonia when pigs were experimentally inoculated with serotype 1, 2, and 5 strains and thiamphenicol-resistant strains of the organism. The combination of trimethoprim and sulfamethoxazole is superior to

a combination of trimethoprim and sulfadimethoxine. Oxytetracycline in the water at 222 mg/L for 7 days beginning 24 hours before experimental challenge reduced the case-fatality rate, lung lesions, and the isolation of the organism compared with the unmedicated group. Treatment of chronically affected pigs did not improve rate or gain, nor did it eliminate the infection. Enrofloxacin at 150 ppm in the feed provided effective control of the experimental disease.

Mass Medication of Feed

In-feed medication with sulfadimethoxine and sulfamethoxazole in combination with trimethoprim has been described.

Oxytetracycline in the feed at 1600 mg/kg of feed for 6 days before experimental challenge and for 9 days after challenge, provided 100% protection from clinical disease, but 400, 800, or 1200 mg/kg of feed did not prevent subsequent shedding and transmission to seronegative animals. Tetracycline should be administered through the feed of all in-contact pigs during the outbreak, but the persistence of the organism in chronically affected pigs may result in clinical disease when the medication is withdrawn.

Doxycycline in feed at 250 ppm for 8 consecutive days is useful for the control of APP.

Tilmicosin fed to pigs at 200 to 400 µg/g is effective in controlling and preventing APP-induced pneumonia, using seeder pigs, when administered in the feed for 21 days. In commercial herds, 400 µg/g of feed for 21 days is no more effective than 200 µg/g of feed for the control of naturally acquired pneumonia caused by APP and *P. multocida*. Sulfathiazole at the rate of 28 g/3.8 L of drinking water for 12 days has also been successful. Tiamulin in the drinking water at a concentration to deliver 23 mg/kg BW for 5 days after an initial individual treatment of affected pigs has also been recommended.

CONTROL

It is impossible to guarantee freedom because the detection of carriers is almost totally impossible. There are no techniques as yet for identifying the animal that may have only a few organisms in the tonsil. You can guarantee freedom from clinical disease at the time of inspection but little else. In a recent study of 980 pigs there was no evidence of an APP clinical or pathologic case until the occurrence of PMWS resulted in the isolation of an APP7 from the series of pigs in a unit that had until that time been considered free.

There are two options for the control and prevention of porcine pleuropneumonia:

1. Control at an economical level using good management combined with the possible use of vaccines
2. Eradication of the infection from the herd

Determining which option to select requires careful consideration of the advantages and disadvantages of each option. With an understanding of the factors that result in clinical disease, it is possible to maintain an infected breeding herd and produce pigs with a small risk of clinical disease.

Control by Management

Management and housing improvements can prevent clinical episodes. One of the most important things to do is to make sure that there is vaccination for enzootic pneumonia.

Control is difficult and unreliable because pigs that recover from clinical disease provide a source of future infection for finishing operations that purchase all of their introductions. The all-in, all-out system of purchasing, feeding, and marketing pigs, with a thorough cleaning between groups of animals in a finishing operation, should be adopted. The disease is highly contagious, and control measures must be directed toward identifying infected pigs and eliminating their introduction into uninfected herds. When moving pigs between herds, it is critically important that the herds be matched according to their infection status. Source herds for feeder animals are serotested, and then pigs of like immune status are commingled to produce a population that is compatible immunologically. By commingling only animals from seronegative herds, the risk of disease is greatly decreased and growth performance improved. The mixing of animals from herds known to be infected with homologous serotypes is also effective.

Every economical effort must be made to identify and isolate infected pigs and to exclude the importation of clinically normal but infected pigs into herds in which the infection is not present. This is a major challenge that is dependent on the availability of a highly sensitive and specific laboratory test. The acquisition of new breeding stock for herds free of the infection should include a period of quarantine and two serologic tests 3 weeks apart. Only seronegative animals should be introduced into the herds. A seropositive animal should be considered a potential carrier. Field trials have shown that it is not possible to rear seronegative animals within a breeding and rearing herd heavily infected with serotype 2 of the organism. Neither medication of the sows and piglets with trimethoprim–sulfonamides, nor a strictly applied all-in, all-out system reduced the percentage of seropositive animals.

Management practices must emphasize the rearing of weaned pigs in pens separate from older stock that are carriers of the organism. Large breeding herds and finishing units should subdivide the total herd into separate units, which minimizes the spread of infection. Early weaning and segregation of gilts from infected stock have been used to develop a seronegative herd.

Herds can be classified into one of three categories depending on their infection status:

CATEGORY 1. Serologically positive for APP without a history of clinical disease. A majority of herds are serologically positive but do not have clinically apparent disease. Good management and environmental quality control can minimize the incidence of clinical disease. Good ventilation, the use of all-in, all-out management practices, and appropriate stocking densities are important.

CATEGORY 2. Serologically negative and clinically free of APP. These herds can be maintained free of infection with good biosecurity practices. New breeding stock must be obtained from herds free of infection. Artificial insemination can be used to limit the introduction of live pigs. Pigs sold from these herds to herds with endemic infection are highly susceptible to infection.

CATEGORY 3. History of clinical disease caused by APP, which has been pathologically and microbiologically confirmed. In these herds, acute disease outbreaks occur most commonly in pigs 9 to 20 weeks of age. Pigs are usually protected by colostral immunity for the first 8 weeks of life. The severity of outbreaks can be reduced by mass medication of the feed, treatment of individual pigs, and good management practices to ensure adequate ventilation.

Eradication

The Danish SPF system was the first to try to eradicate APP. Each month 20 serum samples were tested for APP 2 and 6 and were collected at the monthly clinical inspection. This happened every 3 months also for APP12 and annually for APP 1, 5, 7, and 10. Recurring outbreaks of pleuropneumonia is the most common reason for an eradication strategy. Eradication is done by depopulating the entire herd, followed by repopulation with animals from herds that are clinically and serologically negative. Eradication can be successful but the risk of introducing infections into the herd is high unless biosecurity measures are adopted and strictly implemented.

An alternative to depopulation is medicated early weaning, in which pigs are weaned at 10 to 15 days of age, treated with antimicrobials, and reared in isolation. Transmission of infection between the sow and piglets does not occur before 11 days of age, about half of the piglets are infected at 16 days of age, and if weaned at 21 days of age most of the piglets are infected.

The early weaning program can be expanded to the three-site system of rearing. Adults and nursing piglets are housed in one site. At weaning, piglets are moved to the nursery barn for growth to 25 kg, and then moved to a third site for the final growing period. The adults may be serologically positive for infection, but the nursery pigs, growing pigs, and finishing pigs are negative.

Age segregation, distance that prevents aerosol transmission, and adherence to strict biosecurity practices can reduce the prevalence of infection and the incidence of disease.

Vaccination

A wide range of vaccines have been developed over the years.[50] There are two main groups of vaccines. One is killed organisms the bacterins, and these are serotype specific. The second group are subunit toxin-based vaccines. These contain Apx I, II, III with or without OMP and show a high degree of protection all APP1-12 serotypes. The in vivo ApxIV works well but has not yet been commercialized,[48] although ApxIV is not needed for effective vaccination. An Apx 1A mutant has potential for a live attenuated APP vaccine.[51] Animals vaccinated with bacterins will produce antibodies that will cross-react with ELISA tests that use polysaccharides as antigens. There is a considerable effect of adjuvants in these vaccines.

Natural or experimental infection with a serotype of APP induces a strong immunity to both homologous and heterologous serotypes. Vaccination has been attempted to prevent pleuropneumonia in pigs. However, the protection obtained by parenteral vaccination is serotype specific, and vaccines must therefore contain the serotype existing in the swine population. The mortality rate is lower in vaccinated animals, but they are still carriers of the organism.

Serotype 8 is closely related to serotypes 3 and 6, and parenteral revaccination using a capsular extract or killed APP serotype 8 provides a high degree of protection against challenge with serotypes 3 or 6. A tetravalent vaccine containing serotypes 1, 2, 5, and 7 stimulated titers to all four serotypes and an anamnestic response was induced by a second vaccination. This suggests that the serologic and cross-protective properties of APP serotypes should be identified before they are used as antigen in the complement fixation test and in vaccines.

The protein associated with the capsule of APP is responsible for serotype-specific protection against mortality in mice. Further purification and characterization of this protein antigen is needed to determine whether it is the specific antigen responsible for protection against mortality in swine or if it is a necessary carrier for a serotype-specific capsular disaccharide antigen.

The vaccines that have been evaluated are killed vaccines with an adjuvant. In one experimental trial, two and three vaccinations using a bacterin containing serotypes 1 and 5 prevented mortality following an aerosol challenge with the same serotypes as present in the vaccine. However, all vaccinated pigs had severe signs of respiratory disease and the vaccine did not prevent the development of lung lesions. The use of a formalin-inactivated alum-precipitated vaccine containing serotype 1 was effective in decreasing the morbidity and mortality rates from naturally occurring pleuropneumonia. The adjuvanted vaccines have caused considerable tissue reaction, resulting in abscesses and granulomas. The mineral oil adjuvants are highly irritant and cause granulomas, which are present 8 weeks after vaccination but result in high titers. The aluminum hydroxide adjuvants are less irritating but result in lower titers. Vaccines containing a lecithin-base oil at 5% are nonirritating and stimulate high complement fixation titers.

Subunit vaccines containing purified or partially purified antigens provide better protection than whole cell vaccines. Capsular antigens, outer membrane proteins, lipopolysaccharide, and soluble toxic factors are immunogenic in pigs. An acellular vaccine containing multiple virulence factors provided complete protection from mortality and significantly reduced morbidity to homologous challenge. Pigs vaccinated with the cell extract had fewer clinical signs of pleuropneumonia than pigs vaccinated with three other commercial vaccines and challenged with serotype 1. A vaccine containing the LiCi cell extracts and a crude hemolysin isolated from serotype 1 provided protection against both mortality and morbidity in vaccinated pigs challenged by intratracheal inoculation. An experimental vaccine using bacterial "ghosts," which are empty cells produced by bacteriophage lysis appears to be successful. A better cellular response was observed to inactivated bacteria than to ghost vaccines. Bacteria grown in conditions resulting in high in vitro adhesin levels induced better protection than those grown in NAD rich medium. An APP type 2 vaccine has been described with deletions in the Apx IIA gene, which can then function as a negative marker vaccine, which appears to be capable of protecting pigs without shedding.

Antigenic variation within a capsular serotype, for example in subtypes 1A and 1B, as a result of antigenic variation within the lipopolysaccharide, can result in the failure of whole cell bacterins to provide protection against the same capsular serotype. This lack of cross-protection within a capsular serotype provides a partial explanation for vaccination failures observed under field conditions.

A polyvalent bacterin containing serotypes 1, 3, 5, and 9 provided satisfactory protection against homologous challenge 14 days after the second vaccination. Mortality was reduced, and lung lesions, pleural adhesions, and isolations of the organism from the tonsils and lungs were reduced.

It is possible in the future that a differentiation from vaccinated animals test may be based on the ApxIVA gene.[52]

Live vaccines using laboratory-obtained nonvirulent mutants have also been developed and shown to protect against homologous and heterologous serotypes.[53-55]

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MYCOPLASMA PNEUMONIA (*MYCOPLASMA HYOPNEUMONIAE*)

ETIOLOGY

Mycoplasma hyopneumoniae (once also called *Mycoplasma suipneumoniae*) is the primary causative agent. *M. hyopneumoniae* (MH) inhabits the respiratory tract of pigs, appears to be host specific and survives in the environment for only a very short period of time. The disease has been reproduced with pure cultures, and the organism can be demonstrated directly or indirectly in pigs with enzootic pneumonia worldwide. The isolation of MH is complicated by the presence of other mycoplasmas in the upper respiratory tract of pigs including *M. hyopharyngis, M. hyorhinis, M. sualvi,* and *Acholeplasma* species. The nonpathogenic *M. flocculare* also complicates the culture of *M. hypneumoniae.* The strains of MH are antigenically and genetically diverse. Multilocus sequence typing has been used to estimate genetic diversity,[1,2] and it showed that specific MH strains are responsible for local outbreaks as they are in geographic contact or operative contact.

A wide variety of genetic diversity was found in U.S. strains using comparative genomic hybridization. Significant variation at the genetic level has also been found;[3] and it has not yet been established as to what constitutes cross protection and virulence.[4] MH varies its surface proteins through varied proteolytic events.[5,6] A proteonomic survey of MH identified a total of 31 different coding DNA sequences.[7] Genotyping of MH in wild boar samples showed that variability was high, but there was geographic relatedness; they were related to the domestic pigs, but no matching types were found.[8]

EPIDEMIOLOGY

Occurrence and Prevalence of Infection

Enzootic pneumonia occurs in pigs worldwide, and the incidence is high in intensive pig rearing enterprises. Lesions may be present in 40% to 80% of the lungs of pigs at abattoirs. The peak incidence of pneumonia occurs at 16 to 20 weeks of age, which is likely related to the increased stocking density in this period. In northern climates, the incidence of clinical disease and prevalence of lesions at slaughter are higher in the summer months. The prevalence of lung lesions is often highest in pigs slaughtered in the winter months compared with autumn-slaughtered pigs. The amount of bronchopneumonia lesions in individual lungs ranged from 0% to 69%, with an average of 7.8%. A 2002 survey in the United States showed that 82.3% of finishing sites had at least one animal positive on antibody testing and 94.4% of breeding sites. Seroprevalences were higher in the clinically affected herds, and most of the pigs were infected with MH at a younger age. A study in the United Kingdom showed that geographic location of the finishing unit appeared to be a statistically significant risk factor for EP-like lesions and pleurisy.[9] In addition, they also found that part-slatted floors were a potential risk factor. In a study of colonization at weaning and the infection at slaughter, average lung lesion scores, percentage of affected lungs, presence of MH on the bronchial epithelium, and seroconversion, it was found that the severity of the disease can be predicted by the prevalence at weaning in segregated systems. Strategies focused on reducing colonization at weaning may help to control MH in segregated production systems.[10] Vaccination does not prevent transmission to sentinel pigs in contact with infected animals. Transmission of MH from asymptomatic carriers to unvaccinated and vaccinated sentinels was not different.[11]

Morbidity and Case Fatality

In infected herds, the morbidity rate is high during the growing period, but the case-fatality rate is low. There is however, an increase in the number of treatments of sick pigs in comparison with herds free from the disease. The morbidity rate falls markedly with increasing age, and there is a much lower incidence of pneumonic lesions in sows, even though they may still harbor the organism. However, when MH gains entry into a herd that has been previously free of the disease, all ages of pigs are affected, and mortality, even in adults, can occur.

Methods of Transmission

The organism is an inhabitant of the respiratory tract of pigs, and transmission occurs by direct nose-to-nose contact, which is the main form of transmission. Airborne transmission and fomites are less important. *Mycoplasma* can be transmitted over 1, 75, and 150 m, and recently aerosol has been seen to be transmitted over 9.2 km.[12] *M. hyopneumoniae* was found to travel long distances from an infected experimentally infected group of pigs.[13] Airborne transmission was suggested on 80% of farms where acute respiratory disease was present. No airborne organisms were found on farms without acute respiratory disease. There is no other known host for the organism, although infection and breakdown of closed pneumonia-free herds has occurred without any pig introductions. The number of organisms required for infection is very small, and the possibility of wind-borne infection has been suggested. Transmission is by the respiratory route and in infected herds occurs primarily from the sow to the suckling piglets. In a study of shedding of MH in different parities: gilts were 73% positive, parity 2 to 4 sows were 42% positive, parity 6 to 7 sows 50% positive, and parity 8 to 11 sows were 6% positive. Generally, the nursery is considered the area where transmission occurs and infection spreads slowly. Within pen transmission measured by PCR is very slow. Animals can be PCR positive and not infectious for long periods of time and then can become very infectious up to 119 days, as has been recorded.[11] There is therefore a nonlinear excretion of MH. It is thought that one infected nursery pig will infect on average one littermate. Boars can also infect sows when they are kept together in service areas, but in these areas the disease spreads slowly. The disease is also transmitted and exacerbated during the grouping and stress of pigs that occurs at weaning. Transmission can occur as early as 1 week of age, but usually it is not observed under 6 weeks of age.[14] The highest clinical and pathologic incidence occurs in the postweaning and growing period, and in most herds this is maintained through the growing period to market age. The start of finishing is the critical point. Direct exposure (nose-to-nose contact) of pigs at 9 to 11 weeks of age to seropositive gilts results in seroconversion to the organism by 21 days and is most frequent by about 11weeks after exposure. The presence of gross lesions of pneumonia correlated with the seroconversion.

Frequent coughing by infected, intensively reared pigs suggests that repeated aerosol exposure occurs and is an important natural mode of transmission of respiratory pathogens. There is general agreement that management and environmental conditions considerably influence the severity of the disease.

The reinfection of enzootic pneumonia-free herds, recurrences or so-called breakdowns, occurs at a rate of about 3% of herds every 6 months. In a study of swine herds that had participated in the Pig Health Control Association Scheme in the United Kingdom, the close proximity of the uninfected herds to infected herds appeared to be the most important risk factor that could explain the introduction of the infection. The size of the herd, the density of the pig population in the area, the distance to the next road regularly used for transportation of pigs, and differences in topography were risk factors associated with reinfection. There was little evidence to indicate that unexplained breakdowns occurred in association with long-term latent infection in other herds from which animals had been imported. Clinical signs of enzootic pneumonia in these herds commonly did not occur for several months after the introduction of infected pigs.

MH was not transmitted during a 20-week period when personnel weekly contacted susceptible pigs in a naïve herd after they had been in contact with pigs in an infected herd. A comprehensive herd specific

prevention program is necessary to reduce transmission of disease caused by MH.[15]

Risk Factors

The prevalence, incidence, and severity of pneumonia in swine herds are determined by interactions among infectious agents, the host, the environment, and management practices. This being said a large survey of the seroprevalence of MH in 50 finishing herds showed that there were no risk indicators. Each farm is an individual one, with the farm itself exerting a great effect. A recent study in suckling pigs at the age of weaning in the United Kingdom[16] has suggested that an increase in the number of live pigs born alive was linked to a lower incidence of MH in suckling pigs at weaning. Grinding the piglets' teeth also reduced the incidence of MH. A second dose of iron was also associated with a reduced level of MH. A low environmental temperature also produced an increase in the incidence of pneumonia.

In a study of MH in coughing piglets (3-6 weeks of age) from 50 herds with endemic respiratory disease in Germany,[17] it was found that MH was detected in the lavage fluid in 12.3% of the suckling piglets and 10.6% of the weaned piglets. The study showed that the detection of MH in young piglets is associated with one or two site production and inappropriate gilt acclimatization. In a study of nasal carriage in farmers using PCR it was found that 15% of farmers carried MH in their nose, but it is not possible to say that they were colonized.[18]

Animal Risk Factors

Several factors such as breed, age, presence of diarrhea, the prevalence of atrophic rhinitis, birth weight, and weaning weight, have been examined as animal risk factors. In some herds, the risk of coughing and pneumonic lesions increased with increasing age of pigs within a herd. In a survey of two different groups of pigs slaughtered at different ages, the age-specific prevalence of pneumonic lesions was 2.7% in pigs less than 16 weeks of age at slaughter, but it increased rapidly when pigs were between 16 and 22 weeks of age at slaughter. Infection at an early age has a greater effect than infection later in life. Pigs coughing by 14 weeks of age were, on average, 6.2 to 6.9 kg lighter than those with onset of disease near market age. The highest seroconversion rate occurs between 3 to 4 months of age. In a recent experimental infection, 77.7% of the infected animals were still positive 185 days later, and 100% of the naturally infected animals were still infected at the same time. There may be selective differences in the colonization rates between litters. There may also be a sex effect on colonization. A longitudinal study of the diversity and dynamics of MH infection has been described.[19]

In a study of a large number of sows in northwestern Germany, it was found that the risk of a sow being seropositive was increased in herds with two- or three-site production, when piglets were not vaccinated, when herds had 2-week farrowing intervals, and in herds without AI/AO management of the farrowing units. The lack of an acclimatization period for boars was also associated with the risk of a sow being seropositive.[20]

Immune Mechanisms

Pigs that recover from experimentally induced enzootic pneumonia are resistant to subsequent challenge. The nature of the immunity induced by MH, whether serum or local antibody mediated, T-cell mediated, or a combination of these factors, is not clear. Based on lymphocyte transformation tests of experimentally infected pigs, it is possible that cell-mediated immunity correlates with protective immunity. The median half-life of passively acquired antibodies to MH is 16 days, the persistence of antibodies is related to the initial antibody concentration, and antibodies waned by 30 to 63 days after birth depending on initial concentration. It has been detected as late as 155 days of age. The titer of maternal antibodies is a major concern when pigs are vaccinated. The age of the piglet vaccinated is not the key factor. The level of the sow's antibodies approximately 4 weeks prepartum are at their highest and similar to the levels in colostrum. Immunity is not conferred through colostral immunoglobulins, and thus piglets born from immune dams are susceptible to infection and clinical disease. No significant correlations have been found between the colostral antibody levels and the colonization status of the sows. The level of immunoglobulins to MH can be used to monitor infection in the herd. Pigs usually seroconvert to APP and then MH.

Pigs raised under unfavorable conditions develop pneumonic lesions more frequently than pigs raised under better conditions, regardless of their immune status. Pigs vaccinated with inactivated MH organisms develop both a cell-mediated and humoral immune response, but they are not protected from challenge exposure by natural infection. Local immunity, particularly secretory IgA, is considered to be important in protection against mycoplasma infection. MH may suppress alveolar macrophage function, which may predispose the lung to secondary infection. The organism is very clever in evading the immune response, probably by changing the nature of the immune response to one that is less effective. To do this it causes the production of cytokines IL-1α and beta, IL-6, and TNF-α by macrophages and monocytes and induces local inflammation. This is essentially moving the immune response from a TH1 type response to a T-helper type 2 response.

In an experimental study it was shown that PRRSV vaccine strain and the natural infection were able to induce T-regs in pigs naturally infected with MH. This suggests that the exacerbation of MH following PRRS may be attributable to the ability of PRRSV vaccination and viral infection to induce regulatory T cells.[21]

Pathogen Risk Factors

MH adheres to the tracheal and bronchial mucosae and causes an extensive loss of cilia. An evaluation of the virulence factors of MH field isolates has been made.

Environmental and Management Risk Factors

Pathogenesis

MH colonizes the respiratory epithelium for a long time and produces a prolonged inflammatory response and suppresses and modulates the immune reactions. Little is as yet known about the virulence factors of MH. A wide variety of proteins are produced. *Mycoplasma* have the smallest genomes of organisms capable of separate existence. This genome encodes for several immunogenic proteins including a cytosolic protein p36 (which may have lactic dehydrogenase activity); membrane proteins P46, P65, and P74 (can produce neutralizing antibodies); and an adhesin P97. The P97 adhesin mediates adherence of MH to swine cilia. An adhesin-like protein (P110) composed of a P54 and 2 P28 units has also been found. Attachment is a complex process involving many gene products. A recent study of the total protein profile, glycoprotein profile, and size differences in the amplified PCR product of P97 adhesin genes suggests that there is an intraspecies variation in the MH population in the United States. Combination with the P102 adjacent gene allows the two proteins to contribute to cellular adherence.[5,6] A highly immunogenic MH lipoprotein Mhp366 was identified by peptide–spot array,[22] and this may be a useful method for detecting MH infections. The in vivo virulence of MH isolates does not correlate with in vitro adhesion assessed by a microtiter adherence assay.[23] These observations suggest that mechanisms other than adherence may be responsible for observed differences in virulence.

The *Mycoplasma* penetrate the mucus layer and attach to cilia. They appear only to attach to the cilia. They release calcium^{++} ions from the endoplasmic reticulum of the ciliated cells. As a result there is a clumping and a loss of cilia and excess production of mucus by goblet cells.[24] This results in a dysfunction of mucociliary clearance. The secondary bacteria attach to the damaged epithelium.

In experimental infections of tracheal explants with MH, it was shown that IL-10, IL-6, and IL-8 were produced.[25,26] There is also a production of TNF-α and IL-1[27] and IL-18, but the production of IFN-γ is inhibited.[28] These are possible mechanisms for the down-regulation of cell-mediated immunity

that allows for the enhancement of the duration and severity of pneumonia with PRRSV and a mechanism to modulate the immune response.

Macrophages have an impaired phagocytic activity after MH infection. MH also alters the function of B- and T-cell lymphocytes.

The experimental inoculation of the J strain of MH into piglets causes gross pneumonic lesions that are detectable 7 to 10 days later. Moderately extensive pneumonia is present 6 weeks after inoculation, progressive recovery can be observed after 10 weeks, and residual lung lesions are detectable in a few pigs up to 37 weeks after inoculation. Experimental infections vary in their effects in clinical signs and pathology.[29]

MH causes peribronchiolar lymphoreticular hyperplasia and mononuclear accumulation in the lamina propria, which causes obliteration of the bronchial lumina. There is also perivascular lymphoid hyperplasia. The bronchial mucous glands undergo hypertrophy; there are increased numbers of polymorphonuclear cells in the bronchial lumina and macrophages in the alveoli. Lymphocytes, together with plasma cells and macrophages are responsible for the increase in the thickness of the interlobular septa as the disease progresses. The hyperplastic BALT (bronchial and bronchiolar associated lymphoid tissue) in enzootic pneumonia cases consisted of macrophages, dendritic cells, T- and B-lymphocytes, and IgG^+ and IgA^+ cells. In these aggregates $CD4^+$ predominated over $CD8^+$ cells. The cells in the BALT released IL-2, IL-4 TNF-α, and, to a lesser, extent IL-1α and β. IL-1α and TNF-α were also released in bronchoalveolar lavage fluids, and IL-6 and IL-8 were found in the mononuclear cells of the alveolar septa.

Hyperplasia of type II alveolar epithelial cells is progressive as the disease becomes worse. Affected pigs cough persistently, show labored respiration and reduced exercise tolerance. The lesions are similar to those of chronic bronchitis. After infection, MH multiplies in tracheal and bronchial mucosae, adheres to the ciliated cells, and causes a cytopathic effect and exfoliation of epithelial cells. There is a significant increase in the gland/wall ratio and a decrease in the ratio of respiratory to expiratory resistance.

The effects of this chronic pulmonary lesion have been the subject of considerable investigation. It is thought that the presence of mycoplasmal lesions uncomplicated by secondary bacterial infections has minimal effect on the production of the pig if the environmental conditions are suitable. The lesions will heal, and any loss in production from the initial infection will be regained by compensatory regrowth. Severe lesions or those accompanied by secondary bacterial bronchopneumonia and pleuritis will usually cause a significant decrease in average daily gain and feed efficiency. Secondary infection with *Pasteurella* spp. results in acute episodes of toxemic bronchopneumonia and pleuritis. Dual infections are usually more severe than single infections. For example, SIV and MH together are more severe.

A longitudinal study was made in four herds until slaughter. The percentage of pigs testing positive increased from 35% at 6 weeks to 96% at slaughter at 26 weeks. Within each herd only one distinct strain was detected[19] and was present in the same animal for at least 12 weeks.

The pulmonary and hematologic changes in experimental MH pneumonia cause no significant changes in heart rate, respiratory rate, and rectal temperature, even though at necropsy well-demarcated pulmonary lesions were present. There were several measurable changes in respiratory functions as a result of the atelectasis: partial occlusion of the bronchioles with exudate, localized pulmonary edema, and a reduction in oxygen perfusion to the alveoli leading to a decrease in the partial pressure of oxygen in the arterial blood. There are no remarkable changes in the hematology. The body weight gains are decreased compared with the control animals.

The distribution of lesions is characteristic. They occur in the right middle lobe, the right cranial and left middle lobes, and the left cranial and diaphragmatic lobes, in that order of frequency. The differences in pathogenicity between high- and low-virulence isolates is associated with a faster in vitro growth, a raised capacity to multiply in the lungs, and the induction of a more severe inflammatory process.[30] It has been shown recently that MH- derived lipid-associated proteins induce apoptosis in alveolar macrophages by increasing nitric oxide production, oxidative stress and caspase-3 activation.[31]

In a study to assess the duration of infection with MH 60 pigs were infected and studied until the population became negative on estimation of DNA in bronchial swabs. DNA was detected in 100% of the animals at 94 days postinfection, 615 at 214 days, and 0% at 254 days PI. Experimentally infected pigs transmitted to sentinels at 80 and 200 days post infection.[32]

CLINICAL FINDINGS

The appearance of clinical pneumonia depends on the number of organisms, their virulence, and the involvement of secondary agents. The more pathogenic strains induce more pneumonia.[30] It is also influenced and made more severe by PCV2[33,34] and together with PRRSV is also more severe.

A natural incubation period of 10 to 16 days is shortened to 5 to 12 days in experimental transmission. Two forms of the disease are described. In the relatively rare acute form, a severe outbreak may occur in a susceptible herd when the infection is first introduced. In such herds pigs of all ages are susceptible and a morbidity of 100% may be experienced. Suckling piglets as young as 10 days of age have been infected. Acute respiratory distress with or without fever is characteristic and increased mortality may occur. The usual course of this form of the disease within a herd is usually about 3 months, after which it subsides to the more common chronic form.

The chronic form of the disease is much more common and is the pattern seen in endemically infected herds. Young piglets are usually infected when they are 3 to 10 weeks of age, and clinical signs may be seen in suckling piglets. More commonly, the disease shows greatest clinical manifestation after weaning and in the growing period. The onset of clinical abnormality is insidious and coughing is the major manifestation. Initially only a few pigs within the group may show clinical abnormality, but then the incidence generally increases until coughing may be elicited from most pigs. It may disappear in 2 to 3 weeks or persist throughout the growing period. In affected herds, individual pigs may be heard to cough at any time, but coughing is most obvious at initial activity in the morning and at feeding time. Coughing may also be elicited by exercising the pigs around the pen, and it occurs with greater frequency in the period immediately following the exercise. A dry or crackling, hacking cough, which is usually repetitive, is characteristic. Respiratory embarrassment is rare and there is no fever or obvious inappetence. Subsequently there is retardation of growth that varies in severity between individuals so that uneven group size is common. Clinical disease becomes less obvious with increasing age and is rarely detected in the sow herd, although gilts and young sows frequently harbor MH.

CLINICAL PATHOLOGY

Raised haptoglobin levels have been found in pigs with lung conditions resembling *Mycoplasma* infection but not *A. pleuropneumoniae*–type lesions.[35]

Serologic Tests

Serologic tests are best used to assess the herd status. All the three assays in use in the United States have excellent specificity, but the sensitivity is low, from 37% to 49%. The tests vary in their efficacy in different experimental infections.[36]

Serologic tests have included the CFT (low sensitivity), indirect hemagglutination test (good for early detection as it detects IgM), and the latex agglutination test. The unsatisfactory sensitivities and specificities of these tests led to the development of ELISA systems, DNA probe technology, and PCR to accurately diagnose enzootic pneumonia. The ELISAs detect all classes of IgG and are very sensitive, but they detect the onset of seroconversion rather than infection. An SIgA-ELISA has been developed for

detecting secretory IgA from nasal swabs,[37] and it is capable of detecting MH infection from MH vaccinated pigs.

An ELISA using a commercially available antigen (Auspharm) is highly sensitive (95.6%) and specific (98.8%) for antibodies against MH when pig sera from commercial herds of known infection status were evaluated. An improved ELISA is also available, and the two ELISAs are able to distinguish populations of gross pathology-negative pigs in endemic herds from pigs in true specific pathogen-free (SPF) herds. Pigs from the former group have significantly higher ELISA activity with both tests and would represent recovered or exposed nondiseased pigs, or pigs with only histologic lesions in endemic herds. The ELISA is ideal for diagnostic laboratories and should obviate much of the need for culture and immunofluorescent histopathology, reducing the cost of diagnosis. The ELISA can also detect antibodies in the colostrum of sows with a high specificity. A recent study comparing three ELISAs has shown that the sensitivities of the tests were lower than previously reported especially for vaccinated animals. Animals within 21 days postinfection were also not easily detected. The blocking ELISA was the most sensitive. All three were highly specific. There is also a blocking ELISA against a p40 protein.

Colostrum has also been used for the certification of freedom from MH but must be achieved during the first 2 hours after parturition. High-parity sows are a better source for the detection of antibodies.

Detection of Organism

For the highest level of accuracy in detecting the organism the use of a number of tests would be best.

The organism can be detected in lung tissues by culture, immunofluorescence, PCR, and antigen-ELISA, and all have high sensitivity in the acute stages of pneumonia. A PCR-based assay can differentiate MH, *M. flocculare,* and *M. hyorhinis* and also detect low numbers of organisms. It can also be used on the bronchoalveolar lavage. The identification of the p36 and p46 protein genes has enabled them to be used in a PCR for MH, with a sensitivity of 86.6% and a specificity of 96.7%. Nested PCR is much better. There is a good correlation between the results of nested-PCR and histology. In situ hybridization shows MH on the surface of the epithelial cells, not in the cytoplasm, with an occasional signal in the cytoplasm of the alveolar and interstitial macrophages. A PCR[38] had a diagnostic sensitivity of 97.3% and a specificity of 93.0%.

Herd Certification

The determination of the presence or absence of MH within a herd for certification purposes can be difficult and should be approached with caution. It should not be based on a single examination procedure. It requires a surveillance system that combines regular farm visits and serologic, cultural, and tissue examination of selected pigs and of those sent to slaughter. The herd should be examined clinically for evidence of the disease, and the lungs from several shipments of pigs should be examined at the abattoir and subsequently histologically. There can be seasonal variation in the severity of lung lesions and at certain times market-age pigs may not have visible gross lesions, even though infection may be present in the herd. If doubt exists, the lungs of younger pigs, preferably clinically suspect pigs, or recently weaned pigs, should be examined after elective slaughter. The herd should also be examined for the presence of antibody to MH.

NECROPSY FINDINGS

Except in severe cases, the damage is confined to the cranial and middle lobes, which are clearly demarcated from the normal lung tissue. The lesions are commonly more severe in the right than in the left lung (simply because it is larger, has a larger supply of main-stem bronchi and a greater arterial supply). Plum-colored or grayish areas of lobular consolidation are evident. Enlarged, edematous bronchial lymph nodes are characteristic. In acute cases, there is intense edema and congestion of the lung and frothy exudate in the bronchi. When secondary invasion occurs, pleuritis and pericarditis are common, and there may be severe hepatization and congestion with a suppurative bronchopneumonia.

Evaluation of the pneumonic lesions at slaughter has been used extensively for herd health monitoring. Scoring of the lesions is typically done on both lungs (the entire pluck). To overcome the logistical problems associated with examining entire plucks during the slaughtering procedure, an alternate system based on scoring the right lung only has been investigated. The overall right lung relative sensitivities for the detection of catarrhal pneumonia or chronic pleuritis were 81% and 72%, respectively. It is suggested that an evaluation of the right lung pathology is a useful alternative when the purpose of the survey is to demonstrate the presence or absence of lesions, or when scoring the severity of the lesion is the objective.

The microscopic changes of enzootic pneumonia include lymphohistiocytic peribronchiolar cuffing with increased numbers of mononuclear leukocytes in the bronchial lamina propria. There is hyperplasia of the bronchiolar epithelium and filling of alveoli with macrophages, protein-rich fluid and small numbers of lymphocytes and plasma cells. Hyperplasia of type II alveolar epithelial cells occurs as the disease progresses.

These histologic changes were most marked from 7 to 28 dpi coinciding with a significant increase in the immunohistochemical demonstration of Il-1α, IL-1β, IL-8, TNF-α and INF-γ, lymphoid markers CD4+ and CD8+, muramidase, and IgG and IgA.[39] The lesions and immunohistochemical signals declined in intensity after 35 days.

In one study, a definitive diagnosis of *Mycoplasma* pneumonia of swine was based on the demonstration of MH in lung sections using specific antisera or successful culture of the organism. Utilizing these techniques, it was found that up to 19% of grossly normal lungs may be infected with MH. Conversely, the organism could not be demonstrated in about 33% of the lungs of pigs from herds thought to be affected with MH pneumonia, even though typical gross lesions were present. The sensitivity of these techniques may be surpassed by newer PCR methods. The organism can also be detected in formalin-fixed paraffin-embedded porcine lung by the indirect immunoperoxidase test. The results of immunofluorescence tests performed on piglets with experimentally induced pneumonia revealed that MH organisms are located primarily on bronchial and bronchiolar epithelial surfaces of lungs with gross lesions of pneumonia. Fluorescence was most intense 4 to 6 weeks after infection and began to decrease at 8 to 12 weeks. This suggests a decrease in the number of MH in the more advanced stages of the disease. When assessing plucks at slaughter to determine the severity of pneumonia in a group, it must be remembered that in most instances the lesions observed represent a chronic, partially resolved disease process. Therefore the clinical effects of the infection may have caused a greater degree of respiratory compromise than is apparent at slaughter.

In a recent study the histopathology of lungs in slaughter pigs vaccinated with different vaccines has been described.[40] Lung lesion scores and MH loads differed widely between the three different vaccine groups but were correlated with each other.

DIAGNOSIS

Typical epidemiology and a dry hacking cough are suggestive of MH. Typical lesions need to be investigated at the margins of the lesions and culture attempted. Recently farms have been described that have more than 1 strain of MH.[1]

Tracheal bronchial swabbing associated with RT-PCR could be an accurate diagnostic method.[41]

The most sensitive sampling methods for detecting MH in live, naturally infected pigs were tracheobronchial swabbing or washing compared with oropharyngeal brushing and nasal swabbing.[42]

Samples for Confirmation of Diagnosis

- **Touch preparations** using Giemsa stained slides have been used.

- **Histology**—formalin-fixed lung (LM, IHC). Simple histopathology may not always indicate MH infection. For example, Aujeszky's disease together with *P. multocida* may be difficult to differentiate from MH. Lesions may be characteristic but not pathognomonic.

Detection in lung tissue is either by FA or IHC, and these are rapid and cheap and more often used than ISH. The more fresh the material or immediately fixed material gives better results.

In experimentally infected pigs MH could be reisolated from liver and spleen of experimentally infected pigs and contact pigs.[43]

Indirect immunofluorescence (IF) and indirect immunoperoxidase (IHC) for MH in tissues are extremely useful. However, IF has a lack of sensitivity and IHC is time consuming and expensive.

Mycoplasmology lung (MCULT, FAT, PCR). Isolation of MH is complicated by the overgrowth that occurs from *M. hyorhinis* and *M. flocculare*. The organism is fastidious, and 4 to 8 weeks are sometimes needed for growth. It also requires specialized media, including swine serum. For these two reasons it is not so commonly used now. Many animals that are culture positive do not have gross or microscopic lesions.

PCRs have become a sensitive and specific method for identifying MH.[38,41] Lung tissue, bronchial swabs, or bronchial washings are the best sites. Nested PCRs raise the sensitivity and may detect as few as four to five organisms. A real-time TaqMan PCR that simultaneously detected the proteins P46, P97, and P102 has been designed[44] that can detect 10^8 *Mycoplasma* per pig.

In addition, a multiplex PCR has been developed that can be used on culture broth for several mycoplasma.[45]

A number of RT-PCRs have been developed that allow quantification.[44,46] The PCR can be used as a one-step test but is not good for nasal swabs. The nested PCR can be used for these, but it does tend to produce some false positives. Correct samples give a better diagnosis. Samples from lavage and tracheobronchial sites were the best for nested PCR, and lung tissue and nasal swabs are not the most reliable.[43]

TREATMENT

There is no effective treatment to eliminate infection with MH, although the severity of the clinical disease may be reduced.

Isolates of the organism from the United States were susceptible to lincomycin-spectinomycin, tylosin, and oxytetracycline. Isolates from the United Kingdom were susceptible to doxycycline and oxytetracycline. Doxycycline, a semisynthetic tetracycline, has a greater antimicrobial activity, is better absorbed orally and is more widely distributed in tissues than the first-generation tetracyclines (oxytetracycline, tetracycline, and chlortetracycline). Tetracyclines given as a preventative in-feed are more effective than giving tetracyclines once clinical signs of coughing have started.[47] This is particularly true when using the drug around times of stress and acquisition of the organisms (ie, in the nursery and at weaning). A recent study has shown that CTC when administered at the onset of clinical signs via the feed at a dosage of 500 ppm during two alternate weeks was able to decrease the prevalence of pneumonia lesions and numerically reduced the performance losses and clinical signs.[48]

In some early studies, a mixture of tylosin tartrate at a dose of 50 mg/kg BW and tiamulin at 10 mg/kg BW orally daily for 10 days significantly reduced the pulmonary lesions associated with the experimental disease. However, the use of 60, 120, or 180 mg of tiamulin per liter of drinking water for 10 days was not effective in suppressing the lesions of experimentally induced MH pneumonia or infection in disease-free pigs.

The newer fluoroquinolones have good in vitro activity against MH and exhibit superior activity to tylosin, tiamulin, oxytetracycline, and gentamicin. Ciprofloxacin is particularly active against MH.

Tilmicosin is particularly effective because it appears to prevent the attachment of MH to the surface of the epithelial cells.

Tetracyclines will either prevent transmission or suppress lesion formation in experimental pigs but the levels required are high and in an infected herd continuous administration would be necessary, which would be uneconomic. Treatment is generally restricted to individual pigs showing acute respiratory distress as a result of a severe infection or secondary invaders. Broad-spectrum antimicrobials are used, usually tetracyclines, but the response is only moderately good. The occurrence of severe signs within a group of pigs may necessitate treatment. Tetracyclines, tylosin, or spiramycin fed at 200 mg/kg feed for 5 to 10 days is recommended. A combination of 300 g of oxytetracycline and 30 g of tiamulin per ton of finished feed fed for 2 to 3 days/week over a 16-month period has been used to reduce the incidence of enzootic pneumonia in a large herd. Lung lesions were reduced, average daily gain increased, and efficiency of feed conversion increased, with an overall increase in profitability. Valnemulin may prove to be effective in the treatment of enzootic pneumonia. There is a higher susceptibility to valnemulin and tiamulin when used in conjunction with doxycycline as a treatment.

Tulathromycin administered as a single injection at a standard dosage of 2.5 mg/kg is effective in the treatment of swine pneumonia associated with mycoplasmosis.

Oral florfenicol feed supplementation (20 g/ton) reduces the effects of MH infection.[49]

There is no evidence for resistance to lincomycin/spectinomycin, oxytetracycline, doxycycline, gentamicin, flufenicol, and tiamulin. There is evidence for some resistance from the field to tetracyclines, macroloides, linosamides, and fluoroquinolones.[50,51]

CONTROL

Control strategies have been reviewed.[52] In all cases recommended management procedures such as all in/all out pig flow, medicated and segregated early weaning, and multisite operations further facilitate control of respiratory disease.

MH infects only pigs and transmission requires close pig-to-pig contact. If transmission can be prevented, it is possible to limit or even eradicate the disease from a herd. There are thus two levels at which control can be practiced: (1) Complete eradication of the disease or (2) Controlling the disease and its effects at a low level.

The principles of control of MH include the following strategies:

- Regular inspection of the herd for clinical evidence of disease and slaughter checks of lungs
- Rigorous biosecurity of animals being introduced into the herd and control of visitors
- Provision of adequate environmental conditions, including air quality, ventilation, temperature control, and stocking density
- The use of the all-in, all-out system of production in which groups of pigs by age or stage of production are moved through the herd from the gestation barn, farrowing barn, nursery rooms, and finishing units as groups and the pens previously occupied are cleaned, disinfected, and left vacant for several days before animals are reintroduced. Because most infection is believed to occur between 4 to 12 weeks, nursery depopulation has become an effective way of controlling the infection in nursery pigs.

Control by Eradication

Control by eradication is the most satisfactory and is probably mandatory for large breeding companies, herds supplying replacement stock to other herds, and for large intensive farrow-to-finish enterprises. It is based on the principle that the source of infection for the young pig is the gilt or the sow and this chain of infection must be interrupted to prevent infection. In the past, the 10-month cutoff point has been used in eradication programs, but in view of the colonization studies, this may be too soon. This is especially so in off-site production systems where the time of infection is delayed.

There are three different principle methods. First, there is total depopulation followed by restocking with noninfected stock (Danish SPF system). Second, the test and removal of all positives and inconclusives. Third, complete eradication without total depopulation and restocking.

Eradication without restocking has been described, and here the secret was to wait until farrowing finished, then vaccinate all sows and treat with tiamulin at 6 mg/kg daily for 3 weeks and then monitor with blood tests.

Specific Pathogen-Free or Minimal-Disease Pigs

Several methods of eradication have been attempted, but the most satisfactory is repopulation with specific-pathogen-free (SPF) pigs. The principle underlying this method is that the piglet in utero is free of infection with MH. If it is taken from the uterus at term by suitable sterile hysterectomy or hysterotomy techniques and reared artificially in an environment free of pigs, it will remain free of this infection. In practice this has been carried out in special units, and the piglets have been subsequently used to repopulate existing farms where all pigs have been removed 30 days before the introduction of the SPF pigs and a thorough cleaning program completed. This method was initially developed for the control of MH and atrophic rhinitis. Moreover, if suitable precautions are taken and if the piglets are used to populate new units that have had no previous exposure to pigs, then freedom from other important diseases such as internal and external parasitism, leptospirosis, brucellosis, swine dysentery, and others can be achieved. The progeny of these primary SPF herds can subsequently be used to repopulate other or secondary SPF herds known as minimal disease pigs.

Because of the cost and technical difficulty of this method, other methods of eradicating MH have been attempted, but they are generally less satisfactory and have a higher failure rate. These include "snatching" of pigs at birth and isolated farrowing. In the former the piglets are caught and removed from the sow immediately at birth and reared as previously described or foster-suckled on SPF sows in another environment. Although MH may be eliminated by this method, fecal contamination during parturition of the vulva and vagina and consequently of the piglet is common, and this method is less satisfactory for disease control than removal by hysterectomy.

Isolated Farrowing

Isolated farrowing techniques have proved successful in small herds but have a high failure rate when practiced on a large scale. Older sows believed to be free of infection are farrowed in isolation in individual pens erected outside on pasture and each sow and litter is kept as a separate unit. The litter is inspected clinically at regular intervals, and subsequently a proportion of the litter, usually excess males and gilts undesirable for breeding, will be examined at slaughter for evidence of pneumonia. Any litters with clinical, pathologic, or laboratory evidence of pneumonia are eliminated from the program. Litters that pass inspection are kept for repopulation of the herd. Because of the difficulties in detecting carrier pigs without lesions, eradication by methods using these principles frequently fails.

Minimal Disease Herds

Minimal disease herds have been established in most countries with significant pig populations, either by breeding companies or private purebred breeders. As a result, in most countries there is a nucleus of MH-free stock. The establishment of primary SPF herds is technically difficult and very costly and should not be undertaken lightly. There is also a considerable delay in cash flow between the time of initial population and buildup of herd numbers to the time when significant numbers of pigs are available for sale. If eradication by repopulation is intended, it is preferable to purchase pregnant gilts from established primary SPF herds unless the maintenance of existing genetic lines dictates otherwise. Before recommending eradication by this method it is essential that the pig owner understands the principles of this method of control and the restrictions that will need to apply if it is to be successful. Farrow-to-finish enterprises established by this method should be run as closed herds, and if further genetic material is required it should be introduced by hysterectomy techniques or by purchase from the initial source herd. The use of artificial insemination is an alternate method; however, isolation of MH from semen has been recorded.

The problem of certifying and maintaining herds free of MH is a major task.

Reinfection of Herds

Reinfection of MH-free pig herds occurs despite high standards of isolation and strict precautions when complete protective clothing and showering routines are required for all visitors entering the unit. All visitors are debarred entry if they have been to a possible source of infection during the previous 48 hours and even up to 7 days. Also, the majority of breakdowns occur in herds that have not imported infected stock recently. In reinfected herds that imported stock there was no concurrent evidence of breakdown in the parent herds, which supported the contention that the importation of infected pigs was an unlikely source of the infection. An epidemiologic investigation of these reinfections suggests that close proximity of uninfected herds to infected herds may be an important factor. The organism does not survive for more than a few days under dry conditions; however, it can survive in diluted tap water and rainwater for 2 to 3 weeks, and it has been suggested that the organism may be transported in moist air and that airborne infection between piggeries is a possible method of transmission. In Switzerland, 107 farms were reinfected of the 3983 that were eradicated during the period 1996 to 1999 (2.6%). The significance of known risk factors such as farm size, high density of pigs, and farm type was confirmed in this analysis.

Some preliminary estimates of risk indices based on the proximity of other pig units has indicated that the most important factor was the reciprocal of the square of distance to the nearest other unit. The crucial distance for maximum survival was about 3.2 km. A breakdown was described recently in which a whole variety of measures were included in an attempt to control the disease.

Antimicrobial Prophylaxis

Eradication has also been attempted by antimicrobial treatment of newborn piglets with oxytetracycline on days 1, 7, and 14, which were weaned on day 14 and moved to offsite nursery. This is known as a low-cost modified medicated-early-weaning program. This can be followed by serologic testing of the breeding herd and culling of positive reactors. Control by vaccination on the one hand and by the use of tilmicosin on the other produced similar results when measured by serologic results and the prevalence of macroscopic lung lesions. Lincocin with or without vaccination considerably improves the growth and performance. Doxycycline in the feed at 11 mg/kg BW is effective in controlling pneumonia caused by *P. multocida* and MH in feeder pigs.

Low-Level Disease

The alternative to eradication is to limit the effects of the disease in those herds where eradication is either not desirable or feasible. The effects of the disease are generally less severe in nonintensive rearing situations, in small herds where individual litters are reared separately, and where litters from older sows can be reared separately from other pigs. Where litters are grouped at weaning, a low stocking density with less than 25 pigs in initial pen groups and 100 pigs in a common airspace may also reduce the severity of the disease.

Temperature, humidity and ventilation also have an important influence on the disease. It is possible to determine an optimal air temperature zone for growing-finishing pigs based on the measurement of behavioral and health-related problems. They are interrelated with stocking density and housing. The subject is too broad for treatment here, and the requirements for pigs at different ages and under different housing situations may be found in standard texts on pig

housing and production. The environmental risk factors associated with the incidence of MH should be assessed in each circumstance. Some important environmental variables that should be assessed and modified include the following:

- Number of pigs per shed
- Number of pigs per pen
- Airspace per pig
- Floor space per pig
- Cleaning and disinfection techniques used
- Number of air changes per hour
- Waste disposal system
- Number of temperature fluctuations in a 24-hour period
- Direction of the flow of air in the building
- Concentrations of ammonia and hydrogen sulfide in the building
- Dust levels
- Feeding and watering systems
- Whether or not the all-in, all-out system is being used effectively

Medication of Breeding Stock

The original medicated early weaning program was based on medication of the sows with tiamulin at the time of farrowing and the early weaning of the piglets to an off-site location. A variation of this method is to prevent the spread of infection by the following means:

- Isolation of the breeding stock
- Strategic antimicrobial medication of the breeding stock
- Reintroduction of the breeding stock to the original but empty and disinfected gestation barn
- Separate rearing of the piglets before and after initiating the program
- Regulation of flow of animals through the herd. Farrowing barns are emptied out when possible and cleaned, disinfected, and left empty. After weaning their piglets, sows are transferred to the dry sow barns. Sows about to farrow are treated with tiamulin and moved to the farrowing barn.

Medication and vaccination was used to eradicate MH without total depopulation.[53]

Source of Feeder Pigs

Where possible the purchase of weaners or pigs for finishing units should be from herds free of the disease or from a single source. Purchase through saleyards or the purchase of coughing or uneven litters is not advisable. When pigs from infected herds are purchased it may be necessary to medicate the feed prophylactically with one of the tetracycline group of antibiotics or tylosin or spiramycin at 100 to 200 mg/kg of feed for a 2-week period after introduction. Medication of the feed of finishing pigs with tiamulin at 20 and 30 mg/kg of feed over an 8-week period on farms with histories of severe complicated enzootic pneumonia resulted in improved weight gains and feed efficiency, but the extent and severity of the lung lesions did not change. The level of 30 mg/kg in the feed was superior to the level of 20 mg/kg. Tiamulin at 100 mg/kg combined with chlortetracycline at 300 mg/kg of feed for 7 days was effective in herds with a history of MH complicated by the presence of *P. multocida* and *Actinobacillus pleuropneumoniae.*

Introduced pigs should be isolated from the rest of the herd and preferably they should be reared as a batch through a house on the all-in, all-out system. A high stocking density should be avoided and internal parasites should be controlled.

Vaccination

A general observation was that *Ascaris sum* infection affected the response to vaccination for MH.[54] Vaccination reduces the macrophage infiltration in bronchus associated lymphoid tissue infected with a virulent MH strain.[55] In the same study MH was reduced in the lungs in the vaccinated pigs, and the high-virulence strain was inhibited more than the low virulence strain. Vaccination significantly reduces clinical signs macroscopic and microscopic lung lesions especially infected with avirulent strain.[56] The effect was less pronounced with a less virulent strain. Vaccination does not, however, reduce the transmission to other pigs.[57]

MH vaccines are generally bacterins consisting of outer membrane proteins or whole organisms. The vaccines give little protection against initial infection and often incomplete protection against clinical pneumonia. The vaccines produce a TH1 response and also IgA and IgG in the lavage fluids. Natural maternal antibodies do not seem to inhibit vaccination, but vaccination of sows may inhibit subsequent immunity.

Vaccination with killed MH induces protection in pigs against experimental challenge exposure with the organism. A cost-benefit analysis shows that the vaccination is economically beneficial. The relationships between maternally derived antibodies, age, and other factors in vaccine response have been discussed.

Intranasal vaccination of attenuated MH adjuvanted with bacterial DNA may be effective in evoking the local cellular and humoral response and the systemic immune response.[58]

A killed MH vaccine evaluated in a single herd reduced the prevalence of pneumonic lesions in slaughter pigs from 69% to 36% and the prevalence of pleuritis from 20% to 13%. There was a small decrease in the number of days to market. It usually results in a 2% to 8% increase in daily gain. The mortality rate is usually only better numerically. Feed conversion efficiency increases by about 2% to 3%. Other limited studies indicate that vaccination can reduce the severity and prevalence of lung lesions detected at slaughter (4%–6% compared with 12% in controls). It improves feed efficiency and increases average daily gain during the finishing period. In other studies the average daily gain was not improved. Under experimental conditions the transmission in nursery pigs was only numerically lower in vaccinated pigs and the vaccination does not prevent the establishment of MH in the lung.[59]

Vaccination of piglets improved pulmonary health, but vaccination of sows alone did not prove to be sufficient.[60]

Vaccination of sows against MH reduced the prevalence of positive piglets at weaning and could be used to control MH infections as judged by a nested PCR. PRRS vaccination does not interfere with MH vaccination. Needle-less intradermal vaccination has also been described. Double-vaccinated pigs show a lower percentage of MH-compatible gross lesions and a lower MH prevalence in the URT compared with single vaccinated animals.[61]

Both dual and single injection vaccines are available, but the protection obtained is similar. The single dose vaccine gives protection for up to 23 weeks. The level of protection will probably last 4 months. Vaccination is economically attractive.

DNA vaccination using a p42 heat-stable protein gene has also been used, and this induces rises in IL-2, IL-4, and IFN-γ, which indicates that it induces both a Th1 and a Th2 response. Vaccination for mycoplasma generally induces local mucosal immunity, humoral and cellular immunity. A recent study has shown that inactivated vaccine produced both systemic and mucosal cellular and humoral immune responses.[62] It appears to prime the immune response, but this may not become fully operational until natural exposure takes place.[63]

Sow vaccination strategies are still undergoing study but it has been shown that the severity of the pneumonia in piglets born to vaccinated sows was reduced.[64] It increased the percentage of seropositive sows and piglets at weaning but did not affect the sow or piglet colonization. Maternal antibodies do not interfere with vaccination unless they are very high.

PRRSV infection may reduce the response to vaccination, but this may depend on the strains of both agents. In a study there were no significant differences between the protective efficacy of a combined PRRSV/MH vaccine and the two single vaccines.[65]

Intradermal vaccination was successful in reducing lesions by 10.4% compared with controls, and 6% in the intramuscular injection group. Intradermal vaccination afforded greater protection especially with regard to morbidity, lung lesion, and pleuritis scores.[66]

Subunit vaccines may be developed in the future, and other immunodominant antigens other than P97 should be taken into account.[67]

FURTHER READING

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PORCINE RESPIRATORY DISEASE COMPLEX AND MYCOPLASMAL PNEUMONIA OF PIGS

Mycoplasma hyopneumoniae (MH) is a significant contributor to the porcine respiratory disease complex (PRDC), together with PRRS, PCV2, SIV, and secondary bacterial agents such as *Pasteurella multocida*[1](PM), *Actinobacillus pleuropneumoniae* (APP) *and H parasuis* (HPS)*, E. coli, Klebsiella, Trueperella pyogenes, Bordetella bronchiseptica, streptococci,* and *staphylococci.*[2-6] In the study in Denmark,[3] five bacterial species, five viruses, and two *Mycoplasma* species were found in different combinations. The study in Germany[4] found that among a variety of pathogens, PCV2 and alpha-hemolytic streptococci were most frequently detected. There were also more associations between the organisms in clinical cases than in the healthy pigs. Porcine respiratory disease complex is a better name for what was once called enzootic pneumonia. This term really means pneumonia that occurs naturally in the population and includes a complex of many bacterial and viral agents with the occasional addition of parasites and protozoa.

Some primary pathogens such as MH and APP are not usually isolated from healthy pigs and may be responsible for subclinical infections. A Danish study found that *Actinomyces hyovaginalis* was a common isolate from pyemic lungs in pigs. The authors did a study in the 1960s involving full viral, bacteriologic, and environmental and management analyses, which showed that each farm was an individual with its own set of variables, and that the only significant factor was that MH was associated with clinical disease and economic loss.

Simultaneous occurrence of Aujeszky's disease does increase the severity of acute mycoplasmal pneumonia. The jury is still out as to whether TTV has a role to play in PRDC.[7] In a recent study, lipoteichoic acid from *Staphylococcus aureus* exacerbated respiratory disease in porcine-coronavirus infected pigs.[8]

Normally, the bacteria live in symbiosis with the host. The three major enzootic pig viruses (PRRS, PCV2, and SIV)[9-13] destabilize the situation through direct pathologic effects or disturbances of the immune system. This complex is characterized by slow growth, decreased food conversion efficiency, anorexia, fever, cough, and dyspnea in grower finisher pigs typically around 16 to 22 weeks of age. It corresponds to what was originally called enzootic pneumonia.

ETIOLOGY

Some of the bacteria live happily in the upper respiratory tract, for example, *Bordetella bronchiseptica* (BB), some strains of *Hemophilus parasuis* (HPS), and *M. flocculare,* and, *M. hyorhinis.*[14] Other organisms are inhaled directly or more likely introduced by nose-to-nose contact (MH) or even aerosols, whereas others flare up in times of stress from small numbers normally harbored in the nasopharynx and tonsils, such as APP and PM.

There is variation in the strains of many of these organisms and this determines the outcome of infection in many cases. Similarly, there may be breed dispositions to some of the agents.[15,16]

The presence of PRRS, *P. multocida, H. parasius, M. hyorhinis,* or *S. suis* correlated with a higher probability of also finding MH.[17]

EPIDEMIOLOGY

The combination of pathogens involved in the respiratory disease complex is legendary and varies from country to country, region to region, and even farm to farm.[18] When a new agent enters the field (e.g., the pandemic SIV2009[19-23] or Torque teno virus), then the position becomes even more complicated until the population at large becomes immune.

Secondary bacterial pneumonia can be a significant cause of mortality in the weaning-to-market period. Some of the risk factors for pleuritis and cranioventral pneumonia have recently been reviewed.[24,25] The relationships between the infectious and noninfectious factors in PRDC have been reviewed.[26]

Atrophic rhinitis may also be present along with enzootic pneumonia, and the two diseases in combination may have a greater economic effect than either disease alone. When outbreaks of respiratory disease in pigs occur, they are frequently the result of complex interactions between many agents. The importance of MH is not only its effect as a primary pathogen but also its ability to act synergistically with other infecting agents to produce significant respiratory disease. MH causes a mild pneumonia, whereas *P. multocida* is not pathogenic alone but aggravates the pneumonia initiated by the former pathogen. The epidemiologic associations between MH and *Actinobacillus pleuropneumoniae* antibody titers, and lung lesions in pigs at slaughter have been examined. Only titers to the *Mycoplasma* pneumonia were associated with lesions.

The extent of the lesions produced by MH in PRDC may be influenced by other contributing factors to account for the variations in severity of lesions. Concurrent infection with lungworm, migrating ascarids, and an adenovirus has resulted in lesions of greater severity and secondary invasion of pneumonic lesions by *Pasteurellae, Streptococci, Mycoplasma,* and *Bordetella bronchiseptica;*

Klebsiella pneumoniae is very common and largely influences the outcome of the disease in individual pigs. In some abattoir surveys of lungs, *P. multocida* can be cultured from 16% of normal lungs and from 55% of lungs with lesions resembling those of enzootic pneumonia. *P. multocida* and *Haemophilus* spp. may also be found in conjunction with MH in the lungs of slaughter-weight swine affected with pneumonia and examined at the abattoir. Those lungs with both MH and *P. multocida* had the most macroscopic pneumonia, and those lungs with either of the agents alone had much less pneumonia. MH renders the lungs susceptible to *P. multocida* colonization and infection.

Along with MH, other *Mycoplasma* species, such as *M. hyorhinis, Acholeplasma granularum* and *Acholeplasma laidlawii,* have been isolated from the lungs of pigs at slaughter, but their significance is unclear. MH and *Mycoplasma hyorhinis* have been isolated from 30% and 50% of pneumonic lungs, respectively, from pigs examined at slaughter. MH was also isolated from 12% of lungs with no gross lesions of pneumonia. In a survey in Norway, MH, *P. multocida* and *M. hyorhinis* were detected in 83%, 43%, and 37% of the pneumonic lungs respectively. Most of the macroscopic pneumonia—up to 25%—occurred in lungs with all three pathogens. *M. flocculare* was the most frequently isolated organism in the nonpneumonic lungs.

MH potentiates the severity of PCV2-associated lung and lymphoid lesions and increases the amount and perhaps the presence of PCV2 antigen. It also increases the incidence of PMWS in pigs.

Several environmental and management factors are associated with a high prevalence of pneumonic lesions at slaughter. They include continuous versus all-in, all-out production, open herds, large temperature fluctuations, semisolid pen partitions, and large numbers of pigs in a common airspace. These factors may operate individually or in combination synergistically. Housing pigs in a clean, isolated, disease-free and low-stress environment positively influences the health of pigs. Complex animal production systems in the industrialized world have been reviewed.[27]

The primary and secondary pathogens of the disease produce their most detrimental economic effects and the highest level of morbidity and mortality during the finishing period when the economics of production necessitate indoor housing and intensification.

Four main groups of environmental factors that contribute to high levels of clinical disease and lesions at slaughter include:

1. Meteorologic
2. Population and social
3. Management
4. Airborne pollution

Meteorological factors include wide fluctuations in the temperature indoors, wide variations in relative humidity, irregular ventilation rates, and winter housing. However, experimentally, elevated concentrations of ammonia and fluctuating ambient temperature did not influence the severity of the pneumonia or its effect growth rate. The noninfectious factors associated with pneumonia and pleuritis in slaughtered pigs in 143 farrow-to-finish farms in France were analyzed.[28,29]

Population factors that contribute to an increased prevalence of pneumonia are increasing herd size, increased population density, and decreased airspace and floor space per pig. All management practices influence the microclimate, and the quality of housing and management influences the incidence of pneumonic lesions at slaughter. Larger-than-average swine farms milling their own feed and with characteristics of modern buildings (mechanized inlets, slatted floors) and in close proximity to other farms tend to have a higher risk of enzootic pneumonia. Extensively housed pigs with above-average pen space and air volume have a reduced prevalence of enzootic pneumonia lesions.

Management factors associated with enzootic pneumonia include family farms that feed pigs on the floor and feeder barns that obtain pigs from multiple sources compared with those with good facilities and where the pigs originate directly from breeding units. The disease is a particular problem in continuous-flow herds. In pigs reared in all-in, all-out groups in the farrowing house, nursery, and growing-finishing unit, any *Mycoplasma* transmitted from sows to pigs or between pigs do not necessarily result in clinical signs or lesions of pneumonia. Pigs reared in all-in, all-out systems do not have lesions or minimal lesions at slaughter and gained at a faster rate than litter-mate pigs reared in a continuous system. Risk factors in suboptimal housing in Australia were described.[30]

In small herds, the factors commonly associated with a high prevalence of enzootic pneumonia were larger numbers of pigs per pen section, larger group sizes, and drafty farrowing and weaner accommodation.

A study of housing density on species diversity and number of airborne microorganisms at fattening facilities has shown that the total number of bacteria and fungi did not exceed 10^4 and 10^3 CFU per m^3 respectively. The number of organisms correlated with housing density. The most numerous were gram-positive bacteria and then gram-negative bacteria and fungi.[31]

Airborne pollution in pig houses is thought to contribute to an increased incidence of clinical disease and prevalence of lesions at slaughter.[32] The pollutants include microorganisms, endotoxic cell wall constituents, and ammonia.[29] Ammonia is the most important because it is a powerful ciliotoxic agent in its own right before determining its effects on microorganisms. Toxic levels of ammonia, high concentrations of aerial dust, and high colony counts of aerial bacteria may contribute to an increased incidence and prevalence of pneumonia, but these factors have not been quantified and are commonly based on subjective evaluations by the observer. A large study of 960 pigs has shown that there are no influences of ammonia or dust on the respiratory health of pigs. Environmental air contaminants such as dust, ammonia, carbon dioxide, and microbes in swine barns measured over a period of 12 months were associated with lesions of pneumonia and pleuritis at slaughter.

In a study of experimentally infected animals, it was found that 6/114 long-distance samples were positive for MH. Three samples collected at 3.5, 6.8, and 9.2 km from the herd of origin were infectious.[33]

In large herds, factors associated with a high prevalence were higher pen stocking rate, airspace stocking rate, and a trend toward higher atmospheric ammonia levels in the summer months. The trend to increased herd size has not been accompanied by the satisfactory control of pneumonia. It has been shown that pig-shed air polluted by alpha-hemolytic cocci and ammonia causes subclinical disease and production losses.[34]

Combination and Interaction of Environmental Risk Factors

A computer-based guide can indicate how the prevalence of the disease can be influenced by the combined effect of risk factors. The expected prevalence is estimated by consideration of 11 risk factors that include the following:

1. Number of pigs in the same room
2/3. All-in, all-out versus continuous flow of pigs
4. Type of partitions separating adjacent pens
5/6. Presence or absence of diarrhea as a clinical problem
7/8. Liquid versus solid manure disposal
9. Ascarid control efficiency
10/11. Presence or absence of active Aujeszky's disease.

The temperature and humidity influence the penetration into the lungs of both primary and secondary pathogens by influencing the size of infected aerosol particles and the protective mechanism in the respiratory tract. Temperature and humidity also influence the sedimentation of infected particles in the air and the ventilation and stocking density. Pigs kept at high stocking densities and subjected to environmental temperature fluctuations, cold drafty conditions, and poor nutrition are more likely to suffer greater adverse effects from this disease.

In a study of the effect of different housing and feeding systems considering liquid versus dry feeding in fully slatted and straw-based housing, there were no differences between in the lung lesions.[35]

Economic Losses and Importance

In annual surveys completed by the American Association of Swine Practitioners, pneumonia consistently ranks as the most economically important disease in finishing pigs. The prime importance of enzootic pneumonia is in its economic effects on pig rearing. The disease adversely affects feed conversion efficiency and daily rate of gain under certain circumstances. However, the magnitude of these effects depends on the conditions in which the pigs are reared and has been a subject of much controversy. The complexity of pneumonia and its interactions with the environment make measuring the effect of pneumonia on performance very difficult.

An accurate assessment of the biological and economic effects of enzootic pneumonia has been challenging because of the difficulty of conducting a controlled experiment in which pigs of equivalent genetic merit, both free of the disease and infected, are raised in an identical manner. In addition, studies on the association between performance parameters and the severity of lesions of the lungs have yielded widely variable results dependent on the management and environmental conditions and the different research design and techniques used. In general, there is a proportional relationship between severity of pneumonia and depression of performance but in other observations, this relationship was not found. Where pigs are raised under good management, infection of herds previously free of the disease has resulted in no adverse economic effect other than during the initial period of acute infection in the herd. However, in other situations adverse economic effects are associated with the disease. One study estimated a reduction of feed conversion efficiency as high as 22%, and although the effect of the disease is probably not this severe in most piggeries, a significant economic reduction can occur even under good management conditions.

Because there is no universally accepted method of measuring the extent or prevalence of pneumonia in pigs at slaughter, the results of studies of correlations between the lesions and performance have been difficult to compare. In general, the economic loss associated with respiratory disease ranges from a 2% to 25% reduction in average daily gains. Some methods have been compared and the most informative procedure is to assess the percentage of lung involved and calculate a mean value for the herd sample. The relationship between the weight of pneumonic lesions from pigs at slaughter and their performance indicated that within a range between 3.32% and 74.5% for the weight of a pneumonic lung, a 10% increase in the weight of pneumonic lung was associated with a decrease in mean daily gain of 31.4 g and a 13.2-day increase to slaughter at 104 kg live weight. There is a high correlation between rapid gross lung scores and detailed examination, which indicates that lungs can be visually scored accurately as they pass on a slaughter line. On average, mean daily gain decreases from 23 to 37 g for every 10% of the lung affected by pneumonia. However, the rapid subjective scoring of the lungs, adjusted for lung proportions, is considered adequate for estimating naturally occurring pneumonia and just as informative as detailed dissection of the lungs.

Because the prevalence of pneumonia peaks at about 60 to 65 kg BW and then declines steadily to a very low level in pigs that are 125 kg or more, the age and weight at slaughter must be considered when evaluating the effects of the lesions on performance and when comparing results between different observations. Weight losses are more substantial in pigs affected early in life. In some studies, lung lesion scores detected at slaughter did not significantly correlate to growth indicators during any season. The gross lesions of mycoplasmal pneumonia heal over a 2-month period, which may explain why significant correlations are not found between growth indicators and lung lesions scores. The effects of the lesions on mean daily gains over an entire growth period may vary from one study to another because of the different times during growth when the lesions exerted their effects and in part to compensatory regrowth following recovery from the lesions. Radiographic examination of the lungs of pigs from 21 to 150 days of age, and gross examination of the lungs at slaughter revealed that lesions progress and regress dynamically throughout the life of the animals and examination at slaughter is an inadequate indicator of lifetime pneumonia.

CLINICAL SIGNS

There are very basically four signs of respiratory disease:

- **Sneezing** is indicative of affliction of the nasal cavity gas, dust, or infection (PRCV, PCMV, or PAR).
- **Coughing** is indicative of affliction of the larynx, trachea, and mainstem bronchi and upper bronchial tree because coughing is the only way to clear large amounts of infected debris (SIV, MH).
- **Dyspnea or difficult breathing** is indicative of the terminal bronchioles and alveoli being affected (APP, PM, PRRS, and PCV2)
- **Parameters of growth may be affected when fever is involved or tissue damage is extensive,** in which case the CNS (hypothalamus) instructs the systems to shut down so appetite, and therefore, growth, is reduced. Growth rate is reduced, daily gain falls, days to slaughter increases, and feed efficiency falls as growth is replaced by immunologic recovery.

The principle sign of PRDC is pneumonia manifested as coughing, labored breathing, fever, lethargy, recumbency, anorexia, discoloration of the extremities/cyanosis, weight loss and slow growth, nasal and ocular discharges, and death. In small batches the disease may affect the group over a short period of time, and most may recover, leaving a few to become chronically affected, hospitalized, or having to be euthanized. In the larger batches with different age groups, there may be rolling waves of infection, or pneumonias may progress to pleurisy.

Some pigs affected with the chronic form of mycoplasmosis may later develop acute pneumonia as a result of secondary invasion with *Pasteurella* or other organisms.

A series of investigations has shown that PRRSV does not predispose to MH infection, although lesions are more severe in those pigs that both infections. MH does potentiate PRRSV induced disease and lesions. There may be an association between the seroconversion to PRRSV and the transmission of MH.

PATHOLOGY

Proliferative and necrotizing pneumonia (PNP) is a form of interstitial pneumonia that occurs in weaning and postweaning pigs. In an Italian study of 28 pigs PRRSV was found in 11 pigs, PCV2 in 4 pigs, and both viruses in the lungs of 8 pigs; in the other 5 pigs nothing was detected.[36] A granulomatous lymphadenitis and pneumonia has been associated with *Actinobacillus porcinotonsillarum* in a slaughter pig.[37] This organism was previously thought to be nonpathogenic.

In the study in Denmark,[3] no clear cut associations were found between pathogens and histologic lesions. They came to the conclusion that PRDC was more common than *Mycoplasma* pneumonia in Danish finishing pigs.

TREATMENT

There are many variables in an outbreak of PRDC, and it is essential to approach the problem in a sensible way. The first thing to do is to establish a diagnosis, probably using postmortem examinations and a variety of laboratory aids, such as IHC, ISH, and PCR. The definition of the primary pathogens from the secondaries and opportunists is the next major step. The third step is to treat the pigs quickly and effectively. The fourth step should be to assess the immune status of the herd and how to improve it. The fifth step is to understand the epidemiology of the agents in the herd and the health background of the herd. The last step is use the latest

knowledge, strong biosecurity techniques, and modern vaccines correctly and to manage the units to use the best in management and environmental control.

MONITORING

Monitoring of respiratory disease has been achieved principally by slaughter checks. These involve snout inspection for the presence of progressive atrophic rhinitis. A cross section of the snout at the level of the 1st or 2nd premolar is examined. For the examination of the lungs, the percentage of the lung that is consolidated is calculated that is firm to the touch.[18,38] Recently examination of digital images has been used.[39] There is a significant negative association between pneumonia score and growth.[40]

In addition, the site of pleuritic lesions on the lungs can also be marked on cards and can be recorded as fresh or old fibrotic adhesions.

CONTROL

By definition PRDC is chronic respiratory disease (although there are periods of acute respiratory disease) in the continual production units of breeder/weaner and breeder/feeder herds. In PRDC there are many potential agents, but there are some guiding principles that will help to maintain health. Maternal antibodies from sows on the same farm as the piglets will provide some protection, which wanes quickly. Young pigs then become susceptible, and if the numbers of pathogens are not too high they will develop active immunity without succumbing to disease. Infection follows the usual pattern of colonization, replication, excretion, and immune development. Disease may follow after replication and excretion, and the duration will depend on the level of replication and the agents involved. Older pigs are always a source of infection for younger pigs and will maintain a cycle of infection; therefore. they should be kept away from younger pigs, although sows should not be kept away entirely from young stock because their immunity is not then maintained.

All-in, All-Out

The first rule is all-in, all-out by age by building or by room. Complete disinfection after cleaning is then carried out, followed by drying and resting. The pig flow through the buildings must be established and maintained.

Buildings

- Make the production to suit the building provision, identifying bottlenecks.
- Where necessary, alter the buildings (new divisions, new buildings, etc.).
- Consider what the correct stocking rates are for the buildings.
- Ensure buildings are adequately ventilated to remove polluted air and excess heat without draughts or overventilation.

Production

Review productivity and consider batch production (i.e., a larger number of pigs less often) to enable filling and emptying of buildings. It is to effective to artificially construct a batch of different ages and hospitalized pigs. Evenness of production from the breeding units will prevent overstocking or understocking.

Sick Pigs

The sick pig is a welfare problem and a hazard in itself, so always hospitalize a sick pig as early as possible, treat, and cull it if no response. The hospital area should be well away from other pigs, and recovered pigs should not be returned to the mainstream.

Diagnosis

On farm or laboratory postmortem examinations should be used to achieve diagnosis if there are sudden acute cases. Cross-sectional blood sampling of the herd to establish epidemiologic patterns of pathogens is sometimes necessary. The use of slaughter pig information from the abattoir will indicate patterns of pathology.

Active Control

Lack of sound management cannot be compensated by use of medication and vaccination, but these may help. Partial depopulation with medication in Denmark has been described.[41] Protection of your unit by the imposition of effective biosecurity from without and within the unit can be extremely beneficial in limiting the ingress of pathogens. The use of bird-proofing and rodent control is becoming much more important and in many cases the repair of the buildings is more important than other factors because cleaning and disinfection are pointless if there are areas where the organic matter can collect. Prophylactic or metaphylactic medication will help if targeted at the correct bacterial agent in feed or water.

Vaccination for PRRS, PCV2, ***Mycoplasma hyopneumoniae, Hemophilus parasuis,*** and *A. pleuropneumoniae,* Aujeszky's disease, and *S. suis* will also help.

The effect of vaccination for PCV2 in pigs suffering from PRDC has been described.[42] Additional strategies for PRDC will include partial depopulation and full depopulation as discussed for MH.

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PORCINE CYTOMEGALIC VIRUS (INCLUSION-BODY RHINITIS, GENERALIZED CYTOMEGALIC INCLUSION-BODY DISEASE OF SWINE)

Porcine cytomegalic virus rhinitis (formerly inclusion-body rhinitis), associated with a beta herpesvirus (family Herpesviridae), is an extremely common, but generally minor, disease in young pigs. It was first recognized in 1955. The virus is now called porcine herpesvirus-2. It is associated with the porcine respiratory disease complex-1.

ETIOLOGY

The virus (PCMV; SuHV-2) belongs to the subfamily of beta-herpesviruses of the family Herpesviridae. The virions exhibit typical morphology of herpesviruses. There are believed to be no serotypes or genotypes, although there is some antigenic variability. It has not yet been found as a problem in xenotransplantation although it does grow in human fibroblast cultures.[1]

EPIDEMIOLOGY

The virus is present in the upper respiratory tract of nearly all herds and pigs (in excess of 90%), and the major infection site is the

conchal (turbinate) epithelium. It does not affect other species. SPF herds established by hysterectomy techniques are not necessarily exempt, and congenital transmission of the virus has been demonstrated. High excretion occurs predominantly in the 2- to 4-week period after infection. Transmission is via the respiratory route through direct contact and aerosol infection, possibly also via urine, and usually perinatally.

When the virus first enters a susceptible herd, both transplacental and horizontal virus transmission takes place. Antibody responses start quickly, so there are often no clinical signs but widespread infection.

PATHOGENESIS

The virus invades epithelial cells, especially those of the nasal mucous glands, to produce destruction of acinar cells and metaplasia of the overlying epithelium, and the major clinical manifestation is that of upper respiratory disease. Following infection, the virus may become generalized. In older pigs, generalization is restricted to epithelial cells of other organ systems, especially those of the renal tubules, and is clinically inapparent. However, in very young pigs the virus also shows a predilection for reticuloendothelial cells, and generalization may result in further clinical abnormality.

The virus also crosses the placenta, so it is possible for intrauterine infection to produce fetal death, along with runting after birth and very early pneumonia, rhinitis, and poor piglet weights at weaning. Congenitally infected animals excrete for life.

CLINICAL SIGNS

The incubation period is generally 10 to 21 days. Clinically, the disease affects piglets up to approximately 10 weeks of age, but the age at manifestation in any herd can depend on the method of husbandry. The disease usually occurs when the virus is introduced into the susceptible herd or if for some reason there is a huge increase in the number of susceptible pigs. A wide age-spectrum of involvement may be seen initially when the disease is introduced into the herd for the first time. In most herds the disease affects pigs in the late suckler and early weaner stage. It is at its most severe in pigs under 2 weeks of age. Sneezing is the most prominent sign and frequently occurs in paroxysms and following play fighting. There is a minor serous nasal discharge that rarely may be blood-stained and also sometimes muco-purulent, with a brown or black exudation around the eyes. There may be coughing. The clinical course varies approximately from 2 to 4 weeks. All pigs within the group are affected, but there is usually no mortality. Neonatal pigs may die without showing signs.

Generalized cytomegalic inclusion-body disease may occur in pigs exposed to intrauterine infection and usually occurs as an outbreak involving several litters. The syndrome is characterized by sudden death and anemia. There is often a history of scouring within the group within the first week of life, and affected pigs show skin pallor and often superficially appear plump and well developed as a result of edema, especially in the neck and forequarter regions. Death, resulting primarily from anemia, occurs during the week 2 to 3 of life, and mortality within the group may approach 50%. Petechial hemorrhages have been a feature of the experimentally produced disease in gnotobiotic pigs but do not necessarily occur in field outbreaks. A moderate anemia producing a check to growth, but without significant mortality, which may be seen in recently weaned pigs experiencing the disease. Many survivors may be stunted.

More serious effects from generalized infection are seen when piglets are exposed to heavy infection at a very young age. It also occurs when there are new imports and when intercurrent disease and poor nutrition reduce resistance. This commonly occurs in large herds with high-density continual throughput farrowing and weaning houses. In addition to upper respiratory disease, infection at this age may result in enteric disease, sudden death, anemia, and wasting, with a marked unevenness of growth within the litters.

There may be complete blockage of the nasal passages. It is believed that the olfactory epithelium may be damaged so that there is no sense of smell and that piglets may not then eat, explaining the that so many die.

PATHOLOGY

Gross changes are not seen often in pigs over 3 weeks of age. In pigs under 3 weeks it may be possible to see catarrhal rhinitis, hydrothorax, and edema in various tissues. In fetal infections there may be stillbirths, mummification, embryonic death, and infertility. Interstitial nephritis and random focal gliosis in the CNS with inclusion bodies can be additional findings, with petechiation in the choroid plexuses, cerebellum, and olfactory lobes. In the acute fatal syndrome most of the basophilic inclusions are seen in the capillary endothelium and sinusoidal cells of the lymphoid tissues. Multifocal hemorrhage and edema results from the vascular damage.

DIAGNOSIS

Inclusion-body rhinitis is not a primary cause of atrophic rhinitis. However, it is probably contributory in lowering local resistance to infection and in predisposing to more severe infection with *Bordetella bronchiseptica* and other respiratory pathogens.

The diagnosis of inclusion body rhinitis is commonly made following the demonstration of typical intranuclear inclusion bodies in histologic sections from electively slaughtered piglets. Large basophilic inclusion bodies are found in the mucous gland cells of the conchal mucosa and may also be demonstrated in exfoliated cells obtained via nasal swabs from live pigs. Small intranuclear inclusion bodies are found in the reticuloendothelial cells. These are best taken from several pigs at the height of clinical infection. Diagnosis by virus isolation is uncommon because the virus has proved difficult to grow, but it will establish in porcine lung macrophage cultures and immortalized cells.

Antibody to infection may be detected by indirect immunofluorescent techniques. ELISAs have been developed to show both IgM and IgG responses. Recently a PCR has been developed and this showed that 59% of pigs tested positive. However, only 59% of PCR positive pigs had clinical signs and lesions consistent with inclusion-body rhinitis. The original experimentalists described the presence of intranuclear inclusions, cytomegaly, and karyomegaly as being pathognomonic. Virus isolation and PCRs can be used. The best PM samples are conchal mucosa, lungs, pulmonary macrophages collected by lavage, and the kidneys. PCMV can occasionally be demonstrated in the brain, liver, and bone marrow. Virus isolation is possible on primary or immortalized cells.

Antibodies can be detected by IFA, which peaks at 6 weeks postinfection and remains quite high for 10 to 11 weeks. The development of serum antibody levels coincides with the disappearance of viremia.

DIFFERENTIAL DIAGNOSIS

Differential diagnosis includes CSF, enteroviruses, parvoviruses, PRRSv, PCV2, and PRV.

TREATMENT

There is no effective treatment, and none is warranted in most herds. With severe rhinitis, antibiotics may temporarily reduce the severity of secondary bacterial infection.

CONTROL

Control of severe disease rests with management procedures that avoid severe challenge to very young piglets. It is also possible to produce virus-free pigs from hysterotomy-derived pigs, but it is necessary to monitor.

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SWINE INFLUENZA

SYNOPSIS

Etiology Influenza A virus subtypes H1N1, H1N2, and H3N2 of *Orthomyxovirus.*

Epidemiology United States, England, Japan, Canada, Belgium. Worldwide. Young pigs. High morbidity, low mortality. During cold months. Antigenic diversity of virus. Aquatic birds are natural reservoirs. Spread between pigs, New strains develop,

Continued

Signs High incidence of anorexia, fever, thumps, muscle stiffness; recovery in several days.

Clinical pathology Polymerase chain reaction (PCR) test to detect virus. Hemagglutination test and enzyme-linked immunosorbent assay (ELISA).

Lesions Marked congestion of upper respiratory tract. Exudate in bronchi. Atelectasis. Suppurative bronchiolitis.

Diagnostic confirmation Demonstrate virus in tissues.

Differential diagnosis list:
- Enzootic pneumonia
- Hog cholera
- Inclusion-body rhinitis
- Atrophic rhinitis

Treatment Antimicrobials for secondary infection.

Control No effective measures available. Vaccines are in use in certain parts of the world.

INTRODUCTION

Swine influenza is an important cause of broncho-interstitial pneumonia throughout all pig-keeping areas of the world. Real problems are associated with the changing viruses that cause the disease and the ability of rapid genetic change to occur by genetic drift or shift.

ETIOLOGY

Classical clinical swine influenza is associated with influenza A virus subtypes H1N1, H1N2, and H3N2 belonging to the *Orthomyxovirus* genus of the Orthomyxoviridae family. The three types occur together as in Korea.[1] Other types have been isolated from pigs, but as yet have not established as widespread endemic strains. Only influenza A viruses are important in pigs. They occur in a large number of species, including humans, primates, pigs, horses, sea mammals, and birds. Avian viruses are more stable than mammalian viruses, where the rate of evolution is much greater. Specific subtypes vary in their ability to cross species barriers. Specific gene combinations do have a part to play in influenza virus species specificity.[2] Unstable gene constellations in avian species become stable only in secondary hosts but may then adapt and circulate freely.[3]

The methods by which they cross the species barrier are not well understood and are probably polygenic.[4,5] An isolate of a Korean H1N1 virus was very similar to a U.S. virus, suggesting that it had been transmitted possibly by birds.[6]

When new variants occur in pig husbandry they are usually found in the pig population before they acquire the ability to spread rapidly and become associated with disease. They are named using the following convention: A/species/localization/isolate number/year of isolation, for example, A/Wisconsin/125/98. If no species is indicated, it is a human virus. They are described with reference to the hemagglutinin (HA or H) and the neuraminidase (NA or N) that project from the surface of the viral envelope. There are 16 HA and 9NA forms that can be distinguished antigenically and genetically, and all of these have occurred in waterfowl and shore birds. They provide a permanent source of infection, as does the water on which they float. The H binds to sialic acid and mediates the virus infection of the host and contains most of the antigenic sites. It is the viral receptor-binding protein and mediates fusion with the host-cell membrane. It is an alpha2-3-galactose linkage in avians and an alpha2-6-galactose linkage on the glycocalyx of epithelial cells in mammals.[7] The HA and NA are associated with receptor binding and virus release.[8] No combination of HA and NA has as yet been identified that will increase viral stability during interspecies transmission. The distribution of these receptors and the limited replication of avian viruses in swine complicate the picture.[9] The pig possesses both types of receptor and has therefore been considered as a "mixing vessel" because it can be infected by both avian and mammalian viruses. The N protein catalyzes cleavage of sialic acid and thereby facilitates the virus cell entry by degradation of mucins. The NA and HA are also the main targets of the host immune responses.

The segmented nature of the virus facilitates the changes in the virus. The surface HA and NA antigens undergo two types of change: antigenic drift and antigenic shift. Anitigenic drift involves small changes but the shift may involve whole segments of the genome being changed. If a cell is infected with two or more viruses, interchange of genetic material can take place. The 8 RNA segments encode for 10 or 11 proteins.[10,11]

It may take multiple mutations to make a distinct HA,[12] and then this has to link with other gene segments compatibly to facilitate survival, replication, and transmission.[13] For example, in the spread of the pandemic 2009, it appeared that the M segment was crucial to the transmission of the virus.

It is possible that within the currently circulating strains, a reassortant will occur every 2 to 3 years.[14]

Three types are found worldwide H1N1, H3N2, and H1N2. In Europe three SIV subtypes are cocirculating: (a) an avian-like H1N1 that came from wild birds in 1979, (b) a humanlike H3N2 with HA and NA genes originating from human virus descendants of the Hong Kong/68 pandemic virus, and (c) a subtype H1N2 reassortant that acquired H1 from human influenza in the 1980s.

H1N1—CLASSICAL

In the United States, these were found on their own until 1998. They were very similar to the 1918 pandemic virus.[15] Since the appearance of other viruses, particularly the triple reassortants, there seems to have been an increase in the genetic diversity of the H1N1 strains in the United States (as also in the H1N2).

A typical reassortant found in Ohio[16] had genes from human (PB1), swine (NA, HA, NP, M and NS), and avian (PB2 and PA). Even though the viruses were isolated over only 3 years, there was evidence of antigenic drift.

H1N1—OTHERS

Humanlike H1N1 viruses have been found in Canada,[17] and H1N1 viruses with the human H1 have spread across North America; these have commonly been isolated from swine disease outbreaks in the United States.

A triple-reassortant H1N1 virus was found in China with the NP and NS genes from a classical swine influenza virus, PB1 from a human virus and HA, NA, M, PB2, and PA genes from an avian virus. Five genes were also closely related to H1N2 viruses founds in China (NS, NP, PB2, PB1, and PA).[18]

H1N1 AVIAN-LIKE

Wild bird H1N1 viruses were transmitted to pigs in the late 1970s and established a stable lineage displacing the classical H1N1 swine lineage; once this had happened, interspecies transmission was facilitated. An H1N1 isolated from a turkey farm in northern Germany in 2009 showed a high affinity with avian-like porcine H1N1 viruses circulating and suggested that turkeys may be a possible bridge between avian and mammalian hosts.[19]

The predominance of avian-like swine genes in the Thai pig population has been described.[20] An experimental transmission of avian-like swine H1N1 has been described, and the virus transmitted through naïve and vaccinated pigs without causing clinical signs.[21]

H1N1 HUMANLIKE VIRUSES IN PIGS

Humanlike viruses were reported in pigs in China before the pandemic,[22] and it was concluded that pigs may act as reservoirs for older human H1N1 viruses.

H1N1-PANDEMIC 2009

There is no evidence that the 2009 pig H1N1 pandemic existed in pigs before May 2009[23] and before it was reported in humans. Soon after its discovery in Canada in 2009,[24] it spread rapidly around the world, and most pig cases are believed to originate from humans, although there was often no real proof until 2011.[25] It has been shown that the virus is fully capable of causing a global problem for swine.[26] The initial incursions of this virus into European pigs has been described[27] from separate nonlinked sites, suggesting infection of pigs from humans. The global spread from an animal source has

been described.[28] It has established itself in pig populations in face of relatively high levels of herd immunity to other viruses. In the Norwegian pig population, there was no prevalence of influenza until the infection of pigs from humans with (H1N1) pdm09.[29]

The virus is a reassortant of genes from the most recent triple reassortant in North America and the European avian-like subtype H1N1 viruses.[30]

The precursors of this virus may have existed in swine for a long time, which suggests that the evolution has occurred over a long period.[30,31] A direct precursor has not been recognized.[32,33] The situation was summarized.[34] It transmits very effectively between pigs.[35] The evolutionary characteristics of the H1 gene of the pdm2009 virus are different from the seasonal human viruses and the swine H1N1 viruses.[36]

The pandemic virus seems to cocirculate and interact more intensely with the endemic SIVs lineages and gives rise, it seems, to more reassortants, the properties of which have yet to be seen.[37] A mono-reassortant of the NA from an H1N1 with the pandemic occurred in Hungary.[38] In a study in Germany, the N2 was from three different porcine lineages in an H1N1pdm backbone.

Six new strains of the pdm-like (H1N1) 2009 strain of H1N1 were isolated and characterized in Poland. They belong to one lineage.[39] The pigs in finishing and growing sectors experienced acute onset of respiratory signs. There was anorexia, poor conception rates (50% lower), high morbidity (up to 100%), and low mortality at 2% to 3% in growers and 1% to 2% in finishers. At postmortem there were depressed, well-demarcated. pale purple areas of consolidation in all lobes.

Novel reassortants have followed with this 2009 virus, and it was pointed out in 2010 that although the virus may be of swine origin. significant viral evolution may still be ongoing[40] and others starting with a 2010 virus in Hong Kong.[41] In this virus, only the NA gene of the 2009 pandemic was reassorted. A novel swine reassortant has been described in the United Kingdom with all the internal genes from the 2009 virus and HA and NA genes from a swine subtype H1N2 virus.[42] It is not clear if this virus can be transmitted between pigs. A novel reassortant has been found in Canada from ab H3N2 and a pandemic (H1N1) 2009 virus on several pig farms and also in mink.[43]

In another reassortant, the NA glycoprotein of the pdm09 virus has been replaced by the NA gene from either H1N2 or H3N2 European swine viruses.[44]

Other reassortants of the 2009 virus have been discovered since from a variety of countries Italy, Argentina, Germany, China, Thailand, and the United States.[44-50]

Nine reassortants have been described across the United States.[51]

A reassortant of the pdmH1N1 2009 virus with an H3N2 virus from healthy pigs has been reported in Thailand.[52]

H1N2

Since 2005, the human HA gene in H1N2 has spread across North America.

A novel reassortant in H1N2 had the NA and HA from the recent H1N2 isolates in the United States and four internal genes (PB2, PB1, PA, and NS) from the contemporary swine triple reassortants in circulating strains, known as the TRIG, but the NP and the M genes were derived from the 2009 pandemic H1N1.[53]

An avian-like H1N2 SIV generated by reassortment of circulating avian-like H1N1 and H3N2 subtypes in Denmark has been described.[54] The Danish H1N2 has an avian-like H1 and differs from most other H1N2s in Europe and North America. These have H1 genes of human or classical swine origin, respectively. The variant is also circulating in Italy and Sweden. The infection dynamics are similar to the those of the assorted H1N2s and similar to the older avian-like H1N1 subtype. A novel reassortant influenza A (H1N2) virus derived from A (H1N1) virus Japanpdm09 has been described for the first time in Japan.[219]

H3N2—CLASSICAL

H3N2 variants arrived in the United States from 1998 onward (North Carolina, Iowa, Minnesota, Texas), although they may have circulated previously and had been unable to establish a stable lineage. Most are triple reassortants from human (HA, NA, and PB1), swine (NS, NP, and M), and avian (PB2 and PA) lineages. By 1999 these were widespread in the United States, and a double assortant that had also been found had not spread widely. These are capable of being placed in one of three phylogenetically distinct humanlike lineages (clusters). The third cluster seems to be dominant and some have developed into a fourth cluster.[55] A study of 97 isolates showed that genetic and serologic differences existed between North American isolates[56] and that they show tendencies to reassortment. Once established, these have spread rapidly and evolved.[57]

H3N2—NOVEL

A noncontemporary H3N2 virus was found to be a wholly human H3N2 virus.[58] Triple-reassortant H3N2 SIVs were isolated from pigs[59] and have formed a stable lineage in Canadian swine.

Novel H3N2 viruses in the United States in humans have been linked visits to state fairs and contact with pigs. Similar occurrences have been found in the past, but these have not had a component of H1N1 in the virus, as does this 2011 variant.

Seven novel H3N2 viruses were isolated from U.S. pigs between winter 2010 and spring 2011 containing internal gene segments from the pandemic H1N1 2009.[60] The evolution of novel H3N2 viruses in North American swine has been described.[61]

A novel avian-like H3N2 containing an H5N1 highly pathogenic segment has been described in southern China.[62]

An influenza A (H3N2) virus from pigs was isolated from pigs and its biological properties reported.[63] The virus produced mild interstitial pneumonia with marked oronasal shedding for about 14 days. Because there is likely to be little cross immunity to these strains, they may cause disease in both humans and pigs in the future.

OTHER VIRUSES

Two H5N1 influenza viruses have been isolated from swine in Jiangsu Province in China, and the authors have suggested that swine are naturally infected with H5N1 virus.[220] This was similar to the situation in Indonesia.[64] Quite often these reach pigs from avians, particularly ducks, including H1N1, H3N2, H3N3, H4N6, H5N1,[64-66] and H9N2.

H2N3 viruses were isolated from farms in central United States[67,68] and were probably of waterfowl origin. The ability of this virus to live in three different mammalian hosts suggests that it is well adapted.

An H3N1 SIV has been isolated from pigs with respiratory disease in Korea[69] and also in Italy,[70] where the HA has been acquired from a human virus, and the other genes came from the currently circulating viruses in the swine population.

Novel viruses can occur in pigs at any point in time. An avian H4N6 virus appeared in Canada in 1999 and was associated with a lake on which there were large numbers of waterfowl. Ducks shed large amounts of virus, and this can be recovered from lake water.

An avian-like H4N8 SIV was discovered in southern China.[62]

An assessment of the reassortant rates of the European strains of SIV suggested that there was one reassortment every 2 to 3 years, and we should expect these to occur in the future between the swine strains and the new human pandemic strain (2009).[14]

A high level of genetic compatibility between swine-origin H1N1 and highly pathogenic avian H5N1 influenza virus was shown.[71]

The avian H5N1 viruses in birds in Indonesia have been transmitted to pigs on numerous occasions[72] but appear to become attenuated.

H5N2 reassortant viruses have been characterized from pigs in Korea.[73] A serologic surveillance of H1N1 viruses in China showed that there was no naturally occurring H5N1 infection in pigs.[74] A highly pathogenic turkey H5N1 virus failed to infect pigs cohoused with infected chicks or chickens.[75]

A H6N6 virus was found in swine in China and seems to have adapted from domestic ducks.[76]

The isolation and characterization of two H5N1 influenza viruses from swine in Jiangsu Province in China has been described.[220] The H5N1 virus has spread to a range of avian and mammalian species but has not been fully characterized in the pig. Both swine viruses bound preferentially to avian-type receptors. The findings suggest that pigs are naturally infected with avian H5N1 viruses and are a potential zoonotic threat. In a study of enhanced infectivity of H5N1 highly pathogenic avian influenza virus in pig ex vivo respiratory tract organ cultures following adaptation by in vitro passage.[221] It was suggested that the mutations in the H5N1 virus may provide a replication or infection advantage in pigs in vivo and that pigs may continue to play an important part in the ecology of influenza viruses, including those of avian origin.

An H7N2 virus was isolated in South Korea and was a recombinant from an avian H7N2 and H5N3 virus.[77]

H9N2 SIVs have been described in China,[78] where the six internal genes are from H5 viruses and the HA and NA from the H9 lineages. In a survey in China, 54 genotypes were identified including 19 novel genotypes,[79] and there is a continuing evolution of these viruses. In this study, at least five antigenic groups were recognized, and during the period of 2002 to 2003 there was a considerable antigenic drift.

Human H7N9 IV replicates in swine respiratory tissue explants.[80] Three Chinese isolates all replicated in tracheal and bronchial explants. These viruses were originally avian viruses that appeared in humans in China with over 130 cases, with a mortality of 32%. The surface proteins are probably from ducks and the internal genes possibly from chickens. There are two lineages reported at the moment. Collectively these viruses could lead to another pandemic. The infectivity, transmission, and pathology of these viruses in pigs has been described.[81]

An H10N5 virus has been isolated from pigs in central China.[82] There is no evidence as yet that the "bird" viruses H10N8 and H7N9 poultry viruses that have killed people in China are as yet occurring in pigs in China.

EPIDEMIOLOGY

The segmented nature of the viral genome is a critical structural feature that enables the viruses to be reassorted. Since 1998, H, N, and PB1 polymerase genes from human viruses; M, NS, and NP genes from classical swine viruses; and PA and PB2 polymerase genes from avian viruses have also been found.

Occurrence

Influenza viruses are ubiquitous in pigs worldwide with the exception of Norway until the 2009 pandemic.[83]

A seroprevalence and genetic characterization of five subtypes of influenza A viruses (H1, H3, H5, H7, and H9) in the Chinese pig population has been described.[84] H1 is the most common, followed by H3.

A study in the United Kingdom suggested that at least 52% of farms had antibodies to at least one type.[85]

A Belgian study involving seven European countries[86] showed all had antibodies, but the Czech Republic, Ireland, and Poland had relatively lower levels.

Both H1N1 and H3N2 are found in Poland but at quite low levels.[87]

Chinese studies suggested that there was 31.1% positive to H1 and 28.6% positive for H388. In a recent study in southern China, over 50% of the pigs tested had a HI titer to one or more influenza H1N1 viruses, and most commonly pdm/09-like viruses. One group had Eurasian avian-like swine H1N1 surface genes and pdm/09 internal genes.[50]

The viruses were similarly widespread in Korea[88,89] and also in Malaysia.[90]

In Canada, 83.1% of the sows and 40.3% of the finishing pigs were positive for H1N1[91] but less than 10% to the Colorado and Texas strains of H3N293. In Argentina, over 70% were positive for H1N1 and H3N294. In Brazil, 46% were positive for H1N1.[93]

Swine influenza first appeared in the United States immediately following the 1918 pandemic of human influenza (Spanish flu), and it was generally believed that it was caused by adaptation of the human influenza virus to swine. Nucleotide sequencing of the genes coding for the internal virus proteins indicates that the human pandemic H1N1 strain and the classic swine strain H1N1 have a common avian ancestor. It is suggested that a virulent avian strain H1N1 entered the human population in 1918, causing the pandemic. The pandemic virus was then introduced into the swine population, where it has persisted unchanged. In contrast, this classical swine influenza was seen in the United Kingdom in 1941 but then disappeared until it was seen in Czechoslovakia in 1950 and Germany in 1959. Influenza was not seen again until observed in swine in Europe in 1979, possibly following importation of pigs from North America, associated with a virus antigenically related to contemporary avian H1N1 strains found in ducks. These avian-like strains have been the most common since 1979.

Swine influenza still occurs in the United States, and viruses of the H1N1 lineage were the dominant cause of SIV from 1930 to the 1990s. These were highly conserved (relatively unchanged), but new antigenic and genetic variants did occur. Classical H1N1 viruses have also been isolated from pigs from South America, Europe, and Asia. Wild pigs also have H1N1. In the 1980s there were many genetic mixings between avian-like H1N1 and human-like H3N2 viruses. In 1992 many outbreaks of classical swine influenza occurred in England, associated with a group of H1N1 viruses that were distinguishable from classical swine viruses, the European swine viruses, and human H1N1 viruses, all of which are known to be circulating in pigs. Influenza A virus subtypes H1N1 and H3N2 are endemic in pigs in Great Britain. Two distinct antigenic variants of H1N1 viruses have been associated with outbreaks of swine influenza, one of which was probably transmitted from birds to pigs in the early 1990s. The H1N2 subtypes isolated from pigs in Great Britain appear to have originated from a human H1N1 virus, which was circulating in the pig population in the 1980s, and from swine H3N2. It is suggested that the H1N1 viruses have disappeared from the human population, and the pig population provides a reservoir for the virus. Serologic surveys indicate that a swine H1N1 influenza virus has circulated in the swine population in North America for many years. Recent isolates from Quebec possess a hemagglutinin distinguishable from subtype H1N1.

Transmission of viruses between pigs and humans and vice versa have shaped the current epidemiology of influenza viruses in North America.

Epidemics of swine influenza have also occurred in Japan, Canada, Belgium, and France. In North America, human H3N2 have been found much less often than in the rest of the world, but the very recent introduction of H3N2 from humans to pigs was probably the major factor in the emergence of the recent strains.

Mixtures of human and classical virus genes have been isolated from pigs in Asia and the United States. H3N2 viruses with human H and N genes and avian internal protein genes have been isolated from pigs in Asia. This type of H3N2 has been found in Korea and is currently the dominant H3N2 virus in pigs in Europe. Since 1998 double and triple reassortants have been isolated from pigs in the United States. The North Carolina virus had three human genes and five swine genes. They include human H and N genes, genes from swine H1N1 viruses, and two others from avians.

All the reassortant viruses found in North America have the triple-assortant gene complex (avian PA, PB2; the NS, NP, and M genes of classical swine lineage; and the PB1 of human gene lineage). This suggests that this set of reassortants can more readily accept changes in NA and HA)

Prior infection with swine influenza viruses is a barrier to infection with avian influenza viruses.[94]

SEASONALITY

A study of circulating viruses in five European countries showed that isolation of viruses was possible throughout the year, especially during winter and spring.[95]

Soon after the occurrence of the H3N2 viruses, new H1N2 viruses arose in the United States, where the human H3 had been replaced by a porcine H1 and then spread. They had been known elsewhere in the world for some time: Japan, France, Germany, and Taiwan. They were described in the United Kingdom, where they were found to be the most severe cause of pathology associated with the SIV viruses. These were all reassortants between human H3N2 and classical H1N1.

Human H3N2 and avian H1N1 were isolated in the United Kingdom and were then found to have spread to Europe. They are usually human H and N and the rest avian genes, but one Italian virus has an avian H1. They have shown considerable genetic drift in Europe.

Subtype H3N2 has been isolated in Canada from pigs with severe proliferative and necrotizing pneumonia (PNP), although this PNP is probably associated with PRRS and PCV2. Serologic surveys indicate the infection is widespread in the swine populations in some countries.

The first unusual virus to be found in pigs was an H9N2 introduced to pigs in South East Asia, probably from land-based poultry.

Further problems occurred in the autumn of 1999 when an avian H4N6 was found in pigs with pneumonia on a commercial swine farm in Canada. Since then the avian H5N1 has appeared in pigs in China and is being carried west by bird migrations into Russia. The potential of avian viruses to spread to pigs and persist in pigs is unknown. Even if the viruses do not replicate, they can contribute viral genes to other pig viruses. This is the reason for continual surveillance of SIV viruses. These were wholly avian viruses that were of North American lineage. It was the first report of an interspecies transmission of an avian H4 virus to domestic pigs under natural conditions.

The disease usually affects young pigs, but all ages may be affected. Typically, sudden-onset epidemics occur with a high morbidity rate but with a low case-fatality rate of less than 5%. Loss of body condition is marked, which is usually the important cause of financial loss, although occasionally death losses may be extensive if the pigs are kept under inadequate conditions or if secondary bacterial infections occur. Abortions and deaths of newborn pigs have also been reported as causes of loss in this disease.

A low level of infection was reported in Poland in 2007 in pigs, wild boar, and animal keepers.[96]

The 2009 pandemic first affected pigs in Canada[97] and has since been found worldwide: Norway,[98] Italy,[99] Canada,[100,104] Argentina,[101] South Korea,[102] Thailand,[103] and Europe.[27]

Risk Factors

Animal Risk Factors

In a study in the Netherlands, it was shown that at the end of the finishing period, the seroprevalences in farrow to finish herds and specialized finishing herds were 44.3% and 62.0% for H1N1, 6.6% and 19.3% for H3N2, and 57.2% and 25.6% for H1N2. The incidence for all three types was highest at the beginning of finishing in farrow to finish and at the end in finishing herds.[105]

Risk factors include high pig density, large herd size, high replacement rates, and purchase of pigs.[90,91,106,107]

Young, growing pigs are most susceptible. The viral infection is commonly complicated by bacterial infection caused by *Haemophilus parasuis, A. pleuropneumoniae,* and possibly other opportunists of the upper respiratory tract of the pig. When an epidemic occurs, most of the pigs in the herd are affected within a few days, which suggests that all animals are previously infected and that some risk factor, such as inclement weather, precipitates the epidemic.

The agent also contributes to the PRDCx. In a study in Korea, 14 of 105 cases had SIV, whereas in Iowa it has been reported in 19% of the cases of PRDC.

Environmental Risk Factors

Epidemics occur mainly during the cold months of the year, commencing in the late autumn or early winter and terminating with a few outbreaks in early spring. Several days of inclement weather often precede an outbreak. Three risk factors for SIV were identified on a survey of Belgian finishing farms, where H1N1 was found in 71% and H3N2 was found in 22%. There was a close association between H1N1 and H3N2. H1N1 appeared to be associated with fully slatted floors, increasing numbers of pigs in the locality, and dry feeding. H3N2 was associated with the purchase of pigs from more than two herds, increasing numbers of pigs locally, and natural ventilation.

Pathogen Factors

It has been shown that prior infection with swine influenza viruses in pigs is a barrier to subsequent infection with avian influenza viruses.[94]

Molecular microbiology has now revealed the antigenic diversity of the virus. Several different H and N antigens have been identified and grouped on the basis of serologic tests, which refine the diagnosis and reveal more about the epidemiologic relationships. The H3N2 strain similar to H3N2 strains found in the human population has been isolated from an outbreak in England.

Two antigenically distinct H1N1 influenza A viruses were isolated during an outbreak of respiratory disease in swine in Canada in 1990 to 1991. One is a variant of the swine H1N1 influenza virus that is widespread in the American Midwest, whereas the other is similar to the virus isolated from swine in 1930. This suggests that influenza viruses can be maintained for long periods in swine herds, especially in certain geographic areas. It is proposed that the antigenic diversity of these viruses may be attributable to the result of drifts in the population of circulating swine influenza viruses in an area.[7] The antigenic diversity oligonucleotide analysis of strains isolated from outbreaks in Sweden indicated a similarity with the Danish strain. One of the Swedish strains was closely related to the U.S. strain.

The H1N1 strain of the virus can be found in pig tissues at slaughter but it does not persist for more than 2 to 3 weeks in deep frozen or refrigerated storage.

Virus circulation in weaned pigs may maintain infections in herds,[108] and the introduction of susceptible pigs at regular intervals will maintain this circulation.

Methods of Transmission

Of most importance is that in birds, influenza viruses mainly affect the intestinal tract (without clinical effects), but in mammals, replication occurs mainly in the respiratory tract (with illness).

The right combination of NA and M genes is necessary for the replication and transmissibility of influenza virus infections in pigs.[109]

The natural reservoir of influenza A virus is aquatic birds. Various subtypes have been established in other species, such as influenza A H1N1 viruses, which infect human and different animal species. The influenza viruses may be transmissible between humans and pigs. Swine are the sole animals known to be susceptible to influenza A viruses of human, swine, and avian origin. Swine may become infected with related type A human influenza strains during epidemics of human influenza, but they show no clinical signs of infection. The human strains have been isolated from pigs in Hong Kong, and pigs may serve as a reservoir for pandemics in humans and a source of genetic information for recombination between human and porcine strains. In Japan, pigs may be seropositive to the H1N1 human viruses relative to human H1N1 influenza epidemics and seropositive to human H3N2 viruses unassociated with human epidemics of disease. In Czechoslovakia, influenza A viruses are brought into pig herds by carrier people.

Pigs can be naturally infected with a range of avian influenza viruses. There have been at least three independent introductions of distinct wholly avian viruses into pigs. The virus in the late 1970s spread throughout Europe and the United Kingdom and became a major cause of SI. These viruses also undergo drift.

Elsewhere in the world antibodies against H4, H5, and H9 viruses have been isolated from Asian pigs and avian H4N6, H3N3, and

H1N1 viruses have been recovered from pigs in Canada.

Aerosol transmission is more efficient at low temperatures and low humidity because the virus is more stable under these conditions.[110] Aerosol transmission of a novel swine origin H1n1 virus was shown in China.[111]

In water the avian viruses survive better at low temperature and salinity and high pH.[112,113]

The avian virus survives better on nonporous surfaces rather than porous ones[114] and, if there is mucus, much longer.[115]

Swine are susceptible to both human and avian viruses because they have receptors on their respiratory epithelial cells for both avian (receptor SA 2, 3 Gal) and human (receptor SA α 2, 6 Gal). Several reassortants have been isolated from pigs in the United States and other parts of the world.

Thus swine have an important role in the ecology of influenza A viruses and are regarded as a "mixing vessel" for the introduction of reassorted viruses into the human population.

There is a report claiming that outbreaks of influenza in turkeys followed outbreaks of swine influenza in pigs from nearby swine herds. Swine and other influenza viruses have also been isolated from cattle, and experimental inoculation of calves has been successful. The swine influenza virus may cause natural infection in cattle and the virus can be transferred to uninoculated calves.

The primary route of infection is through pig-to-pig contact[116,117] via the nasopharyngeal route. Peak shedding occurs 2 to 5 days postinfection ($>10^7$ infectious particles/mL at a peak) but also by aerosols and contaminated fomites.[118]

The rapid spread of infection from pig to pig occurs by inhalation of infective droplets. The disease may appear almost simultaneously in several herds within an area following the first cold period in late autumn. The virus can persist in infected swine, which can act as convalescent carriers and be the reservoir of the virus between epidemics. However, the experimental inoculation of a swine influenza virus into specific-pathogen-free (SPF) pigs resulted in a mild disease and the period of viral shedding was shorter than 4 weeks.

Water contaminated with bird droppings has been implicated as a source of influenza virus in several swine outbreaks.[68] Fomites and aerosols[92] are probably important in the transmission of influenza.[119-122]

Insects may be important (certainly in avian influenza[123]) and blowflies have been implicated.[124,125]

Long-distance pig travel via transport may help spread.[126]

International trade may also facilitate the intercontinental spread of viruses.[127]

Immunity

An infection with live virus also stimulates mucosal immunity and cellular immunity, whereas inactivated vaccines only stimulate a limited serologic (HI) response. Preexisting immunity in European pigs to established SIV strains may partially protect against (H1N1) 2009 virus, but the extent of such protection needs to be assessed.[128]

Many of the host defense cells have sensors that ultimately up-regulate the production of interferons, up-regulate other cells, and activate them through cytokines and in general increase the production of host antiviral proteins. The flu virus survives in part by blocking the release of interferons.

Both cell-mediated immunity and humoral responses are important. A high HI titer provides better protection against challenge than a low HI titer. The levels of IgA seem to be more important in providing some protection against heterologous viral strains. It is the antibody-mediated immune reactions at the mucosal level, not the systemic level, that are important in protecting the respiratory tract. Improved adjuvants may aid the efficacy of inactivated vaccines. They do not prevent infection, but they can mediate the killing of infected cells. The immune response is rapid and completes elimination of the virus within 1 week. Antibodies decline by 8 to 10 weeks. The IgA levels in nasal washes are the most important defense. There is limited cross-protection between different viruses, and protection after vaccination is more virus specific.

Maternal antibody rarely prevents infection with influenza viruses and only provides partial protection. Maternal protection will last from 4 to 14 weeks, with no pigs being completely protected from nasal virus shedding upon challenge, but at least the lung is protected. Pigs with a high maternal antibody level did not develop an immune response. It was reported that there was enhancement of pneumonia by inactivated vaccine used in the face of an H1N1 challenge.[129] Maternal antibody does not cross protect between subtypes.

Pigs infected or vaccinated with European SIVs frequently have cross-reactive antibodies to pandemic (H1N1) 2009 virus and related North American SIVs.

Prior infection with an H1N1 SIV partially protects pigs against a low-pathogenic H5N1 avian influenza virus.[130]

ZOONOTIC IMPLICATIONS

Only influenza A viruses are zoonotic. The suspected cases were reviewed.[131] It is highly likely that in the future, further viruses will emerge from animal species to infect humans and vice versa. People who work with pigs are at an increased risk of zoonotic influenza virus infection[132] (including farmers, meat processing workers, and veterinarians)[133,134] and should be vaccinated.[135] The Ohio outbreak of H1N1 at a state fair is an example.

H2 viruses have been absent from the human population since 1968 and as such will present a huge problem if they suddenly turn up as a zoonosis. However, an H2N3 infection in pigs was not transmitted to humans from ill pigs.[136]

In the United States, there were only 11 reported zoonotic cases between 2005 and 2009.[137]

The human pandemic 2009 H1N1 virus has its closest relatives in strains of H1N1 in swine from North America and occasionally from turkeys. There are probably at least two swine ancestors for this 2009 pandemic.

Subclinical infections at Ohio fairs from 2009 to 2011 were described.[138] The influenza A virus (OH07) isolated from humans that attended an Ohio state fair is pathogenic in pigs and fails to cross-react with many swine H1 antisera. The virus gene segments were similar to those circulating in swine viruses, although there were numerous nucleotide changes leading to differences in amino acid composition.[139]

Swine influenzas pose a significant health risk to humans ever since the first human and porcine outbreaks in the United States in 1918. By 1970, there was evidence that people who came into contact with pigs through their jobs became infected with the viruses, and a virus was isolated from pigs and workers. There is very little evidence of maintenance of human H1N1 in the pig populations, but human H3N2 strains have been recovered regularly from pigs in Asia and Europe. The drift that has taken place in pigs of former human H3N2 has also been minimal compared with the rate of drift in the human population. The viruses from pigs found in humans have been reviewed. Poultry and swine workers should be vaccinated in swine pandemic planning.[135]

PATHOGENESIS

Classical swine influenza was originally described as a disease of the upper respiratory tract, the trachea and bronchi being particularly affected, with secondary bacterial pneumonia as a result of *Pasteurella multocida.* However, recent descriptions of the lesions in naturally occurring cases and in the experimental disease indicate that the primary lesion is a viral interstitial pneumonia. Viral replication takes place in the epithelial cells of the nasal mucosa, tonsils, trachea, lungs, and tracheo-bronchial lymph nodes. No other sites have been detected, and viremia is of low titer. Inoculation of the H1N1 strain of influenza virus isolated in England from pigs with clinical disease into 6-week-old pigs caused fever, coughing, sneezing, and anorexia. A widespread interstitial pneumonia, with lesions in the bronchi and bronchioles, and hemorrhagic lymph nodes were characteristic. The H3N2 swine influenza virus isolated in Canada is associated with a proliferative and necrotizing pneumonia (PNP) of pigs, and there is evidence the strain may be related to A/Sw/Hong Kong/76H3N2 swine influenza virus.

There is recent evidence that this PNP is more a feature of PRRS and PCV2 than SIV. A new antigenic variant of H1N1 swine influenza A virus isolated in Quebec has been associated with proliferative and necrotizing pneumonia of pigs.

In the United Kingdom, there has also been recorded an H1N7 that included both equine and human influenza genes. It was of low pathogenicity for pigs, found on only one farm, and did not establish in the pig world. Reassortant H3N1 viruses from human and classical swine H1N1 have also been seen in the United Kingdom and also in Taiwan.

The virus causes an acute infection with shedding beginning on day 1 and finishing by day 7. Infected cells in the respiratory tract are reduced by 2 to 3 days postinoculation. Most of the effects of the infection are caused by the production of proinflammatory cytokines (IFN-α, TNF-α, IL-1, and IL-6.).

Pigs have receptors for both avian (sialic acid–alpha-2,3 terminal saccharides (SA-alpha-2,3) and mammalian viruses (SA-alpha-2,6) in the upper respiratory tract. Both types have been detected in major porcine organs.[140,141] In experimental infections, SIV was widely distributed in bronchi, but it was also present in epithelial cells of the nose, trachea, bronchioles, and alveolar type I and II cells in severely affected animals. The avian virus was found in the lower respiratory tract, especially in alveolar type II cells and occasionally in bronchiolar epithelial cells. Receptor 2,6 was the predominant receptor in all levels of the tract, but the 2,3 was found only in small numbers in the bronchioles and in the alveoli. The receptor expression of both types of receptors was reduced in influenza-affected areas compared with nonaffected areas.[142] The distribution of receptors is similar in the pig to that of humans, and as in humans, avian viruses prefer to infect the alveolar cells. The in vitro attachment of virus to the upper and lower respiratory tract tracts of pigs has been characterized.[143]

The pathogenicity of SIV lies in its ability to elude host antiviral immune responses. In pigs SIV infection induced long-lived increase of CD8+ T cells and local lymphoproliferative responses.[144] The activation of cell-mediated immunity or cytotoxic T-lymphocytes depends on the efficient delivery of signals by antigen presenting cells. Dendritic cells are the most potent APCs. A study on porcine dendritic cells (DCs) has recently been published.[145] In one study,[146] it was shown that DCs could infect susceptible cells by close contact. The swine, human, or avian viruses differentially activate porcine dendritic cell cytokine profiles.[147]

There is an important role for IFN-α (induces fever and a transient rise in neutrophil counts) with IL-6 and IL-12 induction and an important role of these three cytokines in the symptoms of swine influenza.[148] There is a strong up-regulation of additional cytokines (IFN-α and IL-12) and several acute-phase proteins during the acute stages of a swine influenza virus infection. These produce inflammation, fever, malaise, and loss of appetite. The depth of infection in the lung probably determines how much of these cytokines are produced. Contrary to widespread belief, there is no evidence that the virus causes reproductive failure in swine. The experimental inoculation of seronegative pregnant gilts did not reveal any evidence of transplacental transmission of the virus to the fetus.

The pandemic H1N1 influenza virus causes disease in pigs and up-regulates genes related to inflammatory and immune responses. The virus is effectively shed from the nasal passages. Pigs infected with the pandemic virus mounted an early potent immune response, and it has been shown that such a response is associated with an increased viral pathogenesis. It also produced a higher proinflammatory cytokine response when given to macaques.[149] The PB1-F2, which is expressed from a +1 reading frame of the viral RNA polymerase subunit PB1, is able to induce apoptosis and promote inflammation.[150] Dysregulation of lipid metabolism also occurs at the site of primary infection.[151]

The pandemic 2009(H1N1) virus was shown to be more pathogenic in ferrets than the standard seasonal H1N1 virus with more extensive viral replication taking place in trachea, bronchi, and bronchioles and the more normal nasal cavity.[152] The virus replicates to higher titers in the lung tissues. It showed less efficient respiratory droplet transmission in ferrets.[153]

In patients with pandemic A (H1N1) pdm09, it was found that the numbers of dendritic cells and T cells were significantly reduced compared with controls. On the other hand, the frequency of natural killer cells and T-regulatory cells increased. The concentrations of plasma interferon (IFN-α/γ) and interleukin (IL-15) were significantly higher than in the control group.[154]

CLINICAL FINDINGS

The patterns of disease in farms may vary considerably from an endemic form, with waves of infection to single epidemic outbreaks depending on the strains of virus involved.[155]

It is essentially a herd disease. The signs have not changed over the 80 years. After an incubation period of 1 to 7 days (usually 1-3), the disease appears suddenly, with a high proportion of the herd showing fever (up to 41.5° C [107° F]), anorexia, and severe prostration. The animal is disinclined to move or rise because of muscle stiffness and pain. Labored, jerky breathing ("thumps") is accompanied by sneezing and a deep, painful cough that often occurs in paroxysms. There is congestion of the conjunctivae with a watery ocular and nasal discharge. Sometimes there is open-mouth breathing and dyspnea, especially if the pigs are forced to move. Morbidity is usually 100%, but mortality is rarely above 1%. In general, the severity of the illness appears greater than it truly is, and after a course of 4 to 6 days, signs disappear rapidly, depending, in part, on the level of colostral antibody. However, there is much loss of weight, which is slowly regained. Clonic convulsions are common in the terminal stages in fatal cases. The condition may continue to affect the herd for several weeks as the disease spreads, especially so if the herd is outdoors and the population dispersed. The new H3N2 reassortants in the United States have been associated with respiratory disease but also spontaneous abortion in sows and death of adult pigs. The clinical signs are dependent on immune status but are also influenced by age, infection pressure, concurrent infections, climatic conditions, housing, and, most of all, by the secondary infections. particularly bacteria.

The clinical and epidemiologic characteristics of pdmH1N1 2009 virus in pigs have been described.[156] There are differences in disease presentation, spread, and duration of infection. These factors include whether they were outdoors or housed, age of the pigs, intercurrent disease, and management. In breeding pigs the infection was mild or inapparent, with a more typical clinical appearance detected in their progeny. Mortality was low unless complicated by other diseases, especially *S. suis* infections. The virus transmitted very easily. The clinical signs were usually sneezing and coughing.

CONCURRENT INFECTIONS

There is some question as to whether other viruses can predispose to SIV, but experimentally infection with PRCV and H1N1 or H3N2 SIV has not shown this. Pigs with both *M. hyopneumoniae* and SIV coughed more and had more pneumonia than either of the two agents on their own.

Preinfection with *M. hyopneumoniae* modifies the outcome of infection with SIV H1N1 but not H1N2. The H1N2 was more pathogenic than the H1N with an earlier shedding and greater spread in the lungs. The *M. hyo* and H1N1 seemed to act synergistically, but the *M. Hyo* and H1N2 seemed to compete because H1N2 appeared to eliminate *M. hyo* in the caudal lobes.[157]

The occurrence of SIV in pigs presents opportunities for an increased impact of bacterial infections such as *H. parasuis* (HPS). It has been shown that coinfection between H3N2 and both a virulent and nonvirulent strain of HPS and porcine bone marrow dendritic cells was heightened because it raised the levels of Il-1β, TNF-α, IL-6, IL-12, and IL-10 compared with SIV or mock infections.[158] With the virulent strain of HPS, Il-12 and IFN-α increased differentially.

CLINICAL PATHOLOGY

Experimental Infections

Following experimental H1N1 infection, it was found that IFN-α, IL-6, IL-1, and TNF-α peaked in bronchoalveolar lavage fluid (BALF) at 24 to 30 hours postinfection, when virus titers and the severity of the clinical signs were maximal.[159] Serum cytokine concentrations were not detectable or 100-fold lower than the BALF readings, but IFN-γ and IL-12 in serum followed the lavage pattern. The acute-phase protein(APP), C-reactive protein, and haptoglobin were raised 24 hours after the cytokine response, and the lipopolysaccharide binding protein only increased in the BALF. The findings suggested that IFN-α and IL-12 play an important part in the pathogenesis of SIV and that APPs are induced by cytokines.[164] Acute-phase proteins and serum amyloid were raised when pigs were simultaneously infected with H1N1 virus and *P. multocida*.[160]

Experimental infections with the human 1918 pandemic influenza virus produced only a mild disease and pigs, and they did not become moribund, whereas in other mammalian species the effects were lethal.[161] The findings suggested that the virus entered the swine population from humans and then established the classical H1N1 lineage in pigs.

Experimental infection with H1N1 European swine influenza virus protects pigs from infection with the 2009 pandemic H1N1 human virus.[162] Experimental infections with the U.S. isolates of the p(H1N1) 2009 were described,[163] and all the pigs developed clinical signs similar to those induced by endemic SIV viruses.

Within 24 hours of the onset of clinical signs there is a switch of cells in the bronchial lavage from macrophages to over 50% neutrophils.

Serologic Tests

After infection has ceased to circulate in the herd, SIV AB could still be demonstrated after 28 months postinfection.

It is extremely important to make sure that the antigens that are used in the serologic tests are contemporary to the viral strains that may be found in the country. Diagnosis of acute SIV infections requires the use of paired serum samples.

The hemagglutination inhibition test has been the recommended test for many years and still remains so. However, it is tedious and has only moderate sensitivity but high specificity. It has been adapted and modified. One HI test for H1N1 will detect other H1N1 strains, but this is not true for H3N2 when the Midwest strains are compared with the North Carolina strains because they differ considerably. Above 1:80 is usually considered positive, and within 5 to 7 days the titers may reach 1:320 to 1:640 by 2 to 3 weeks postinfection. An ELISA-based test is now available to estimate the hemagglutination titer and can be used at the herd screening level.[164] Antiinfluenza A nucleoprotein antibodies have been detected in pigs using a commercial ELISA developed for avian species.[165]

Detection of Virus

Virus is likely to be found in the nasopharyngeal area during the acute phase of the disease. Swabs should be taken on Dacron, placed in transport medium, and stored at 4° C for no more than 48 hours; if storage will be longer, samples should be frozen at −70° C (−94 F). Viruses can also be isolated from trachea or lung tissues of pigs. They can be grown in hens' eggs or increasingly in tissue culture. Samples need to be cool and moist. The virus is then detected by hemagglutinating activity in egg fluids about 5 days after inoculation. There are some strains that may not grow in hens' eggs or require more than one cell line to isolate and identify the virus, which may require 1 to 2 weeks.

Oral Fluids

Pen-based oral fluids provide an easy, effective, and safe collection method for the detection of SI with rapid testing methods, such as RT-PCR.[166] Virus isolation from nasal swabs was more successful than using oral fluids.[167]

The sensitivity of oral fluids for detecting influenza A virus in populations of vaccinated and nonvaccinated pigs has been described. The overall sensitivity of oral fluids was 80%, and virus was isolated from 51% of RRT-PCR positive oral fluids. The method can detect SIV even when pen prevalence is low and when pigs have been vaccinated.[168]

Antigen Detection

A PCR test can be used to detect virus in nasal swab specimens and gives results similar to virus isolation. Recently a gel-based multiplex RT-PCR assay was developed to detect H1 and H3 subtypes of SIV. An RT-n-PCR for the identification of SIV in clinical samples has been described.[71] A real-time RT-PCR assay for differentiating the pandemic H1N1 2009 pandemic from SIVs has also been described.[170] A real-time RT-PCR has been developed for the detection of p(H1N1)2009 and European SIV A infections.[171] A real-time RT-PCR for pandemic influenza A virus (H1N1) 2009 matrix gene has been described.[172]

A multiplex RT-PCR assay for differentiating European SIV subtypes H1N1, H1N2, and H3N2 has been described[169,173] and used in North American pigs.[174]

Loop-mediated isothermal amplification has been used for the rapid and specific detection of H3 SIV.[175]

There are rapid detection methods for the 2009p(H1N1) using multiplex rtRT-PCR.[176,177,222]

The virus can be detected by direct immunofluorescence of lung tissue or lavage fluids.

Immunohistochemistry on fixed tissue is also useful. The positivity is mainly in the bronchial and bronchiolar epithelial cells and less intense in the interstitial cells and alveolar macrophages.

NECROPSY FINDINGS

Swelling and marked edema of cervical and mediastinal lymph nodes are evident. There is congestion of the mucosae of the pharynx, larynx, trachea, and bronchi. A tenacious, colorless, frothy exudate is present in the air passages. Copious exudate in the bronchi is accompanied by collapse of the ventral parts of the lungs. This atelectasis is extensive and often irregularly distributed, although the apical and cardiac lobes are most affected, and the right lung more so than the left. It may reach 50% by 4 to 5 days postinfection. The affected tissue is clearly demarcated, dark red to purple, and often reminiscent of enzootic pneumonia. Surrounding the atelectatic areas the lung is often emphysematous and may show many petechial hemorrhages.

Histologically, in acute swine influenza the major feature is necrotizing bronchiolitis. There is a suppurative bronchiolitis and widespread interstitial pneumonia characterized by the early appearance of neutrophils followed by the accumulation of macrophages and mononuclear cells in the alveolar walls. After a few days there is a peribronchial and peribronchiolar infiltration of lymphocytes. In the variant of H1N1 swine influenza in Canada, there is more diffuse damage to the respiratory epithelium, resulting in firm to meaty lungs that appear thymus-like on cut surface.

Microscopically, there is marked proliferation of type II pneumocytes, in addition to the presence of macrophages and necrotic inflammatory cells in the alveoli. The influenza type A virus can be demonstrated by indirect immunofluorescence staining using monoclonal antibody directed to certain protein parts of the human type A influenza virus. The influenza type A virus can be detected and differentiated from the virus of porcine reproductive and respiratory syndrome in formalin-fixed, paraffin-embedded lung tissue using immunogold staining.

Samples for Confirmation of Diagnosis

These are best collected from animals with high fevers and clear nasal discharge. Most pigs may excrete virus for 5 to 7 days postinfection, but the peak load may be around 24 hours postinfection

- **Histology**—formalin-fixed lung, trachea, turbinate (LM, IHC). After 72 hours there is little IFA or IHC positivity. Histopathology may help in the diagnosis for 2 weeks postinfection.
- **Virology**—nasopharyngeal swab in viral transport media; lung and

trachea (ISO, FAT, PCR) fresh chilled but not frozen. Keep cool. Do not use cotton.

DIFFERENTIAL DIAGNOSIS

The explosive appearance of an upper respiratory syndrome, including conjunctivitis, sneezing, and coughing, with a low mortality rate, serves to differentiate swine influenza from the other common respiratory diseases of swine.

Enzootic pneumonia of pigs is most commonly confused with swine influenza, but it is more insidious in its onset and chronic in its course.

Hog cholera is manifested by less respiratory involvement and a high mortality rate.

Inclusion-body rhinitis in piglets may resemble swine influenza quite closely.

Atrophic rhinitis has a much longer course and is accompanied by characteristic distortion of the facial bones.

TREATMENT

No specific treatment is available. Treatment with penicillin, sulfadimidine, or, preferably, a broad-spectrum antibiotic may be of value in controlling possible secondary invaders. The provision of comfortable, well-bedded quarters, free from dust, is of major importance. Clean drinking water should be available, but feed should be limited during the first few days of convalescence. Medication of the feed or water supplies with a broad-spectrum antibiotic for several days is a rational approach to minimizing secondary bacterial pneumonia.

A novel monoclonal antibody was shown to be effective against lethal challenge with swine lineage and 2009 pandemic H1N1 influenza viruses.[178]

CONTROL

Treatment of human influenza is possible with oseltamivir, but some viruses have become resistant; however, there is no evidence that natural oseltamivir resistance in swine and wild waterbirds is common.[179]

There are only two options: vaccination and biosecurity. Biosecurity is difficult because there is always the possibility of aerosol infections and wild fowl/poultry infections It should be aimed at preventing transmission from people to pigs and vice versa.

Eradication following herd closure and partial depopulation has been achieved.[180] There was no introduction of replacement animals, replacement gilt deliveries were seronegative and went to quarterly instead of monthly, and the nursery was totally depopulated along with the finishing sites once shedding had finished.[181]

The perceptions of the pig producers in Australia in response to the occurrence of the pandemic[182] suggests that ongoing communications about biosecurity are very important when new outbreaks occur.

Vaccination against swine influenza in a herd experiencing an outbreak of PCVAD is of questionable value.[183]

A study of vaccination in pigs infected with PRRS at the time of vaccination against SIV showed increased levels of macroscopic and microscopic lesions and also increased clinical disease and shedding of the virus.[184]

All-in, all-out systems may remove infection with each group of pigs, and the subsequent disinfection may wipe out the virus. Good housing and protection from inclement weather help to prevent the occurrence of severe outbreaks. Once the disease has appeared on a unit, there is little that can be done to prevent spread to other pigs. Recovered animals are immune to subsequent infection for up to 3 months.

The air filtration systems proposed for PRRSV and *M. hyopneumoniae*[185] may also be able to control SIV.

VACCINATION

Whole inactivated virus may not be the best adjuvant for the induction of cross-reactive cellular and mucosal immunity against antigenic variants.

Live attenuated vaccines could prime pigs for better cross-reactivity. One method of achieving this is to use truncation of the NS1 gene[200] that encodes an immune-modulating interferon antagonist. It replicates poorly but elicited neutralizing serum antibodies and mucosal antibodies and provided robust protection against homologous challenge given a single intranasal (IN) application. These vaccines provide in a single IN dose a better protection than an inactivated vaccine given intramuscularly (IM). A concern with inactivated adjuvanted vaccines is the phenomenon of vaccine-associated enhanced respiratory disease.[186,187]

Another obstacle is the presence of maternally derived immunity. It can reduce clinical disease, but passive antibodies are less effective in blocking viral shedding from the upper respiratory tract because the main Ig in colostrum is IgG. Pigs with maternally derived antibodies have suppressed adaptive antibody responses to homologous infection or vaccination. This interference affects IgM and HI titers in serum or nasal mucosa. The cellular response is less susceptible to maternally derived antibodies. The perception is that live attenuated IN vaccines are less likely to be interfered with by MDA.[188]

Virus transmission is reduced in neonatal pigs with homologous maternal immunity compared with seronegative neonatal pigs and pigs with heterologous maternal immunity.[189] Vaccine development has been described.[190]

The genetic homology of the vaccine and the challenge virus is not the ultimate predictor for swine influenza vaccine performance.[191]

Vaccination with currently approved commercial vaccines in the United States did not fully prevent transmission, but certain vaccines may provide a benefit by limiting shedding, transmission, and zoonotic spillover at agricultural fairs.[192]

Vaccination decreases lesions and clinical signs and may eliminate virus shedding.[193] Vaccines may well reduce transmission but do not eliminate infection.[194]

In the United States, a large number of producers vaccinate sows (~67%), and many vaccinate weaner pigs (20%). A large proportion vaccinated breeding pigs with autogenous vaccines, not commercial vaccines, and these by law are prepared by inactivating virus cultures. The main reason is that commercial vaccines are not upgraded fast enough. Vaccines (1) need to be developed quickly to keep pace with the virus changes, (2) need to have better cross-protection against new isolates, and (3) need to be able to overcome maternal antibody, which may negate vaccine use.

Vaccines may use only one or two circulating strains of H3N2 in the vaccine, but the wide variation in H3N2 present in the swine population may mean that only a small percentage of currently circulating strains may be protected against by the current vaccine[194] and that regular challenge studies may be necessary to determine the effectiveness of vaccines.

Vaccination with influenza A virus decreased transmission rates in pigs,[195] but it was not completely prevented when a heterologous vaccine was used.

INACTIVATED VACCINES

Inactivated whole-virus vaccines have limited ability or complete failure to protect against homologous challenge and even poorer cross-protection to heterologous strains.[196] They can stimulate both humoral and cellular immunity.[197]

A trivalent inactivated swine flu vaccine was shown to be protective for all three strains (H1N1, H1N2, and H3N2).[198]

Inactivated vaccines from U.S. viruses and the new pandemic showed partial protection, but none was able to prevent all shedding or clinical disease.[199]

MODIFIED LIVE

Modified live vaccines or vectored subunit vaccines induce a balanced immune response (humoral and cell -mediated) and will improve homologous and heterologous protection. All vaccinated pigs developed a significant level of HI titer and serum IgG and IgA antibodies.[200,201]

A modified live vaccine as a master donor strain has been developed for the 2009 pandemic virus,[202] and a pandemic virus vaccine was developed that was superior to commercial vaccines.[203]

Adjuvanted and nonadjuvanted A(H1N1) pdm/09 influenza vaccines were shown to produce strong antibody responses and included high levels of specific IgG1 and HI titers to H1 virus. The adjuvanted vaccines produced a greater response.[204]

An eight-segment SIV with H1 and H3 was found to be attenuated and protective against both H1N1 and H3N2 subtypes in pigs.[205]

Vaccines, both commercial inactivated and adjuvanted SIV for IM use, are available in the United States and Europe. Active immunization occurs in the face of maternal derived antibody when titers are less than 10 for H1N1 and less than 40 for H3N2. Some of the vaccines contain the original H1N1 viruses, but others such as those used in the United States, contain a monovalent H1N1 virus. Following the outbreaks of H3N2 in the United States in 1998, both monovalent and bivalent H1N1/H3N2 SIV vaccines became available. Autogenous vaccines are used in the United States.

In Europe, although the viruses have changed, the old vaccines are still used because they produce high antibody titers. There is a need to add H1N2 to the vaccines, however, because there is no cross protection between the European H1N2 and H1N1 and H3N2 viruses and because it was shown that there is no current vaccine protection against H1N2. There is evidence from the United States showing that there is cross protection with the U.S. strain of H3N2 for H1N2 infections. Most animals with titers greater than160 are probably protected against viral replication in the lungs and disease. Sow vaccination is important in controlling infection in suckling pigs and often controls the infection in nursery pigs. Intranasal or IN/IM vaccination of pigs with formalin-inactivated SIV induces very specific IgM, IgG, and IgA antibodies in their nasal secretions and sera, resulting in complete protection.

A recent trial of a new H1N1/H3N2 vaccine was successful, with reduced viral shedding and reduced clinical signs and pneumonia.

Experimental vaccines continue to be produced, including a human adenovirus 5 recombinant expressing the hemagglutinin and nucleoprotein of H3N2 SIV that has been used experimentally to provide protection against challenge with H3N2. Complete protection was shown by lack of nasal shedding and by lack of lung lesions following subsequent challenge.

A DNA vaccine elicited robust serum antibody and cellular responses after three immunizations and conferred significant protection against influenza virus challenge.[206] Vaccination with human adenovirus vector vaccines has been shown to induce both cell-mediated and humoral immunity, making them more effective than inactivated vaccines and nearly as good as live vaccines. They can also prime the immune response in the presence of maternal antibody.[207]

Recently an avian-like H1N1 influenza virus was shown to be able to transmit efficiently through four pairs of vaccinated pigs at antibody levels that were thought to be protective.[208]

Immunity induced by infection with European avian-like H1N1 SIV affords protection for pigs against North American SIVs with a classical H1 and possibly also protects against the pH1N1.[209]

Pandemic (H1N1)2009 influenza virus-like particles are immunogenic.[210] The vaccinated pigs were protected and showed reduced lung lesions, reduced viral shedding, and inhibition of viral replication in the lungs.

NEWER OPTIONS

Elastase-dependent SIV mutants can be used as live-virus vaccines against swine influenza in pigs.[211,212]

Use of the M2 conserved matrix protein may have potential as a vaccine but requires an immune response to the HA protein to reduce shedding.[213]

Replicon particle vaccine protects swine against influenza.[214-216]

Vaccination with NS1-truncated H3N2 SIV primes T cells and confers cell-mediated cross-protection against a H1N1 heterosubtypic challenge in pig.[217] In addition, there was a significantly lower level of Th1-associated cytokines in infected lungs. A similar vaccine can be used to differentiate between infected and vaccinated animals.[218]

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PORCINE RESPIRATORY CORONAVIRUS

Infection with coronavirus causes a rapid seroconversion to some of the tests for TGE and is responsible for "vaccinating" large populations of pigs worldwide against the threat of TGE. This has coincided with the great reduction in TGE in most countries. It was first identified in Belgium in 1986 and since then has spread worldwide.

ETIOLGY

The virus is very similar to TGE, and the major difference is a 621- to 628-base-pair deletion in the S protein gene causing a truncated S protein and loss of the ability of the TGE to bind sialic acid. It has a tropism for the respiratory tract. It is one of the four swine coronaviruses and is a mutant of TGE, first isolated in 1984. The virus has been fully or partially sequenced and has 96% to 98% homogeneity with TGE.[1] Lipoteichoic acid from *S. aureus* exacerbates respiratory disease in PRCV-infected pigs,[2] and coinfection with *B. bronchiseptica* is reported.[3] PRRSV-induced immunosuppression exacerbates the inflammatory response to PRCV in pigs.[4] PRCV-infected pigs produce antibodies that neutralize TGE virus.

EPIDEMIOLOGY

The virus distribution is affected by the season and the density of pig farms, and in a dense area there is rapid local spread probably by aerosol. The virus infects pigs of all ages by contact or airborne transmission and in areas of high density can probably spread several kilometers. The virus circulates in the herd, infects pigs less than 10 to 15 weeks of age after the maternal antibodies have declined, and becomes endemic. Experimentally, infected pigs shed virus from the nose for less than 2 weeks. The infection can be maintained in herds, cycle regularly, or appear in waves. In Europe, these waves often coincide with the rainy season. There is no evidence of fecal/oral transmission.

PATHOGENESIS

The virus has a tremendous ability to replicate in the respiratory tract in most of the airway but rarely the alveolar macrophage.[5-8] The main targets are type 1 and type 2 alveolar epithelial cells, and it induces necrosis in these cells, causing a rise in cytokines that induces a rise in nitric oxide and IFN-α. The shedding from the nose lasts 4 to 6 days. The pneumonia produced and the viral replication peak at 7 to 10 days postinoculation and then resolve with the increasing levels of neutralizing antibody.

CLINICAL SIGNS

Most infections are inapparent, but in a susceptible population there may be respiratory signs such as labored breathing and coughing, followed by depression, anorexia, and decreased growth rates.

LESIONS

The lesions are usually self-limiting. The major lesions are broncho-interstitial pneumonia with cuffing and syncytial formation from type 2 hyperplasia, followed by necrosis and lymphoid hyperplasia. Necrotic cells and inflammatory cells may obstruct the lumen of the alveoli.

DIAGNOSIS

Virus isolation in PK and swine testicle cells is necessary using nasal fluid or lung homogenates, and frequently PRCV produces syncytia in culture.

Respiratory samples are required for diagnosis of PRCV. Currently, RT-PCR or qRT-PCR is needed to differentiate TGEV and PRCV. The primers target the S protein. Multiplex PCR has now been developed for TGEV, PRCV, and PEDV[9] and up to eight viruses. Multiplex microarray has also been developed for the rapid differentiation of eight coronaviruses.[10]

Blocking ELISAs have been developed to differentiate antibodies of PRCV from TGE and should be used on a herd basis. Recently, new ELISAs have been developed that will also differentiate TGE, PRCV, and the new TGE-like coronaviruses.[11,12]

TREATMENT

There is no treatment for PRCV infections except supportive therapy and control of secondary infections.[13]

CONTROL

Neonatal pigs require 6 to 8 days after PRCV exposure to produce partial immunity to TGE. Sows naturally exposed to PRCV reinfected with PRCV during pregnancy secreted TGEV antibodies in milk and provided a high degree of protection.

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LUNGWORM IN PIGS

ETIOLOGY

The lungworms that infest pigs are *Metastrongylus apri* (*M. elongatus*), *M. salmi,* and *M. pudendotectus. M. apri* is the most common species, but mixed infestations are not uncommon.

LIFE CYCLE

Adult *Metastrongylus* spp. appear much like *D. viviparus* in the bronchi of their host. Their life cycles are also similar, except that *Metastrongylus* spp. eggs are passed in the feces and earthworms act as intermediate hosts. Here development to infective larvae takes about 2 weeks, and transmission occurs when the earthworm is eaten by a pig.

SYNOPSIS

Etiology The nematode parasites *Metastrongylus apri (M. elongatus), M. salmi,* and *M. pudendotectus.*

Epidemiology Transmission is by ingestion of the earthworm intermediate host.

Signs Check in growth rate; barking cough.

Clinical pathology Characteristic eggs in feces.

Lesions Grayish nodules near the ventral border of the diaphragmatic lobes of the lung.

Diagnostic confirmation Characteristic eggs in feces.

Treatment Doramectin, ivermectin, fenbendazole, flubendazole, levamisole.

Control Difficult, unless pigs reared on concrete.

EPIDEMIOLOGY

The disease is most prevalent in pigs 4 to 6 months of age in husbandry systems that allow access to earthworms. The eggs first appear in the feces 3 to 4 weeks after infestation and at their peak reach levels of 25 to 50 eggs per gram of feces. The eggs are very resistant to cold temperatures and can survive for over 1 year in the soil. Larvae may survive in the earthworm for up to 7 years. The primary host must ingest an intermediate host to become infested, and this is an important factor influencing the spread of the disease. Once ingested the infective larvae migrate to the lungs in much the same manner as do *D. viviparus* larvae. Many infestations are asymptomatic and induce immunity against reinfection.

PATHOGENESIS

The pathogenesis is similar to that of *D. viviparus.* These worms may provide a route of transmission for swine influenza virus, and possibly hog cholera virus, from pig to pig, but this is unproven.

CLINICAL FINDINGS

Lungworm infection in pigs can cause a marked check in growth rate. The bronchitis is accompanied by sporadic bouts of a barking cough, which is easily stimulated by exercise. Pneumonia is a feature of severe cases. Fatal bronchopneumonia can occur in coinfections of porcine circovirus type 2 and *Metastrongylus* spp.[1]

CLINICAL PATHOLOGY

Laboratory diagnosis is by demonstration of the characteristic eggs in feces.

NECROPSY FINDINGS

Early lesions comprise small areas of consolidation as a result of verminous pneumonia. More chronic cases have bronchitis, emphysema, peribronchial lymphoid hyperplasia, and bronchiolar muscular hypertrophy, often accompanied by areas of overinflation. The lesions are small and discrete, appearing as grayish nodules up to 1 cm in diameter, and are present particularly at the ventral border of the diaphragmatic lobes.

DIAGNOSTIC CONFIRMATION

The *Metastrongylus* egg is embryonated (larvated) and has a thick shell and a wavy outline. They may be missed on routine screening as they are usually passed in small numbers and do not float well in saturated salt (NaCl) solution. A flotation fluid with a higher specific gravity should be used. There will always be a history of access to yards or paddocks where earthworms exist.

DIFFERENTIAL DIAGNOSIS

- Other swine pneumonias
- Migrating larvae in heavy *Ascaris* infestation

TREATMENT

TREATMENT

Abamectin (0.1 mg/kg, PO) (R1)

Ivermectin (0.3 mg/kg, SC) (R2)

Fenbendazole (9 mg/kg, PO qd for 3 days) (R2)

Flubendazole (4.0 mg/kg, PO) (R2)

Levamisole (8 mg/kg, PO)

A number of anthelmintics are effective at normal pig dose rates, including abamectin,

ivermectin,[2] doramectin, fenbendazole, and flubendazole. Levamisole (8 mg/kg) has been used in the water or feed.

CONTROL

Rearing pigs on concrete reduces the risk considerably but, in view of the longevity of the eggs and larvae in the earthworm, little can be done if pigs are kept on contaminated land. Pastures that are known to be contaminated should be left for at least 6 months before restocking, although infested earthworms may persist in hog lots for up to 4 years.

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Respiratory System Toxicoses

FURAN (IPOMEANOL AND 3-METHYLINDOLE) TOXICOSIS

4-Ipomeanol (4-IPO) is a furanoterpinoid mycotoxin produced by *Fusarium solani* (synonym *F. javanicum*) and *F. semitectum* growing on garden refuse. It has the effect of causing lesions indistinguishable from those of atypical interstitial pneumonia. Other known causes of these lesions are 3-methylindole and the ketone produced by *Perilla frutescens, Zieria arborescens,* and one of the fungi *Fusarium solani* or *Oxysporum* spp. on *Ipomoea batatas* (sweet potatoes) tubers and tryptophan-containing plants.[1] Catabolism by the fungus of phytoalexins induced in the tubers produces four closely related ipomeanols: ipomeanine (IPO), 4-ipomeanol (4-IPO), 1-ipomeanol (1-IPO), and 1,4-ipomeadiol (DIOL).[2] These are not toxic until activated by pulmonary microsomal enzymes; 4-IPO and IPO are ultimately the most toxic. Experimental administration of infected potatoes to calves is associated with bronchiolitis and interstitial pneumonia. Unweaned, nursing calves may not be affected.[3]

Animals are exposed to these toxins in a number of ways. Cows gain access to moldy sweet potatoes by grazing plowed potato fields or being fed spoiled sweet potatoes. The toxic dose is 7.5 mg IPO/kg BW, which converts to about 6 kg of spoiled sweet potatoes per adult cow.[1] The mortality rate is often high.[4] Perilla mint (purple mint or beefsteak plant) is widespread in the southeastern United States[4] and found in Asia and several other parts of the world.[1,5] All large animal species are susceptible, but poisoning is most widely reported in cattle. Cows are exposed by eating the leaves and seeds; toxicity is highest in the seed portion of the plant.[4]

In a similar fashion, tryptophan toxicosis occurs in cows grazing on lush pastures with elevated concentrations of tryptophan. Outbreaks often develop several days to a week after cows are moved from poor pastures or forage to early summer pastures with high tryptophan content in the grasses. Rumen microflora convert tryptophan to 3-methylindole, which is then activated by cytochrome p450 in the lung to a reactive compound.[4]

The clinical signs present in ipomeanol and 3-methylindole toxicosis are similar to acute respiratory distress syndrome and atypical interstitial pneumonia. The reactive compounds produced in the lung damage the pulmonary endothelial cells and result in acute pulmonary emphysema and edema.[1,4] Affected animals have labored breathing, frequently standing with an open mouth and extended neck. Frothy foam from the nostrils or a foam-covered tongue may be present. Treatment is aimed toward reducing edema, supporting respiration, and reducing physical stress. Animals living longer than 48 hours have a good prognosis for survival.[4]

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GALEGINE TOXICOSES

Galegine, an isoprenoid guanidine, is found in the following plants:

Galega officinalis: French honeysuckle[1]
Schoenus asperocarpus: poison sedge (Australia)
S. rigens (Australia)
Verbesina encelioides: crown beard (North America and Australia)[1]

Ingestion of galegine-containing plants is associated with a syndrome of severe dyspnea, frothing from the nose, convulsions, and sudden death in ruminants as a result of pulmonary edema with large fluid accumulations in the thoracic cavity, the result of a direct effect on pulmonary vascular permeability.[1] Sheep may find access via these plants being mixed in with hay or among a standing crop.

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MANURE GAS POISONING AND CONFINEMENT EFFECTS

ETIOLOGY

Confinement housing of cattle and swine is accompanied by manure storage for varying periods of time, often in large holding pits under slatted floors. Oxygen is excluded from the storage so that anaerobic bacteria degrade the organic and inorganic constituents of manure, yielding hydrogen sulfide, ammonia, methane, and carbon dioxide as major gases.[1,2] When diluted with water to facilitate handling, liquid manure in storage separates by gravity. The solid wastes form sediment, the lightweight particles float to the top, leaving a middle layer that is relatively fluid. Thorough remixing is necessary before pits are emptied to prevent the fluid fraction from flowing out and the solids remaining. The remixing or agitation results in the release of large quantities of toxic gases from the slurry.[2]

Besides the well-established gaseous toxicants listed, certain other agents with detrimental inhalation risks are present in confinement operations and have been best characterized for swine confinement operations. Total dust is a major contaminant in swine barns[3] and may range from 2 to 7 mg/m. Particulates may adsorb gases and be part of the objectionable odors released and reaching neighbors near confinement operations. Respirable dusts may be 10% or more of the total dusts generated in swine barns. Such dust is contaminated with bacteria, fungi, endotoxins, and glucans.[3] Dusts are primarily composed of feed or fecal material. Both endotoxins and glucans have been suggested as potential contributors to swine respiratory disease and respiratory complications for workers in swine buildings. So far, however, high mortality and acute death losses in confinement operations are most commonly caused by excessive concentrations of hydrogen sulfide and carbon dioxide, whereas subacute or chronic irritation and disease of the upper respiratory tract may also be contributed by elevated ammonia levels. Methane is explosive and may act as an asphyxiant, but is not implicated as a toxicant.

Additional factors that must be considered in a differential diagnosis include possible power loss during electrical storms or equipment failure; this results in the cessation of the artificial ventilation required to cool the building and exhaust carbon dioxide from the animals' respiration. In these situations, CO_2 levels build rapidly, and environmental temperatures increase dramatically as well, especially when weather conditions

are hot and humid.[1] Acute loses from hyperthermia or heat stroke may be mistaken for manure gas poisoning.[1] This is important for veterinarians because they may be called to establish a diagnosis that affects insurance claims for many thousands of dollars. Besides overheating and CO_2 accumulation, electrocution should be considered whenever there are large numbers of acute losses in a confinement building.

PATHOGENESIS

The exposure of humans, cattle, and swine to high concentrations (above 700 ppm of H_2S) of manure gases, particularly hydrogen sulfide, can be associated with peracute deaths in cattle and swine. Hydrogen sulfide is both an irritant and an acute toxicant. Fatal or severe exposure often is associated with respiratory distress and pulmonary edema. Exposure to low concentrations of hydrogen sulfide over long periods is thought to be associated with reduced performance in cattle and swine. At high concentrations, from 500 to 1000 ppm, carotid-body receptors are stimulated, causing rapid breathing. As high concentrations continue or increase, the respiratory center is depressed, and animals become depressed and die. High concentrations of H_2S depress olfactory sensors, and the offensive rotten-egg odor is no longer detected as a warning sign.

Ammonia is either an irritant or corrosive agent depending on the concentration. Ammonia combines with tissue moisture to produce ammonium hydroxide, a strong alkali capable of causing tissue necrosis.

CLINICAL FINDINGS

In acute hydrogen sulfide poisoning the animals die suddenly. Affected animals may be found dead throughout a building in various postures of lateral or sternal recumbency. There may be little or no evidence of struggle or excitement because high concentrations can be associated with nearly immediate respiratory paralysis. In acute ammonia poisoning the syndrome includes conjunctivitis, sneezing, and coughing for a few days, but pigs will soon acclimatize, after which no effects may be detectable. An increased incidence of pneumonia and reduced daily weight gains in pigs are associated with exposure to a combination of gaseous ammonia at levels of 50 to 100 ppm and the presence of atmospheric dust in barns. Higher concentrations of ammonia (100-200 ppm) are associated with irritation to the conjunctiva and respiratory mucosa. At very high ammonia concentrations (>500 ppm), there is pharyngeal and laryngeal irritation, laryngospasm, and coughing. Concentrations above 2000 ppm can be associated with death within 30 minutes. Carbon dioxide overexposure first is associated with mild to moderate excitement, followed by depression, weakness, coma, and death. Concentrations above 30% in air are serious, and 40% CO_2 for more than a few minutes can cause death.

NECROPSY FINDINGS

In cattle that have died from acute hydrogen sulfide poisoning, lesions include pulmonary edema, extensive hemorrhage in muscles and viscera, and bilaterally symmetric cerebral edema and necrosis. Ammonia exposure results in lacrimation, conjunctivitis, corneal opacity, tracheal hyperemia or hemorrhages, and pulmonary edema. Secondary bacterial pneumonia may be evident in exposed animals. For carbon dioxide, the principal lesions are of cyanosis.

CONTROL

Production of hydrogen sulfide in manure can be inhibited by aeration using air as the oxidizing agent or the use of chemical oxidizing agents. The use of ferrous salts virtually eliminates hydrogen sulfide evolution. Adequate ventilation with all doors and windows wide open during remixing and agitation of the slurry will reduce the concentration of hydrogen sulfide to nontoxic levels. Animals and personnel should not enter closed barns when the pits are being emptied. In confinement buildings, ammonia usually does not accumulate to fatal levels, but much of the economic loss is from reduced feed consumption and possibly increased susceptibility to acute or chronic respiratory disease. Limiting protein supplementation to actual needs has been considered a means for reducing nitrogen losses and the resultant production of ammonia in feces and urine.

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PLANTS CAUSING PULMONARY DISEASE (UNIDENTIFIED TOXINS)

The following plants have been associated with pulmonary disease. The toxins currently are unidentified.

Dyspnea and pulmonary edema:

- *Glechoma hederacea* (= *Nepeta hederacea*: ground ivy)
- *Gyrostemon* spp.: camel poison

Pulmonary consolidation and fibrosis, characterized by dyspnea and cough (horses):

- *Eupatorium* (= *Ageratina*) *adenophorum*: crofton weed
- *E. riparium:* mist flower
- *Lactuca scariola:* prickly lettuce

Neoplastic Diseases of the Respiratory Tract

Neoplasms arising as a result of viral infection (nasal adenocarcinoma of sheep, ovine pulmonary adenocarcinoma) and nonneoplastic tumors (equine ethmoidal hematoma) are dealt with under those headings in this chapter.

PULMONARY AND PLEURAL NEOPLASMS

Primary neoplasms of the lungs, including carcinomas and adenocarcinomas, are rare in animals and metastatic tumors also are relatively uncommon in large animals. Primary tumors reported in lungs or pleura of the farm animal species include the following:

Horses

- Granular cell tumors are the most common tumor arising in the pulmonary tissue of horses.
- Malignant melanomas in adult gray horses
- Pulmonary adenocarcinoma (either primary or as metastatic disease)
- Pulmonary leiomyosarcoma
- Bronchogenic carcinoma, pulmonary carcinoma, bronchogenic squamous-cell carcinoma, pulmonary chondrosarcoma, and bronchial myxoma are all rare tumors in lungs of horses.
- Mesothelioma arise from the visceral or parietal pleura.

Cattle

- Pulmonary adenocarcinoma is the most commonly reported primary lung tumor in cattle. The ultrastructure and origin of some of these have been characterized.
- Lymphomatosis in young cattle may be accompanied by pulmonary localization

Sheep

- Ovine pulmonary adenocarcinoma (jaagsiekte sheep retrovirus) is locally common in some areas.

Goats

- An asymptomatic, squamous-cell-type tumor, thought to be a benign papilloma, has been observed in 10 of a series of 1600 adult Angora goats. The lesions were mostly in the diaphragmatic lobes, were

multiple in 50% of the cases, and showed no evidence of malignancy, although some had necrotic centers.
- Bronchoalveolar carcinoma not related to ovine adenocarcinoma virus is reported.[1]

A wide variety of tumors metastasize to the lungs, and these tumors can originate in almost any tissue or organ. A series of thoracic neoplasms in 38 horses included lymphosarcoma, metastatic renal cell carcinoma, primary lung carcinomas, secondary cell carcinoma from the stomach, pleural mesothelioma, and malignant melanoma.

The etiology of the tumors is unknown in most cases, apart from those arising from viral infections. Equine granular cell tumors arise from the Schwann cells of the peripheral nervous system in the lungs.

Characteristically, primary pulmonary or pleural tumors arise in middle-aged to old animals. The prevalence of these tumors is not well documented, although they are rare in abattoir studies of horses. The tumors occur sporadically, with the exception of those associated with infectious agents (bovine lymphomatosis, ovine pulmonary adenocarcinoma).

The pathogenesis of pulmonary tumors includes impairment of gas exchange, either by displacement of normal lung with tumor tissue and surrounding atelectasis and necrosis or by obstruction of the large airways (e.g., granular cell tumor in horses).

CLINICAL FINDINGS

Clinical findings are those usually associated with the decrease in vital capacity of the lungs and include dyspnea that develops gradually, cough, and evidence of local consolidation on percussion and auscultation. There is no fever or toxemia, and a neoplasm can be mistaken for a chronic, encapsulated pulmonary abscess. Major clinical findings included weight loss, inappetence, and dyspnea and coughing. An anaplastic small-cell carcinoma of the lung of a 6-month-old calf located in the anterior thorax caused chronic bloat, anorexia, and loss of body weight. Some tumors, notably mesothelioma and adenocarcinoma, cause accumulation of pleural fluid. Hypertrophic pulmonary osteopathy occurs in some animals with pulmonary tumors.

Ovine pulmonary adenocarcinoma can metastasize to liver, kidneys, skeletal muscle, gastrointestinal tract, spleen, skin, and adrenal glands.[2]

Granular cell tumors in horses present as chronic coughing and exercise intolerance in horses without signs of infectious disease. As the disease progresses, there is increased respiratory rate and effort and weight loss, suggestive of severe heaves. However, horses are unresponsive to treatment for heaves. The disease can progress to cor pulmonale and right-sided heart failure. A bronchial mass is evident on endoscopic or radiographic examination (Figs. 12-34 and 12-35). There are no characteristic hematologic or serum biochemical changes.

Hemangiosarcomas of the thoracic cavities of horses occur and are evident as excess pleural fluid with a high red blood cell count.[3]

Thymoma, or **lymphosarcoma** as a part of the disease bovine viral leukosis, is not uncommon in cattle and can resemble pulmonary neoplasm, but there is usually displacement and compression of the heart, resulting in displacement of the apex beat and congestive heart failure. The presence of jugular engorgement, ventral edema, tachycardia, chronic tympany, and hydropericardium can cause a mistaken diagnosis of traumatic pericarditis. Mediastinal tumor or abscess (cranial thoracic masses) can have a similar effect. Metastasis to the bronchial lymph nodes can cause obstruction of the esophagus with dysphagia, and in cattle chronic ruminal tympany. This tumor is also common in goats, many of which show no clinical illness.

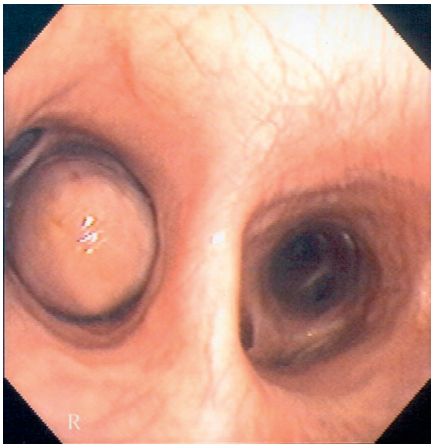

Fig. 12-34 Endoscopic view of a granular cell tumor in a horse.

Radiographic or ultrasonographic examination is useful in demonstrating the presence of a mass in the lungs or thorax. **Endoscopic examination** is useful for detection of tumors that invade the larger airways, such as granular cell tumors of horses. Thoracoscopy and pleural biopsy can be useful in the diagnosis of lesions at the pleural surfaces.

The nature of the tumor can sometimes be determined by examination of **pleural fluid**, into which some tumors shed cells, or of tumor tissue obtained by biopsy. Examination of pleural fluid for the presence of tumor cells is not very sensitive because many tumors do not shed sufficient numbers of cells to be detectable, but it is quite specific in that detection of abnormal cells is diagnostic.

TREATMENT

There is no effective treatment, with the exception of resection of localized tumors. Granular cell tumors in horses have been successfully treated by lung resection or transendoscopic electrosurgery.[4,5]

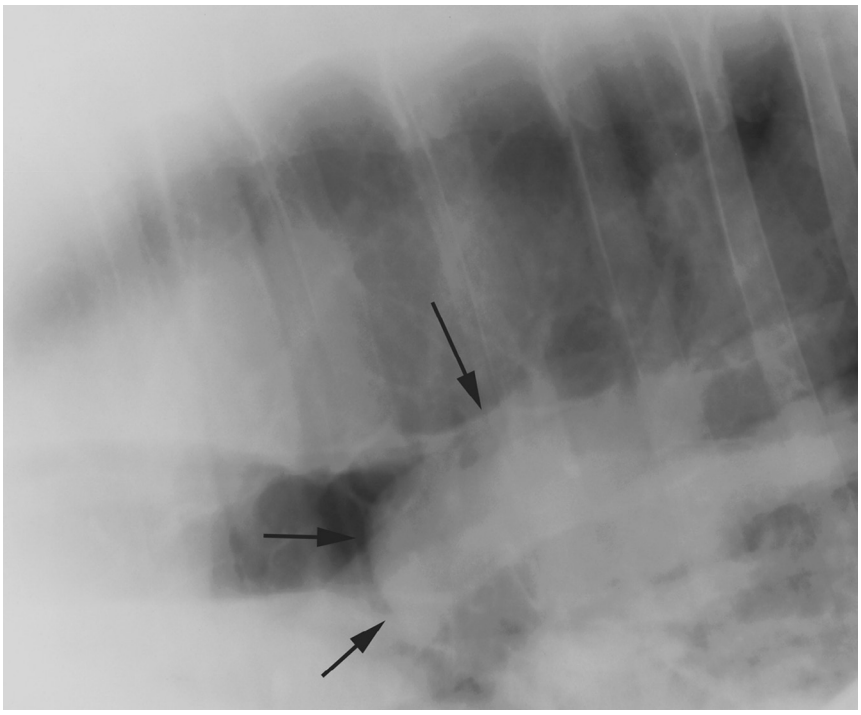

Fig. 12-35 Lateral thoracic radiograph of an adult horse demonstrating presence of a granular cell tumor (outline by black arrows).

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Congenital and Inherited Diseases of the Respiratory Tract

CONGENITAL DEFECTS

Primary congenital defects are rare in the respiratory tracts of animals. Congenital defects of the soft palate of foals have been sporadically reported; horses with minor defects can grow normally and may be able to have a successful athletic career for their intended use.[1] Hypoplasia of the epiglottis is detected occasionally in horses. Tracheal hypoplasia is recognized in calves and Miniature horses. Bronchogenic cysts are rare in foals[2] and calves[3] and result from the abnormal development of the tracheobronchial system during the embryonic period. Bronchogenic cysts can cause respiratory distress and dysphagia, particularly when located in the cervical region. Secondary defects, which are associated with major defects in other systems, are more common. Most of the defects are associated with defects of the oral cavity, face, and cranial vault, particularly cleft palate. Accessory lungs are recorded occasionally, and if their bronchi are vestigial, the lungs can present themselves as tumor-like masses occupying most of the chest. Pulmonary hypoplasia has been associated with congenital diaphragmatic hernia. Retrosternal hernia (Morgagni hernia), which is a right ventral diaphragmatic defect, has been surgically corrected in adult horses as a result of incarceration of the large colon; in all cases the defect was thought to be congenital.[4]

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