Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

18 Diseases Primarily Affecting the Reproductive System

Infectious Diseases Primarily Affecting the Reproductive System

This chapter presents information related to important and selected livestock pathogens that affect not only the fertility but also the health of animals, and in some cases, such as bovine brucellosis, the health of humans. It is worth noting that national control and eradication campaigns against *Brucella abortus* infection in cattle played, and continue to play, an important global role in expanding the veterinary profession. More recently, the worldwide spread of porcine reproductive and respiratory syndrome (PRRS) virus during the last 20 years has had a marked economic impact on the swine industry. As a consequence, PRRS is currently one of the most intensively researched diseases of livestock.

Readers seeking detailed information related to reproductive performance, the estrous cycle, conception, pregnancy, and parturition are directed to the many excellent textbooks that address these subjects.

INDUCTION OF PARTURITION

CALVES

The induction of parturition in pregnant cows during the last 6 weeks of gestation by the parenteral injection of corticosteroid with or without prostaglandin F2α (PGF2α) has raised the question of animal welfare and of the possible effects of prematurity on the disease resistance of the newborn calf. The induction of premature parturition in cattle has found application in the following areas:

- With pastoral-based dairy production, synchronization of the calving period has allowed maximal utilization of seasonally available pastures by the synchronization of peak demand for dry matter intake with spring flush in pasture growth. In pastoral-based herds with breeding for seasonal calving, late-calving cows will be induced and these average approximately 8% of the herd.
- Ensuring that calving coincides with the availability of labor to facilitate observations and management of calving and to overcome the inconvenience caused by late-calving cows.
- Minimizing dystocia in small heifers and animals with exceedingly long gestation periods (past due).
- The therapeutic termination of pregnancy for various clinical reasons.
- As an aid in the control of milk fever in combination with parenteral administration of vitamin D analogs.

A variety of short-acting and long-acting corticosteroids have been used. A single injection of a short-acting formulation is used when it is desirable to induce calving within the last 2 weeks of gestation. Earlier in pregnancy short-acting corticosteroids were found to be insufficiently reliable to induce parturition, which has led to the common use of long-acting corticosteroid formulations. A variety of protocols to induce premature parturition (3–6 weeks before due date) are used in practice; the main issue is the poor predictability of the time of calving relative to treatment when using long-acting corticosteroids. Common protocols use a second treatment with short-acting corticosteroids or the administration of PGF2α 50 to 10 days after the initial treatment. The use of PGF2α at least 9 days after treatment with long-acting corticosteroids was found to reliably narrow down the calving time, with the great majority of all cows calving within 72 hours of PGF2α treatment.[1] The use of PGF2α did not improve the viability of the premature neonates or their survival rate.

For cattle near term (within 2 weeks of due date) the use of short-acting corticosteroid formulation is more appropriate with parturition generally occurring within 2 to 4 days posttreatment.[2]

The **mortality rate** of induced calves is considerable and can exceed 30%, particularly when dams are induced at or before the eighth month of gestation.[2] Mortality in calves born as a result of induced parturition is primarily a result of prematurity, and

calf mortality is generally low when calving is induced within 12 days of parturition, although there are welfare concerns. The calves born earlier in pregnancy after using long-acting corticosteroid are usually lighter in weight, lethargic, and slow to stand and to suck properly. The serum immunoglobulin concentration was found to be lower in calves born from dams induced with long-acting corticosteroids because of interference with intestinal absorption by the corticosteroid. Up to 60% of calves born following induction with long-acting corticosteroids are at risk for failure of transfer of passive immunity. The colostrum available to such calves also has a reduced immunoglobulin content, and there may also be a reduction in the total volume of colostrum available from the induced-calving cows. Immunoglobulin absorption rates were not impaired when short-acting corticosteroids are used to induce calving close to term.

Artificial induction of parturition is an important risk factor for retention of the placenta, and the incidence is reported to vary from 20% to 100%. Subsequent reproductive performance of induced cows can be impaired. A risk for acute gram-negative bacterial infections is reported in a low (0.3%) proportion of cows following induction with dexamethasone. The use of long-acting corticosteroids was also associated with a higher incidence of photosensitization in treated heifers.[2]

In a study where partus induction was systematically used in cows that exceeded a gestation length of 282 days, no detrimental effects on calf viability, cow health, and productive and reproductive performance during lactation were found compared with untreated control animals. The incidence of retained fetal membranes in untreated animals was not recorded in this study and could thus not be compared with treated animals.[2]

When parturition is induced in large herds of beef cattle, particularly with a high percentage of heifers, increased surveillance will be necessary after the calves are born to avoid mismothering. Every attempt must be made to establish the cow–calf pair (neonatal bond) and move them out of the main calving area. Heifers that disown their calves must be confined in a small pen and be encouraged to accept the calf and let it suck, which is sometimes a very unrewarding chore. Calf mortality can be very high where calving is induced earlier than 35 weeks of pregnancy.

LAMBS

The induction of parturition in sheep is not a common practice, but it can be used to synchronize lambing in flocks where there are accurate dates of mating for individual ewes. Unless accurate dates are available, there is risk of prematurity. Also, ewes that are more than 10 days from their normal parturition date are unlikely to respond.

Induction of parturition is also used as a therapeutic ploy to terminate pregnancies in sheep with pregnancy toxemia. Induction is usually performed with dexamethasone and less commonly with betamethasone or flumethasone, which is more expensive. Lambing occurs 36 to 48 hours later, and there may be breed differences in response. Variability in lambing time can be reduced by the use of clenbuterol and oxytocin.

FOALS

The induction of parturition in mares for reasons of economy, management convenience, concern for prolonged gestation, or clinical conditions such as prepubic tendon rupture or research and teaching is now being practiced.

Foaling can be induced with oxytocin, ideally administered as an intravenous (IV) drip over 15 to 30 minutes, and occurs within 15 to 90 minutes of its administration. High doses of oxytocin are potentially dangerous to the foal and low doses (10–20 IU) are preferred. Glucocorticoids, and antiprogestagens that are effective in inducing pregnancy in other species, are either ineffective in the mare or capricious in their efficacy and can also be associated with adverse effects on the foal.

Prostaglandin F2α and its analogs have been used for partus induction in the mare and low doses (5–12 mg intramuscularly [IM]) may be effective at term, but repeated treatments may be required. The time interval between treatment and delivery is difficult to predict and can range from 1 to 48 hours. The use of PGF2α for partus induction in mares has been discouraged because considerable risks such as premature placental separation and foal death that have been associated with this treatment.[3]

Induction of parturition in the mare is not without risk and has been associated with the birth of foals that are weak, injured, or susceptible to perinatal infections. The period of fetal maturation is relatively short in the horse and is considered to be the last 2 to 3 days' gestation. Because spontaneous parturition in healthy mares can occur between 320 and 360 days, there is the risk of delivering a foal that is premature and nonviable. Fetal maturity is the major prerequisite for successful induced parturition, and the three essential criteria are

- A gestational length of more than 330 days
- Substantial mammary development and the presence of colostrum in the mammary gland with a calcium concentration greater than 10 mmol/L
- Softening of the cervix

The rise in calcium concentration is the most reliable predictor of fetal maturity and milk calcium concentrations above 10 mmol/L, in combination with a concentration of potassium that is greater than sodium, are indicative of fetal maturity. Commercial milk test strips are available for estimating mammary secretion electrolyte concentrations; however, it is recommended that testing be done in an accredited laboratory.

In mature foals, head lifting, sternal recumbency, and evidence of suck reflex occurs within 5 minutes of spontaneous full-term deliveries. The foal can stand within 1 hour and suck the mare within 2 hours. The behavior and viability of the premature foal after induced parturition have been described. The overall survival rate of foals delivered from induced parturition before 320 days' gestation was 5%. Four patterns of neonatal adaptation were observed on the basis of righting, sucking, and standing ability. If the suck reflex was weak or absent and the foals were unable to establish righting reflexes, the prognosis of survival was poor. Foals born before 300 days' gestation did not survive for more than 90 minutes; foals born closer to 320 days' gestation had a better chance of survival and exhibited behavioral patterns of adaptation.

In addition to the potential delivery of a premature or weak foal, other adverse effects of induction can be dystocia, premature placental separation, and retained placenta.

PIGLETS

The induction of parturition of gilts and sows on days 112, 113, or 114 of gestation is highly reliable and can be achieved by a single IM injection of 175 μg of cloprostenol or 5 to 10 mg of PGF2α. The sows farrow approximately 20 to 36 hours later. Synchronization of farrowing can be improved by administration of oxytocin (5–30 IU) 20 to 24 hours after injection of PGF2.

Induction of parturition has been used on large-scale farms to allow a concentration of labor, to improve supervision and care at the time of farrowing, to reduce the incidence of the mastitis/metritis/agalactia syndrome, and to reduce the percentage of stillborn piglets. The end day of a batch farrowing system can be fixed and weekend farrowing avoided. The subsequent fertility of the sows is not impaired. Induction on day 110 may be associated with a slight increase in perinatal mortality.

TREATMENT

Premature partus induction cattle (>2 weeks before due date):

Dexamethasone trimethyl-acetate (or other long-acting formulation) (25–30 mg/animal IM as single dose) (R-1)

Dinoprost (or other PGF2α-analogon) (25 mg/animal IM as a single dose 5–10 days after dexamethasone treatment) (R-2)

Partus induction cattle (<2 weeks before due date):

Dexamethasone sodium-phosphate (or other short-acting formulation) (40 mg/animal IM as a single dose) (R-1)

Continued

Cloprostenol (500 µg/animal IM 36–48 h after dexamethasone treatment) (R-1)

Dinoprost (25 mg/animal IM as a single dose 36–48 h after dexamethasone treatment) (R-2)

Partus induction mare:

Oxytocin (10–20 IU/animal as IV drip over 15–30 min, several repetitions possible) (R-1)

Prostaglandin F2α (or analogon) (R-3)

Partus induction sow:

Prostaglandin F2α (or analogon) (10–25 mg/animal IM) (R-1)

Cloprostenol (175 µg/animal IM) (R-1)

Oxytocin (5–30 IU/animal IM 20–24 h after treatment with PGF2α) (R-1)

Partus induction ewe:

Dexamethasone (15–20 mg/animal IM) (R-1)

IM, *intramuscularly;* IV, *intravenously.*

FURTHER READING

Ingoldby L, Jackson P. Induction of parturition in sheep. *In Pract*. 2001;23:228231.

MacDiarmid SC. Induction of parturition in cattle using corticosteroid: a review. Part 1. Reasons for induction, mechanisms of induction and preparations used. *Anim Breed Abstr*. 1983;51:40319.

MacDiarmid SC. Induction of parturition in cattle using corticosteroid: a review. Part 2. Effects of induced calving on the calf and cow. *Anim Breed Abstr*. 1983;51:499508.

Pressing AL. Pharmacologic control of swine reproduction. *Vet Clin North Am Food Anim Pract*. 1992;8:70723.

REFERENCES

1. Mansell PD, et al. *Aust Vet J*. 2006;84:312.
2. Villarroel A, Lane VM. *Can J Vet Res*. 2010;74:136.
3. Olsey J. *Equine Vet Educ*. 2003;15:164.

FREEMARTINISM IN CALVES

A freemartin is defined as a sterile female partner of a pair of heterosexual twins. In cattle, 92% of females born cotwins to males are freemartins.

In normal calves, the chromosomal identification of females is 60,XX (60 chromosomes, both X chromosomes) and of males is 60,XY (the Y being smaller and not readily paired with its opposite X chromosome).

The freemartin is the classical example of the chimera in cytogenetics. They are the individuals that contain two or more cell types that originated in separate individuals. The only way in which a chimera can develop is via the fusion of circulations or zygotes in utero. Sex chromosome chimerism is also reported in goats, sheep, and pigs, and, although the male partners of female twins are usually anatomically normal, they often have reduced fertility. Bulls born cotwin with freemartin females may also be chimeric and have low reproductive efficiency.

The diagnosis of freemartinism has been based on physical examination, karyotyping, or blood typing, and each has its limitations. There is variation in the degree of reproductive tract abnormalities in freemartins. The external genitalia may appear normal, the vulval hair may be coarser than usual, or the clitoris may be enlarged. The vagina is generally expected to be shorter than normal. The cervix, uterus, uterine tubes, and ovaries may be absent, present in underdeveloped form, or may appear normal on rectal palpation.

Special cytogenetic techniques are also available that facilitate the diagnosis of freemartinism in a female calf of a male–female twinning. In freemartins (phenotypically female, but also carrying male cells) there is a mixture of mostly 60,XX chromosomes to a cell and a small proportion of 60,XY cells. A large number of cells need to be analyzed if only the freemartin calf is available, because the proportion of abnormal cells present may be as low as 2%. It is, however, possible to make a diagnosis on the examination of 10 to 20 cells, provided the male twin is also analyzed; the female may have very few XY chromosomes, but the male will have a very high proportion of XX chromosomes. This technique is much more accurate than blood group analysis or clinical observations of a short vagina, enlarged clitoris, and the presence of a vulval tuft of hair. Karyotyping is a definitive method of freemartin diagnosis, but it is tedious, time-consuming, and expensive. Blood typing analysis may be performed on both the male and female cotwins to demonstrate two blood group populations, but it is expensive and requires blood samples from both cotwins.

The **polymerase chain reaction** (PCR) method of freemartin diagnosis using sex-specific DNA sequences is rapid, accurate, relatively simple, and inexpensive to perform, and a blood sample is required only from the female cotwins. It allows for the accurate decision of freemartinism down to a level of 0.05% of male chimeric cells present.

FURTHER READING

Padula AM. The freemartin syndrome: An update. *Anim Reprod Sci*. 2005;87:93-109.

BULLER STEER SYNDROME

SYNOPSIS

Etiology Unknown. Behavioral problem of steers in feedlots.

Epidemiology Prevalence varies and increases with increasing age and weight at entry.

Clinical findings and lesions Areas of denuded hair, subcutaneous hematomas, and other traumatic injuries.

Treatment Symptomatic.

Control Removal from pen.

ETIOLOGY

The buller steer syndrome is a **behavioral** problem in cattle confined in feedlots[1] of unknown etiology. Within a pen of cattle, one or more cattle persistently ride a particular individual or individuals of the group. The ridden animals are referred to as bullers. There have been several suspect etiologies. Improper placement of hormonal growth implants has been suspected as being associated with this behavioral problem.

EPIDEMIOLOGY

Occurrence

The syndrome occurs only in cattle in feedlots. A recent survey conducted among U.S. feedlots revealed a feedlot prevalence of 68.8% of all surveyed feedlots and an animal level prevalence of 2.8%.[1] The prevalence increases with increasing weight and age. The case fatality has been estimated at 1%. The incidence of occurrence is higher in the summer and the fall and during the first 30 days of the feeding period.

Epidemiologic studies indicate that bullers occur as a point source epidemic with the cause occurring soon after cattle arrive in the feedlot and mingle into pen groups. The peak incidence of bullers occurs much sooner after arrival and declines much quicker in older cattle. Bullers occur significantly sooner after mixing in older cattle than in younger cattle. The pen prevalence also increases as cattle become older on arrival at the feedlot and are more aggressive. As the prevalence of intact bulls increases in pens of cattle, so does the prevalence of bullers, presumably caused by more aggressiveness in the bulls.

Risk Factors

Postulated causative and risk factors include the incorrect timing and administration of hormonal growth implants, reimplantation and double dosing, estrogenic substances in feeds, pheromones in the urine of certain cattle, improper or late castration of young cattle, daily feedlot management, weather and seasonal factors, disease, group size, and dominance behavior. However, these factors have not been well substantiated, and controlled studies have found little influence of implant type and implant timing on buller incidence.

The mixing and confinement of **unfamiliar cattle** into pen groups, with subsequent agonistic interactions because these cattle established a social hierarchy, are considered as important risk factors. Both riding behavior and antagonistic behavior cease once cattle establish a stable social hierarchy. This suggests that riding behavior and subsequent identification of bullers is associated with this dominance behavior. It is possible that when a dominant animal becomes ill in a pen, other more subordinate animals in the pen that were previously subdued in

dominance contests may want to fight the sick animal to achieve higher social status.

Economic Importance

The syndrome has been ranked along with acute undifferentiated bovine respiratory disease and foot rot as **one of the three most important disease syndromes** in beef feedlots in North America. In addition to the economic loss from decreased weight gain, injury, treatment, death, and carcass condemnation, there are economic losses associated with extra handling necessary to accommodate affected cattle, the disruption of uniform marketing of cattle, especially in custom feedlots, and the need for extra pens in which to house the bullers. The importance of the syndrome includes animal welfare aspects.

Bullers may be at significantly greater risk of illness and mortality (from bacterial pleuropneumonia) than other steers. The association between illness, mortality, and bullers among individuals was greatest among the oldest yearling steers.

CLINICAL FINDINGS

Two types of bullers are identified. **Type 1 or true bullers** stand as if they were a heifer in estrus and do not move away or show agonistic behavior when being mounted by rider cattle. There can be several rider cattle in a pen and type 1 bullers are rapidly damaged. **Type 2 bullers** are animals that appear low in social dominance. They use aggression to discourage riders and will lie down to avoid being ridden.

Affected animals show areas of denuded hair and have extensive subcutaneous hemorrhage. The hematomas may become infected and develop to subcutaneous pockets of pus and gas. Other traumatic injuries, such as limb fractures, also occur.

CONTROL

Management of the syndrome has usually involved identification and removal from the pen to prevent injury and even death from riding-related injuries. The high rate of risk of illness and mortality in bullers relative to other feedlot steers suggests that bullers should always be checked for evidence of illness in addition to their removal from their designated pen to prevent severe riding-related injuries. Treating sick bullers may improve the chance of settling them back into their designated pen by allowing them to resume their original position in the social hierarchy.

FURTHER READING

Blackshaw JK, Blackshaw AW, McGlone JJ. Buller steer syndrome review. *Appl Anim Behav Sci.* 1997;54:97.

REFERENCE

1. USDA-APHIS. Feedlot 2011.Part IV. 2014. <http://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot2011/Feed11_dr_PartIV.pdf>; Accessed 20.01.2014.

Infectious Diseases Primarily Affecting the Reproductive System

BRUCELLOSIS ASSOCIATED WITH *BRUCELLA ABORTUS* (BANG'S DISEASE)

SYNOPSIS

Etiology *Brucella abortus*

Epidemiology Major cause of abortion in cattle in countries without a national control program. Undulant fever in humans; is an important zoonosis. Sexually mature animals susceptible; outbreaks occur in first-calf heifers, older cows are infected but do not abort. Transmitted directly from the infected animal to the susceptible animal by uterine discharges. Congenital infection occurs. Infection in wildlife species but significance to domestic animals unknown. Infection introduced into herd by unknown infected carrier animal. Natural infection and vaccination result in immunity to abortion but not infection, and infected animals remain serologically positive for a long time.

Signs Abortion epidemics in first-calf unvaccinated heifers after fifth month of pregnancy. Subsequent pregnancies carried to term. Orchitis and epididymitis in bulls. Synovitis (hygroma) occurs. Fistulous withers in horses.

Clinical pathology Serology. Serum agglutination test is standard test. Rose Bengal test (rapid screening test). Complement fixation test. ELISA test. Milk ring test. False-positive reactors are a major problem.

Lesions Necrotizing placentitis, inflammatory changes in fetus.

Diagnostic confirmation Culture organism from fetus. Positive serologic test in unvaccinated animal.

Treatment No treatment.

Control Test and reduce reservoir of infection. Quarantine. Depopulation. Vaccination to reduce incidence of abortion and percentage of infected animals. Eradication on herd and area basis by test and cull.

ELISA, *enzyme-linked immunosorbent assay.*

ETIOLOGY

Brucella abortus, a gram-negative, facultative intracellular coccobacillus of the family Brucellaceae, is the organism responsible for **bovine brucellosis**. *B. abortus* is one of 10 species with validly published names, including *B. melitensis, B. abortus, B. suis, B. ovis, B. neotomae, B. canis, B. ceti, B. pinnipedialis, B. microti,* and *B. inopinata,* each of which has specific host preferences.[1] *B. abortus* is responsible for bovine brucellosis, *B. melitensis* is the main causative agent of brucellosis in small ruminants and men, *B. suis* for brucellosis in swine, and *B. ovis* in sheep. *B. abortus* has eight recognized biovars (1–7, 9) of which the most prevalents are 1–4, and 9.[2] Approximately 5% of infections are from biovar 1. Biovar 2 was isolated in an outbreak of brucellosis in cattle in Canada in 1986. In the United States, biovars 1 to 4 are found.

EPIDEMIOLOGY

Occurrence and Prevalence of Infection

Bovine brucellosis has a worldwide occurrence and, according to the Food and Agriculture Organization (FAO), the World Health Organization (WHO), and the World Organization for Animal Health (OIE), is still one of the most important and widespread bacterial zoonoses in the world. The prevalence of infection varies considerably among herds, areas, and countries. Many countries have made considerable progress with their eradication programs, and some have eradicated the disease. However, in other countries brucellosis is still a serious disease facing the veterinary and medical professions. Currently, Australia, New Zealand, Canada, Japan, and 16 member states of the European Union (EU) have a status as officially brucellosis free.[2,3] Bovine brucellosis remains prevalent in several southern European countries and the Mediterranean basin. The seroprevalence of bovine brucellosis in the Kars district of Turkey between 2004 and 2006 was determined to be around 34%.[4] In Greece 0.97%, in Italy 0.51%, in Portugal 0.19%, in Spain 0.07%, and in the UK 0.09% of all cattle herds were positive for brucellosis in 2012.[3] Although bovine brucellosis has been reported from Egypt (biovar 1), Iran (biovar 2), and Sudan (biovar 6), little is known about the infection prevalence in the region.[5]

In the United States, the entire country is classified as class free for bovine brucellosis. Notwithstanding, infection remains highly prevalent in the wildlife population in the Greater Yellowstone area, with occasional spread to cattle. Repeated incidents of brucellosis-infected cattle in Montana, Idaho, and Wyoming have been reported in recent years.[2] Bovine brucellosis remains an important bacterial disease in Mexico, with biovars 1–6 the most prevalent. Although limited epidemiologic data are available for Central America, the disease seems to prevail widely, with an estimated animal prevalence of between 4% and 8% and a (dairy) herd prevalence between 10% and 25%.[5] In South America Chile made great progress toward eradicating the disease, but it remains prevalent in Venezuela (animal seroprevalence 3%–4%), Argentina (animal seroprevalence 2%–3%), and Brazil.[5]

The livestock prevalence was estimated at 8.2% in East Africa, 15.5% in West Africa,

14.2% in South Africa, and 13.8% in North Africa.[6]

Cattle

Infection occurs in cattle of all ages but is most common in sexually mature animals, particularly dairy cattle. **Abortions are most common during outbreaks and primarily occur in unvaccinated heifers over 5 months pregnant.** Bulls are affected with orchitis, epididymitis, and seminal vesiculitis.

Camelids

Brucellosis has been reported in the one-humped (*Camelus dromedaries*) and two-humped camel (*C. bactrianus*) and New World camelids such as llama (*Lama glama*), alpaca (*lama pacos*), guanaco (*lama guinicoe*), and vicuña (*Vicugna vicugna*) and was related to contact with small and large ruminants infected with either *B. abortus* or *B. melitensis.*[7]

Wildlife Species

The infection has been observed in American and European bison (*Bison bison, B. bonasus*); domestic buffalo (*Bubalus bubalus*); elk (*Cervus elaphus canadensis*); deer; coyotes; wild opossums; and raccoons, moose, and other wild and domesticated ruminants. Infection of moose with *B. abortus* biovar 1 is highly fatal, and it is likely that the moose is a dead-end host for brucellosis. Experimental inoculation of the organism into badgers results in the development of antibodies and elimination of the organism, which indicates that the badger is relatively resistant to infection and unlikely to be a reservoir of the organism.

Bison and elk are potential reservoirs of bovine brucellosis and have been associated with recurrence of bovine brucellosis in the Greater Yellowstone area in the United States. Brucellosis associated with *B. abortus* was first detected in bison (*B. bison*) in Yellowstone National Park in 1917 and has been present ever since. Bison can remain latently infected with virulent *B. abortus* until attainment of reproductive age despite extensive use of vaccination and serologic testing.

Cattle and bison appear to maintain *B. abortus* at higher seroprevalence than other ungulate species. The seroprevalence in the Yellowstone bison and elk population is estimated with 40% to 60% and 22%, respectively.[8,9] This has been associated with physiologic and immunologic characteristics common to bovine species but is probably also caused by typical behavioral patterns of large social groups and the periparturient behavior of bison dams that tend to calve within groups that facilitate disease transmission through direct contact around parturition.[10] In contrast elk dams segregate themselves during the periparturient period and meticulously clean the birthing site, considerably reducing the risk of disease transmission through direct contact.[11] Disease transmission may, however, be common during the abortion period in the last trimester of pregnancy from February to April, when many elk congregate in large groups on lower elevation winter habitat that overlaps with cattle-grazing areas.[11] From 2009 to 2011 eight infected cattle or captive bison herds were detected in Wyoming and Montana and all episodes were genetically or epidemiologically linked to elk, suggesting that spillover transmission from elk to cattle is epidemiologically more important than transmission from bison to cattle.[11] This has been explained with the continuously increasing elk population, which is currently above management target values in many areas of the Greater Yellowstone area and the greater mobility of free-ranging elk.[10]

Horses

In horses the organism is often found in chronic bursal enlargements as a secondary invader rather than a primary pathogen. It is commonly present with *Actinomyces bovis* in fistulous withers and poll evil. It has also been identified as a cause of abortion in mares. A serologic survey of horses over a period of 8 years revealed that 8% to 16% of serum samples were positive. However, experimentally infected horses do not excrete the organism in sufficient numbers to infect susceptible in-contact cattle.

Pigs and Sheep

The organism can be recovered from naturally infected pigs and, although not normally pathogenic in this species, may occasionally cause abortion. The disease occurs naturally in sheep exposed to infected cattle, which has significant implications for brucellosis eradication.

Dogs

Naturally acquired *B. abortus* infection can occur in dogs associated with infected cattle. Although farm dogs are not generally considered to be a major reservoir of *B. abortus,* the organism has been isolated from dogs on a farm in which several cattle were serologically positive for brucellosis, and dogs should be included in any investigation and eradication of the disease.

Methods of Transmission

Parturition/Abortion

The **risk posed to susceptible animals** following parturition or abortion of infected cattle depends on three factors:

- Number of organisms excreted
- Survival of these organisms under the existing environmental conditions
- Probability of susceptible animals being exposed to enough organisms to establish infection

The organism achieves its greatest numbers in the contents of the pregnant uterus, the fetus, and the fetal membranes, all of which must be considered as major sources of infection. The numbers of organisms in the tissues of two naturally infected cows and their fetuses were as follows: umbilicus $2.4 \times 10^8 - 4.3 \times 10^9$/g – 1.4×10^{13}/g. This illustrates the potentially large numbers of organisms that can be shed and to which other animals and humans are potentially exposed. However, the numbers of organisms decrease when uterine discharges are cultured at sequential parturitions, and a substantial number of uterine samples from infected cows are culture negative at the second and third parturition following challenge.

Transmission

The disease is transmitted by ingestion, penetration of the intact skin and conjunctiva, and contamination of the udder during milking. The organism does not multiply in the environment but merely persists, and the viability of the organism outside the host is influenced by the existing environmental conditions. Grazing on infected pasture, or consuming feedstuffs or water supplies contaminated by discharges and fetal membranes from infected cows, and contact with aborted fetuses as well as infected newborn calves are the most common methods of spreading the disease.

Intraherd spread occurs by both vertical and horizontal transmission. **Horizontal transmission is usually by direct contamination** and, although the possibility of introduction of infection by flies, dogs, rats, ticks, infected boots, fodder, and other inanimate objects exists, it is not significant relative to control measures. The organism is ingested by the face fly but is rapidly eliminated, and there is no evidence for a role in natural transmission. Evidence exists for horizontal, dog-to-dog, cattle-to-dog, dog-to-cattle, and dog-to-human transfer of infection. The most likely and effective means of cattle-to-dog transfer is exposure to aborted fetuses or infected placental membranes, because dogs commonly ingest the products of parturition.

Spread Between Herds

Movement of an infected animal from an infected herd to a susceptible noninfected herd is a common method of transmission. The rate of spread will depend on the level of surveillance testing. In Great Britain, which is officially brucellosis free, 20% or more of both beef and dairy cattle more than 24 months old are tested routinely. A simulation model indicates that reducing the level of testing would have a major effect on the rate of spread of infection, should it be imported.

Spread Between Countries (Breach of Biosecurity)

A quantitative risk assessment model to determine the annual risk of importing brucellosis-infected breeding cattle into Great

Britain from Northern Ireland and the Republic of Ireland, which are not brucellosis free, was developed. Predictions estimated that brucellosis could be imported from Northern Ireland every 2.63 years and from the Republic of Ireland every 3.23 years. Following this assessment, the Department of Environment, Food, and Rural Affairs introduced postcalving testing for all imported breeding cattle. Under this system, all imported animals are issued a passport that records their age and pregnancy status. This information enables identification of animals that require testing and provides an additional safeguard in maintaining official brucellosis status.

Congenital Infection

Congenital infection may occur in calves born from infected dams but its frequency is low. The infection occurs in utero and may remain latent in the calf during its early life; the animal may remain serologically negative until its first parturition, when it then begins to shed the organism. Calves born from reactor dams are serologically positive for up to 4 to 6 months because of colostral antibodies and later become serologically negative even though a latent infection may exist in a small proportion of these calves. The **frequency of latent infections** is unknown, but may range from 2.5% to 9%. Latent infections in serologically negative animals are of some concern because they remain unnoticed and can potentially serve as a source of infection later. However, latent infections in calves born from infected cows are infrequent. The organism could not be isolated from any of 150 calves born to infected cows, 135 of which were experiencing their first pregnancy after infection. In one report, a heifer from a herd affected with widespread infection with *B. abortus* biotype 2 was moved to a brucellosis-free herd and remained apparently free from brucellosis until 9 years later, when the same animal produced a strongly positive serologic reaction and the same biotype was isolated from its milk. Such observations have resulted in the recommendation that calves from seropositive dams should not be used for breeding. Even vaccinated heifers from seropositive dams can harbor a latent infection. There is a risk that 2.5% of heifer calves born from serologically positive dams will react in early adulthood and constitute a threat to a reestablished herd.

Survival of Organism

The organism can survive on grass for variable periods depending on environmental conditions. In temperate climates, infectivity may persist for 100 days in winter and 30 days in summer. The organism is susceptible to heat, sunlight, and standard disinfectants, but freezing permits almost indefinite survival. The activity of several disinfectants against *B. abortus* has been examined, and representatives of the phenolic, halogen, quaternary ammonium, and aldehyde groups of disinfectants at 0.5% or 1.0% concentrations in the absence of serum generally inhibited a high concentration of the organism.

Uterine Discharges and Milk

A cow's tail heavily contaminated with infected uterine discharges may be a source of infection if it comes in contact with the conjunctiva or the intact skin of other animals. In the same way that the more common forms of mastitis can be spread during milking, *B. abortus* infection can be spread from a cow whose milk contains the organism to an uninfected cow. This may have little significance in terms of causing abortion, but it is of particular importance in its effects on agglutination tests on milk and the presence of the organism in milk used for human consumption.

Bulls and Semen

Bulls do not usually transmit infection from infected to noninfected cows mechanically. Infected bulls may discharge semen containing organisms but are unlikely to transmit the infection. The risk of spread from the bull is much higher, however, if the semen is used for artificial insemination. Some infected bulls are negative on serum agglutination tests and their carrier status can only be detected by the isolation of organisms from the semen or agglutination tests on seminal plasma.

Carrier Cows

Few infected cows ever recover from infection completely and should be considered as permanent carriers whether or not abortion occurs. Excretion of the organism in the milk is usually intermittent, is more common during late lactation, and can persist for several years. In cattle vaccinated before infection, the degree of excretion of *B. abortus* in the milk is less than in nonvaccinated animals. Embryo transfer from infected donors may be achieved without transfer of infection, and superovulation is unlikely to reactivate the release of *Brucella* into the uterus during the period when embryos are normally collected. Thus embryo transfer is a safe procedure for salvaging genetic material from infected animals.

The herd characteristics and the results of the first herd test may be used as predictors of the potential presence or absence of *B. abortus* in herds with reactors to the tube agglutination test. The presence of only single suspicious reactors on the first test is a reliable predictor of lack of infection. The presence of one or more positive reactors on the first herd test is a reliable predictor of the presence of infection.

Risk Factors

The risk factors that influence the initiation, spread, maintenance, and/or control of bovine brucellosis are related to the animal population, management, and the biology of disease. The variables that contribute significantly to seropositive animals are

- Size of farm premises
- Percentage of animals on a premises that are inseminated artificially
- Size of investment in livestock
- Number of cows that aborted in the previous year, whether or not dairying is the major agricultural activity of the premises
- Policy of the owner regarding disposal of reactor animals

The longer infected animals are in contact with the remainder of the herd, the greater will be the ultimate number of seropositive animals. In a defined geographic area in northern Mexico where a brucellosis control program did not exist, the greatest percentage of seropositive animals was related to larger farms, poor artificial insemination technique, and small financial investment in the farm.

From a practical viewpoint, the factors influencing the transmission of brucellosis in any given geographic region can be classified into two fundamental categories: those associated with the transmission of disease between herds and those influencing the maintenance and spread of infection within herds. Factors influencing interherd transmission include the purchase of infected replacement animals, which is influenced by frequency of purchase, source of purchase, and brucellosis test history of purchased animals. The proximity of infected herds to clean herds is an important risk factor. Cattle contacts at fence lines, sharing of pastures, and strays of infected animals into clean herds are common methods by which transmission occurs to adjacent herds.

The risk factors associated with spread of the disease within a herd include unvaccinated animals in infected herds, herd size, population density, method of housing, and use of maternity pens. Large herd sizes are often maintained by the purchase of replacement cattle, which may be infected. It is also more difficult to manage large herds, which may lead to managerial mistakes that allow the disease to spread. There is a positive association between population density (number of cattle to land area) and disease prevalence, which is attributed to increased contact between susceptible and infected animals. The use of maternity pens at calving is associated with a decrease in the prevalence of infection, presumably from decreasing the exposure of infected and susceptible animals.

Animal Risk Factors

Susceptibility of cattle to *B. abortus* infection is influenced by the age, sex, and reproductive status of the individual animal. **Sexually mature, pregnant cattle are more susceptible to infection with the organism than sexually immature cattle of either sex.** Natural exposure to field strains occurs

primarily at the time of parturition of infected cows. The greater the number of infected cows that abort or calve, the greater the exposure risk to the other cattle in the herd. An important application of this observation is that infected cows need to be removed from the herd before parturition. Young cattle are less susceptible to *B. abortus* than older, sexually mature cattle. Susceptibility appears to be more commonly associated with sexual maturity than age. Young, sexually immature cattle generally do not become infected following exposure, or recover quickly. Susceptibility increases with pregnancy and as the stage of gestation increases. The probability of isolation of the organism at parturition increased from 0.22 to 0.90 as fetal age at exposure of nonvaccinated heifers increased from 60 to 150 gestation days.

Management Risk Factors

The spread of the disease from one herd to another and from one area to another is almost always caused by the movement of an infected animal from an infected herd into a noninfected susceptible herd. The unregulated movement of cattle from infected herds or areas to brucellosis-free herds or areas is the major cause of breakdowns in brucellosis eradication programs. A case-control study of brucellosis in Canada indicated that herds located close to other infected herds and those herds whose owners made frequent purchases of cattle had an increased risk of acquiring brucellosis. Once infected, the time required to become free of brucellosis was increased by large herd size, by active abortion, and by loose housing.

Pathogen Risk Factors

Brucella spp., in contrast to other pathogens, do not possess typical virulence factors such as a capsule, flagella, exotoxins, or inducers or host cell apoptosis. They express a **lipopolysaccharide (LPS)** that, in contrast to LPS from other gram-negative pathogens, is nonendotoxic but is important for the protection from complement-mediated bacterial killing and the resistance against antimicrobial peptides such as defensins and lactoferrin.[12]

Brucella spp. possess a number of **outer membrane proteins (OMPs)**, some of which are required for full virulence, and that are recognized as antigen by immunity receptors such as Toll-like receptors (TLRs), triggering proinflammatory cytokine release.[13] Certain mutants of *B. abortus* lack a major 25-kDa OMP (Omp25), which renders them unable to replicate efficiently in bovine phagocytes and chorionic trophoblasts. Expression of OMPs is regulated through **the BvrR/BvrS two-component regulatory system**, which also modulates the host cell cytoskeleton on invasion, contributing to pathogen virulence.[12] The BvrR/BvrS two-component regulatory system furthermore regulates the expression of the **type IV secretion system (T4SS)**, which is crucial for intracellular survival in host cells and virulence in vivo. T4SS is required for *Brucella* spp. to reach their intracellular replication niche.[12]

Immune Mechanisms

Brucellas are able to survive within host leukocytes and may use both neutrophils and macrophages for protection from humoral and cellular bactericidal mechanisms during the periods of hematogenous spread.

Immunity against brucellosis is principally mediated by cellular immune responses because it is an intracellular pathogen. *B. abortus* is an efficient inducer of type 1 cellular immune responses, and interferon gamma (IFN-γ) is crucial for control of brucellosis. Infections are chronic and often lifelong. The bovine T lymphocyte in brucellosis is a critical component of host defense based on mononuclear phagocyte activation by IFN-γ. The killing of *Brucella*-infected mononuclear phagocytes and IFN-γ–mediated activation of mononuclear phagocytes are the major mechanisms of host defenses against brucellosis in cattle.

The antibody response to *B. abortus* in cattle consists of an early IgM isotype response, the timing of which depends on the route of exposure, the dose of the bacteria, and the health status of the animal. The IgM response is followed almost immediately by production of IgG_1 antibody and later by small amounts of IgG_2 and IgA. Most cross-reacting antibody from exposure to bacteria other than *Brucella* spp. or environmental antigens consists mainly of IgM. Serologic tests that measure IgM are therefore not desirable, because false-positive results occur. Because IgG_2 and IgA antibodies accumulate later after exposure and are usually present in small and inconsistent amounts, the main isotype for serologic testing is IgG_1.

Naturally infected animals and those vaccinated as adults with strain 19 remain positive to the serum and other agglutination tests for long periods. The serum of infected cattle contains high levels of IgM, IgG_1, IgG_2, and IgA isotypes of antibody. Most animals vaccinated between 4 and 8 months of age return to a negative status to the test within a year. All are considered to have a relative immunity to infection. Calves from cows that are positive reactors to the test are passively immunized via the colostrum. The half-life of colostral antibodies to *B. abortus* in calves that have received colostrum from either vaccinated noninfected or infected dams is about 22 days. It is possible that some calves remain immune sufficiently long to interfere with vaccination. After vaccination of cattle with strain 19 of the organism, IgM antibodies appear after about 5 days, reaching peak values after 13 days. IgG_1 antibodies appear a little later or simultaneously with IgM, and peak values are reached at 28 to 42 days, after which they decline. The same general pattern follows experimental infection with virulent strains and also in chronic field cases, except that IgM antibody declines to low levels and residual activity resides in IgG_1 and IgG_2 as well as in IgA, which remain at higher levels.

Economic Importance

Losses in animal production caused by this disease can be of major importance, primarily because of decreased milk production in aborting cows. The common sequel of infertility increases the period between lactations, and in an infected herd the average intercalving period may be prolonged by several months. In addition to the loss of milk production, there is the loss of calves and interference with the breeding program. This is of greatest importance in beef herds where the calves represent the sole source of income. A high incidence of temporary and permanent infertility results in heavy culling of valuable cows, and some deaths occur as a result of acute metritis following retention of the placenta.

Zoonotic Implications

According to the Food FAO, the WHO, and OIE, brucellosis is still one of the most important and widespread zoonoses in the world. Of the six *Brucella* spp. known to cause human disease (*B. melitensis, B. abortus, B. suis, B. canis, B. ceti,* and *B. pinnipedialis*), *B. melitensis* is the one with the largest public health impact because it is the most virulent species and has the highest prevalence in small ruminant populations in many areas of the world. *B. abortus* and *B. suis* serovars 1, 3, and 4 are also important human pathogens; *B. suis* serovar 2 and *B. canis* are uncommon human pathogens. Most cases in humans are occupational and occur in farmers, veterinarians, and slaughterhouse personnel after direct contact with infected animals or animal material contaminated with the pathogen. The organism can be isolated from many organs other than the udder and uterus, and the handling of a carcass of an infected animal may represent severe exposure. Brucellosis is also one of the most easily acquired laboratory infections.[15] Infection can also occur after ingestion of raw milk or raw milk products. Officially approved methods of commercial pasteurization render naturally *Brucella*-contaminated raw milk safe for consumption.

In endemic regions, the reported incidences of human brucellosis range from less than 0.01 per 100,000 population to more than 200 per 100,000 population.[15] In the United States, where approximately 100 cases of human brucellosis are reported annually, the incidence rate is less than 0.05 per 100,000 population.[15] In Europe the highest incidences were reported from Greece (1.09 per 100,000 population), Portugal (0.36 per 100,000 population), and Spain (0.13 per 100,000 population), which together

accounted for 67.7% of all confirmed cases of human brucellosis in member states of the EU in 2012.[14] Of the human cases reported within the EU in 2012 where species information was available 83.9% were caused by *B. melitensis,* 10.1% *B. abortus,* 3.0% *B. suis,* and 3.0% by other *Brucella* spp.[14] The importance of the disease in humans is an important justification for its eradication. The cost-effectiveness to human health and the potential net economic benefits of a nationwide mass vaccination program for livestock over a period of 10 years has been evaluated using Mongolia as the model. If the costs of vaccination of livestock against brucellosis were allocated to all sectors in proportion to the benefits, the intervention would be cost-effective and would result in net economic benefits.

PATHOGENESIS

The successful coexistence of *Brucella* spp. with its preferred host is the outcome of coevolutionary relationships and selection pressures, which result in a stalemate where the pathogen has evolved to survive within the biologic system of the host, and the host has evolved innate and acquired immune systems that allow controlled survival of infection by the pathogen, ultimately supporting the survival of the host–pathogen system.

Following ingestion most commonly through the digestive or respiratory tract *Brucella* spp. can invade epithelial cells of the host, allowing infection though intact mucosal surfaces. Once invasion successfully occurs the organism may be phagocytized by host immune cells and may also invade nonphagocytic host cells through a mechanism that is not entirely understood. Following cell invasion the organism is contained in a membrane-bound modified phagosome, the *Brucella*-containing vacuole (BCV), and interferes with intracellular trafficking, preventing fusion of the BCV with lysosome markers and directing the BCV to the rough endoplasmic reticulum, which is highly permissive for intracellular replication of *Brucella.*[12] Invaded polymorphonuclear leukocytes then transport the pathogen to regional lymph nodes, other sites such as the reticuloendothelial system, and organs such as the udder and when present the fetal placenta. In the draining lymph node, *Brucella* infection causes cell lysis and eventual lymph node hemorrhage 2 to 3 weeks following exposure. Because of vascular injury, some of the bacteria enter the bloodstream and subsequent bacteremia occurs, which disseminates the pathogen throughout the body.

B. abortus has a predilection for the placenta; udder; testicle; and accessory male sex glands, lymph nodes, joint capsules, and bursae. Erythritol, a substance produced by the fetus and capable of stimulating the growth of *B. abortus,* occurs naturally in greatest concentration in the placental and fetal fluids and is responsible for localization of the infection in these tissues. Invasion of the gravid uterus results in a severe ulcerative endometritis of the intercotyledonary spaces. The allantochorion, fetal fluids, and placental cotyledons are invaded, and the villi are destroyed. The organism has a marked predilection for the ruminant placenta. In acute infections of pregnant cows, up to 85% of the bacteria are in cotyledons, placental membranes, and allantoic fluid. The resulting tissue necrosis of the fetal membranes allows transmission of the bacteria to the fetus. The net effect of chorionic and fetal colonization is abortion during the last trimester of pregnancy. The characteristic pneumonia in aborted fetuses is caused by localization of perivascular foci in the interlobular septa of the lung, indicative of hematogenous spread in the fetus rather than aspiration of contaminated fetal fluids. Fetuses inoculated with sufficient numbers of *B. abortus* will abort 7 to 19 days postinoculation. With experimental conjunctival exposure of pregnant heifers with the organism, the numbers of infected animals and the number of tissue samples positive for the organism are increased as fetal age at exposure increases from gestation days less than 127 to more than 157. **Abortion occurs principally in the last 3 months of pregnancy**, and the incubation period is inversely proportional to the stage of development of the fetus at the time of infection.

Congenital infection can occur in newborn calves as a result of in utero infection, and the infection may persist in a small proportion of calves, which may also be serologically negative until after their first parturition or abortion.

In the adult, nonpregnant cow, localization occurs in the udder, and the uterus, if it becomes gravid, is infected from periodic bacteremic phases originating in the udder. Infected udders are clinically normal, but they are important as a source of reinfection of the uterus, as a source of infection for calves or humans drinking the milk, and because they are the basis for the agglutination tests on milk and whey. Variable disease expression may occur in the male reproductive tract and musculoskeletal system, particularly affecting large joints, of either sex.

CLINICAL FINDINGS

Abortion

The clinical findings are dependent on the immune status of the herd. In highly susceptible nonvaccinated pregnant cattle, abortion after the 5th month of pregnancy is a typical feature of the disease in cattle. In subsequent pregnancies, the fetus is usually carried to full term, although second or even third abortions may occur in the same cow. Retention of the placenta and metritis are common sequelae to abortion. Mixed infections are usually the cause of the metritis, which may be acute, with septicemia and death following, or chronic, leading to sterility.

The history of the disease in a susceptible herd can usually be traced to the introduction of an infected cow. Less common sources are infected bulls, or horses with fistulous withers. In a susceptible herd, it is common for the infection to spread rapidly and for an abortion "storm" to occur. The storm might last for a year or more, at the end of which time most of the susceptible cows are infected and have aborted and then carry their calves to full term. Retained placentae and metritis could be expected to be common at this time. As the abortion rate subsides, the abortions are largely restricted to first-calf heifers and new additions, because other animals of the herd acquire partial resistance.

In recent years, particularly in areas where vaccination is extensively practiced, an insidious form of the disease may develop, which spreads much more slowly and in which abortion is much less common.

Orchitis and Epididymitis

In the bull, orchitis and epididymitis occur occasionally. One or both scrotal sacs may be affected, with acute, painful swelling to twice normal size, although the testes may not be grossly enlarged. The swelling persists for a considerable time, and the testis undergoes liquefaction necrosis and is eventually destroyed. The seminal vesicles may be affected and their enlargement can be detected on rectal palpation. Affected bulls are usually sterile when the orchitis is acute but may regain normal fertility if one testicle is undamaged. Such bulls are potential spreaders of the disease if they are used for artificial insemination.

Synovitis

B. abortus can often be isolated from the tissues of nonsuppurative synovitis in cattle. Hygromatous swellings, especially of the knees, should be considered with suspicion. Progressive and erosive nonsuppurative arthritis of the stifle joints has occurred in young cattle from brucellosis-free herds that had been vaccinated with strain 19 vaccine. The calves may or may not be serologically positive, but synovial fluid and joint tissue samples contain immunologic evidence of strain 19 *B. abortus* antigenic material. The synovitis has been reproduced by intraarticular injection of the vaccine.

Fistulous Withers

In horses, the common association of *B. abortus* is with chronic bursal enlargements of the neck and withers, or with the navicular bursa, causing intermittent lameness, and the organism has been isolated from mares that have aborted. When horses are mixed with infected cattle, a relatively high proportion can become infected and develop a positive reaction to the serum agglutination test

without showing clinical illness. Some horses appear to suffer a generalized infection with clinical signs including general stiffness, fluctuating temperature, and lethargy.

CLINICAL PATHOLOGY

The major objective in the laboratory diagnosis of brucellosis is to identify animals that are infected and potentially shedding the organism and spreading the disease. Most infected animals are identifiable using the standard serologic tests, but latent infection occurs in some animals that are serologically negative. Furthermore, vaccinated animals may be serologically positive and uninfected, and transitory titers occur sporadically in a small percentage of animals, for which there is no clear explanation. These diagnostic problems make control and eradication programs difficult to administer and difficult to explain to animal owners.

The collection and submission of samples to the laboratory must be done with care, and careful attention must be given to recording the identity of the animal and the corresponding sample, which should be uniquely identified. For blood samples, it is recommended that silicone-coated evacuated glass tubes without additives be used to collect the blood sample, because they ensure effective clotting and clot retraction, to provide an easy source of serum without the need for centrifugation. Clotting is also aided by maintaining the sample at 25°C to 37°C for 1 to 2 hours.

Laboratory tests used in the diagnosis of brucellosis include isolation of the organism and serologic tests for the presence of antibodies in blood, milk, whey, vaginal mucus, and seminal plasma. The organism may be present in the cervical mucus, uterine flushings, and udder secretions of experimentally infected cows for up to 36 days after abortion.

Identification of *Brucella* spp.

Staining

The appearance of specifically stained smears and the colonial morphology can lead to a presumptive diagnosis of brucellosis. *Brucella* bacteria are not really acid-fast but are resistant to decolonization by weak acids, and the presence of a weakly acid-fast intracellular organism, stained with the Stamp-modified Ziehl–Neelsen method may be suggestive for the presence of *Brucella* spp. in the smear. However, staining has a very limited sensitivity because of the low number of organisms present that may be present in some tissues and body fluids of infected animals. Positive results must be interpreted carefully because of the morphologic similarities of *Brucella* organisms with other pathogens associated with abortion, such as *Coxiella burnetii* or *Chlamydia abortus*.[7] Results, positive or negative, should only be considered presumptive and always need to be confirmed ideally by culture.

Culture

The gold standard diagnostic test continues to be based on isolation and characterization of the organism from the organs and lymph nodes of the fetus, the placenta, milk, vaginal mucus, or uterine exudate. Bacteriologic methods detect the organism directly and thus limit the possibility of false-positive results. Isolation of the organism from the udder secretion of a cow is conclusive evidence of infection. Culture methods are reliable and usually definitive. A range of specific culture media are commercially available. A disadvantage is the long time required for definitive identification. Most culture results are positive between the 7th and 21st day and rarely become positive before the 4th day of culture.[16] Incubation for at least 45 days has been advised before declaring a blood sample negative for *Brucella* spp.[16] Furthermore *Brucella* organisms are among the most dangerous bacteria with which to work in terms of the risk of producing laboratory-acquired infections.[7]

Detection by Polymerase Chain Reaction (PCR)

The PCR-based assays for *Brucella* spp. have been developed and are simple. PCR has been applied to tissues such as aborted fetuses and associated maternal tissues, blood nasal secretions, semen, and food products such as milk and soft cheeses. *Brucella* spp. can be detected in the milk of naturally infected cattle, sheep, goats, and camels using a PCR assay that is more sensitive than the culture method. A further diagnostic advancement of recent years is the Bruce-ladder PCR, which is a multiplex PCR assay that helps to identify and differentiate several *Brucella* spp., including vaccine strains, in a single step.[17]

Serologic Tests

In the absence of a positive culture of *B. abortus*, a presumptive diagnosis is usually made based on the presence of antibodies in serum, milk, whey, vaginal mucus, or seminal plasma.

The antibody response following infection depends on whether or not the animal is pregnant and on the stage of gestation. On average, the agglutinins and complement fixation antibodies become positive 4 weeks following experimental infection during the fourth to sixth months of gestation and not until about 10 weeks if experimental infection occurs 2 months before or after insemination. The serologic diagnosis is considered to be unreliable when applied 2 to 3 weeks before and after abortion or calving.

Any of the currently available serologic tests or combination of tests measures the response of a single animal at one point in time and does not describe the status of the herd. When the tests are used in the recommended sequence and in combination, along with a consideration of accurate epidemiologic data, the limitations of each test can be minimized. None of the tests is absolutely accurate, and there are varying degrees of sensitivity. The result has been the development of a very extensive range of tests, each of which has its own special applicability. The salient features are as follows.

Agglutination Tests

Serum Agglutination Test

The serum (tube) agglutination test (SAT) or microtiter plate variants of it are some of the traditional standard tests, which are widely used, but are not recognized as prescribed or alternative tests. The main limitations are

- Detect nonspecific antibodies as well as specific antibodies from *B. abortus* infection and vaccination
- During the incubation stage of the disease these tests are often the last to reach diagnostically significant levels
- After abortion caused by *B. abortus* they are often the last tests to reach diagnostically significant levels
- In the chronic stage of the disease, the serum agglutinins tend to wane, often becoming negative when the results of some other tests may be positive.

Rose Bengal Test (Buffered Plate Antigen or Card Test)

The rose Bengal test (RBT) is a simple, rapid spot-agglutination test using antigen stained with rose Bengal and buffered to low pH. The test detects early infection and can be used as an initial screening test. False-positive reactions are caused by residual antibody activity from vaccination, colostral antibody in calves, cross-reaction with certain bacteria such as *Yersinia enterocolitica,* and laboratory error. False-negative reactions are observed during early incubation of disease and immediately after abortion. However, the RBT is an excellent test for the large-scale screening of sera. The application of the RBT as a screening test, followed by a confirmatory or complementary test, can markedly increase the proportion of infected cattle that test positive.

For **beef cattle**, screening of herds can be achieved by collecting blood at abattoirs and submitting it to the RBT or tube agglutination test. Reactors are traced back to the herd of origin, and the herd is tested. In heavily infected herds, it is best to remove all cows positive to the RBT even though it is highly sensitive and there will be a small percentage of false-positive cows. In herds where the prevalence of infection is low and where vaccination has been used, this procedure will eliminate too many false-positive cows. In this situation the sera positive to the RBT are submitted to a more definite confirmatory test such as the complement fixation test (CFT), and only those animals reacting to the test are discarded.

Complement Fixation Test

The CFT is one of the prescribed tests for international trade and is widely accepted as a confirmatory test. It rarely exhibits nonspecific reactions and is useful in differentiating titers of calfhood vaccination from those caused by infection. The reactions to the CFT recede sooner than those to the serum agglutination test after calfhood vaccination with the strain 19 vaccine. The CFT titers do not wane because the disease becomes chronic and often the CFT reaches diagnostic levels sooner than the serum tube agglutination test following natural infection. In addition, recent technical laboratory advances have allowed much greater speed and accuracy in doing the CFT and it is now considered to be the nearest approach to a definitive test for infection. Nonetheless, because of its complexity the CFT requires good laboratory facilities and skilled laboratory personnel.

Enzyme-Linked Immunosorbent Assays

Two main types of immunosorbent assay have been used: the indirect and competitive formats.

Indirect Enzyme-Linked Immunosorbent Assay

The indirect enzyme-linked immunosorbent assay (iELISA) has been a useful test during an eradication program, after vaccination has ceased; for screening; or as a supplementary test to the CFT. Several variations of the assay using either whole-cell, smooth lipopolysaccharide (sLPS), or O-polysaccharide (OPS) as an antigen have been validated.[7] The iELISA has gained wide acceptance for serologic diagnosis of bovine brucellosis because of its ability to detect antibody of all isotopes, unlike the conventional tests. The iELISA can be useful in conjunction with the CFT during the later stages of an eradication program, when it is important to reduce the number of false-negative serologic reactions that contribute to the persistence of problem herds. The iELISA has an excellent sensitivity and specificity but cannot distinguish between the antibody response induced by vaccination with *B. abortus* strain 19 and natural infection.

The iELISA has also been developed and validated for milk, and several different variations of this assay are currently commercially available.

Competitive Enzyme-Linked Immunosorbent Assay

The competitive ELISA (C-ELISA) uses monoclonal antibody specific for one of the epitopes of the *Brucella* spp., which makes it more specific than assays using cross-reacting antibody. The C-ELISA is thus more specific but less sensitive than the iELISA. It eliminates most but not all reactions caused by cross-reacting organisms and in most but not all cases, eliminates reactions with residual antibody in animals vaccinated with strain 19.[7] The OIE therefore recommends the further investigation of positive reactors with the C-ELISA using appropriate complementary or confirmatory diagnostic tests.[7]

Fluorescence Polarization Assay

This test can be done outside the diagnostic laboratory, allowing for rapid and accurate diagnosis. The fluorescence polarization assay (FPA) can be done almost anywhere using a portable analyzer, which receives power from a laptop computer, using serum, milk, or ethylenediaminetetraacetic acid (EDTA) anticoagulated blood. The FPA technology has been developed and validated for the serologic diagnosis of brucellosis in cattle, pigs, sheep, goats, bison, and cervids. Sufficient cross-reactivity of the common epitopes of *B. abortus, B. melitensis,* and *B. suis* OPS has allowed for the use of a single antigen for all species of smooth *Brucella* and animals. The FPA was initially developed for testing serum; however, the technology has been extended to testing whole blood and milk from individual animals or bulk tank samples pooled from 2000 or fewer animals. The accuracy results of the FPA equals or exceeds those obtained using other serologic tests such as the buffered antigen plate agglutination test, the milk ring test, the CFT, the IELISA, and the C-ELISA. Validation of studies of the FPA and the C-ELISA for the detection of antibodies to *B. abortus* in cattle sera and comparison to the standard agglutination test, the CFT, and the iELISA, found that the FPA is highly superior. It offers a clear advantage because it is easy to use. Full implementation and acceptance of FPA methods for the diagnosis of brucellosis will necessitate the use of an International Standard Serum panel containing at least a low titer-positive sample and a negative sample.

Brucellin Skin Test

The brucellin skin test presents an alternative immunologic test that can be used to test unvaccinated animals. Tested animals are injected intradermally with 0.1 mL of a standardized brucellin preparation consisting of purified, sLPS-free *Brucella* antigen. The skin thickness at the injection site is measured with Vernier calipers before and 48 to 72 hours after injection. Skin thickening of at least 1.5 to 2 mm at the injection site are considered a positive reaction. This test is among the most specific brucellosis tests available, provided it is conducted with a purified, standardized antigen preparation; serologically negative unvaccinated animals with a positive reaction to the skin test are therefore considered as infected.[7] Because not all infected animals show a positive reaction the test is not recommended as a stand-alone test for the purpose of international trade.

Sensitivity and Specificity of Serologic Tests

Serologic tests must have high sensitivity to ensure that all true serologic reactors are detected. However, with a high sensitivity, a high rate of false-positive reactions may be expected and hence the need for the use of a confirmatory test to identify false-positive reactors. Confirmatory tests must therefore demonstrate a high level of diagnostic specificity and yet maintain an effective diagnostic sensitivity.

It has been recommended to use a buffered *Brucella* antigen test, such as the buffered plate antigen test or the RBT as a screening test. Either the CFT or the indirect enzyme immunoassay is appropriate for use as a confirmatory test in situations requiring a high specificity. The relationships between the quantitative serology and infection status of brucellosis in bison in Yellowstone National Park have been evaluated and found to be similar to those in chronically infected cattle.

Antibodies in Milk

The **milk ring test** is a satisfactory inexpensive test for the surveillance of dairy herds for brucellosis. A small sample of pooled fresh milk or cream, from no more than 25 cows, is tested and the herd is classified only as suspicious or negative. Final determination of the status of a suspicious herd and each animal in it is accomplished by blood testing. The more frequently a herd is tested with the milk ring test, the more effective the test becomes as a method to detect early infections, preventing serious outbreaks in susceptible herds. At least three tests done annually are now required by some regulatory agencies. The major limitation of the test is the dilution factor, which occurs in large dairy herds where large quantities of milk are stored in bulk tanks. To adjust for this dilution effect, larger sample volumes are used with increasing herd size. Although 1 mL of bulk milk is required for herds with up to 150 head, the use of 2 mL for herds between 150 and 450 head and 3 mL for herds with 450 to 700 head has been advised.[7] False-positive reactions have been observed with cattle vaccinated less than 4 months before testing and in samples containing colostrum or mastitic milk.

The milk iELISA test is a sensitive, specific, and inexpensive method for screening large numbers of individual or bulk milk samples for the antibody to *B. abortus.* An ELISA using potassium chloride extract of the organism used on bulk tank milk samples of dairy herds was highly specific and is considered as a highly reliable test for monitoring brucellosis control programs. The combined use of an ELISA and PCR on milk samples gives a sensitivity of 100%.

False-Positive Reactors

A major problem in brucellosis eradication programs has been the false-positive animals

or singleton reactor, which may remain persistently suspicious or positive in a herd that is otherwise considered to be free of brucellosis. It is of some concern because of the unnecessary slaughter of uninfected animals.

Cross-reacting antibodies usually result from exposure to antigen(s) that share antigenic determinants with *Brucella* spp., which are found in a large number of bacteria. The most prominent cross-reaction is with *Yersinia enterocolitica* O:9, which shares the major OPS almost completely with *B. abortus*. Serologic cross-reactions have also been demonstrated between smooth *Brucella* spp. and *Escherichia coli* O116:H21 and *E. coli* O157:H7, *Francisella tularensis, Salmonella* serotypes of Kauffman–White group N, *Pseudomonas maltophilia, Vibrio cholerae,* and *Y. enterocolitica* serotype O:9. Only rarely will naturally occurring *E. coli* O157:H7 infections cause false-positive reactions with standard serologic tests for bovine brucellosis. The standard serologic tests are unreliable in differentiating between *Y. enterocolitica* and *Brucella*-infected cows, but both the lymphocyte transformation and brucellin skin tests could be used to differentiate them.

Other causes of false positives include a *B. abortus*-infected animal, strain 19 residual vaccination titer, and naturally occurring nonspecific agglutinins, which may occur in some cattle populations. These agglutinins are EDTA labile and can be differentiated from agglutinating antibodies by the addition of EDTA to the diluent used in the standard serum agglutination test. The serologic cross-reactions are of major significance when the prevalence of infection has decreased to a very low level. At this stage it becomes much more important to correctly identify the status of animals reacting to the serologic tests for brucellosis.

The incorrect attribution of such reactions to factors other than *Brucella* infection is likely to result in herd breakdowns and failure to control the disease. On the other hand, the misinterpretation of cross-reactions as evidence of brucellosis results in the imposition of unnecessary restrictions and waste of resources. The problem of serologic cross-reactions has resulted in considerable research and an investigation to find laboratory tests, which will accurately distinguish positive, infected animals from positive, noninfected animals. Differentiation of cross-reacting antibodies can be difficult to achieve, especially in the case of *Y. enterocolitica* O:9 antigen, but immunodiffusion, immunoelectrophoresis, and primary binding tests and cross-absorption procedures are useful. The DNA homology of *B. abortus* strains 19 and 2308 has been examined using restriction enzyme analysis. Strain 19 is the official U.S. Department of Agriculture (USDA)-attenuated *Brucella* vaccinal strain for cattle, and strain 2038 is a virulent laboratory-adapted strain that is pathogenic to cattle. The DNA differences between the two strains are small and will require analysis at the DNA sequence level.

The serologic assay of choice for screening samples for antibody to *B. abortus* is the FPA. It is robust, very rapid, and field-adaptable, without subjective results. The C-ELISA is a useful confirmatory assay. The sera from cattle naturally infected with *B. abortus,* vaccinated with *B. abortus* S19, or immunized with *Y. enterocolitica* O:9 or *E. coli* O157:H7, were compared for antibody content to the same bacteria by iELISA, FPA, and C-ELISA. The serologic assay of choice for screening samples for antibody to *B. abortus* is the FPA. Between the two tests, nearly all reactivity to *E. coli* O157:H7 and more than one-half of the sera with antibody to *Y. enterocolitica* O:9 could be eliminated as *Brucella* reactors. These assays, perhaps in combination with a brucellin skin test, may be capable of distinguishing virtually all reactions caused by *Y. enterocolitica* O:9.

NECROPSY FINDINGS

The host responses at the organ and tissue levels have been described and are summarized here. Lymph nodes draining the sites of the early stages of infection have marked germinal center hyperplasia and hypertrophy, accompanied by acute neutrophilic and eosinophilic lymphadenitis. In the later stages of the infection, lymph nodes draining mammary gland, head, and reproductive tract develop chronic granulomatous lymphadenitis, which is usually associated with cortical and paracortical T-cell–dependent lymphoid depletion, germinal center expansion, and deep histiocytic expansion. The spleen may develop lymphoid hyperplasia and histiocytic and plasmacytic expansion in the germinal centers, and the mammary gland usually has a pronounced interstitial lymphoplasmacytic mastitis. In the uterus, there is usually an endometritis, fibrosing mural lymphocytic metritis, and caruncular necrotizing vasculitis, whereas the placenta is colonized with *B. abortus* and has extensive desquamation of fetal chorioallantoic trophoblasts with subsequent hematogenous spread to villous trophoblastic epithelium, and **necrotizing fibrinopurulent cotyledonary placentitis of the placental arcades** accompanied by granulation and intercotyledonary inflammation exudation. The placenta is usually edematous. There may be leathery plaques on the external surface of the chorion, and there is necrosis of the cotyledons. The key microscopic feature of this inflamed chorioallantois is the presence of **intracytoplasmic coccobacilli within chorionic trophoblasts**. The use of modified Ziehl–Neelsen stains on impression smears from fresh placentas can provide a rapid presumptive diagnosis. The fetal lesions consist of marked fibrinopurulent necrotizing bronchopneumonia; monocytic and neutrophilic alveolitis; thromboembolic necrotizing arteritis and lymphangitis; fibrinopurulent pleuritis; and granulomata of the liver, spleen, kidney, and lymph nodes. In **fetuses naturally and experimentally infected** with *B. abortus*, the tissue changes include lymphoid hyperplasia in multiple lymph nodes, lymphoid depletion in the thymic cortex, adrenal cortical hyperplasia, and disseminated inflammatory foci composed mainly of large mononuclear leukocytes.

The affected joints usually develop a fibrinous and granulomatous synovitis with proliferative villous projection formation, proliferative tendovaginitis with lymphoplasmacytic nodule formation, and arthritis with articular erosions, which may be associated with suppurative, granulomatous bursitis. In the testes there are unilateral or bilateral visceral to parietal tunica adhesions, interstitial lymphocyte orchitis with seminiferous tubular degeneration, necrotizing intratubular orchitis, and acute fibrinopurulent periorchitis with infarction. The ampulla may have a unilateral or bilateral granulomatous epididymitis with focally necrotic purulent and calcified sperm granulomata, and the seminal vesicles have unilateral or bilateral necrotizing fibrinopurulent seminal vesiculitis and interstitial lymphocytic, plasmacytic seminal vesiculitis with necrosis.

The distribution of *B. abortus* in experimentally and naturally infected cattle has been examined. In experimentally infected pregnant cows, the most frequently infected specimen was the mammary lymph node; the organism could also be found in other lymph nodes, uterine caruncles, cotyledons, or fetal tissues. In naturally infected heifers, the most frequently infected specimen was the mandibular lymph node. In bulls, the most frequently infected tissues were the mandibular, caudal superficial cervical, subiliac, and scrotal lymph nodes.

The lesions in *Brucella*-positive aborted fetuses and placentas in bison are similar to those in experimental infections of *B. abortus* in bison and cattle. Both *B. abortus* biovar 1 and *B. abortus* biovar 2 were isolated from specimens collected from aborted bison fetuses or stillborn calves and their placentas. The infection can also be associated with death in calves at least 2 weeks of age.

Samples for Confirmation of Diagnosis

- Bacteriology: maternal caruncle; placenta, fetal stomach content, lung (culture, has special growth requirements; cytology, Stamp's or Koster's stain on placental smears)
- Histology: fixed placenta, lung, spleen, brain, liver, kidney; maternal caruncle (light microscopy, immunohistochemistry)

Note the zoonotic potential of this organism when handling carcasses and submitting specimens.

DIFFERENTIAL DIAGNOSIS

The diagnosis of the cause of abortion in a single animal or in a group of cattle is difficult because of the multiplicity of causes that may be involved. When an abortion problem is under investigation, a systematic approach should be used. This includes a complete laboratory evaluation and follow-up inquiries into each herd.

The following procedure is recommended:

- Ascertain the age of the fetus by inspection and from the breeding records.
- Take blood samples for serologic tests for brucellosis and leptospirosis.
- Examine uterine fluids and the contents of the fetal abomasum at the earliest opportunity for trichomonads, and subsequently by cultural methods for *B. abortus, Campylobacter fetus,* trichomonads, *Listeria* spp., and fungi.
- Supplement these tests by examination of urine for leptospires, and of the placenta or uterine fluid for bacteria and fungi, especially if the fetus is not available.
- Examine placenta fixed in formalin for evidence of placentitis.

It is most important that all examinations are done in all cases because coincident infections with more than one agent are not uncommon.

In the early stages of the investigation, the herd history may be of value in suggesting the possible etiologic agent. For example, in brucellosis, abortion at 6 months or later is the major complaint, whereas in trichomoniasis and vibriosis, failure to conceive and prolongation of the diestrual period is the usual history.

Of special interest is epizootic bovine abortion, which is a major disease of rangeland cattle in the western United States. A spirochete has been isolated from the soft tick *Ornithodoros coriaceus* and from the blood of fetuses with lesions of epizootic bovine abortion. The disease occurs at a very high level of incidence but only in cattle introduced to a certain area; resident cattle are usually unaffected. Cattle returned to the area each winter are unaffected after the first abortion. The cows are unaffected systemically. Aborted fetuses show characteristic multiple petechiae in the skin, conjunctiva, and mucosae; enlargement of lymph nodes; anasarca; and nodular involvement of the liver.

In most countries where brucellosis is well under control and artificial insemination limits the spread of vibriosis and trichomoniasis, leptospirosis may be the most common cause of abortion in cattle.

However, surveys in such countries reveal that in about two-thirds of the abortions that occur no causative agent is detectable with routine laboratory techniques. In only 35% of cases was the cause determined, and brucellosis accounted for less than 1% of the total. In an Australian experience, the cause of abortion was determined in only 37% of cases in spite of the submission of the fetus, placenta, and maternal serum. The general procedures for submission of specimens to the laboratory and laboratory methods are available.

Bulls

Infected bulls may be serologically positive or negative, and their semen may be culturally positive or negative, but the organism may be isolated at slaughter. Clinical examination may reveal the presence of epididymitis, orchitis, seminal vesiculitis, and ampullitis. All bulls from known infected herds should therefore be considered as suspicious, regardless of their serologic status, and not be used for artificial insemination.

TREATMENT

Treatment is unsuccessful because of the intracellular sequestration of the organisms in lymph nodes, the mammary gland, and reproductive organs. *Brucella* spp. are facultative intracellular bacteria that can survive and multiply within the cells of the macrophage system. Treatment failures are considered to be caused by the inability of the drug to penetrate the cell membrane barrier instead of the development of antimicrobial resistance.

CONTROL AND ERADICATION

Most countries with brucellosis have programs designed to control and ultimately eradicate the infection in cattle to reduce economic losses and protect the public from the disease. These programs usually have several components, and to ensure effectiveness each component needs to be scientifically sound and accepted by all concerned. The major components of a control and eradication program are as follows.

Test and Reduction of Reservoir of Infection

All breeding cattle in the herd are tested, and those that are positive are culled and sent for slaughter. This removes infected cows from the herd and reduces exposure and transmission within the herd. Of particular importance is the detection and removal of infected cows before parturition.

Quarantine

This is a period of time during which cattle movement is restricted and the cattle are tested. This will prevent interherd transmission by infected cattle, especially those that are test negative and incubating the disease. The quarantine period should be sufficiently long that all cattle have had sufficient time to develop brucellosis and ensure that the remaining cattle will not be a source for interherd transmission. The time will usually range from 120 days to 1 year, or until all breeding animals have completed a gestation without test evidence of infection.

Depopulation

Depopulation is slaughter of all cattle in a herd when all animals have been exposed and are capable of becoming infected and acting as a source of new infection.

Vaccination

Properly vaccinated cattle are less likely to be infected and, therefore, are less likely to shed field strains of the organism. Vaccination strategies will be discussed in more detail below.

Education

All participants in a program must understand and adopt the scientific basis for the program. This includes livestock producers, veterinarians, and regulatory officials.

Guidelines

To be successful, any program needs guidelines and policies, which must be followed and modified to meet the needs of certain areas or herds.

Apart from the question of human exposure to infection, the cost and economic benefits of an eradication program must be assessed against the costs and benefits from a vaccination control program. Certain basic considerations apply to all programs aimed at the eradication of brucellosis.

- The control programs indigenous to any given area must receive primary recognition, and any plan or plans must be adapted to that area
- Cooperation at all levels of government from local to the national is essential for the success of a program. This is attained only after an intensive program of education has been performed. The individual owner of an infected herd must recognize the problem of brucellosis and express a willingness to cooperate. Experience has shown that the owner must be impressed with the hazards of the disease for human health and with the economic losses in the herd
- A reliable and uniform diagnostic procedure must be generally available.
- If disease is detected in a herd, established procedures should be available for handling the disease. If immunization is to be used, a standardized and effective vaccine must be readily available. The disposal of infected animals may create a serious economic threat for the owner and the possibilities of financial compensation must be explored
- Finally, and of major importance, the movement of animals from one area to another must be controlled at a high level, because a rigid eradication program in one area may be nullified by neglect in a neighboring area.

Sufficient information exists about bovine brucellosis that it can be eradicated. The

disease was considered to have been eradicated from Great Britain in 1981; in 1985, having met certain European Community criteria for national surveillance and with over 99.8% of the cattle herds free from brucellosis, all herds within the country not under restrictions were designated as being officially brucellosis free for trade purposes. However, small foci of infection persisted, and following the prohibition of the use of *Brucella* vaccines the national herd was becoming fully susceptible to brucellosis. This was followed by outbreaks of brucellosis in southwest England from 1984 to 1986. The movement of cattle through premises owned by dealers who specialized in the purchase and sale of newly calved cattle was a significant epidemiologic feature of these herd breakdowns.

Control by Vaccination

Because of the serious economic and medical consequences of brucellosis, efforts have been made to prevent the infection through the use of vaccines. Historically brucellosis vaccines were composed of attenuated strains of *B. abortus* and *B. melitensis*. These vaccines were shown to be effective in reducing pathogen transmission and production loss, but were less effective in preventing infection. Another inconvenience of these whole-cell vaccines was that they interfere with diagnostic assays detecting antibody against the O-side chain of the *Brucella* LPS.[18] Currently vaccines used to protect livestock against infection with *B. abortus* contain one of three attenuated live strains of *B. abortus:* strain 19, RB51, and strain 82.

Brucella abortus Strain 19 Vaccine

Vaccines containing the live *B. abortus* strain 19 are the most widely used vaccines to prevent bovine brucellosis and are considered the reference vaccines to which any other vaccine is compared.[7] The vaccine protects uninfected animals living in a contaminated environment, enabling infected animals to be disposed of gradually. This overcomes the main disadvantage of the test and disposal method of eradication, in which infected animals must be discarded immediately to avoid spread of infection. *B. abortus* strain 19 has a low virulence and is incapable of causing abortion except in a proportion of cows vaccinated in late pregnancy. Strain 19 is a smooth *B. abortus* strain expressing the O-antigen on its LPS. Antibody produced in response to vaccination will interfere with diagnostic assays identifying this antigen, which is a major problem with the use of these vaccines. Another weakness of the vaccines is that it cannot completely prevent infection.[18]

Strain 19 vaccines are normally administered to female calves between 3 and 8 months old as a single subcutaneous dose of 5 to 8 $\times$ 10^{10} organisms (**calfhood vaccination**). There is no significant difference between the immunity conferred at 4 and at 8 months of age. Calves vaccinated with strain 19 at 2 months of age have resistance comparable to those vaccinated at 4 to 8 months of age. However, generally, calves under 75 days of age are immunologically immature in response to strain 19 vaccine. Vaccination of calves with a single dose at 3 to 5 weeks of age does not provide protection compared with vaccination at 5 months of age.

In calves vaccinated between the recommended ages, the serum agglutination test returns to negative by the time the animals are of breeding age, except in a small percentage (6%) of cases. The LPS with an O-chain on *B. abortus* strain 19 explains the appearance and persistence of antibodies in serum following vaccination. These antibodies are detectable in the serologic assays used for the diagnosis of brucellosis and are the major problem with strain 19 vaccination, because they prevent easy differentiation of vaccinated from infected cattle. The appearance and persistence of these antibodies depends on age, dose, and route of vaccination. This situation makes the continued use of the vaccine incompatible with simultaneous application of test and slaughter procedures for the control of brucellosis.

In brucellosis-free herds where heifers are vaccinated between 4 and 9.5 months of age, positive titers may persist for up to 18 months if they are tested with screening tests such as the RBT. This supports the official policy in some countries not to test vaccinated heifers before 18 months of age and to retest positive cases with the CFT.

In most control programs, vaccination is usually permitted up to 12 months of age, but the proportion of persistent postvaccinal serum and whey reactions increases with increasing age of the vaccinates. Such persistent reactors may have to be culled in an eradication program unless the reaction can be proved to be the result of vaccination and not due to virulent infection.

Vaccination of adult cattle is usually not permitted if an eradication program is contemplated, but it may be of value in reducing the effects of an abortion storm. Under specific circumstances vaccination of adult cattle with a reduced single subcutaneous dose of 3 $\times$ 10^8 to 3 $\times$ 10^9 viable organisms can be used but will result in persistent antibody titers in some animals. Furthermore, the risk of abortion when vaccinating pregnant animals and the risk of excretion of the vaccine strain in milk has been reported.[7] An alternative vaccination protocol for adult cattle consists in the single or repeated subconjunctival administration of a dose of 5 $\times$ 10^9 living organisms. This latter protocol was reported to reduce the risk of abortion and shedding in milk while providing similar protection.[7]

Vaccination of bulls is of no value in protecting them against infection and has resulted in the development of orchitis and the presence of *B. abortus* strain 19 in the semen. For these reasons the vaccination of bulls is discouraged.

Efficiency of Brucella abortus Strain 19 Vaccine

Calfhood Vaccination. This can be assessed by its effect on both the incidence of abortion and the prevalence of infection as determined by testing. Field tests show a marked reduction in the number of abortions that occur, although the increased resistance to infection, as indicated by the presence of *B. abortus* in milk, may be less marked. Vaccinated animals have a high degree of protection against abortion and 65% to 75% are resistant to most kinds of exposure. The remaining 25% to 35% of vaccinated animals may become infected but usually do not abort. Experimentally, 25% of cattle vaccinated with strain 19 will become infected following challenge. Vaccinated animals continually exposed to virulent infection may eventually become infected and act as carriers without showing clinical evidence of the disease.

In summary, vaccination with a single dose of *B. abortus* strain 19 vaccine given subcutaneously at 3 to 8 months of age confers adequate immunity against abortion for five or more subsequent lactations under conditions of field exposure. Multiple or late vaccinations have no appreciable advantage and increase the incidence of postvaccinal positive agglutination reactions. When breakdowns occur, they are caused by excessive exposure to infection and not by enhanced virulence of the organism. In herds quarantined for brucellosis, calfhood vaccination reduces reactor rates, duration of quarantine, and the number of herd tests.

Adult Vaccination. Vaccination of adult cows with strain 19 vaccine is highly successful in reducing the number of infected cows in large dairy herds in which it is impossible to institute management procedures for the ideal control of brucellosis.

The vaccination of adult cattle with a reduced dose of vaccine is efficacious and results in an agglutinin response that declines more rapidly after vaccination than when the full dose is used. The reduced dose also provides protection comparable to the standard dose. Vaccination eliminates clinical disease and reduces exposure of infection to susceptible cattle. The reduction of infected adult cattle may vary from 60% to 80% in 6 to 9 months following vaccination. The CFT becomes negative sooner than the standard tube agglutination test following vaccination and can be used to distinguish postvaccine titers from culture-positive cows. The use of reduced doses of strain 19 vaccine in adult cows will also help to eliminate the problem of postvaccine titers.

The protection provided by **subcutaneous and conjunctival routes of vaccination** is the same but the subcutaneous route may result in a persistent serologic response, which requires complement fixation testing and milk culture to identify infected animals.

The principal advantages of adult vaccination include the following:

- An effective method of control of abortion
- Reduction in the reactor losses in herds
- Reduction of the number of tests required to eliminate brucellosis from infected herds

The major disadvantages of adult vaccination are

- Residual vaccine titers
- Persistent positive milk ring test
- Persistent strain 19 infection in a small percentage of adult vaccinates
- The stigma attached to adult vaccinates, which identifies them with infected herds, even though brucellosis has been eliminated and the herd released from quarantine

B. abortus strain 19 has been recovered from the supramammary lymph nodes of cattle at slaughter that were vaccinated with a low dose of the vaccine 9 to 12 months previously and had persistent titers to the CFT. The stage of gestation affects the immune responses of cattle to strain 19 vaccination. Cattle that are late in the first or early in the second trimester of gestation (84 to 135 days) at the time of administration of a low dose of strain 19 are at greater risk of being positive by official tests for brucellosis. Vaccination of cattle during the third trimester with a low dose of the vaccine is not as efficacious as when performed earlier. Although reduced-dose strain 19 vaccination is a possible alternative to the total depopulation of problem herds, its use during pregnancy should be avoided because of the risk of abortion and positive serologic titers and positive bulk milk ring tests.

The results expected following adult vaccination depend on the disease situation. In herds vaccinated in the acute phase of the disease, abortion may continue for 60 to 90 days but the incidence begins to decline by 45 to 60 days. A large number of serologic reactors will be present for the first 120 days following vaccination, and testing is usually not done for the first 60 days. The rate of reactors declines rapidly after 120 days and with good infected herd management most adult vaccinated herds can be free of brucellosis 18 to 24 months following vaccination.

The prevalence of *B. abortus* strain 19 infection in adult vaccinated cattle is low and is often not permanent. The prevalence is lower among cattle given the reduced dose of the vaccine subcutaneously. Bacteriologic examination of the milk and serologic examination of the infected cattle are necessary to identify strain 19 infected cattle, which can be retained for milk production because the infections are temporary.

Adult vaccination, even with a low dose, should **not be used in uninfected herds** because of persistent titers, which may last for more than 12 months in up to 15% of vaccinated animals, and because of the potential for abortion. The illegal or unintentional use of the standard dose of strain 19 vaccine in adult cattle will result in a sudden steep antibody titer response in the CFT, which declines in 6 to 11 months. In herds where adult vaccination with a reduced dose of vaccine is used, blood samples should be collected about 4 months after vaccination and subsequently at intervals of 2 months. Those positive to the CFT should be culled. In one study of three large dairy herds in California, the CFT at 2 and 4 months after vaccination was used to identify and cull pregnant reactor cows that were at risk of aborting or calving. The prevention of parturition of infected cows is an effective management technique.

Systemic Reactions to Vaccination With Strain 19

These occur rarely in both calves and adults, and may be more severe in Jersey calves than in other breeds. A local swelling occurs, particularly in adult cattle, and there may be a severe systemic reaction manifested by high fever (40.5–42° C; 105–108° F) lasting for 2 to 3 days, anorexia, listlessness, and a temporary drop in milk production. An occasional animal goes completely dry. The swellings are sterile and do not rupture, but a solid, fibrous mass may persist for many months.

Deaths within 48 hours of vaccination have been recorded in calves after the use of lyophilized vaccine.

B. abortus strain 19 vaccine has been associated with lameness in young cattle with synovitis following vaccination. Experimentally, the intraarticular injection of the vaccine strain can produce synovitis similar to that which occurs following vaccination.

Septicemia due to *B. abortus* may cause some deaths but in most cases the reaction is anaphylactic, and vaccinated calves should be kept under close observation. Immediate treatment with epinephrine hydrochloride (1 mL of 1:1000 solution subcutaneously) or antihistamine drugs is recommended and is effective provided it can be administered in time.

Cows in advanced pregnancy may abort if vaccinated, but the abortion rate is only about 1%; although *B. abortus* strain 19 organisms can be recovered from the fetus and placenta, their virulence is unchanged and they do not cause further spread of infection. Vaccination with strain 19 does not have a deleterious effect on the subsequent conception rate.

Brucella abortus Strain RB51 Vaccine

Brucella abortus strain RB51 (SRB51) is a live, stable, rough mutant of *B. abortus* strain 2308 that lacks much of the LPS O-side chain, therefore, it does not interfere with serologic surveillance tests. Since 1996 vaccines containing SRB51 have become the official vaccines for prevention of brucellosis in several countries.[7] The results of studies comparing the efficacy of SRB51 and strain 19 vaccines in the literature are inconsistent. Generally, SRB51 vaccines are administered subcutaneously to female calves between 4 and 12 months old with a dose of 1 to 3.4×10^{10} living organisms.[7] Heifer calves vaccinated at 3 months, 5 months, or 7 months of age with the SRB51 vaccine were protected when challenged against infection and abortion during their first pregnancy. None of the heifers developed antibodies that reacted in the standard agglutination test. A reduced dose of 1×10^9 viable organisms administered as calfhood vaccine does not protect against *B. abortus* infection.

Vaccination of cattle over 12 months of age may be permitted under some circumstances and is performed by subcutaneous administration of a single dose of 1 to 3×10^9 viable organisms. The use of SRB51 vaccines in pregnant cows is discouraged. The strain RB51 has a tropism for the bovine placental trophoblast and has been associated with placentitis and abortion under field conditions.[7] A reduced dose of an SRB51 vaccine containing 1×10^9 viable organisms given to pregnant cattle was protective against infection with *Brucella abortus* without causing placentitis or abortion but resulted in shedding of the vaccine strain in a significant proportion of vaccinated animals.[7] Vaccination of mature sexually intact bulls and heifers with a standard calfhood dose of SRB51 is not associated with shedding or colonization in tissues, and does not appear to cause any reproductive problems when administered to sexually mature cattle. Use of the vaccine in cattle already vaccinated with strain 19 vaccine will not cause positive responses on confirmation tests and does not interfere with brucellosis surveillance.

Studies with strain RB51 vaccine indicate that it is as efficacious as *B. abortus* strain 19 vaccine but is much less abortigenic in cattle. It does not produce any clinical signs of disease after vaccination and does not produce a local vaccination reaction at the injection site. The organism is cleared from the bloodstream within 3 days and is not present in nasal secretions, saliva, or urine. Immunosuppression does not cause recrudescence, and the organism is not spread from vaccinated to nonvaccinated cattle. The vaccine is safe in all cattle over 3 months of age.

In the United States, strain RB51 vaccine was licensed by the USDA's Animal and Plant Health Inspection Service (APHIS) in 1996 for use in cattle and was approved for use in

the Cooperative State–Federal Brucellosis Eradication Program. Strain RB51 vaccine must be administered by an accredited veterinarian or by a state or federal animal health official. Calves must be vaccinated with the calf dose ($1–3.4 \times 10^{10}$ organisms) between 4 and 12 months of age. Only animals in high-risk areas should be vaccinated over 12 months of age.

Vaccinates must be identified with the standard metal vaccination eartag and a vaccination tattoo. The tattoo will be the same as the tattoo for *B. abortus* strain 19 vaccination except the first digit for the quarter of the year will be replaced with an R to distinguish animals vaccinated with RB51 from those vaccinated with strain 19. Recording and reporting are the same as with strain 19 vaccine. The diagnosis requires special diagnostic tests that are not routinely available in most hospitals. Both strains are sensitive to a range of antimicrobials. Physicians deciding to initiate a metaphylactic treatment in a human patient exposed to the RB51 vaccine strain must be advised that this strain is resistant to rifampin, one of the antimicrobials of choice for the treatment of human brucellosis.

Brucella Vaccines in Wildlife

A reservoir of *B. abortus*-infected bison in the Greater Yellowstone area in the United States is an obstacle in the effort to eradicate brucellosis from the United States and a source of potential reinfection for livestock in the states of Wyoming, Idaho, and Montana. The free-ranging and infected bison in the area migrate from public land on to private lands and may come into contact with cattle. *Brucella*-induced abortions in bison have occurred under experimental and field conditions, and infected bison can transmit brucellosis under range conditions. Wild and free-ranging bison in parts of western Canada have also been shown to be infected with bovine brucellosis. Therefore a safe and effective vaccine suitable for delivery to free-ranging bison in the Greater Yellowstone area and in Canada is considered useful in reducing the risk of transmission and an aid in the prevention and control of the disease.

Brucella abortus Strain 19 in Bison

The use of strain 19 vaccine has been evaluated in pregnant bison and 10-month-old calves, and the results have been unsatisfactory. In adult bison, strain 19 was found to be highly abortigenic, and animals vaccinated as calves were not protected from infection after experimental inoculation in later life.[18]

Brucella abortus Strain RB51 in Bison

The vaccine is safe for vaccination in herds of naive and previously exposed bison calves, young growing bison, adult males, and adult pregnant and nonpregnant females. Fetal lesions do not appear to be significant with bison cows vaccinated with RB51 in early gestation, but placentitis and abortion have occurred incidentally in advanced stages of pregnancy. Limited data from efficacy studies indicate that booster vaccination with strain RB51 vaccines may increase the protection after experimental challenge.[18]

Calfhood vaccination of bison with SRB51 vaccines is efficacious in protecting against intramammary, intrauterine, and fetal infection following exposure to a virulent strain of *B. abortus* during pregnancy. However, these vaccines appear to be less effective in bison than in cattle in protecting from experimental infection. Limited data from efficacy studies indicate that booster vaccination with strain RB51 vaccines may increase the protection after experimental challenge.[18] Calfhood vaccination with SRB51 would be beneficial in a program to reduce the prevalence of *B. abortus* field stains in American bison. As with cattle, SRB51 calfhood vaccination provides a method to prevent transmission and reduce the numbers of susceptible individuals in a bison herd without interfering with serologic identification of *Brucella*-infected animals. Brucellosis management programs in bison and elk are unlikely to be successful if capture and hand vaccination is necessary. The effect of hand vaccination versus ballistic vaccination for vaccination of bison and elk on the immunologic responses to SRB51 has been evaluated. Ballistic delivery may require a greater dose of SRB51 to induce cell-mediated immune responses in bison that are comparable to those induced by hand injection.

Brucella abortus Strain RB51 in Elk (*Cervus elaphus canadensis*)

Several studies conducted in elk using strain 19 and SRB51 vaccines have yielded disappointing results with poor or no protection against experimental infection. Neither single nor repeated doses provided significant protection against *B. abortus*–induced abortion. Following vaccination, elk remain bacteremic for a prolonged period of time, rapidly develop high antibody titers while the cellular immune response is poor or lacking.[18]

Control Programs on a Herd Basis

The following recommendations are based on the need for flexibility depending on the level of infection that exists and the susceptibility of the herd and the disease regulations in effect at the time.

During an Abortion Storm

Test and disposal of reactors may be unsatisfactory during an outbreak because spread occurs faster than eradication is possible. Vaccination of all nonreactors is recommended in some countries or, if testing is impracticable, vaccination of all cattle. It is preferable to retest the herd before the second vaccination and to cull cows with a threefold rise in agglutination titer.

Heavily Infected Herds in Which Few Abortions Are Occurring

These do not present an urgent problem because a degree of herd resistance has been reached. All calves should be vaccinated immediately, and positive reactors among the remainder should be culled as soon as possible. Periodic milk ring tests (preferably at 2-month, and no more than 3-month, intervals) on individual cows are supplemented by complement fixation and culture tests.

Lightly Infected Herds

These present a special problem. If they are situated in an area where infection is likely to be introduced, calfhood vaccination should be implemented and positive reactors immediately culled. If eradication is the goal in the area, culling of reactors will suffice, but special market demands for vaccinated cattle may require a calfhood vaccination policy. When a herd is declared free of brucellosis on the basis of serum agglutination tests, its status can be maintained by introducing only negative-reacting animals from brucellosis-free herds and annual blood testing. In areas where dairying predominates, semiannual testing of milk may be substituted for blood testing.

In all of the previously mentioned programs, the careful laboratory examination of all aborted fetuses is an important and necessary corollary to routine testing. There are many difficulties achieving control and eventual eradication on a herd basis. These relate mainly to the failure of owners to realize the highly infectious nature of the disease and to cooperate fully in the details of the program. Particularly, they may fail to recognize the recently calved cow as the principal source of infection. In a herd control program, such cows should be isolated at calving and blood tested at 14 days, because false-negative reactions are not uncommon before that time.

Hygienic Measures

These include the isolation or disposal of infected animals; disposal of aborted fetuses, placentas, and uterine discharges; and disinfection of contaminated areas. It is particularly important that infected cows be isolated at parturition. All cattle, horses, and pigs brought on to the farm should be tested, isolated for 30 days, and retested. Introduced cows that are in advanced pregnancy should be kept in isolation until after parturition, because occasional infected cows may not show a positive serum reaction until after calving or abortion. Chlorhexidine gluconate is an effective antiseptic against *B. abortus* and is recommended for washing the arms and hands of animal attendants and veterinarians who come into contact with contaminated tissues and materials.

Eradication on an Area Basis by Test and Slaughter and Cessation of Calfhood Vaccination

Following a successful calfhood vaccination program, eradication on an area basis can be considered when the level of infection is below about 4% of the cattle population. Brucellosis control areas must be established and testing and disposal of reactors and their calves at foot is performed. Financial compensation is paid for disposal of reactors. Infected herds are quarantined and retested at intervals until negative; in heavily infected herds complete depopulation is often necessary. Brucellosis-free areas are established when the level of infection is sufficiently low, and the movement of cattle between areas is controlled to avoid the spread of infection.

Farms with a low incidence may find it possible to engage in an eradication program immediately provided the incidence on surrounding farms is low. Breakdowns may occur if there are accidental introductions from nearby farms, and in these circumstances it is hazardous to have a herd that is not completely vaccinated. When the area incidence is low enough (about 5%) that replacements can be found within the area or adjoining free areas, and immediate culling of reactors can be performed without crippling financial loss, compulsory eradication by testing and disposal of reactors for meat purposes can be instituted. Compensation for culled animals should be provided to encourage full participation in the program.

The work of testing can be reduced by using screening tests to select herds for more intensive epidemiologic and laboratory investigation. In dairy herds, the milk ring test conducted on bulk milk samples is useful. In beef herds, the favored procedure is the collection of blood from drafts of cattle at the abattoir and use of the RBT. The same technique has also been used to screen shipments of beef destined for countries with an aversion to meat infected with *B. abortus*. An additional means of reducing labor costs in an eradication program is the use of automated laboratory systems such as the one available for the RBT and the one based on agglutination and CFT. An educational program to promote herd owners to voluntarily submit all aborted fetuses to a laboratory for bacteriologic examination is also deemed necessary in any eradication scheme. When an area or country is declared free, testing of all or part of the population needs to be performed only at intervals of 2 to 3 years, although regular testing of bulk milk samples (milk ring test) and of culled beef cows in abattoirs and examination of fetuses should be maintained as checks on the eradication status. In all eradication programs, some problem herds will be encountered in which testing and disposal do not eliminate the infection. Usually about 5% of such herds are encountered and are best handled by a "problem herd" program. Fifty percent of these herds have difficulty because of failure to follow directions. The other half usually contain infected animals that do not respond to standard tests. Supplementary bacteriologic and serologic tests as set out previously may occasionally help these spreader animals to be identified and the disease to be eradicated.

United States

Efforts to eradicate brucellosis associated with *B. abortus* in the United States began in 1934 as an economic recovery program to reduce the cattle population because of the Great Depression. Brucellosis was considered the most significant livestock disease at that time, with a reactor rate of 11.5%. In 1954, a cooperative federal and state program was launched based on calfhood vaccination and test and slaughter with compensation. Two very effective surveillance programs for detecting brucellosis were the market cattle testing and milk ring testing of dairy herds. On July 10, 2009, all 50 states, Puerto Rico, and the U.S. Virgin Islands were officially classified as class free for bovine brucellosis.[19] The number of human cases of brucellosis declined with the decline in number of cases in animals. As of 2013, about 100 human cases per year are reported of which most cases are associated with consumption of unpasteurized milk and milk products of goat origin infected with *B. melitensis*.

Bison and elk in the Greater Yellowstone area are the last known remaining reservoir of *B. abortus* in the United States. Control of brucellosis in these species on public lands requires special consideration to preserve the largest wild, free-ranging population of bison in the United States. Vaccination trials are under way.

The primary surveillance methods for testing eligible cattle in the United States have been the **market cattle testing** program in the beef industry and the **milk ring testing** in the dairy industry. In 2009, the National Surveillance Unit USDA-APHIS identified considerable redundancies in bovine brucellosis surveillance in regions classified as class free for bovine brucellosis for at least 5 years.[19] Consequently, slaughter surveillance was reduced, and brucellosis milk surveillance was eliminated in 2011.

Market Cattle Testing

Surveillance by this method is part of the marketing process. Testing is done at livestock markets, slaughterhouses, livestock buying stations, or dealer premises. This type of testing is very effective, especially if required at the first point of assembly of cattle after leaving the farm of origin. Until 2011 95% or more of cows and bulls 2 years of age or older were required to be tested for brucellosis at slaughter in the United States. As of 2011 the number of slaughter plants participating in slaughter surveillance testing was reduced to 13 of the 40 top establishments and two bison slaughter plants. These slaughter plants are located in 13 states, representing all regions of the country.

Milk Ring Testing

Surveillance by this method involves the regular, periodic testing of milk or cream from commercial dairy herds. Milk ring testing is required twice annually in commercial dairy herds in states officially declared free of brucellosis, and four times annually in states not officially free of brucellosis. This test is very sensitive and is done on a small sample of milk from the entire herd. The milk ring test itself is simple and inexpensive. A well-managed testing program is important to public health and can reduce the exposure potential of contaminated dairy products to humans by quickly identifying affected herds. Routine brucellosis ring testing was discontinued in the United States in 2011, following the recommendation of the National Surveillance Unit of the USDA-APHIS that had identified redundancies in the diagnostic surveillance of bovine brucellosis in regions free of brucellosis for over 5 years.[19]

Australia

In Australia, under range conditions, considerable progress toward eradication of brucellosis in large beef herds has been possible. Management must be motivated and confident that the disease can be permanently eradicated. All cattle should be permanently identified, security between subherds must be good, vaccination histories must be accurate, and accurate round-up (mustering) of cattle must be possible. Quarantine facilities for infected subherds must be strict and absolutely reliable, and fence lines must be impenetrable. The development of a two-herd system, based on segregation of weaned heifer calves from adult cows and maintenance of testing pressure on the adults, will reduce the chance of infection of heifers. All calves from reactor dams are discarded, which necessitates positive identification. Only bulls or semen from brucellosis-free herds should be used in clean herds. In some situations, a laboratory is established on the ranch and equipped to do RBT and CFT. This increases the efficiency of the testing program and creates an excellent team effort between management, laboratory personnel, and the field veterinarian.

New Zealand

In New Zealand, the brucellosis status of accredited herds is monitored by a triennial CFT with a sensitivity of greater than 95%. Slaughterhouse surveillance, as performed in Australia, has a low probability of identifying infected herds. A skin test for brucellosis is attractive because it could be used at the same time as routine tuberculin testing.

Canada

In Canada, the bovine brucellosis eradication program is a success story that began in 1950 when the national prevalence of infection was about 9%. With the cooperative Federal–Provincial Calfhood Vaccination Program, the prevalence of infection was reduced to 4.5% by 1956. In 1957, a test and slaughter program was begun in which brucellosis control areas were established and mandatory testing of all cattle was done using the tube agglutination test. Reactors were identified and ordered to be slaughtered, and compensation was paid. Infected herds were quarantined and retested until negative or in some cases completely depopulated. When the infection rate was reduced to below 1% of the cattle population and 5% of the herds, the area was certified for a period of 3 years. When the infection rate was reduced to below 0.2% of the cattle in the area and 1% of the herds, the area was designated brucellosis free and certified for a period of 5 years. In the 1960s, the milk ring test and the market cattle testing programs were introduced as surveillance procedures. These are done on a continuing basis, are effective in locating infected herds, and have reduced the volume of on-farm testing required to recertify areas.

When the national level of infection was reduced to below 0.2%, calfhood vaccination was deemphasized to overcome the problem of distinguishing between persistent vaccination titers and titers caused by natural infection. Thus all seropositive animals could be disposed of and no vaccination privileges allowed. In 1973, an increase in the incidence of brucellosis occurred, which necessitated some modifications in the eradication program. The intensity of milk ring testing was increased, herds adjacent to infected herds were tested, the length of quarantine of infected herds was increased, and calves from reactor dams were ordered to be slaughtered. In heavily infected herds and in those in which it is not possible to maintain effective quarantine, it was preferable to completely depopulate a herd rather than conduct tests and successive retests. In the Canadian experience, brucellosis-free herds usually became infected when the owner unknowingly purchased an infected animal. The uncontrolled movement of infected animals from infected herds to brucellosis-free herds was a major obstacle in the final stages of the eradication.

The rate of progress in an eradication program is determined mainly by the rate at which herds that are accredited free of the infection become reinfected. The severity of reinfection (or **breakdown**) is dependent on the proportion of the herd that has been vaccinated as calves. The cessation of compulsory calfhood vaccination results in a large proportion of cattle that are fully susceptible to *B. abortus* infection. The prevention of reinfection requires a constant surveillance system.

Canada was declared free of bovine brucellosis in 1985. In 1997, a comprehensive review of Canada's bovine brucellosis surveillance program was undertaken. As a result of the findings of this review, a number of modifications to the surveillance program were introduced in 1999. The routine serologic testing of market and slaughter cattle and the routine milk ring testing of all dairy cattle were discontinued in 1999. However, auction market testing of cattle 24 months and older continues in the five markets in northern Alberta and British Columbia in response to the disease risk associated with the infected free-roaming bison herds in and around Wood Buffalo National Park.

In April 2000, the vaccination of calves with reduced dosage strain 19 *B. abortus* vaccine was discontinued. Strain RB51 *B. abortus* vaccine is not licensed for use in Canada.

Bovine brucellosis in wildlife is restricted to free-roaming bison in and around Wood Buffalo National Park in northern Canada. Information on this occurrence is found in Canada's report to the OIE Wildlife Diseases Working Group.

FURTHER READING

DelVecchio VG, Wagner MA, Eshenbrenner M, et al. *Brucella* proteomes: a review. *Vet Microbiol.* 2002;90:593-603.

Ragan VE. The Animal and Plant Health Inspection Service (APHIS) brucellosis eradication program in the USA. *Vet Microbiol.* 2002;90:11-18.

Lapaque N, Moriyon I, Moreno E, Gorvel J-P. *Brucella* lipopolysaccharide acts as a virulence factor. *Curr Opin Microbiol.* 2005;8:60-66.

OIE. Bovine brucellosis. OIE Terrestrial Manual. At: <http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.03_BOVINE_BRUCELL.pdf>; 2009 Accessed 25.01.14.

WHO. Brucellosis in humans and animals. At: <http://www.who.int/csr/resources/publications/Brucellosis.pdf>; 2006 Accessed 27.01.14.

REFERENCES

1. Scholz HC, Vergnaud G. *Rev Sci Tech.* 2013;32:149.
2. Díaz-Apparicio E. *Rev Sci Tech.* 2013;32:53.
3. EFSA. *EFSA J.* 2014;12(3547):175.
4. Otlu S, et al. *Acta Vet Brno.* 2008;77:117.
5. Lopez LB, et al. *Open Vet Sci J.* 2010;4:72.
6. McDermott J, et al. *Rev Sci Tech.* 2013;32:249.
7. OIE 2009. At: <http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.03_BOVINE_BRUCELL.pdf>; 2014 Accessed 25.02.14.
8. White PJ, et al. *Biol Conserv.* 2011;144:1322.
9. Scurlock BM, Edwards WH. *J Wildl Dis.* 2010;46:442.
10. Cross PC, et al. *Rev Sci Tech.* 2013;32:79.
11. Schumaker B. *Rev Sci Tech.* 2013;32:71.
12. Poester FP, et al. *Rev Sci Tech.* 2013;32:105.
13. Baldi PC, Giambartolomei GH. *Rev Sci Tech.* 2013;32:117.
14. EFSA. *EFSA J.* 2014;12:3547.
15. The Center for Food Security and Public Health 2007. At: <http://www.cfsph.iastate.edu/Factsheets/pdfs/brucellosis.pdf>; 2014 Accessed 27.01.14.
16. WHO 2006. At: <http://www.who.int/csr/resources/publications/Brucellosis.pdf>; 2014 Accessed 27.01.14.
17. McGiven JA. *Rev Sci Tech.* 2013;32:163.
18. Olsen SC. *Rev Sci Tech.* 2013;32:207.
19. USDA. At: <http://www.aphis.usda.gov/animal_health/animal_diseases/brucellosis/downloads/natl_bruc_surv_strategy.pdf>; 2010 Accessed 27.01.14.

BRUCELLOSIS ASSOCIATED WITH *BRUCELLA OVIS*

SYNOPSIS

Etiology *Brucella ovis.*

Epidemiology Organism carried by sexually mature rams with spread by direct contact or passive venereal infection. Predominantly a disease of sheep, but red deer stags can be naturally infected.

Clinical findings Complete or partial infertility in rams caused by epididymitis. Epididymal abnormality can be detected by palpation in some affected rams. Occasionally abortion in ewes and neonatal mortality in lambs.

Clinical pathology Serology of most value including complement fixation, gel diffusion, and ELISA; semen examination.

Diagnostic confirmation Physical palpation of scrotal contents; serology; culture or PCR of semen, testes, and seminal vesicles, aborted material.

Treatment Oxytetracycline in valuable rams.

Control Total segregation of normal and young rams. Initial culling of rams with palpable scrotal abnormality and subsequent repeated serologic testing and culling of seropositive rams. Where permitted, vaccination with live *B. melitensis* strain Rev. 1 is an alternative.

ETIOLOGY

Brucella ovis has significant DNA homology with other members of the *Brucella* genus and shares antigenic and other characteristics. However, it has a permanently rough phenotype, whereas *B. melitensis* and *B. abortus* colonies are smooth.[1]

EPIDEMIOLOGY

Geographic Occurrence

Brucellosis of sheep associated with *B. ovis* has been reported in most of the major sheep-producing regions of the world, including Australia, New Zealand, North and South America, Central Asia, Russia, South Africa, and Europe, but is not a major cause of ram wastage in Great Britain. When the disease is first diagnosed in a country, and before control procedures are established, the flock prevalence of infection can be as high as 75% and as many as 60% of rams may be infected. The prevalence of infection is generally much lower in

countries and in flocks that have established control programs.

Host Occurrence

In nature mainly sheep are affected, with the ram more susceptible than the ewe. A small number of natural cases occur in farmed red deer (*Cervus elaphus*) in New Zealand, but most infections resolve after 340 days and it is regarded as a self-limiting disease.[2] It is difficult to establish infection in laboratory animals. However, white-tailed deer and goats can be infected experimentally and develop epididymitis. There is no evidence of natural infection in goats, even in those that graze with infected sheep.

The Merino breed and Merino-derived crossbreeds show a much lower incidence of the disease than do British breeds. The disease is most important in large flocks where there is multisire breeding.

Source of Infection

The infected ram is the source of infection and perpetuates the disease in a flock. The majority of infected rams excrete the organism in semen, and in most rams the active excretion in semen probably persists indefinitely. Ewes are more resistant to infection, but the organism can be isolated from them in infected flocks. After being bred by an infected ram, the majority will not carry infection for more than one or two heat cycles. Infection may result in early embryonic death and occasionally abortion or the birth of weak and poorly viable lambs. In ewes where the infection does persist to produce abortion, the organism is present in the placenta, vaginal discharges, and milk.

Transmission

Transmission between rams occurs via passive venereal infection and by direct ram-to-ram transfer. Passive venereal infection occurs from ewes that have been bred by an infected ram in the same heat cycle. Under natural conditions, this may be the major form of transmission from ram to ram during the breeding season. Infection can also be transmitted between rams in the nonbreeding season when housed or grouped together on pasture. This occurs as they sniff and lick each other's prepuce and by homosexual activity. Submissive rams may lick the prepuce of dominant rams as a trait in the dominance hierarchy. Spread of infection in a group of virgin rams is recorded. Lambs born from infected ewes and drinking infected milk do not become persistently infected.

The organism can survive on pasture for several months, but transmission by fomites appears to have no practical significance. However, transmission from infected rams to infection-free red deer stags grazed on the same pasture can occur, and it is not known if this results from direct contact between the animals or indirectly via environmental and pasture contamination.

Host Risk Factors

All postpubertal rams are susceptible to infection, but disease is more common in adult rams and disease prevalence increases with age, probably because of greater exposure to infection. Differences between flocks in the prevalence of disease suggest that environmental factors and stress may modulate susceptibility, but the risk factors are poorly defined. When the number of affected rams in a flock is greater than about 10%, the fertility of the flock is appreciably decreased.

Experimental Reproduction

Experimentally, rams can be infected by the IV, subcutaneous, intratesticular, oral, conjunctival, and preputial routes, but the latter two are the most effective. The first observable abnormality is the presence of inflammatory cells in the semen, which appear at 2 to 8 weeks. *B. ovis* appears in the ejaculate at approximately 3 weeks, but it is not always present in an infected ram after that.[3] Testicular and epididymal lesions can be palpated at about 9 weeks after infection but may occur earlier in some rams. A significant proportion of infected rams have no palpable lesions but still excrete the organism.

Ewes in early pregnancy can also be infected by the oral and IV routes, but many of these infections are transient and do not result in abortion. Abortion caused by placentitis has been produced experimentally. Intrauterine infection produced experimentally also causes lesions in and death of the fetus, but the significance of this to natural cases is undetermined.

Economic Importance

The economic effects of the disease are subtle but significant. The effect of the disease on ram fertility can influence the number of rams that are required in a flock, with the required ram to ewe ratio significantly reduced in *B. ovis*–free flocks. The percentage of lambs born early and within the first 3 weeks of the lambing period is also markedly increased. Lambing percentage may be reduced by 30% in recently infected flocks and by 15% to 20% in those where the infection is endemic. The loss of rams of high genetic potential and the need for repeat serologic testing are additional costs. In the United States, the advantage in a control program has been calculated as an additional return of $12 per ewe mated.

Zoonotic Implications

B. ovis is not a zoonosis, but live *Brucella* vaccines used for prevention of this infection in some countries, such as Rev. 1 *B. melitensis* vaccine, are pathogenic to humans and should be handled and used with care.

PATHOGENESIS

There is an initial bacteremia, often with a mild systemic reaction, and the organism can be isolated from the internal organs of animals slaughtered after experimental infection. However, systemic disease is not a feature of the natural disease, and clinical disease results from localization and inflammation in the epididymides, typically in the tail. Inflammation in this area results in sperm stasis and extravasation with a subsequent immunologic reaction that is often unilateral, causing a spermatocele and reduced fertility. Not all infected rams have palpable lesions in the epididymis, and infection can also establish in the seminal vesicles and ampullae. In either case the organism is shed in the ejaculate.

Generally, *B. ovis* has low pathogenicity for ewes. The primary effect is a placentitis, which interferes with fetal nutrition, sometimes to the point of causing fetal death, but more commonly producing lambs of low birth weight and poor viability.

Analysis of the immune response by microarray hybridization and reverse transcription (RT)-PCR found that infection with *B. ovis* causes upregulation of genes involved in phagocytosis and downregulation of host defense mechanisms, both of which probably contribute to the chronic nature of the infection.[4]

CLINICAL FINDINGS

The first reaction in rams is a marked deterioration in the quality of the semen together with the presence of leukocytes and *Brucella*. Acute edema and inflammation of the scrotum may follow. A systemic reaction, including fever, depression, and increased respiratory rate, accompanies the local reaction.

Regression of the acute syndrome is followed, after a long latent period, by the development of palpable lesions in the epididymis and tunicae of one or both testicles.

The palpation of both testicles simultaneously is the best method of examination. The epididymis is enlarged and hard, more commonly at the tail; the scrotal tunics are thickened and hardened; and the testicles are usually atrophic. The groove between the testis and epididymis may be obliterated.

The abnormalities are often detectable by palpation, but many affected rams show no acute inflammatory stage and others may be actively secreting *Brucella* and poor-quality semen in the chronic stage in the absence of palpable abnormalities. Palpable abnormality of the scrotal contents may be present in less than 50% of serologically positive rams. Affected rams have normal libido.

There are usually no clinical signs in the ewe but in some flocks infection causes abortion or the birth of weak or stillborn lambs, associated with a macroscopic placentitis.

In red deer, only a small proportion of stags infected with *B. ovis* develop epididymitis detectable by scrotal palpation.[5] In contrast to rams, in most stags the infection resolves within 12 months following infection.[2]

CLINICAL PATHOLOGY

Semen examination, including culture of the ejaculate, and serologic tests are used in suspect individuals and in groups of rams. The complement fixation and ELISA tests are by far the most useful; many infected rams have palpably normal scrotal contents and microbiologically negative semen. Ultrasound examination of the scrotal contents can reveal anechoic areas that correspond to foci of fibrosis, but these appear no earlier and are nonspecific, offering no real advantage over scrotal palpation.

Multiplex PCRs to differentiate *B. ovis* from *Actinobacillus seminis* and *Histophilus ovis* have been described for use on semen or urine.[6,7] Real-time PCR has also been used to type *Brucella* from field material, such as ovine placenta, without the need for culture.[8]

Semen Examination

A combination of semen examination and palpation of the testicles for abnormalities will identify approximately 80% of infected rams. In affected animals the findings are a general reduction in semen quality, a reduced total sperm output, poor motility, and a high proportion of spermatozoa with secondary morphologic abnormalities.

Culture

B. ovis is fastidious in its growth and requires special cultural techniques. The examination of the semen for the presence of leukocytes has been used to determine those sheep that should be cultured for *B. ovis,* but it is not a highly sensitive screening test. PCR for detection of *B. ovis* in semen has an equivalent sensitivity to culture.

Serology

The CFT, the standard test in many countries, is the prescribed test for international trade, and when used in conjunction with genital palpation has allowed the eradication of *B. ovis* from flocks. However, a small proportion of infected rams are negative to CFT, which can compromise or delay eradication programs. The sensitivity and specificity of the various serologic tests depend mainly on the antigens used and the serologic cut points, which may vary between countries and laboratories. A UK study reported the sensitivity of an ELISA, gel diffusion, and CFT as 97.6, 96.4, and 92.7%, respectively, with all tests 100% specific. Studies in other countries support this ranking, but others suggest that the ELISA has no advantage over the classic complement and gel diffusion tests. A combination of serologic tests may increase the sensitivity closer to 100%, but will obviously increase testing costs. Seroconversion occurs slightly earlier with the ELISA, compared with the complement fixation and gel diffusion tests, so it may be useful in situations where infection is rapidly spreading within a group of rams.[9]

Serologic tests will not differentiate vaccinated from infected sheep or sheep infected with *B. melitensis.*

NECROPSY FINDINGS

In the acute stage, there is inflammatory edema in the loose scrotal fascia, exudate in the tunica vaginalis, and formation of granulation tissue. In the chronic stage, the tunics of the testes become thickened and fibrous and develop adhesions. There are circumscribed indurations in the epididymis and these granulomata may also be present in the testicle. In advanced stages, they undergo caseation necrosis. As the epididymis enlarges the testicle becomes atrophied. *B. ovis* can usually be isolated from the genital organs, especially the tail of the epididymis, and rarely from internal organs and lymph nodes. Similar lesions are described in red deer stags.[5]

The abortus is characterized by thickening and edema, sometimes restricted to only a part of the placenta, with firm, elevated yellow-white plaques in the intercotyledonary areas and varying degrees of cotyledonary necrosis. Microscopically, organisms are visible within the cytoplasm of trophoblasts of the inflamed placenta. A vasculitis is often present. The organism can be isolated from the placenta and the stomach and lungs of the lamb.

Samples for Confirmation of Diagnosis

- Bacteriology and PCR: epididymal granuloma, seminal vesicle, inguinal lymph node/fetal lung, stomach content, placenta (culture, has special growth requirements; cytology, Stamp's or Koster's stain on placental smear; PCR)
- Histology: formalin-fixed epididymis, testicle, seminal vesicle, inguinal lymph node from rams; in abortions placenta, fetal lung, liver, spleen, kidney, heart, brain

DIFFERENTIAL DIAGNOSIS

Infection with *Actinobacillus seminis* and *Histophilus ovis* can cause similar scrotal lesions, although many rams with abnormalities of intrascrotal tissues do not have brucellosis or infectious epididymitis.

Abortion in ewes may be associated with a number of infectious diseases, which are summarized in Table 18-1.

TREATMENT

Treatment of naturally occurring cases is rarely undertaken. IM administration of long-acting oxytetracycline at 20 mg/kg body weight (BW), given every 3 days for 24 days, along with the daily IM administration of 20 mg/kg of dihydrostreptomycin sulfate, resulted in bacteriologic cure of 90% of experimentally infected rams. Oxytetracycline alone is less effective, but the use of dihydrostreptomycin is prohibited in food-producing animals in many countries. Treatment is economically feasible only in valuable rams and must be instituted before irreparable damage to the epididymis has occurred. The treatment of rams that are infected but without palpable lesions results in a significant improvement in breeding soundness classification on examinations subsequent to treatment.

CONTROL

Control is by preventing the spread of infection between rams and detecting and culling infected rams. In small flocks, culling of all rams and replacement with *B. ovis*–free rams may be the most cost-effective approach. Some control can be achieved using scrotal palpation to detect infected rams, but this must be combined with repeated serologic testing if eradication is the goal. Vaccination may be the most economical and practical means of controlling the disease in areas with a high incidence of infection and in regions of the world where eradication by test and slaughter is impractical.

Eradication

In a flock where the diagnosis has been confirmed all rams are palpated and those with scrotal abnormalities are culled. The remaining rams are tested serologically and reactors culled. Serologic tests are repeated at monthly intervals, with culling of reactors, until all rams are serologically negative. Further tests, 6 and 18 months later, are used to confirm eradication.

Infection spreads rapidly during the mating season, so eradication should be delayed until after the breeding season. During breeding it may be wise to run two breeding flocks, with virgin rams and rams known to be free of infection separated from older or suspicious rams (seropositive and/or those with scrotal lesions). Strict separation of the two ram flocks must be maintained at all times, and the clean group must not mate ewe flocks that have been mated to the suspect rams.

Several countries have voluntary accreditation schemes based on inspection of boundary fencing, restricting the introduction of new rams to those from accredited flocks and serologic testing.

Vaccination

A number of vaccines have been used, but none is fully effective. In some countries, vaccination is not permitted and eradication by test and slaughter is the only method of control.

Table 18-1 Diagnostic summary of infectious abortion in ewes

		Epidemiology and diagnosis			Laboratory findings	
Disease	*Transmission*	*Time of abortion*	*Clinical data*	*Fetus*	*Serology*	*Vaccination*
Brucellosis (*Brucella ovis*)	Passive venereal, ram to ram	Late or stillbirth, weak lambs	Abortion in ewes, epididymitis in rams	Organisms in fetal stomach and placenta	CFT or ELISA	In some countries simultaneous *B. abortus* strain 19 and killed *B. ovis* vaccine, or *B. melitensis* Rev. 1 vaccine
Campylobacter fetus or *C. jejuni*	Ingestion High stocking rate, intensive grazing, and supplementary feeding on the ground increases risk	Mainly young ewes; last 6 weeks of pregnancy, stillbirths, weak lambs	Metritis in ewes after abortion	*Campylobacter* in stomach, large necrotic foci in liver	Agglutination test, flock only	Formalin-inactivated bivalent vaccine can increase live lambs by around 10%; variable efficacy depending on which strains are present
Enzootic abortion of ewes (*Chlamydophila abortus*)	Ingestion	Last 2–3 weeks. Stillbirths, weak lambs	No sickness in ewes, neonatal mortality	*Chlamydophila* in fetal cotyledons Degenerative changes in placenta	ELISA, CFT, PCR	Killed vaccine gives moderate immunity. Live attenuated vaccine
Listeriosis (*Listeria monocytogenes*)	Probably ingestion	After 3 months	Retained placenta and metritis Septicemia in some ewes	Organisms in fetal stomach Autolysis, necrotic foci in liver	Agglutination and complement fixation of doubtful value	In some countries killed or live attenuated vaccines
Salmonellosis (*Salmonella abortusovis*)	Probably ingestion Carrier sheep	Last 6 weeks	Metritis after abortion	Organisms in fetal stomach Not in the United States	Agglutination test	Doubtful efficacy
Salmonellosis (*S. dublin, S. montevideo, S. typhimurium*)	Ingestion	Last month	Abortion: fetal metritis, neonatal mortality	Organisms in stomach	Agglutination test	—
Toxoplasmosis	Ingestion	Late or stillbirths Live-born weak lambs	Abortion, stillbirths, and neonatal mortality; no illness in ewe	Multiple small necrotic foci in fetal cotyledons Toxoplasma in cells of trophoblast epithelium	Modified agglutination test, ELISA of limited value in adult; test pleural fluid of fetus PCR	Live S48 tachyzoite in some countries (e.g., UK, New Zealand); single dose 3 weeks before mating
Rift Valley fever	Insects	—	Important cause of abortion in all species in Central Africa Heavy mortality in young animals	Acidophilic inclusions hepatic cells	Hemagglutination inhibition and ELISA Fluorescent antibody for tissues	Available in endemic countries
Coxiellosis (Q-Fever)	Inhalation, ingestion	Later term and weak lambs	No illness in ewe, neonatal mortality	Fetus fresh, Intercotyledonary necrotizing placentitis	Fluorescent and PCR; serology of limited value	Vaccine available in Europe but not in most other countries
Tick-borne fever	Ticks	Late, following systemic disease	Fever and abortion	None specific	Giemsa smear of blood, PCR Counterimmunoelectrophoresis	None
Border disease	Ingestion	All stages, stillbirth	Infertility in ewes, hairy shaker lambs	Virus isolation	See text description	None that are specific for sheep strains

CFT, *complement fixation test;* ELISA, *enzyme-linked immunosorbent assay;* PCR, *polymerase chain reaction.*

Killed *B. ovis* vaccines, even with adjuvants, have poor efficacy. The use of a killed vaccine may be inadvisable in flocks where eradication is being attempted, because it may protect against clinical disease but allow a carrier state in some rams in which there is excretion of the organism in animals that become seronegative. An experimental vaccine prepared from enriched OMPs and rough LPS of *B. ovis* gave equivalent protection in challenge studies to that given by *B. melitensis* Rev. 1 vaccine.

A combined vaccine containing killed *B. ovis* in an adjuvant and *B. abortus* strain 19 also provided durable immunity but had several disadvantages. Vaccinated animals become seropositive, which compromises the subsequent use of serologic tests for eradication. Strain 19 also can cause epididymitis, and vaccinated rams may excrete strain 19 in their semen.

Live *B. melitensis* strain Rev. 1 has been found to be most effective and is the most widely used vaccine, where permitted. This strain was developed in the 1950s from a virulent isolate that had become streptomycin dependent. It is avirulent for rams, and subcutaneous or conjunctival vaccination provides protection against experimental and field challenge. Vaccinated animals become positive to the complement fixation and ELISA tests, but titers are low and can be minimized by using the conjunctival route for vaccination. However, vaccinated animals can excrete *B. melitensis* strain Rev. 1, and it can cause abortions, so alternative vaccine candidates are being evaluated. These include an OMP extracted from *B. melitensis* (Omp31) and an attenuated strain of *B. ovis* (Delta abcBA). The latter protects against experimental challenge with virulent *B. ovis* and is considered a potential vaccine strain for rams.[10]

If vaccination is used there should also be a program of culling clinically abnormal rams, and ram replacements should be yearlings vaccinated at 4 to 5 months.

FURTHER READING

Ridler AL, West DM. Control of *Brucella ovis* infection in sheep. *Vet Clin North Am Food Anim Pract*. 2011;27:61-66.

REFERENCES

1. Whatmore AM. *Infect Genet Evol*. 2009;9:1168.
2. Ridler AL, et al. *New Zeal Vet J*. 2006;54:85.
3. Carvalho Júnior CA, et al. *Small Rumin Res*. 2012;102:213.
4. Galindo RC, et al. *Vet Immunol Immunopathol*. 2009;127:295.
5. Ridler AL, et al. *New Zeal Vet J*. 2012;60:146.
6. Saunders VF, et al. *Aust Vet J*. 2007;85:72.
7. Moustacas VS, et al. *BMC Vet Res*. 2013;9:51.
8. Gopaul KK, et al. *Vet Rec*. 2014;175:282.
9. Ridler AL, et al. *New Zeal Vet J*. 2014;62:47.
10. Silva AP, et al. *PLoS ONE*. 2015;10:e0136865.

BRUCELLOSIS ASSOCIATED WITH *BRUCELLA SUIS* IN PIGS

Brucella suis infection may be inapparent or may result in stillbirths, abortion, and infertility in both sexes. In boars it causes infection of the testicles and accessory sex glands. It will cause disease in man[1].

SYNOPSIS

Etiology Disease in pigs is caused by *Brucella suis* biovars 1–3. Biovars 1–4 cause rare disease in cattle.

Epidemiology Disease in pigs is transmitted by contact, ingestion, and venereally.

Clinical findings
Sows: Infertility, irregular estrus, small litters, and abortion.
Boars: Orchitis, lameness, incoordination, and posterior paralysis.
Piglets: Mortality.

Clinical pathology Isolation of organism. Several serologic tests available but none with good sensitivity.

Necropsy Metritis, orchitis, osteomyelitis. Granulomatous inflammation and foci of caseous necrosis.

Diagnostic confirmation Isolation of *B. suis* and herd serology tests.

Treatment None satisfactory.

Control Serologic testing and disposal of reactors. No effective vaccine. Humans, and occasionally cattle. Transmission congenital or by ingestion or contact with infected placenta, vaginal discharge, or milk.

Clinical findings Abortion storms, abortions often in last 2 months of pregnancy. Weak-born lambs.

Clinical pathology Culture of organism. Serologic tests and skin hypersensitivity testing for herd diagnosis.

Necropsy findings Placentitis.

Diagnostic confirmation Only by isolation of the organism.

Control Slaughter eradication. Vaccination with Rev. 1 vaccine, which will produce abortion in pregnant animals.

ETIOLOGY

It is a small, aerobic, gram-negative *Bacillus*. Remember that *B abortus* and *B. melitensis* will also occasionally infect the pig, however, only *B. suis* will cause systemic and generalized infections in pigs. The other species will infect pigs, but the infection is self-limiting and the infection is usually restricted to the local lymph nodes. There are five biovars.

EPIDEMIOLOGY

Geographic Occurrence

Biovar 1

Biovar 1 is important in pigs and occurs worldwide, but the disease has not been recorded in the UK, Canada is disease free, and the prevalence is very low in the United States. It is particularly important in the Philippines and the Pacific islands and Africa.

Biovar 2

Biovar 2 occurs in pigs in west central Europe, particularly Croatia and Czechoslovakia, and also in hares. There appears to be a close relationship between pigs and wildlife in this strain and wild boar in particular.[2] Occasionally it appears in cattle, dogs, and horses.

Biovar 3

Biovar 3 has a close similarity to *B. melitensis* biovar 2 and requires phage typing, oxidative metabolic testing, or PCR for differentiation. It also occurs in pigs in the United States, South America, and southeast Asia. It is a problem in wild boar where it may reach 8% to 32% prevalence,[3,4] and particularly in Italy[5] and Spain[6] the spill over from wild boar to domestic pigs is a particular problem.[7]

Biovar 4

Biovar 4 is a cause of rangiferine brucellosis (reindeers, caribou, bison, moose, etc.) and can transmit to cattle but does not appear to be a disease of pigs. It will transmit to humans.

Biovar 5

Biovar 5 is murine brucellosis. It may also include *B. microti,* which has been isolated from voles and wild rodents in Russia.[8]

Host Occurrence

Domestic, wild, or feral pigs are the host for biotypes 1 and 3, and widespread infection in feral pigs is recorded in Queensland, Australia, and the southern states of the United States. Bison may remain reservoirs. Incursion to domestic pigs from wild boar is an increasing problem.

Cattle and horses may be infected, especially if they share a range with feral pigs, and this association adversely affects the status of cattle herds undergoing brucellosis eradication programs. Cattle are noncontagious hosts, but an outbreak in Switzerland where the disease had not appeared since 1946 has been attributed to a spread of infection from horses.

Biovar 1

Biovar 1 has been isolated from the semen of a ram. Infection in dogs, usually symptomless but occasionally producing orchitis or epididymitis, or granulomas can result from eating raw pig meat.

Biovar 2

In addition to the pig, the European hare (*Lepus capensis*) is also a major host for biovar 2, and this biovar is common in central Europe. Some studies have suggested that the type found in hares in Europe is a different strain from the wild boar.[9]

Biovar 4

Biovar 4 can transmit to cattle in contact with infected reindeer. Wild canids can also be naturally infected with biovar 4, presumably by ingestion.

Source of Infection

Infected boars can shed 10^4 to 10^7 colony-forming units (CFU) of *B. suis* per milliliter of semen. The bacterium is also shed in the milk.

The introduction of infected pigs, usually a boar or the communal use of an infected boar, is the common means of introduction of the bacterium into a pig unit. Artificial insemination using noncertified or untreated semen can also spread the disease as can ova. Transmission usually requires direct or close contact and is usually oral. Discharges in milk and uterine secretions are infectious. Sows may be carriers and piglets can spread the disease horizontally. It is thought that infection through the conjunctiva is also a possibility. It probably does not survive in the environment unless contained in organic matter under cold conditions. Within a piggery the disease is spread by ingestion and by coitus. The ingestion of food contaminated by infected semen and urine and discharges from infected sows are also important methods of spread. Dried secretions, if frozen, may remain infective. Most disinfectants and sunshine kill the virus.

The feeding of kitchen waste containing raw pig meat also presents a risk. Domestic herds are also at risk when they are kept under extensive husbandry methods in areas where there is a high prevalence of infection in feral pigs. Cattle infected with biovar 1 are noncontagious to other livestock and can have normal pregnancies and give birth to uninfected calves.

Wild animals, including hares and rats, may provide a source of infection with biovar 2, and ticks are also suspected of transmitting the disease.

Host and Pathogen Risk Factors

The fact that *B. suis* survives so well in raw meat, e.g., 128 days in sausage meat, means that prepared pork products are always a source of infection. They can survive freezing for over 2 years. Environments and pastures can be infected for a long period of time.

B. suis is more resistant to adverse environmental conditions than *B. abortus,* although its longevity outside the body has not been fully examined. It is known to survive in feces, urine, and water for 4 to 6 weeks. As the environmental temperature rises, the survival in the environment decreases. It is also deactivated by bright sunlight. It has also been known to survive desiccation.

Among pigs, susceptibility may vary with age. The prevalence of infection is much higher in adults than in young pigs, although this may represent an exposure risk rather than an age-related risk. Susceptibility is much greater in the postweaning periods and is the same for both sexes, but there may also be genetically determined differences in susceptibility. Some piglets acquire infection from the sow, either from the ingestion of infected milk or by congenital infection.

Lateral spread through a herd is rapid because of the conditions under which pigs are kept. No durable herd immunity develops and, although a stage of herd resistance is apparent after an acute outbreak, the herd is again susceptible within a short time and the bacteria can spread rapidly on entry to a herd. Within a few months 50% may be infected and 70% to 80% may be involved at the start of the outbreak. Further outbreaks may occur if infection is reintroduced.

In an enzootic area, the proportion of herds infected is usually high (30%–60%). The prevalence of seropositivity in an infected herd varies but can be as high as 66%. Seroprevalence in feral pigs is also high, is higher in adult pigs than pigs under 6 months of age, and varies between populations of feral pigs.

Economic Importance

The disease is economically important because of infertility and reduction in numbers of pigs weaned per litter. Mortality in live-born piglets, which occurs during the first month of life, may be as high as 80%. The mortality rate is negligible in mature animals, but sows and boars may have to be culled because of sterility, and occasionally pigs are culled because of posterior paralysis. In addition, eradication involves a great deal of financial loss if complete disposal of a registered herd is undertaken.

Zoonotic Implications

Biovar 2 is not a zoonosis, but biovars 1, 3 (as pathogenic as *B. melitensis*), and 4 have considerable significance for public health and are very pathogenic to humans. In countries where pigs are a significant part of animal farming and the human diet, *B. suis* is the major cause of human brucellosis (e.g., South America).[10,11]

B. suis presents an occupational hazard, particularly to abattoir workers, and to a lesser extent to farmers and veterinarians and hunters.[12] *B. abortus* and *B. melitensis* may also be found in pig carcasses and present similar hazards. *B. suis* can be widespread in the carcass of infected pigs, and undercooked meat can be a source of human infection. This is particularly true for wild boar and feral pig meat. A recent experiment described infection with biovar type 1 and its transmission to negative pigs after 4 to 6 weeks. Antibody was detected in blood samples from farmers and abattoir workers.

In infected cattle, *B. suis* localizes in the mammary gland without causing clinical abnormality and, where cattle and pigs are run together, the hazard to humans drinking unpasteurized milk may be significant. Biovar 4 causes human disease associated with consumption of caribou.

Human brucellosis at a pig slaughterhouse in Argentina has been described.[13] The median age of the slaughterhouse workers was 40 (23–65) and they had worked for 1 to 9 years in the slaughtering or butchery part of the plant. A systemic or localized disease with recurrent episodes was described. The chronic disease may be progressive. The patients' serum antibody titers (SAT) titers ranged from 1:25 to 1:12.800 and CFT from 1:10 to 1:1280. Of the pigs tested, 11% of the males (7/62) and 18% of the females (25/138) were positive. It is suggested that the swine keepers did not send infected animals for incineration but sent them to slaughter. Diagnoses are rarely made on farms that breed pigs. Such pigs arriving at packing plants have high levels of organisms but rarely have lesions and genital infections that may be a major source of infection. Protective clothing, such as gloves, protective clothing, eye protection, and protection of any bare skin, is essential.

PATHOGENESIS

Infection is followed by multiplication in the local lymph nodes. Only $10^{4\text{-}7}$ organisms will produce an experimental infection, but the severity of the infection is not correlated with either the dose or the route of infection. As for the other species, *B. suis* requires the *vir*B operon-encoded T455 for intracellular invasion and multiplication within host cells. The T455 mutants are not able to survive and multiply in macrophages or epithelial cells.

As in brucellosis associated with *B. abortus,* there is initial systemic invasion possibly through the M cells of the lymphoid tissue in the gut, but also possibly the oral, nasopharyngeal, conjunctival, or vaginal mucosa. There is generally a long period of incubation before clinical signs appear. In young animals these are not necessarily visible and will depend mainly on the age, sex, and physiologic state of the animals at the time they are infected. The organism then appears in the bloodstream, usually within 1 to 7 weeks, and often lasts for 5 weeks but can persist for up to 34 weeks. However, infection with *B. suis* differs from that associated with *B. abortus* in that localization occurs in several organs in addition to the uterus and udder, and the organism is found in all body tissues and produces a disease similar to undulant fever in humans. The organisms persist in lymph nodes, joints, bone marrow, and the genital tract. The more common manifestations of localization are abortion and infertility caused by localization in the uterus; lymphadenitis, especially of the cervical lymph nodes; arthritis and lameness caused by bone and joint localization; and posterior paralysis caused by osteomyelitis. In boars, involvement of the testicles

often leads to clinical orchitis, and the boars are probably infected for life. Widespread infection makes handling of the freshly killed carcass hazardous and creates a risk for brucellosis in humans eating improperly cooked pork.

CLINICAL FINDINGS

Do not forget that clinical signs in pigs may also be produced by *B. abortus* and *B. melitensis*. Porcine brucellosis is usually a more generalized and chronic disease than bovine brucellosis.[14]

The clinical findings in swine brucellosis vary widely, depending on the site of localization. The signs are not diagnostic, and in many herds a high incidence of reactors is observed with little clinical evidence of disease. Reproductive inefficiency is the common manifestation.

Sows

Infection at service usually results in early abortion, sometimes as early as 17 days after natural service with infected boars, with return to estrus at 5 to 8 weeks after service, which may be the only sign that infection has taken place.

Infertility, irregular estrus, small litters, and abortion occur. Later infection will give rise to mummification and stillbirths. The incidence of abortion varies widely between herds but is usually low and is usually early. Infection of the fetus may lead to abortion. As a rule, sows abort only once in a lifetime, and this is most common during the third month of pregnancy. Affected sows usually breed normally thereafter. Sows may remain carriers and may shed organisms in milk and uterine discharges, which may be extremely bloody and may be accompanied by endometritis and retained fetal membranes.

Boars

Orchitis with testicular swelling, epididymitis, and necrosis of one or both testicles is followed by sterility usually within 7 weeks of infection. Lameness, incoordination, and posterior paralysis are fairly common. The onset is gradual, and signs may be caused by arthritis or, more commonly, osteomyelitis of lumbar and sacral vertebral bodies. Testicular atrophy may result at around 19 weeks. Boars have a low rate of recovery (less than 50%). After infection, enough animals remain infected to perpetuate the disease.

In both sows and boars, the bones and joints may be involved, and in these cases there may be posterior paralysis and lameness. Nodules may be seen in the spleen and liver and abscesses may be seen in boars.

Piglets

A heavy mortality in piglets during the first month of life is sometimes encountered, but most piglet loss results from stillbirths and the death of weak piglets within a few hours of birth. Up to 10% may contract infection when they are young and retain the infection until adulthood.

CLINICAL PATHOLOGY

Culture

Laboratory identification of the disease is difficult. It should be routine to use more than one culture method.[15] Isolation of the organism should be attempted if suitable material is available. Such material for culture includes aborted fetuses, testicular lesions, abscesses, blood and lymph nodes (particularly the submandibular, gastrohepatic, and external iliac nodes).[16] The organism is a small, slender, aerobic gram-negative organism that produces 1- to 2-mm colonies on blood agar after 2 to 4 days. A new method of culture has been described for *B. suis* called LNIV-M.[17] Interestingly, in a study of wild boar the organism was isolated from 93% of males but only 61% of females.[18]

PCRs using the *omp* 2b gene or RT-PCR may be more reliable.[19] *B. suis* can be differentiated from the other species by PCR,[20-22] although it may be less successful than culture.[23]

A fingerprinting technique based on a PCR method for multilocus variable number tandem repeat analysis (MLVA) has been developed.[24]

There is no PCR test for differentiating the five biovars from each other.[25]

Serology

Antibodies are usually developed 6 to 8 weeks after infection. These tests are only useful on a herd basis. There is no satisfactory serologic test. Some animals remain seronegative to all tests. Recently indirect or competitive ELISAs have been developed and may be 98% and 100% specific.[26]

An ELISA compared with complement fixation was found to be just as sensitive and as specific a test for both pigs and hares for *B. suis* infections. A meat juice ELISA has also been shown to be a valuable method for testing both hares and wild boars. There is considerable individual variation in the antibody response of pigs following infection, and some may be culture positive but have negative or indefinite titers to the common tests. Pigs under 3 months of age have a poor antibody response to infection.

Serologic tests in common use include the rose Bengal plate agglutination test, Rivanol test, rose Bengal card test, complement fixation, agar gel immunodiffusion, and tube agglutination. The preferred test varies between countries but most use the rose Bengal plate or card test. *B. abortus* antigens are used for diagnosis because *B. suis* has the same surface LPS antigens. Estimates of the sensitivities of the complement fixation and tube agglutination tests range from 40% to 51%, and they range from 62% to 79% for the rose Bengal plate test. The immunodiffusion test has poorer sensitivity than the standard serologic tests. The sensitivity and specificity of all the tests have been shown to vary with the stage of infection in the experimental disease, and it has been recommended that more than one test should be used for diagnosis. A recent study showed a range of sensitivity from 84% to 100% with the CFT low at 84% and the serum agglutination test high at 100%. The sensitivities ranged from 79.7% to 100%, with the serum agglutination test low at 79.7% and iELISA and C-ELISA high at 100%. A recent validation of the polarization assay as a serologic test for the presumptive diagnosis of porcine brucellosis has shown promise. Tests have been reviewed,[26,27] and both authors say that the problem is cross-reaction with *Yersinia* O9.

NECROPSY FINDINGS

On necropsy, there may be arthritis, posterior paralysis, spondylitis, and abscess formation in both sexes. The lesions are usually granulomatous as a result of persistent cytokine release, and these may be in the liver, kidney, spleen, and reproductive tracts.

Many organs may be involved in chronic cases. Chronic metritis manifested by nodular, white, inflammatory thickening, 2 to 5 mm in diameter, and abscessation of the uterine wall is characteristic with or without hemorrhage and necrosis. Arthritis may be purulent, and necrosis of vertebral bodies in the lumbar region may be found in lame and paralyzed pigs. The clinical orchitis of boars is revealed as testicular enlargement or atrophy and testicular necrosis, often accompanied by lesions in the epididymis and seminal vesicles. Splenic enlargement and pronounced lymphadenopathy, caused by hyperplasia of mononuclear phagocytes, occur in some cases. Typical histologic changes consist of granulomatous inflammation with neutrophils, macrophages, and giant cells and hyperplasia of reticular tissues and foci of caseous necrosis in the liver, kidney, spleen, and reproductive tract.

DIAGNOSIS

Diagnosis is suggested by the clinical signs, the necropsy findings, clinical pathology, and epidemiologically by the presence of wild boar locally. None of the tests is capable of diagnosing disease in the individual animal. The real problem of diagnosis is the cross-reactions with *Y. enterocolitica* O:9 infection.[28] In a survey of slaughter pigs in the UK 10% were found to have *Y. enterocolitica* in their gut.[29] There are false positives caused by this organism in initial screenings as the antibody lasts 2 to 9 weeks following *Y. enterocolitica* infection. They can be eliminated by testing for cellular immunity by measuring the IFN-γ generation by leukocytes.

Internationally accepted tests for swine brucellosis include ELISAs, FPA, RBT, buffered plate agglutination test, and the CFT.

Samples for Confirmation of Diagnosis

- Bacteriology: *adults,* culture swab from joint, lymph nodes, spleen, uterus, epididymis, or other site of localization; *fetus,* lung, stomach content, placenta (has special growth requirements)
- Histology: formalin-fixed samples of above tissues (light microscopy)

Note the zoonotic potential of this organism when handling carcasses or submitting specimens.

DIFFERENTIAL DIAGNOSIS

The protean character of this disease makes it difficult to differentiate. Syndromes that need differentiation include:

- Abortion and infertility in sows
- Posterior paresis diseases of spinal cord
- Mortality in young pigs is also caused by many agents, and the important entities are listed in Chapter 19 in the section on Perinatal Disease—General Epidemiology.

TREATMENT

Treatment with a combination of streptomycin parenterally and sulfadiazine orally, or with tetracycline, is ineffective, although combinations of oxytetracycline, streptomycin, and possibly gentamicin have been used.[30] It is unlikely that treatment will ever be attempted on a commercial scale.

CONTROL

Vaccination

No suitable vaccine is available.[31] Strain 19 *B. abortus, B. abortus* "M" vaccine, living attenuated *B. suis* vaccines, and phenol and other extracts of *B. suis* are all ineffective. In a recent study, a natural rough mutant of *B. suis* that does not induce adverse clinical effects or tissue localization but does induce significant humoral and cellular immune responses after vaccination in swine has been observed.[32] The antibody responses to infection in any case are often not powerful enough to eliminate infection.

Test and Disposal

In herds where the incidence of reactors is high, complete disposal of all stock as they reach marketing age is by far the best procedure because of the difficulty in detecting individual infected animals. This is most practicable in commercial pork-producing herds. Restocking the farm should be delayed for 6 months after thorough disinfection is complete. The existing serologic tests can be used for certifying herds free of infection that can then provide replacement stock. Repopulation programs can also use specific pathogen-free pigs.

The alternative is to commence a two-herd segregation program, and this is recommended for purebred herds that supply pigs for breeding purposes. Total disposal is not usually economical in these herds. Once a herd diagnosis has been established, all the breeding animals must be considered to be infected; all piglets at weaning are submitted to the serum agglutination, Rivanol, or other test and, if negative, go into new quarters to start the nucleus of a free herd. It is probably safer to wean the pigs as young as possible and test again before mating. If complete protection is desired, these gilts should be allowed to farrow only in isolation, should then be retested, and their piglets used to start the clean herd. A modified scheme based on the previously mentioned method of weaning and isolating the young pigs as soon as possible but without submitting them to the serum agglutination test has been proposed, but its weakness is that infections may occur and persist in young pigs.

After eradication is completed, breakdowns are most likely to occur when infected animals are introduced. All introductions should be from accredited free herds, should be clinically healthy, and be negative to the serum agglutination test twice at intervals of 3 weeks before introduction.

Eradication of swine brucellosis from an area can only be achieved by developing a nucleus of accredited free herds and using these as a source of replacements for herds that eradicate by total disposal. Sale of pigs for breeding purposes from infected herds must be prevented.

With the advent of infection in wild boar and feral pigs, it is essential to maintain an effective separation from them when there are domestic pigs, and this is especially true where there are outdoor pig units. Recently contaminated wood has been shown to be a problem.[33]

REFERENCES

1. Meirelles-Bartolli RB, et al. *Trop Anim Health Prod.* 2012;44:1575.
2. Wu N, et al. *J Wildl Dis.* 2011;47:868.
3. Koppel C, et al. *Eur J Wildl Dis.* 2007;53:212.
4. Munoz PM, et al. *BMC Infect Dis.* 2010;10:46.
5. Bergagna S, et al. *J Wildl Dis.* 2009;45:1178.
6. Closa-Sebastia F, et al. *Vet Rec.* 2010;167:826.
7. Cvetnic Z, et al. *Rev Sci Tech.* 2009;28:1057.
8. Audic S, et al. *BMC Genomics.* 2009;10:352.
9. Lavin S, et al. *Informacion Veterinaria.* 2006;10:18.
10. Lucero N, et al. *Epidem Infect.* 2008;136:496.
11. Ariza J, et al. *PLoS Med.* 2007;4:e317.
12. Irwin MJ, et al. *N S W Public Health Bull.* 2009;20:192.
13. Escobar GL, et al. *Comp Immunol Microbiol Infect Dis.* 2013;36:575.
14. Megid J, et al. *Open Vet Sci.* 2010;4:119.
15. De Miguel MJ, et al. *J Clin Microbiol.* 2011;49:1458.
16. Abril C, et al. *Vet Microbiol.* 2011;150:405.
17. Ferreira AC, et al. *Res Vet Sci.* 2012;93:565.
18. Stoffregen WC, et al. *J Vet Diagn Invest.* 2007;19:227.
19. Hinic V, et al. *BMC Vet Res.* 2009;5:22.
20. Garin-Bastuji B, et al. *J Clin Microbiol.* 2008;46:3484.
21. Lopez-Goni I, et al. *J Clin Microbiol.* 2008;46:3484.
22. Mayer-Scholl A, et al. *J Microbiol Methods.* 2010;80:112.
23. Bounaadja L, et al. *Vet Microbiol.* 2009;137:156.
24. Garcia-Yoldi D, et al. *J Clin Microbiol.* 2007;45:4070.
25. Ferrao-Beck L, et al. *Vet Microbiol.* 2006;115:269.
26. McGiven JA, et al. *Vet Microbiol.* 2012;160:378.
27. Praud A, et al. *Prev Vet Med.* 2010;104:94.
28. Jungersen G, et al. *Epidemiol Infect.* 2006;134:347.
29. Milnes A, et al. *Epidemiol Infect.* 2008;136:739.
30. Grillo MJ, et al. *J Anim Chemother.* 2006;58:622.
31. Stoffregen WC, et al. *Am J Vet Res.* 2006;67:1802.
32. Stoffregen WC, et al. *Res Vet Sci.* 2013;95:451.
33. Calfee MW, Wendling M. *Lett Appl Microbiol.* 2012;54:504.

BRUCELLOSIS ASSOCIATED WITH *BRUCELLA MELITENSIS*

SYNOPSIS

Etiology *Brucella melitensis.*

Epidemiology Disease of goats, sheep, humans, and occasionally cattle. Transmission congenital or by ingestion or contact with infected placenta, vaginal discharge, or milk.

Clinical findings Abortion storms, abortions often in last 2 months of pregnancy. Weak-born lambs. Important zoonotic disease in humans.

Clinical pathology Polymerase chain reaction (PCR) and culture of organism. Serologic tests and skin hypersensitivity testing for herd diagnosis.

Necropsy findings Placentitis.

Diagnostic confirmation Isolation of the organism, PCR.

Control Slaughter eradication. Vaccination with *B. melitensis* Rev. 1 vaccine, but this can cause abortion in pregnant animals.

ETIOLOGY

B. melitensis causes brucellosis in goats and sheep, is capable of infecting most domestic animal species, and is the primary cause of brucellosis of humans (Malta fever) in many countries. There are three biovars of the organism that have differing geographic distribution, but no difference in pathogenicity or animal species affected. There is a close relationship to other members of the genus, which currently has 10 species but is expanding with the advent of molecular typing.[1]

EPIDEMIOLOGY

Geographic Occurrence

The distribution of *B. melitensis* is more restricted than that of *B. abortus* and its primary area of occurrence is in the Mediterranean region, including southern Europe. Infection is also present in west and central Asia, Mexico, countries in Central and South America, and in Africa. Northern Europe is free of infection, except for periodic incursions from the south, as are Canada, the United States, southeast Asia, Australia, and New Zealand.

The prevalence of infection varies between countries and regions, but in many

countries the prevalence has declined in the past 20 years in association with mandatory vaccination policies. However, in many others it is not effectively controlled because of the low incomes or nomadic nature of those who farm small ruminants. Hence it is regarded as a neglected but very important disease of livestock and humans in developing countries.[2,3]

Host Occurrence

Goats and sheep are highly susceptible. Susceptibility in sheep varies with the breed, with Maltese sheep showing considerable resistance. The organism is capable of causing disease in cattle and has been isolated from buffalo, yaks, camels, and pigs.

Source of Infection

The source of infection is the infected carrier animal. Introduction to a naive herd or flock occurs with the introduction of an infected animal, and persistence results from sheep or goats that are prolonged excreters. Excretion is from the reproductive tract and in milk.

Reproductive Tract

Infected does and ewes, whether they abort or give birth normally, discharge many brucellas in their uterine exudates and placenta. The organism can be present in uterine discharge for at least 2 months following parturition in infected goats. The vaginal exudate of infected virgin or open animals may also contain the bacteria, but transmission between animals is most likely from the massive exposure provided by an infected placenta.

Milk

The majority of goats infected during pregnancy will excrete the organism in milk in the subsequent lactation and many will excrete it in all future lactations. In sheep, the period of excretion of the organism from the uterus and in milk is usually less than in goats, but the organism can be present in milk throughout lactation. The duration of excretion in cattle is not known.

Transmission

Routes of infection for both adults and young are via ingestion, by nasal or conjunctival infection, and through skin abrasions, with infected placenta and uterine discharge as a major source.

In Utero Infection

Infection of the fetus during pregnancy does not necessarily result in abortion: infected kids and lambs may be born alive but weak, or they may be quite viable. In some cases the infection persists in a latent form until sexual maturity, when pregnant animals may abort the first pregnancy. However, others, if weaned early from their dams and from the infected environment, become free from the infection as adults.

Colostrum and Milk

Latent infection can also be acquired from the ingestion of infected colostrum and milk. This is a major route of transmission and perpetuation of infection in a herd or flock.

Host and Pathogen Risk Factors

The organism is reasonably resistant to environmental influences and under suitable conditions can survive for over 1 year in the environment. *B. melitensis* is susceptible to disinfectants in common use at recommended concentrations.

In goats and sheep, the infection of a naive herd or flock will produce an abortion storm, following which most animals are infected but immune, and further abortions are usually limited to young or introduced animals. Because of the limited periods of excretion in sheep the disease tends to be self-limiting in small flocks that have few new introductions. It can be a continuing problem in large flocks because of massive environmental contamination of areas used for pregnant and lambing ewes. In some areas the prevalence of brucellosis associated with *B. melitensis* is linked to the practice of animal movement to summer and mountain pastures in which there is commingling of sheep and goats from a variety of sources on the same pasture.[3]

Spread in beef cattle is slow, presumably because they are usually farmed at lower stocking rates, whereas spread in dairy herds can be more rapid and extensive.

Economic Importance

Brucellosis has major veterinary and human importance in affected countries. Costs include production loss associated with infection in animals, the considerable cost of preventive programs, and human disease. There is further loss from restriction in international trade in animals and their products.

The occurrence of *B. melitensis* in the sheep and goat population of countries that have eradicated *B. abortus* poses a threat for the continuing occurrence of brucellosis in cattle herds.

Zoonotic Implications

B. melitensis is the most invasive and pathogenic for humans of the three classical species of the genus, and is the cause of Malta or Mediterranean fever in humans, which is an extremely debilitating disease. It is an important zoonosis in areas of the world in which *B. melitensis* is enzootic in goats and sheep. The disease in humans is severe and long-lasting and often occurs in communities with limited access to antimicrobial therapy. Control and eradication of the infection in animal populations has high priority in all countries.

Large numbers of organisms are excreted at and following parturition, providing a source of infection for humans managing the herd or flock and also for people in the immediate vicinity from aerosol infection with contaminated dust. The risk of infection is high in cultures that cohabit with their animals or when weak, infected newborn animals are brought into the house for warmth and intensive care. Milking of sheep and goats is usually manual, often with poor sanitation and milking-time hygiene. Raw milk and cheese products from infected goats, sheep, or cattle also provide a risk and were the mechanism for the occurrence of Malta fever that initiated the definition of the disease.

Abattoir workers, shearers, and people preparing goat and sheep skins are also at risk. The risk for veterinarians is primarily from assisting birthing in infected animals and herds, but is also the examination of any animal that is subclinically infected. There is also the risk of accidental self-inoculation with live vaccine.

Vaccination of small ruminants with *B. melitensis* Rev. 1 vaccine is a primary method in controlling the human disease. In Greece, a 15-year period of vaccination was associated with a drop in the incidence of human brucellosis, but when this program was stopped the prevalence of abortions in animals and the incidence if brucellosis in humans increased dramatically, only to be controlled by the reinstitution of vaccination of animals as an emergency mass vaccination program. However, although the Rev. 1 vaccine is attenuated compared with field strains, it retains some virulence and incorrect selection from the seed stock can result in vaccines with considerable virulence for both vaccinated animals and in-contact humans.

Because of its pathogenicity to humans and animals, *B. melitensis* is listed as an agent of bioterrorism and agroterrorism. It is thought that fewer than 10 CFU are capable of infecting humans via aerosols. This would require mass therapy of human populations and destruction of animal populations.

PATHOGENESIS

The organism is a facultative intracellular parasite. As in other forms of brucellosis, the pathogenesis depends on localization in lymph nodes, udder, and uterus after an initial bacteremia. In goats, this bacteremia may be sufficiently severe to produce a systemic reaction, and blood culture may remain positive for a month. Localization in the placenta leads to the development of placentitis, with subsequent abortion. After abortion, uterine infection persists for up to 5 months, and the mammary gland and associated lymph nodes may remain infected for years. Spontaneous recovery may occur, particularly in goats that become infected when they are not pregnant. In sheep, the development of the disease is very similar to that in goats. In cattle, *B. melitensis* has a similar pathogenesis and produces a persistent

infection in the mammary gland and the supramammary lymph node, with obvious significance for public health.

CLINICAL FINDINGS

Abortion during late pregnancy is the most obvious sign in goats and sheep, but as in other species there may be a storm of abortions when the disease is introduced, followed by a period of flock resistance during which abortions do not occur. Abortion is most common in the last 2 months of pregnancy. The excretion of the organism in milk is not accompanied by obvious signs of mastitis. Infection in males may be followed by orchitis, which is frequently unilateral.

In experimental infections, a systemic reaction occurs with fever, depression, loss of weight, and sometimes diarrhea. These signs may also occur in acute, natural outbreaks in goats and may be accompanied by mastitis, lameness, and hygroma; however, they are uncommon in the natural disease and their occurrence in the experimental disease reflects a massive challenge dose. Osteoarthritis, synovitis, and nervous signs may occur in sheep.

In pigs, the disease is indistinguishable clinically from brucellosis associated with *B. suis*.

In many instances, *B. melitensis* infection reaches a high incidence in a group of animals without signs of obvious illness, and its presence may be first indicated by the occurrence of disease in humans infected from the herd or flock. This is so in cattle where the infection is subclinical and does not produce abortion, but the organism is shed in milk.

CLINICAL PATHOLOGY

Culture and Molecular Tests

Positive blood culture soon after the infection occurs and isolation of the organism from the aborted fetus, vaginal mucus, or milk are the common laboratory procedures used in diagnosis. The organism is moderately acid fast, and staining smears from the placenta and fetus with a modified Ziehl–Neelsen method may give a tentative diagnosis; however, this does not distinguish this infection from *B. ovis* or the agent of enzootic abortion (*Chlamydia abortus*), and culture is required.

The organism can be detected by PCR in the abomasal fluid of aborted fetuses and, compared with culture, PCR has a sensitivity and specificity of 97.4% and 100%, respectively. PCR can also be used to detect the organism in tissues, semen, and milk. A real time RT-PCR has been used to type Brucella from field samples, such as ovine placenta, without the need for culture.[4]

Multilocus variable-number tandem repeats analysis (MVLA) is an alternative to classical biotyping and may be useful in analyzing the epidemiology and source of outbreaks. For example, in 2011 a strain of *B. melitensis* in a single infected flock in Sardinia, a region of Italy free from this disease since 1998, was confirmed as being a rare America lineage and probably originating from Spain.[5] Multiplex PCR and high-resolution melt point analysis has also been used to differentiate *Brucella* spp.

Serology

The conventional serologic tests for the diagnosis of *B. melitensis*—agglutination, CFT, and the rose Bengal or card test—use the same antigens that are used for the diagnosis of *B. abortus* infections (either whole cells or sLPS).

The RBT and CFT are the most widely used. These, plus iELISA and FPA are prescribed tests for international trade.[6] The RBT is not 100% specific, but is typically used as a screening test with the CFT applied in series or parallel. RBT or CFT is not sufficiently sensitive to accurately detect infection in an individual animal. Nevertheless, they can be used to detect infected herds for slaughter eradication of the disease. They can be used for test and slaughter programs within an infected herd, but their reduced sensitivity makes this strategy less effective in sheep and goats compared with cattle. A combination of these tests and tests performed on several occasions may increase the accuracy of detection of infected animals. If only one test is possible, the CFT is recommended, but it suffers from the requirement for a sophisticated laboratory, which is not always available in affected areas.

Conventional serologic tests will not differentiate infection with different species of *Brucella* and will not differentiate infection associated with *Y. enterocolitica* type O:9.

Several ELISA tests have been evaluated for use in small ruminants, some using recombinant antigens such as Omp31 and others using whole-cell antigens. These include indirect, competitive, and blocking ELISAs. A C-ELISA had a diagnostic sensitivity ranging from 74% to 89%, depending on cutoff values, and a specificity from 93% to 97%.[7] Comparisons of the FPA and commercial ELISA tests with the RBT and CFT have shown no great advantages over the older tests, with the iELISA often having a slightly greater sensitivity. Overall testing sensitivity may be improved if these tests are used in parallel.[7,8]

Brucella-free animals are serologically positive for long periods following vaccination with *B. melitensis* Rev. 1, with varying persistence in different serologic tests. The period of seropositivity is shorter in animals vaccinated conjunctivally.

Milk Tests

The milk ring test used for testing pooled (bulk) milk in cattle is not useful in small ruminants. Other tests include whey CFTs, whey Coombs or antiglobulin test, whey agglutination tests, and a milk ELISA. They have no apparent advantage over serologic tests, and in many cases they are less sensitive, hence, they are not suitable as screening tests using pooled milk samples.

Allergic Tests

An intradermal allergic test using 50 mg of brucellin INRA (purified and free from LPS) can be used for diagnosis. The injection sites in goats are the neck or caudal fold and in sheep the lower eyelid, with reactions read in 48 hours. The test has high specificity in flocks that are free of infection and not vaccinated. However, it has little advantage over conventional serologic tests in infected herds, and Rev.-1–vaccinated animals can react for at least 2 years. It has particular value in identifying some animals that are false-positive reactors, differentiating infections with *Y. enterocolitica* but not *B. ovis*. Anergy occurs between 6 and 24 days after injection.

NECROPSY FINDINGS

There are no lesions that are characteristic of this form of brucellosis. The causative organism can often be isolated from all tissues but the spleen, lymph nodes, and udder are the most common sites for attempted isolation in chronic infection.

Samples for Confirmation of Diagnosis

- Bacteriology: *adults,* spleen, lymph node, udder, testicle, epididymis; *fetus,* lung, spleen, placenta (culture: has special growth requirements; cytology: Stamp's or Koster's stain on placental smear; PCR); *fetus,* PCR of fetal abomasal fluid
- Histology: formalin-fixed samples of the previously listed tissues

The zoonotic potential of this organism means care needs to be taken when handling potentially infected material, and specimens should be properly packaged when submitted to a laboratory.

DIFFERENTIAL DIAGNOSIS

The primary differential is from other forms of brucellosis (seen in this chapter) and other causes of abortion in small ruminants.

TREATMENT

Treatment is unlikely to be undertaken in most animals because it is unlikely to be economically feasible or therapeutically effective. For example, a dose of 1000 mg per animal of long-acting tetracycline given every 3 days for 6 weeks achieved a cure rate of 75%.

CONTROL

Hygiene

Control measures must include hygiene at kidding or lambing and the disposal of infected or reactor animals. Separate pens for kidding does that can be cleaned and

disinfected, early weaning of kids from their does and their environment, and vaccination are recommended. In endemic areas, all placentas and dead fetuses should be routinely buried.

Eradication

Where a group is infected for the first time it may be most economical to dispose of the entire herd or flock, because eradication by test and slaughter is prolonged by the lack of sensitivity of the serologic tests.

Many countries that have this disease have statutory control measures and the disease can be eradicated, such as from Cyprus. *B. melitensis* also can be eradicated, with difficulty, from dairy cattle. However, vaccination may be the only practical method of control in areas in which there is a high prevalence of the disease, extensive management systems, communal and nomadic grazing, and a low socioeconomic level.

Rev. 1 Vaccination

Rev. 1 vaccine is a live, attenuated *B. melitensis* strain derived from a virulent *B. melitensis* isolate that is resistant to dihydrostreptomycin. It is the reference vaccine strain that provides protection against infection with *B. melitensis* in sheep and goats and against infection with *B. ovis* in rams. However, this vaccine has significant disadvantages, including persistent serologic response and, although attenuated compared with field strains, it retains some virulence. Incorrect selection from the seed stock can result in vaccines with considerable virulence for both vaccinated animals and in-contact humans.

Vaccination with Rev. 1 produces a bacteremia that is cleared by 14 weeks in goats and a shorter time in sheep. Vaccination at 3 to 8 months of age confers a high degree of immunity that lasts for more than 4 years in goats and 2½ years in sheep. The initial recommendations were to vaccinate replacement animals with the expectation that herd/flock immunity would develop over time. However, this has proved ineffective in some regions, and whole-flock/herd vaccination is now recommended in certain countries.

Vaccination of pregnant goats and sheep, especially in the second and third month of pregnancy, will result in abortion and the excretion of the living *B. melitensis* vaccine organism in the vaginal discharge and the milk. Consequently, the vaccine should not be used in pregnant animals or for 1 month before breeding. Vaccination of lactating animals may be followed by excretion of the organism in the milk for a short time. Reduced dose vaccination or conjunctival vaccination does not significantly reduce the risk of vaccine-induced abortions in pregnant animals, although reduced-dose Rev. 1 vaccination has been shown to provide protection for at least 5 years in endemically infected areas.

Conjunctival vaccination does decrease the period of seropositivity following vaccination. Vaccine efficacy and safety can vary with the manufacturer. National policies promoting widespread vaccination of sheep and goats with Rev. 1 vaccine have resulted in a significant reduction in the prevalence of small ruminant brucellosis and in the incidence rates of human brucellosis. However, Rev. 1 vaccine is also pathogenic to humans and its excretion, and persistence in milk following vaccination can result in human infection.

The general approach in endemically infected countries is to institute a whole-flock vaccination scheme followed by a young-stock vaccination until the prevalence of the disease is reduced, at which time test and slaughter can be implemented to eradicate the disease. This ignores the risk of adverse disease in the vaccinated animals and the risk for human infection from the vaccine strain. There is an urgent need for a nonvirulent vaccine that induces seropositivity that can be differentiated from the seropositivity resulting from natural infection.

Other Vaccines

To circumvent the problem of persistent serologic response, ongoing efforts have been made to develop defined rough mutant vaccine strains that would be more effective against *B. melitensis*. Various studies have examined cell-free native and recombinant proteins as candidate protective antigens, with or without adjuvants. However, limited success has been obtained in experimental models with these, or with DNA vaccines encoding known protective antigens.[9]

B. abortus strain 19 has been used for vaccination and appears to give protection that is as good as that achieved with the attenuated *B. melitensis* vaccine.

FURTHER READING

Blasco JM, et al. Control and eradication of *Brucella melitensis* in sheep and goats. *Vet Clin North Am Food Anim Pract.* 2011;27:95-104.

Whatmore AM. Current understanding of the genetic diversity of *Brucella,* an expanding genus of zoonotic pathogens. *Inf Genet Evol.* 2009;9:1168-1184.

REFERENCES

1. Whatmore AM. *Inf Genet Evol.* 2009;9:1168.
2. Ducrotoy MJ, et al. *PLoS Negl Trop Dis.* 2014;8(7):e3008.
3. Kasymbekov J, et al. *PLoS Negl Trop Dis.* 2013;7(2):e2047.
4. Gopaul KK, et al. *Vet Rec.* 2014;175:282.
5. De Massi F, et al. *Transbound Emerg Dis.* 2015;62:463.
6. OIE. Terrestrial Manual. 2012 Ch 2.7.2; 968.
7. Minas A, et al. *Vet J.* 2008;177:411.
8. Fiasconaro M, et al. *Small Rumin Res.* 2015;130:252.
9. Da Costa Martins R, et al. *Expert Rev Vaccines.* 2012;11:87.

ABORTION IN EWES ASSOCIATED WITH *SALMONELLA ABORTUSOVIS*

Salmonella abortusovis (*S. enterica* serovar Abortusovis) is a gram-negative rod-shaped aerobic bacterium of the family Enterobacteriaceae. The pathogen is highly adapted to sheep and is considered to be host specific for this species in which it can cause abortion. *S.* Abortusovis infection has a worldwide occurrence with a generally low prevalence. The infection appears to be more common in some European and Western Asian countries.

Transmission and spread of the infection occurs through infected animals that are introduced to flocks naive to the pathogen. The reservoir of infection is infected animals that do not abort. The organisms persist in internal organs of the **asymptomatic carriers** for up to 6 months and are excreted in the feces and vaginal mucus for periods up to 4 months. Infection can occur through the oral, conjunctival, or respiratory route, but oral ingestion is thought to be the main mode of infection. Venereal spread has been postulated, and rams certainly become infected, but all the evidence is against spread at coitus. Intrapreputial inoculation results in infection of rams and the passage of infected semen for up to 15 days.

The only significant clinical sign of *S.* Abortusovis infection is abortion, which is common during the second half to last third of gestation. Lambs may also be stillborn or die within the first day of life. Mortality in lambs is common from either weakness and ensuing hypothermia and hypoglycemia or to the development of acute pneumonia in previously healthy lambs up to 2 weeks old.

In flocks naive to the infection, introduction of the pathogen can cause abortion storms, with up to 60% of ewes aborting generally in the last trimester of gestation. Ewes rarely develop clinical signs, although some may transiently have a fever or develop postabortive endometritis with vaginal discharge. Septic metritis and peritonitis in dams has been associated with deaths among ewes. Spread of the disease is strongly associated with the presence of aborting ewes and subsequent heavy environmental contamination. In flocks where the pathogen is endemic, abortion occurs sporadically, mainly affecting primiparous and newly introduced ewes. The infection appears to induce a strong immune response preventing abortion during the following pregnancies.[1]

Identification of the disease depends on isolation of the organism, which is present in large numbers in the fetus, placenta, and uterine discharges. Use of PCR to identify *S.* Abortusovis is feasible because the organism has an IS200 element in a distinct chromosomal location. The resulting PCR assay has high specificity for *S.* Abortusovis, effectively

discriminating it from other *S. enterica* serovars. The disease can be diagnosed in fetuses by using a coagulation test on fetal stomach contents. The test had a sensitivity and specificity of 100% and 90% in a small number of samples.

Serologic tests to detect antibody to *S. abortusovis* include the SAT, hemagglutination inhibition, complement fixation, indirect immunofluorescence, gel immunodiffusion, and ELISA.

A strong immunity develops after an attack, and an autogenous vaccine has shown good results in the control of the disease.[1] The results of vaccination need to be very carefully appraised because flock immunity develops readily and the disease tends to subside naturally in the second year.

The clinical findings in *S.* Dublin infections in ewes are very similar, and infection has become more important as a cause of abortion in ewes in the UK than *S.* Abortusovis. *S.* Ruiru has also been recorded as a cause of abortion in ewes, and ewes with salmonellosis associated with *S. typhimurium* may also lose their lambs. *S.* Brandenburg is a cause of illness and abortion in sheep, horses, calves, goats, and humans in New Zealand. Other **differential diagnoses** for abortion in ewes include chlamydiosis, brucellosis, campylobacteriosis, listeriosis, coxiellosis (Q-fever), and toxoplasmosis.

The administration of broad-spectrum antibiotics might aid in controlling an outbreak, but available reports are not generally encouraging. Chloramphenicol and the trimethoprim and sulfadiazine combination are considered effective for treatment, but use of chloramphenicol in animals intended for human food production is not permitted in many countries. A live *S. typhimurium* vaccine with optimal level of attenuation for sheep constructed by means of "metabolic drift" mutations was highly effective in preventing *S.* Abortusovis–induced abortions under field trial conditions. Subcutaneous and conjunctival vaccination with a live attenuated strain of *S.* Abortusovis confers immunity for at least three lambing periods. More recent vaccines, including those containing plasmid-cured strains of *S.* Abortusovis, are effective in preventing pregnancy loss in response to experimental challenge with wild-type *S.* Abortusovis.

To contain the spread of the infection during an outbreak aborted ewes should be isolated and abortion products that contain large amounts of bacteria must be destroyed. Disinfection of stalls and fomites with an agent with proven efficacy against *Salmonella* spp. is important.

FURTHER READING

Jack EJ. *Salmonella* abortion in sheep. *Vet Annu.* 1971;12:57.

REFERENCE

1. Cagiola M, et al. *Vet Microbiol.* 2007;121:330.

ABORTION IN MARES AND SEPTICEMIA IN FOALS ASSOCIATED WITH *SALMONELLA ABORTUSEQUI* (*ABORTIVOEQUINA*) (EQUINE PARATYPHOID)

This is a specific disease of Equidae characterized by abortion in females, testicular lesions in males, and septicemia in the newborn.

ETIOLOGY

Salmonella abortusequi (*abortivoequina*) (also known as *Salmonella enterica* serovar Abortusequi) is a host-adapted serovar causing abortion in mares and donkeys. *S.* Abortusequi strains vary in virulence, with more virulent strains having greater in vitro cytotoxigenicity. It is possible to determine the origin and progression of outbreaks of the disease by determining pulsed-field gel electrophoretic patterns of *S.* Abortusequi.

EPIDEMIOLOGY

The infection appears to be limited to horses and donkeys. Although widely reported in the early 1900s, this disease is rarely encountered and is one of the less common causes of either abortion or septicemia in horses. Recent reports of the disease are from Austria, Brazil, Croatia, Japan, and India, although the disease occurs in other countries. However, in the early 1990s, an outbreak of abortion occurred in a herd of 38 horses, in which 21 mares aborted between 5 and 10 months of gestation.

Natural infection may be caused by the ingestion of foodstuffs contaminated by uterine discharges from carriers or mares that have recently aborted. Transmission from the stallion at the time of service is also thought to occur. The infection may persist in the uterus and cause repeated abortion or infection of subsequent foals. Transmission from a female donkey to mares is reported with abortion a result in both species.

PATHOGENESIS

When infection occurs by ingestion, a transient bacteremia without marked systemic signs is followed by localization in the placenta, resulting in placentitis and abortion. Foals that are carried to term probably become infected in utero or soon after birth by ingestion from the contaminated teat surface or through the umbilicus.

CLINICAL FINDINGS

Abortion usually occurs at about the seventh or eighth month of pregnancy. The mare can show signs of impending abortion followed by difficult parturition, but other evidence of illness is usually lacking. Retention of the placenta and metritis are common sequels and may cause serious illness, but subsequent sterility is unusual. A foal that is carried to term by an infected mare may develop an acute septicemia during the first few days of life or survive to develop polyarthritis 7 to 14 days later. Polyarthritis has also been observed in foals from vaccinated mares that showed no signs of the disease.

Infection in the stallion has also been reported with clinical signs including fever, edematous swelling of the prepuce and scrotum, and arthritis. Hydrocele, epididymitis, and inflammation of the tunica vaginalis are followed by orchitis and testicular atrophy.

CLINICAL PATHOLOGY

The organism can be isolated from the placenta, the uterine discharge, the aborted foal, and the joints of foals with polyarthritis. A high titer of *Salmonella* agglutinins in the mare develops about 2 weeks after abortion. Vaccinated mares will give a positive reaction for up to a year.

NECROPSY FINDINGS

The placenta of the aborted foal is edematous and hemorrhagic and may have areas of necrosis. The nonspecific changes of acute septicemia will be manifested in foals dying soon after birth; polyarthritis is found in those dying at a later stage.

Samples for Confirmation of Diagnosis

- Bacteriology: placenta, fetal stomach content, lung, culture swabs of joints (culture)
- Histology: formalin-fixed placenta, various fetal tissues including lung, liver (light microscopy)

TREATMENT

The antimicrobials recommended in the treatment of salmonellosis should also be effective in this disease.

CONTROL

Careful hygiene, including isolation of infected mares and disposal of aborted material, should be practiced to avoid spread of the infection. Infected stallions should not be used for breeding. In the past, when this disease was much more common than it is now, great reliance was placed on vaccination as a control measure. An autogenous or commercial bacterin, composed of killed *S.* Abortusequi organisms, was injected on three occasions at weekly intervals into all mares on farms in which the disease was enzootic, commencing 2 to 3 months after the close of the breeding season. A smaller dose (5 mL) of vaccine of higher concentration is as effective as a larger dose (20 mL) of vaccine of lower concentration. A formol-killed, alum-precipitated vaccine is considered to be superior to a heat-killed, phenolized vaccine. In China, a virulent strain vaccine is credited with effective protection after two injections 6 months apart.

The widespread use of vaccines and hyperimmune sera is credited with the almost complete eradication of the disease in developed countries.

CHLAMYDIAL ABORTION (ENZOOTIC ABORTION OF EWES, OVINE ENZOOTIC ABORTION)

SYNOPSIS

Etiology *Chlamydia abortus.*

Epidemiology Prevalence varies within regions and between countries. Oral route of infection, with the placenta and uterine discharge of aborting ewes the major source of infection. Pregnant sheep infected by contact with aborting ewes usually do not abort until the next lambing season. Zoonotic.

Clinical findings Abortion, stillborn and weak-born lambs.

Necropsy findings Necrotic and hemorrhagic placental cotyledons, intercotyledonary areas thickened, edematous, and leathery.

Diagnostic confirmation Demonstration of the organism in the placenta by polymerase chain reaction, rising titer in paired serum samples.

Control Isolation of aborting ewes. Killed vaccine with adjuvant gives short-term protection and can be used in pregnant ewes during an outbreak in an attempt to reduce the number of abortions. Live attenuated vaccines may be more effective but cannot be used during pregnancy.

ETIOLOGY

Chlamydia abortus (previously known as *Chlamydophila abortus* and *Chlamydia psittaci* biotype 1/serotype 1) has a tropism for ruminant placenta and causes the disease commonly referred to as ovine enzootic abortion (OEA). The organism causes a similar disease in goats, and although this organism also can produce abortion in cattle, pigs, and horses, abortion associated with this organism is not common in these species. There is considerable genetic diversity among strains that cause abortion.

EPIDEMIOLOGY

Occurrence

The disease is one of the most common causes of diagnosed abortion in sheep and goats in the UK, Europe, Asia, the United States, and other countries. In the UK, it accounts for approximately 45% of abortions, and it is particularly common in lowland flocks that are intensively managed at lambing. However, its importance varies from country to country. It is an uncommon cause of abortion in Northern Ireland, and the disease does not occur in Norway, Australia, or New Zealand.

There have been several studies of seroprevalence in Europe that show a high seroprevalence in both domestic and wild ruminants but, until recently, most surveys have used the complement fixation test (CFT), which is not specific for *C. abortus;* therefore, the true seroprevalence of *C. abortus* in many countries is not well established.

Source of Infection and Transmission

Infection is introduced into a flock by the purchase of latently infected replacements that usually abort at the end of their first pregnancy. Within a flock, the major source of infection is the placenta and the uterine discharge of aborting ewes. The main routes of transmission of *C. abortus* are oral or nasal: either ingestion of organisms shed in vaginal fluids and placental membranes at the time of abortion or lambing, or the inhalation of aerosols from contaminated areas. Pasture and the environment are contaminated by vaginal discharges, placenta, and aborted fetuses, and infected ewes shed the organism for a week before aborting and 2 weeks afterward. The elementary body of *C. abortus* is resistant to both physical and chemical influences, because it is metabolically inactive and the rigid cell envelope is osmotically stable and poorly permeable. Consequently, the organism is thought to survive for several days on pasture and longer in cold weather.

Infection of the ewe lamb may occur at birth, shortly following, or at subsequent lambing periods. Infection of pregnant ewes in early or midgestation results in either abortion in the final 2 to 3 weeks of gestation or the birth of stillborn or weak lambs that frequently die in the first few days of their life. Abortion always appears in the last weeks of gestation regardless of the time of infection. Infection of ewes in the last 5 to 6 weeks of pregnancy usually leads to the development of a latent infection, in which ewes appear to be uninfected until the next lambing season, when they abort. Thus late pregnant sheep may be infected by contact with aborting ewes, but usually do not abort until the next lambing season.

The common pattern of infection and disease is the small number of abortions in year 1 following the introduction of infected replacement ewes and then an epidemic abortion storm, in which up to 35% of ewes abort in the last 3 weeks of gestation or give premature birth to weak or dead lambs. After aborting, ewes develop a protective immunity and, in endemically infected flocks, 5% to 10% of the ewes abort annually. Surviving lambs born to infected mothers may be affected by enzootic abortion in ewes (EAE) in their first pregnancy.

Sheep that have aborted, subsequently rebreed successfully, do not have further abortions, and the organism is not present in the placenta or vaginal discharge of subsequent pregnancies. However, levels of immunity vary and some may excrete organisms at estrus or seasonally for up to 3 years.

In chronically infected sheep, persistent infection can be demonstrated in the endometrial cells of the reproductive tract, and the organism is excreted in vaginal fluids during estrus.

Vaginal challenge of ewes at breeding time will result in infection and subsequent abortion. Thus venereal or passive venereal transmission is a possible route of infection but is not common. Chronic infection of the male genital tissues has been recorded, and infection may impair fertility in both rams and bulls.

The epidemiology of abortion with this agent in cattle is unknown, but it may transmit to cattle from infected sheep on the same farm.

Experimental Reproduction

The disease is readily reproduced experimentally. Following subcutaneous injection there are no signs of clinical disease other than a modest increase in rectal temperature for 2 days after infection. There is a systemic antibody response that peaks 2 weeks after infection and then decreases until just before abortion or parturition, during which there is a second increase in antibodies to *C. abortus.* Experimental infection at 70 to 75 days pregnancy can cause abortion in the last 2 to 3 weeks of pregnancy or the birth of stillborn or live lambs. There is variation in the severity of the placental lesions in experimental infections. Abortion is associated with severe placental lesions, but the reason for the variation in severity and fetal manifestations is not known.

Economic Importance

In the UK, enzootic abortion is the most common infectious cause of abortion in lowland flocks that are intensively managed at lambing time and has a major economic impact on agricultural industries worldwide. There are no recent estimates, but losses in the UK were estimated in the early 1990s at £15 to £20 million per annum.

Zoonotic Implications

There is some risk for people working with livestock, such as shepherds and abattoir workers, for respiratory infection with this organism. However, the major zoonotic risk is to pregnant women because of the ability of *C. abortus* to colonize the human placenta. Human infection in early pregnancy results in abortion, whereas later infection can result in stillbirth or preterm labor. Infection is probably oral, from infected hands or food following handling of infected sheep or goats, or contaminated fomites such as clothing. Practices at lambing, such as mouth to mouth resuscitation of weak lambs or bringing weak lambs into the house to be warmed, promote zoonotic spread. Consequently,

infected placentas and dead lambs should be handled using gloved hands and disposed of by burning or burial. The organism can be detected in the milk of both sheep and cattle, so consuming raw milk also poses a risk for zoonotic infection.

PATHOGENESIS

Following infection, it is thought that the organism resides first in the tonsil and is then disseminated by blood and lymph to other organs, although the site of latent infection is not definitely known. Release from the latent state during pregnancy is thought to be caused by immune modulation and leads to bacteremia and infection of the placenta. Despite being a key feature of infection with *C. abortus,* little is understood about the underlying mechanisms that result in latent infections. However, experimental intranasal infection of nonpregnant ewes with a low or moderate dose of organisms induced latent infection and subsequent abortion, whereas a higher dose stimulated protective immunity.[1]

The organisms invade the trophoblast cells of the fetal cotyledon then spread to the intercotyledonary regions of the chorion, producing a necrotic suppurative placentitis and impairment of the maternal–fetal exchange of nutrients and oxygen, hence, fetal death and abortion. An inflammatory response in the fetus may also contribute to fetal death.

It is not known why, regardless of the time of infection, pathologic changes in the placenta do not commence before 90 days' gestation, or even as late as 120 days, although this coincides with the commencement of rapid fetal growth.

CLINICAL FINDINGS

There are generally no premonitory indications of the impending abortions, which occur in late pregnancy. Ewes suffer no obvious systemic effects, but retained placenta and metritis can occur in goats. A vaginal discharge, lasting up to 3 weeks following the abortion, is common. Additional losses are caused by stillbirths and weak-born lambs and kids that die soon after birth.

In cattle, the infection causes abortion in the last third of pregnancy. Infected calves born alive may show lethargy, depression, and may be stunted. Mixed infections with *C. abortus, C. suis* and Chlamydia-like organisms (*Parachlamydia* and *Waddlia* spp.) are recorded in cattle and associated with abortions featuring necrotic placentitis, but the true prevalence and significance of these infections is not clear.[2,3]

CLINICAL PATHOLOGY

If the flock history and placental lesions suggest OEA, smears from affected and adjacent chorionic villi of the placenta can be appropriately stained (e.g., Giemsa or modified Ziehl–Neelsen) and examined under high magnification. Single or clumps of small, coccoid elementary bodies (300 nm) will stain red compared with blue cellular debris. Vaginal swabs from recently aborted ewes and smears of the fleece of uncleaned lambs or fetal abomasal contents can also be examined but contain fewer organisms. The organisms appear similar to *Coxiella burnetii,* the agent of coxiellosis (Q fever), so this is not a definitive test.

Commercial antigen detection kits (fluorescent antibody test [FAT] and ELISA) are available but do not discriminate between Chlamydial species.

Chlamydial DNA can be amplified by conventional or real time RT-PCR. These are highly sensitive, but can result in false positives if samples are cross-contaminated or false negatives if samples contain PCR-inhibitory substances. RT-PCR is rapid and relatively easily standardized and can demonstrate the DNA of *C. abortus* in tissues and swabs, such as of vaginal fluid, conjunctivae, and fetal membranes.[4,5] A number of multiplex PCR tests are described and can differentiate between Chlamydial species and other agents of infectious abortion, such as *Toxoplasma gondii* and *C. burnetii.*[6]

C. abortus can be isolated in embryonated chicken eggs or cell culture, but most diagnostic laboratories do not do this because of the zoonotic risk and requirement for level 2 biocontainment.

Infection in aborting animals can be demonstrated by rising serologic titers in paired serum samples collected 3 weeks apart. The CFT is commonly used but has only moderate sensitivity and is not specific because of common antigens shared with other Chlamydiae and some gram-negative bacteria. It will also be positive in vaccinated animals. Ambiguous results, such as suspected false-positive tests in flock accreditation programs or export testing, can be analyzed further by a Western blot using specific antigens.

A number of research and commercial ELISA tests have been developed. Those based on whole-cell or extracts of chlamydial elementary bodies have better specificity than the CFT but are less sensitive. Those based on segments of the outer membrane protein (OMP) or synthetic peptide antigens have greater sensitivity and specificity and are now more frequently used in diagnostic, epidemiologic, and seroprevalence studies.[7]

Vaccinated animals will react to the currently used serologic tests, but wild-type and vaccine strains of *C. abortus* can be differentiated by PCR-restriction fragment length polymorphism (RFLP).[8] This has provided evidence that the temperature sensitive mutant strain 1B used in vaccines is associated with ovine abortions in Scotland.[9,10]

NECROPSY FINDINGS

Aborted fetuses typically have no gross abnormalities. Fetal fluid may contain chlamydial antibody and, although less sensitive than either isolation in McCoy cells or detection of chlamydial LPS antigen, can be useful when placenta is not available**.** Histologically, there may be mononuclear cell infiltration of hepatic portal areas and multifocal areas of hepatitis. The placenta is critical for diagnosis of chlamydial abortion in both cattle and sheep.

Placental cotyledons are necrotic and hemorrhagic, and the intercotyledonary areas are thickened, edematous, and leathery. This is in direct contrast to the targeting of cotyledons seen with toxoplasmosis. Chlamydial organisms can be seen in tightly packed sheets within the cytoplasm of swollen trophoblasts in formalin-fixed tissues or in direct placental smears using modified Gimenez, Koster's, or other appropriate stains. Well-preserved, fresh placenta should be examined because the organisms are difficult to demonstrate in the fetus. Immunohistochemical stains perform well on formalin-fixed specimens. Most laboratories are reluctant to culture *Chlamydia* spp. because of their zoonotic potential.

Samples for Confirmation of Diagnosis

- **Bacteriology:** chilled liver, lung, placenta (cytology, PCR, ELISA)
- **Histology:** fixed placenta, liver (light microscopy, IHC)

The zoonotic potential of this organism means care needs to be taken when handling potentially infected material, and specimens should be properly packaged when submitted to a laboratory.

DIFFERENTIAL DIAGNOSIS

Other causes of abortion in cattle and ewes are given in Tables 18-1 and 18-2.

CONTROL

Ewes that have aborted should be isolated from the rest of the flock. There should be proper hygiene of the lambing areas, including disposal of bedding and aborted materials, and disinfection of pens with intensive lambing systems. Long-acting oxy tetracycline has been used at 20 mg/kg IM in early pregnant sheep within an aborting flock to reduce subsequent abortions. However, treated ewes still shed the organism in vaginal discharges, and treatment at 10-day intervals may be needed.

Vaccines

Killed and live attenuated vaccines are available, but none are fully protective. **Killed vaccines**, composed of egg-derived or tissue culture organisms of one or two strains have been used for several decades. They are variably effective, but can reduce the frequency of abortion and shedding of the organism. However, outbreaks have occurred in vaccinated sheep, with strain variation a possible

Table 18-2 Diagnostic summary of causes of abortion in cattle

				Field examination		Laboratory diagnosis	
Epidemiology disease	*Clinical features*	*Abortion rate*	*Time of abortion*	*Placenta*	*Fetus*	*Isolation of agent*	*Serology*
Brucellosis (*Brucella abortus*)	Zoonotic disease, chronic infection, abortion, retained placenta, and metritis	High, up to 90% in susceptible herds	5 months +	Severe placentitis, thickened placenta with surface exudate	Possibly pneumonia	Culture of fetal stomach, placenta, uterine fluid, milk, and semen	Serum and blood agglutination test, milk ring test, whole milk plate agglutination test; whey plate agglutination, semen plasma, and vaginal mucous agglutination test
Trichomoniasis (Trichomonas *foetus*)	Venereally transmitted disease resulting in early embryonic loss with occasional abortion and pyometra	Moderate, 5%–30%	Primarily first 5 months	Flocculent material and clear, serous fluid in uterine exudate	Usually no gross lesions, histologically fetal giant cell pneumonia may occur	Hanging drop or culture examination of fetal stomach and uterine exudate within 24 hours of abortion; isolation, best source in female pyometra fluid if pyometra exists; best method is InPouch; in male bull's preputial smegma with InPouch	Cervical mucous agglutination test; serology rarely performed, mucus agglutination or complement fixation hemolytic assay
Neosporosis (*Neospora caninum*)	Worldwide distribution of infection in both dairy and beef cattle, most abortions reported in dairy cattle In addition to abortion, mummification and birth of full-term infected calves can occur with or without clinical signs Chronic infection in which congenital transmission commonly occurs during pregnancy, acquired postnatal infection may also occur	Sporadic or outbreaks common (20%–40%) Repeat abortions from same cows can occur	3–8 months of gestation (mean 5.5 months)	No characteristic gross lesions in placenta Parasite may be present	Autolyzed midgestation fetus, widespread histologic inflammatory lesions in fetus including nonsuppurative necrotizing encephalitis and myocarditis	Identify parasite in fetal tissues by immunohistochemistry stain or PCR	Antibodies in fetus and cow IFAT and ELISA antibodies used for serologic detection Positive result supports infection in cow and/or fetus but is not causal proof; negative result in dam strong evidence that neosporosis not involved in abortion; serologic comparison of groups of aborting and nonaborting herdmates useful in establishing the role in herd outbreaks of abortion
Vibriosis (*Campylobacter fetus* subsp. *venerealis*)	Venereally transmitted, resulting in infertility, irregular, moderately prolonged diestrus with occasional abortion Epidemiology similar to trichomonosis except for a longer vaginal carrier state (up to 4 months after uterus has cleared organism) Significance: fertility returns but is still a threat to any uninfected bull	Low, up to 5%, may be up to 20%	46 months	Semiopaque, little thickening Petechiae, localized avascularity and edema	Flakes of pus on visceral peritoneum Fibrin may be present in serosal cavities Usually associated with suppurative pneumonia in fetus	Culture of fetal stomach, placenta, and uterine exudate Sporadic abortion, not venereally transmitted, can be associated with *C. fetus* subsp. *fetus* and *C. jejuni,* which need to be differentiated from *C. fetus* subsp. *venerealis*	Blood agglutination after abortion (at 3 weeks) Cervical mucous agglutination test at 40 days after infected service

Table 18-2 Diagnostic summary of causes of abortion in cattle—cont'd

				Field examination		Laboratory diagnosis	
Epidemiology disease	*Clinical features*	*Abortion rate*	*Time of abortion*	*Placenta*	*Fetus*	*Isolation of agent*	*Serology*
Leptospirosis (*Leptospira interrogans* serovar pomona and *Leptospira hardjo*) *L. borgpetersenii* serovar hardjo (formerly serovar hardjo-*bovis*) occurs worldwide; *L. interrogans* serovar hardjo (formerly hardjo-prajitno) primarily in the UK	Abortion may occur at acute febrile stage, later, or unassociated with illness	25%–30%	Abortions may occur throughout gestation; Late, 6 months +	Avascular placenta, atonic yellow-brown cotyledons, brown gelatinous edema between allantois and amnion	Fetus usually autolyzed, occasional icterus Fetal death common	Fluorescent antibody stain of smears of fetal kidney or PCR Direct examination of urine of cow by dark-field or fluorescent antibody stain	Positive serum agglutination test 14–21 days after febrile illness Titers usually at or near maximum at time of abortion Chronically infected *L. hardjo* dams may have low or negative titers
Infectious bovine rhinotracheitis (IBR)	Abortion storms in inadequately vaccinated animals. May be associated with upper airway disease in one or several animals	Variable	Most in second half of gestation	No significant gross lesions	Autolyzed fetus, rarely may have pale foci of hepatic necrosis Histopathology characteristic with multifocal necrosis	Virus isolation or PCR on placenta or fetal tissues Immunohistochemistry or fluorescent antibody stain on fetal tissues	Acute and convalescent sera
Mycoses (*Aspergillus, Absidia*)	Variable incidence, more common in cooler moist climates, retained placenta may occur	Unknown. 6%–7% of all abortions encountered	3–7 months	Necrosis of maternal cotyledon, adherence of necrotic material to chorionic cotyledon causes soft, yellow, cushion-like structure Small yellow, raised, leathery lesions on intercotyledonary areas	Minority of fetuses have skin lesions May be small raised, gray-buff, soft lesions, or diffuse white areas on skin Resemble ringworm	Direct examination of cotyledon and fetal stomach for hyphae, suitable cultural examination Histopathology on placenta	

Continued

Table 18-2 Diagnostic summary of causes of abortion in cattle—cont'd

				Field examination		Laboratory diagnosis	
Epidemiology disease	*Clinical features*	*Abortion rate*	*Time of abortion*	*Placenta*	*Fetus*	*Isolation of agent*	*Serology*
Listeriosis (*Listeria monocytogenes*)	May be an associated septicemia (Cows that abort may die of septicemia near term.) Retained placentas and metritis may also occur	Low, rare abortion storms related with poorly fermented silage	About 7 months	—	Autolysis Foci of necrosis in liver and other organs	Organisms in fetal stomach, liver, and throughout fetus placenta and uterine fluid	Agglutination titers higher than 1:400 in contact animals classed as positive
Epizootic bovine abortion	Tick-transmitted bacterial infection, occurs in dry foothill pastures in the western United States in which tick vector resides No clinical signs in aborting cattle Herd immunity develops Incubation period ≈3 months after exposure to agent	Abortion storms may occur, usually in heifers and newly introduced cattle High, 30%–40%	Third trimester abortion or birth of premature weak calves	Negative	Fresh fetus with petechiae in mucosa, enlarged lymph nodes and spleen, subcutis edema, ascites, nodular swollen liver	Diagnosis based on typical histologic lesions, etiologic bacterial agent has been identified by DNA analysis but is not culturable on artificial media Bacterial rod can be detected with special stains (Steiner silver stain and immunohistochemistry)	No serology test, elevated fetal IgG levels
Bovine viral diarrhea (BVD)	Variable outcome of fetal infection depending on timing of infection and other factors. Persistent bovine viral diarrhea virus infection in full-term live calves a significant problem for exposure of other animals	Less than 10%	Any time during gestation Most common in first trimester	No obvious gross lesions	Mummification, variable fetal lesions possible including deformities (cerebellar, pulmonary, or renal hypoplasia), myocardial lesions with congestive heart failure, thymic depletion or no lesions	Immunohistochemical or fluorescent antibody examination of tissues to detect virus Virus isolation or PCR also available Animals affected early with congenital lesions may no longer be positive for virus at time of abortion	Fetal antibody, evidence of seroconversion in dam and/or herd

Nutritional: *Ingestion of excessive amounts of performed estrogens in the diet may cause abortion. There are usually accompanying signs due to increased vascularity of the udder and vulva. Possible dietary factor in so-called lowlands abortion.*
Isoimmunization: *Has not been observed to occur naturally in cattle. It has been produced experimentally by repeated IV injections of blood from the one bull of pregnancy. Intravascular hemolysis occurs in the calves.*
Unknown: *30%–75% of most abortions examined are undiagnosed. The ingestion of large quantities of pine needles is suspected as a cause of abortion in range cattle in the United States. Infection with* Ureaplasma *and* Mycoplasma *spp. are other causes of undetermined relative importance.*
ELISA, *enzyme-linked immunosorbent assay;* IFAT, *indirect fluorescent antibody test;* PCR, *polymerase chain reaction.*

explanation when using monovalent vaccines. The addition of Freund's incomplete adjuvant provides better protection, and some other adjuvants may improve the efficiency of killed vaccines against naturally occurring enzootic abortion. Killed vaccines can be used in pregnant ewes and have been used in the face of an outbreak in an attempt to reduce the prevalence of abortion.

A live vaccine containing a temperature-sensitive attenuated strain of *C. abortus* (strain 1B) provides reasonable, but not complete, protection against *C. abortus*. It is registered for use in sheep (not goats). Live attenuated vaccines should not be used in pregnant ewes because they may pose a risk of zoonotic infection and have been associated with abortions.[8-10]

Recombinant and DNA vaccines have shown little protection against experimental challenge with *C. abortus*.

FURTHER READING

Radostits O, et al. Chlamydophila abortion (enzootic abortion of ewes, ovine enzootic abortion). In: *Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Goats and Pigs*. 10th ed. London: W.B. Saunders; 2007:1435-1437.

Stuen S, Longbottom D. Treatment and control of *Chlamydial* and *Rickettsial* infections in sheep and goats. *Vet Clin North Am Food Anim Pract*. 2011;27:213-233.

REFERENCES

1. Longbottom D, et al. *PLoS ONE*. 2013;8(2):e5790.
2. Ruhl S, et al. *Vet Microbiol*. 2009;135:169.
3. Reinhold P, et al. *Vet J*. 2011;189:257.
4. Sachse K, et al. *Vet Microbiol*. 2009;135:2.
5. Gutierriez J, et al. *Vet Microbiol*. 2011;147:119.
6. Gutierriez J, et al. *J Vet Diagn Invest*. 2012;24:846.
7. Wilson K, et al. *Vet Microbiol*. 2009;135:38.
8. Laroucau K, et al. *Vaccine*. 2010;28:5653.
9. Wheelhouse N, et al. *Vaccine*. 2010;28:5657.
10. Sarginson N, et al. *New Zeal Vet J*. 2015;63:284.

COXIELLOSIS (Q-FEVER)

SYNOPSIS

Etiology *Coxiella burnetii.*

Epidemiology High seroprevalence in ruminants. Latent infection with recrudescence and excretion at parturition. Infection by direct contact and inhalation. Persists in the environment. Important zoonotic disease.

Clinical findings Infection in ruminants is common. Clinical disease is less common and presents mainly as abortion in sheep and goats.

Necropsy findings Placentitis. Organisms demonstrable in placental trophoblast cells by fluorescent antibody.

Diagnostic confirmation Fluorescent antibody staining and PCR of aborted material and vaginal discharge. Acid-fast rodlike organisms in stained impression smear of placenta. Serology (ELISA, CFT, immunofluorescent antibody) or bulk tank milk test to establish herd infection status.

Control Vaccination possible in many countries. Isolation of aborting ruminants. Destruction of bedding and straw contaminated with birth fluids.

Zoonotic aspects Infection of humans can vary from asymptomatic to severe and even fatal. Presents mainly as a mild influenza-like illness with pneumonia, but chronic infections can have serious outcomes, including endocarditis and osteoarticular disease. Mainly follows contact with sheep and goats around parturition rather than cattle.

CFT, *complement fixation test;* ELISA, *enzyme-linked immunosorbent assay;* PCR, *polymerase chain reaction.*

ETIOLOGY

Coxiellosis (Q) fever is a zoonosis associated with *C. burnetii,* which is an obligate intracellular parasite classified within the family Coxiellaceae (formerly Rickettsiaceae). It can be divided into six genotype clusters on the basis of RFLP, although different methods can be used such as multispacer sequence typing (MST) or MLVA, which can identify up to 17 different microsatellite markers.[1] The presence or absence of specific genotypes could explain inconsistencies in reports on the effects of coxiellosis, particularly on reproduction in cattle. Coinfection with multiple genotypes can occur, although a large study of milk samples in the United States identified predominantly genotype ST20 in bovine milk and mainly ST8 in caprine milk,[2] whereas ST20 was associated with an abortion storm in a goat dairy in the UK[3] and ST33 with the large outbreak in the Netherlands.[4] Unlike other rickettsiae, *C. burnetii* it is quite resistant to environmental influences and is not dependent on arthropod vectors for transmission. It displays two antigenic phases or phenotypes: Phase 1 is more infectious and able to replicate in the host, and Phase 2, which is unable to replicate (these phases correspond to the smooth and rough phases of other gram-negative bacteria, respectively).

EPIDEMIOLOGY

Occurrence

The organism has worldwide distribution, although serologic surveys have found no evidence of infection in New Zealand.

C. burnetii cycles in a wide variety of wildlife species and their ectoparasites. The infection also cycles in domestic animals; cattle, sheep, and goats are the main livestock reservoirs of infection for humans. Rates of infection in farm animals vary considerably between locations, between countries, and with time, because there appears to be cycles of infection within regions.[5]

There can be a wide range in the seroprevalence of Q fever within regions and within individual herds or flocks. In cattle, from 4% to 100% of herds have been reported as positive (either seropositive or bulk milk test), and the within-herd prevalence varies from 0% to 49%.[6] The flock and within-flock prevalence in sheep and goats shows similar ranges and, as for cattle, varies according to year and region.[5,6] There is little information on management or other factors that might influence this variation in prevalence, but one study found a significantly higher prevalence in housed cattle compared with cattle kept on pasture. Analysis of data from 69 publications found the overall mean prevalence to be slightly higher in cattle (estimate of 20% and 38% for herd and within-herd prevalence, respectively) than for small ruminants (sheep and goat; 15% and 25% for flock and within-flock prevalence, respectively).[6] The prevalence in flocks of dairy goats and dairy sheep is much higher than in nondairy flocks.

Source of Infection and Transmission

Infection and transmission is by direct contact and by inhalation. Infection of nonpregnant animals is clinically silent and is followed by latent infection until pregnancy, at which time there is recrudescence with infection in the intestine, uterus, placenta, and udder and excretion from these sites at parturition. The organism is present in high concentration in the placenta and fetal fluids and subsequent vaginal fluids, is also excreted in urine, and is present in the feces of sheep from 11 to 18 days postpartum. In a longitudinal study in a naturally infected sheep flock in France, the number of *C. burnetii* was higher in vaginal mucus and feces compared with milk, peaked 3 weeks after abortion or birth, and was highest in primiparous and aborting ewes.[7] Shedding of *C. burnetii* in the feces can be persistent, so this can contribute significantly to environmental contamination with the organism. Infection can result in abortion, stillbirths, or poorly viable lambs, but the neonates of infected, excreting ewes are often born clinically normal.

Abortion usually does not occur at successive pregnancies. However, there can be recrudescence of infection and excretion at these pregnancies, especially the one immediately following, and reproductive failure at a second consecutive pregnancy is recorded in goats.[8] Goats excrete the organism in vaginal discharges for up to 2 weeks, it is present in milk for up to 52 days after kidding, and is also found in the feces. It also strongly adheres to the zona pellucida not removed by standard washing procedures. Thus the possibility of transfer of *C. burnetii* by embryo transfer cannot be ruled out.[9]

In cattle, maximum shedding also occurs at and 2 weeks following parturition, the organism is excreted in milk for at least

several months and can be detected for up to 2 years in bulk tank milk. Abortion in cattle is less common than in goats and sheep and is sporadic rather than occurring as abortion storms like occur with sheep and goats. The organism is present in the semen of seropositive bulls, and venereal transmission is suspected.

There is a significant contamination of the environment of infected animals at the time of parturition and abortion. This is a major risk period for transmission of the disease within herds and flocks and presents a significant zoonotic risk. The organism is still present in large concentrations in soil 12 months after outbreaks of coxiellosis on goat farms.[10]

Pathogen Risk Factors

C. burnetii is very resistant to physical and chemical influences and can survive in the environment, manure, and soil for several months. It can resist common chemical disinfectants but is susceptible to sodium hypochlorite, 1:100 Lysol solution, and formalin fumigation provided a high humidity is maintained.

There is strain variation in the organism, and differences in genotypes and DNA sequences have been correlated with differences in the type of disease occurring in humans and domestic ruminants. The organism is highly infectious, with the infective dose for humans estimated to be one organism.

Zoonotic Implications

In humans infection is primarily by inhalation. Sources of infection include such diverse materials as soil; airborne dust; wool, bedding; and other materials contaminated by urine, feces, or birth products of animals. The potential for human infection from these sources is substantial (e.g., ovine manure used as a garden fertilizer has been incriminated).

Sheep and goats have traditionally been identified as the major reservoir of infection for humans, and the location of urban populations in proximity to large dairy goat herds was a significant reservoir in the Dutch outbreak from 2007 to 2010.[1]

The organism is present in the milk of infected cattle, sheep, and goats. A significant proportion of seropositive cattle excrete the organism in milk, and periods and duration of excretion are variable but may persist at least 2 years. *C. burnetii* is destroyed by pasteurization but there is a risk for people who consume raw milk, particularly unpasteurized milk from sheep and goat.

Rates of seropositivity in humans vary markedly between surveys, but there is a higher rate of seropositivity in people that are associated with domestic animals and their products and with farm environments (such as farm workers, veterinarians, livestock dealers, dairy plant and slaughterhouse workers, and shearers).[5]

Many instances of infection in humans have been linked to exposure to parturient sheep and goats. A spectacular example is the 2007 to 2010 epidemic in the Netherlands, in which over 3000 notifications of human disease were analyzed and only 3.7% of people had worked in agriculture or slaughterhouses.[11] This outbreak was attributed to airborne transmission of contaminated dust originating from dairy goat farms located in densely populated areas. The number of human cases abruptly declined after control measures were implemented on the goat and sheep farms, including vaccination, the mass culling of more than 50,000 pregnant does and ewes on infected farms to reduce shedding of the organism, and mandatory PCR testing of bulk tank milk for *C. burnetii*.[11] Living close (<2 km) to a large dairy goat farm that had an abortion storm caused by *Coxiella burnetii* was identified as the major risk factor for human cases during the Netherlands epidemic.[12]

Coxiellosis in humans is referred to as Q-fever and is often asymptomatic, but can result in acute disease characterized by fever, general malaise, headache, and less commonly, pneumonitis, hepatitis, and meningoencephalitis. Endocarditis, hepatitis, and osteoarticular diseases are manifestations of chronic disease in around 2% of human infections.[11] Those at most risk of chronic disease are immunocompromised individuals and pregnant women. There is a concern that the prevalence of infection in farm animals is increasing and spreading geographically, so that there is a greater risk for human infection, particularly when dairy farms are located near urban populations. Epidemics of human infection have been documented in several countries including Australia, France, Germany, the United States, Bulgaria, the UK, and the Netherlands.[11]

C. burnetii is considered a potential agent for bioterrorism because of its survival in the environment, the ease with which it can be transmitted by aerosol and wind, and the very low infectious dose.

CLINICAL FINDINGS

Infection of ruminants can occur at any age and is usually clinically inapparent. In the experimental disease in cattle, anorexia is the only consistent clinical finding. *C. burnetii* is a cause of abortion storms and sporadic abortion in sheep and goats but only rarely associated with sporadic abortion in cattle.[13] Abortion occurs during the latter part of pregnancy in individual does or ewes, usually with no sign of impending abortion.

In the 2007 to 2010 epidemic in the Netherlands, abortion storms were reported on 28 dairy goat and 2 sheep dairy farms, with up to 60% of goats aborting compared with an average of 5% abortions on the sheep farms.[11]

CLINICAL PATHOLOGY

There are a number of serologic tests, including complement fixation, microagglutination, ELISA and indirect immunofluorescence, and PCR (both conventional and quantitative real-time PCR). A comparison of these tests concluded a combination tests was preferable, such as ELISA for serology and PCR for detection of DNA of the organism.[14]

The immunofluorescence assay is used as the seroreference test for the serodiagnosis of coxiellosis. It can detect antibody to phase variants and can provide epidemiologic information because Phase 1 antibody is associated with recent and acute infections and Phase 2 antibody with chronic infections.

Conventional and quantitative real-time PCR tests can be conducted on bulk tank milk and are a useful means of monitoring herd or flock prevalence within regions and outbreaks within herds or flocks.[15,16]

NECROPSY FINDINGS

There are seldom gross lesions in aborted fetuses, but foci of necrosis and inflammation are occasionally seen in the liver, lung, and kidney microscopically.[13] The placenta from aborting animals is usually thickened and a purulent exudate or large, red-brown foci of necrosis is typically seen in the thickened intercotyledonary areas. Microscopically, large numbers of necrotic neutrophils are usually visible on the chorionic surface, and swollen trophoblasts filled with the organisms can also be found in well-preserved specimens. This is consistent with bacterial replication occurring only in the trophoblasts of placenta, and not in other organs, of experimentally infected pregnant does and their kids.[17] Examination of placental impression smears stained with Gimenez, Koster's, or other appropriate techniques provides a means of rapid diagnosis. However, care must be taken to avoid confusing *Coxiella*-infected trophoblasts with cells containing *Chlamydophila* organisms. Coxiellosis can be confirmed by PCR, fluorescent antibody staining of fresh tissue, or immunohistochemical staining of formalin-fixed samples. In most laboratories, culture is not attempted because of the zoonotic potential of this agent.

Samples for Confirmation of Diagnosis

- **Bacteriology and detect DNA:** chilled placenta (fetal liver and spleen) (cytology, FAT, PCR)
- **Histology:** fixed placenta and fetal lung, liver, kidney (light microscopy, immunohistochemistry)

Note the zoonotic potential of this agent when handling aborted material and packaging and submitting specimens.

DIFFERENTIAL DIAGNOSIS

- Other causes of abortion in sheep and goats (*Campylobacter, Chlamydophila,* and *Toxoplasma*, Table 18-1).
- The diagnosis of the disease in farm animals, other than abortion, suspected as associated with this agent is difficult and relies on the detection of the organism.
- A positive serologic test in an animal or herd is indicative of infection at some time but does not indicate an association with the problem at hand.
- PCR or PCR-ELISA has been used for detection of the organism in milk.

ELISA, *enzyme-linked immunosorbent assay;* PCR, *polymerase chain reaction.*

CONTROL

Aborting animals should be isolated for 3 weeks and aborted and placenta-contaminated material burnt. Ideally, manure should be composted for 6 months before application to fields to inactivate the organism, and closed composting with CaO or $CaCN_2$ has been practiced following outbreaks in the Netherlands, France, and Germany.[5] Feed areas should be raised to keep them free from contamination with feces and urine.

Although Q fever has significant implications for human health, until recently it has not been regarded as important enough to justify national or regional control strategies based on control in the animal population. In the Netherlands, a seasonal epidemic of animal and human *C. burnetii* infections occurred in 2007, but was no different from several previous isolated outbreaks in Europe. Subsequently, the unexpected scale of the outbreak in 2008 meant that national and regional public health authorities were largely unprepared for the expanding epidemic.[5,18]

The response in the Netherlands was to cull all pregnant dairy goats on affected farms before the 2010 kidding season, without reference to individual testing, and to vaccinate dairy goats and dairy sheep.[18,19] A retrospective analysis identified that testing pregnant goats by PCR or ELISA, followed by culling only the positive goats, would not have effectively reduced the massive bacterial shedding on these farms because many infected goats would not have been detected.[20]

Inactivated Phase 1 vaccines show a good and persistent antibody response, suggesting that vaccination should limit the excretion of the organism. However, there is little economic incentive for vaccination of livestock when an outbreak of coxiellosis is not occurring, and a vaccine for livestock is not available in many countries. A systematic review and meta-analysis of investigations into the use of inactivated Phase 1 vaccines, such as used in the Dutch outbreak, found a significantly reduced risk of shedding in vaginal mucus, uterine fluids, milk, and feces of vaccinated goats exposed to infection compared with controls. However, it was concluded that there was no reduction in the risk or amount of shedding in vaccinated ewes compared with unvaccinated ones.[21]

Vaccination of humans has reduced infection rates in high-risk groups and is used in the appropriate circumstances in Australia, such as workers on goat and sheep dairy farms, abattoir workers, veterinarians, and veterinary students.

During a natural outbreak of coxiellosis in a dairy flock, two treatments with oxytetracycline at days 100 and 120 of pregnancy failed to reduce shedding of the organism in vaginal fluids, milk, or feces compared with untreated ewes.[22] In this flock, vaccination for three consecutive seasons reduced the proportion of ewes shedding the organism to around 4%.

Pasteurization of milk that is consumed fresh is preferable, but veterinarians dealing with herds that provide raw milk should ensure that these herds are seronegative or bulk tank milk is PCR negative for *C. burnetii.* In a study of manufactured dairy products in France (mainly cheese, but also yogurt, cream, and butter), the DNA of *C. burnetii* was detected in 64%, but no viable organisms were recovered. A greater proportion of food products from large-scale manufacturers were positive compared with artisan food.[23]

FURTHER READING

Agerholm JS. *Coxiella burnetii* associated reproductive disorders in domestic animals—a critical review. *Acta Vet Scand.* 2013;55:13.

Georgiev M, et al. Q fever in humans and animals in four European countries, 1982 to 2010. *Euro Surveill.* 2013;18:pii 20407.

Hogerwerf L, et al. Reduction of Coxiella burnetii prevalence by vaccination of goats and sheep, the Netherlands. *Emerg Infect Dis.* 2011;17:379-386.

O'Neil TJ, et al. A systematic review and meta-analysis of phase I inactivated vaccines to reduce shedding of Coxiella burnetii from sheep and goats from routes of public health importance. *Zoonoses Public Health.* 2014;61:519-533.

Roest HIJ, et al. The Q fever epidemic in the Netherlands: history, onset, response and reflection. *Epidemiol Infect.* 2011;139:1-12.

REFERENCES

1. Roest HIJ, et al. *Emerg Infect Dis.* 2011;17:668.
2. Pearson T, et al. *BMC Microbiol.* 2014;14:41.
3. Reichel R, et al. *Res Vet Sci.* 2012;93:1217.
4. Tilburg JJHC, et al. *Emerg Infect Dis.* 2012;18:887.
5. Georgiev M, et al. *Euro Surveill.* 2013;18:pii 20407.
6. Guatteo R, et al. *Vet Microbiol.* 2011;149:1.
7. Joulié A, et al. *Appl Environ Microbiol.* 2015;81:7253.
8. Berri M, et al. *Res Vet Sci.* 2007;83:47.
9. Alsaleh A, et al. *Theriogenology.* 2013;80:571.
10. Kersh GJ, et al. *Appl Environ Microbiol.* 2013;79:1697.
11. Djikstra F, et al. *FEMS Immunol Med Microbiol.* 2012;64:3.
12. Schimmer B, et al. *BMC Infect Dis.* 2010;10:69.
13. Agerholm JS. *Acta Vet Scand.* 2013;55:13.
14. Niemczuk K, et al. *Vet Microbiol.* 2014;171:147.
15. Garcia-Pérez AL, et al. *J Dairy Sci.* 2009;92:1581.
16. Bauer AE, et al. *BMC Vet Res.* 2015;11:186.
17. Roest H-J, et al. *PLoS ONE.* 2012;7:e48949.
18. Roest HIJ, et al. *Epidemiol Infect.* 2011;139:1.
19. Hogerwerf L, et al. *Emerg Infect Dis.* 2011;17:379.
20. Hogerwerf L, et al. *Vet J.* 2014;200:343.
21. O'Neill TJ, et al. *Zoonoses Public Health.* 2014;61:519.
22. Astobiza I, et al. *Vet J.* 2013;196:81.
23. Eldin C, et al. *Am J Trop Med Hyg.* 2013;88:765.

DISEASES OF THE GENITAL TRACT ASSOCIATED WITH *MYCOPLASMA* SPP.

Vulvovaginitis in cattle, sheep, and goats may be associated with ***Mycoplasma agalactiae* var. *bovis.*** The same infection, when introduced with semen into the uterus, can cause endometritis and salpingitis, resulting in temporary infertility and failure to conceive. Persistent infection of the genital tract of bulls has also been produced experimentally.

Although **ureaplasmas** and ***M. bovigenitalium*** are considered to pertain to the normal flora of the lower urogenital tract of ruminants, these organisms have also been associated with reproductive disease.[1] In healthy individuals *Ureaplasma diversum* is usually limited in its distribution to the vestibule and vulva. Both microorganisms have been isolated from the vulva of animals with granular vulvovaginitis, and the disease could be transmitted experimentally. *M. bovigenitalium* infection has further been associated with infertility, abortion, and birth of weak calves.[1] These infections adversely affect reproduction when they are either acute or chronic; along with producing granular vulvovaginitis, some strains can, if introduced to the upper reproductive tract, cause transitory endometritis and salpingitis. Because *U. diversum* is a normal inhabitant of the upper respiratory tract and the lower urogenital tract of ruminants, contamination of fetal membranes or fetal tissue submitted to the diagnostic laboratory should be considered.[2] The diagnosis of *U. diversum* as a causative agent for abortion should therefore ideally be based on isolation of the pathogen from fetal lung tissue, stomach content, or placenta, coupled with the presence of compatible lesions in the lung and placenta.[2]

Ureaplasmas, *M. bovis,* and *M. bovigenitalium* have been found in the reproductive tract of bulls and their semen. Using PCR for the detection of mycoplasma in semen, *M. mycoides* subsp. *mycoides* SC (the causative agent of the contagious bovine pleuropneumonia) has been found in semen of yearling bulls with seminal vesiculitis. Mycoplasmas in semen can be transmitted through in vitro fertilization (IVF) and infect embryos, and

supplementation of culture media with standard antibiotics and washing embryos as recommended by the International Embryo Transfer Society are not effective in making IVF embryos free from *M. bovis* and *M. bovigenitalium*. *M. bovis* in frozen semen can survive the antibiotic combination of gentamicin, tylosin, and lincomycin and spectinomycin.

In horses, ***M. equigenitalium, M. subdolum***, and ***Acholeplasma* spp.** have been associated with infertility, endometritis, vulvitis, and abortion as well as with reduced fertility and balanophostitis in stallions.[3] Notwithstanding, these microorganisms are also commonly isolated from clinically healthy horses, which has raised questions about their direct association with genital disease. Two microorganisms frequently isolated from the upper respiratory tract of horses, ***M. equirhinis*** and ***M. felis***, have also been isolated from the genital tract of stallions but have not been associated with any clinical disease.[3]

In pigs infection with ***M. suis,*** the causative agent of eperythrozoonosis, has also been associated with infertility, abortion, stillbirth, and birth of weak piglets.[1]

REFERENCES

1. Given MD, Marley MSD. *Theriogenology*. 2008;70:270.
2. Anderson ML. *Theriogenology*. 2007;68:474.
3. Spergser J, et al. *Vet Microbiol*. 2002;87:119.

EQUINE COITAL EXANTHEMA

Equine coital exanthema is a **venereal disease** associated with infection of equids by equine herpesvirus-3 (EHV-3). The genome of EHV-3 has been sequenced.[1] The disease is highly contagious and has economic importance because of disruptions to breeding programs on stud farms when stallions have clinical signs or there is an outbreak of disease among mares. The economic impact is greatest in those breeds or studbooks, such as Thoroughbreds, that do not permit artificial insemination of mares.[2]

Transmission is usually venereal from affected or clinically normal carrier animals in which the infection is thought to be latent in sciatic ganglion.[3] The virus is highly contagious, and outbreaks among mares in an embryo transfer facility in which both donor and recipient mares were affected is strongly suggestive of iatrogenic spread by personnel or on equipment such as ultrasound probes.[2] Inapparent or latent infection is apparently common, with 14% of 220 Thoroughbred mares on a stud farm having EHV-3 genome detectable by PCR in swabs of the perineum and vagina and 48% having serum antibodies to the virus.[3] The virus can be excreted intermittently by infected mares, although the factors determining reactivation have not been determined.[3] Efforts to demonstrate that administration of corticosteroids induces reactivation of EHV-3 shedding are inconclusive.[4]

The disease can be reproduced experimentally with more severe disease and longer shedding of the virus in mares that are seronegative at the time of infection than in seropositive mares.[5]

The disease is relatively mild and causes only local signs manifested by papular, then pustular, and finally **ulcerative lesions** of the vaginal mucosa, which is generally reddened. The ulcers can be as large as 2 cm in diameter and 0.5 cm deep and are surrounded by a zone of hyperemia. In severe cases the lesions extend onto the vulva and the perineal skin to surround the anus. There can be pain on passage of feces, and the anorectal lymph nodes are enlarged.[2] In the male, similar lesions to those on the perineum of the mare are found on the penis and prepuce. Many mild cases are unobserved because there is no systemic disease and affected horses eat well and behave normally. The effect on fertility is equivocal although there might be a loss of libido during the active stage of the disease in stallions. The **incubation period** is 2 to 10 days and the course up to complete healing of ulcers is about 14 days, although depigmented lesions on the perineum can persist for months.[3]

EHV-3 has been associated with **unilateral rhinitis** in adult horses examined with the same endoscope. All horses were affected unilaterally and in the nostril through which the endoscope was passed.[6]

Diagnosis can be achieved by use of virus isolation or demonstration of viral DNA in skin lesions or swabs of the vaginal or perineal regions.[3] Secondary bacterial infection can lead to suppurative discharge and a longer course. In some outbreaks lesions occur on the skin of the lips, around the nostrils, and on the conjunctiva and can also be present on the muzzle of the foal. Ulcerative lesions of the pharyngeal mucosa also occur in infections with EHV-2 and with equine adenovirus. Ulcerative lesions of the oral mucosa are of great importance because of the necessity to diagnose vesicular stomatitis early.

Treatment is symptomatic and can include cleaning of lesions, although this might not be necessary in uncomplicated disease. Mares with severe inflammation of the perianal tissues with or without enlargement of the anorectal lymph nodes and signs of pain on defecation might benefit from administration of fecal softening agents (mineral oil) or diets.

Control can be achieved by use of artificial insemination, but careful attention must be paid to biosecurity measures that minimize the opportunity for iatrogenic spread on stud farms or embryo transfer facilities. Recommendations for control in embryo transfer facilities and stud farms include the following[2,7]:

- Strict adherence to hygiene procedures designed to prevent both the direct and indirect transmission of the virus.
- Personnel with direct contact with mares should wear long, disposable examination sleeves and short latex gloves, which should be changed between subsequent inspections.
- Ultrasonography probe should be covered with a disposable glove or be carefully disinfected before the inspection of each mare.
- All instruments and other devices used during the inspection procedure, artificial insemination, and embryo collection must be either disposable or washed and sterilized between uses.

FURTHER READING

Barrandeguy M, Thiry E. Equine coital exanthema and its potential economic implications for the equine industry. *Vet J*. 2012;191(1):35-40.

REFERENCES

1. Sijmons S, et al. *Genome Announc*. 2014;2:e00797.
2. Barrandeguy M, et al. *J Equine Vet Sci*. 2010;30:145.
3. Barrandeguy M, et al. *Theriogenology*. 2010;74:576.
4. Barrandeguy M, et al. *Equine Vet J*. 2008;40:593.
5. Barrandeguy M, et al. *Vet Microbiol*. 2012;160:319.
6. Barrandeguy M, et al. *Vet Rec*. 2010;166:178.
7. Barrandeguy M, et al. *Vet J*. 2012;191:35.

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS)

SYNOPSIS

Etiology Porcine reproductive and respiratory syndrome virus belonging to the Arteriviridae family.

Epidemiology Highly contagious disease of swine manifested by reproductive failure, and respiratory disease in young pigs. Worldwide occurrence; spreads rapidly in swine-raising areas during the last 20 years. Subclinical infection endemic in most swine herds; incidence of clinical disease lower but causes severe economic losses. Pigs become infected in nursery from older infected pigs; persistent infection for several months is common. Different antigenic strains with variable virulence. Natural infection or vaccination results in immunity, but viremia still common. Infection with virus may predispose to secondary infections of respiratory tract. Transmitted by direct contact, feces and discharges, importation of infected pigs into herds, aerosol infection, and semen.

Signs Highly variable clinical syndrome.

Reproductive failure Outbreaks of late gestation abortions, stillbirths, mummified fetuses, weak neonates, and high rate of return to estrus. Problem may persist and recur for many months.

Respiratory form Anorexia, fever, dyspnea, polypnea, coughing, unthriftiness, high mortality in young pigs and low in older pigs and breeding stock. Deaths occur in acute phase.

Lesions Interstitial pneumonia with reduction in alveolar macrophages. Aborted and mummified fetuses, stillbirths, and weak neonates with pulmonary lesions.

Diagnostic confirmation Serologic testing for viral antibody titers. Detection of virus in tissues and alveolar macrophages using immunofluorescent microscopy and other techniques.

Differential diagnosis list Major differential is porcine circovirus infections

Respiratory disease:

Pneumonia caused by

- *Mycoplasma hyopneumoniae*
- *Actinobacillus pleuropneumoniae*
- *Pasteurella multocida*
- Glasser's disease (*Haemophilus parasuis*)
- *Streptococcus suis*

Reproductive failure

- Leptospirosis
- Parvovirus
- Brucellosis
- Aujeszky's disease
- Hog cholera virus

Treatment Must clinically manage outbreak to minimize mortality in young pigs.

Control Segregation and off-site rearing of recently weaned pigs. Nursery depopulation and clean up protocol. Import only virus-free breeding stock into breeding herds. Both live attenuated and dead vaccines available for sows and piglets.

INTRODUCTION

PRRS is a significant cause of respiratory disease in its own right but is also a significant contributor to the porcine respiratory disease complex (PRDC).[1] The ever-expanding diversity of porcine reproductive and respiratory syndrome virus (PRRSV) infections has been emphasized.[2] This agent is one of the three major contributors to the continuing evolution of respiratory disease in pigs (swine influenza virus [SIV], PRRS, and porcine circovirus type 2 [PCV2]).

ETIOLOGY

PRRS is caused by an RNA virus morphologically, structurally, and genomically similar to members of the genus *Arterivirus* of the family **Arteriviridae** belonging to the Order Nidovirales[3] including equine arteritis virus. The virus was first isolated in Lelystad in the Netherlands in 1991 (it was initially called the "Lelystad virus"). The mystery swine disease in North America was then shown to be a similar virus. These two strains are considered to be one virus but differ genetically and antigenically. The North American and European strains are only 60% identical at the nucleotide level.[4]

In terms of evolution, it is possible that lactic dehydrogenase virus of mice infected wild boar in Central Europe and became adapted. It then went to North Carolina in the United States possibly in wild boar. It is thought that the most likely date for a common isolate of the European strains is before 1981. The two species of PRRSV then developed separately on the two continents. Some evidence of this comes from a study of the number of nucleotides in open reading frame 7 (ORF7) of the virus from the United States (372 nucleotides), and the European virus (Lelystad types) had 387 nucleotides, but the Lithuanian strains that were collected had 378 nucleotides.

The European viruses (Lelystad type) have become known as type 1, and the North American viruses as type 2 viruses (ATC-2332 was the first).

There is the implied existence of two distinct genotypes derived from a common ancestor.[5] New clinical cases may occur because of other microbes interacting with the virus but also because new viral variants escape the neutralizing responses of pigs to the previous pig strains of PRRSV.[6]

Genome

The virus has a genome of approximately 15.4 kb consisting of 10 ORFs. ORF1a and ORF1b comprise 80% of the genome and encode polyproteins that are processed to 14 nonstructural proteins (nsps)[7] by viral proteases. ORF 2a, ORF2b, ORF3, ORF4, ORF5a, and ORF5-7 encode eight structural proteins: GP2a, GP2b, GP3, GP4, ORF5a, GP5, matrix protein (M), and nucleocapsid (N).[8,9]

All of these structural proteins have been shown to be important for virus infectivity because of their critical roles in virion assembly and/or interaction with cell-surface receptors.[8] N-linked glycosylation of GP5 is critically important for virus replication.[10] The heterodimer consisting of the GP5 and M proteins is required for infectivity of arteriviruses. GP5 plays a key role in viral entry by interacting with the host cell receptor.[11] ORF2b is also essential for virus infectivity and is likely to function as an ion channel to facilitate uncoating of the virus.[12,13] GP3 is found in type 1 and 2 viruses.[14]

One other protein, (N) or nucleocapsid, synthesized by ORF7 is highly immunogenic and has been used for most antibody studies. It is found in the cytoplasm and the nucleus in which it has an important role in antagonizing cellular gene function. The more recent type 2 strains still exhibit variability in sequence and pathogenicity.[15]

A novel structural protein in PRRSV has been discovered to be encoded by an alternative ORF5, and this protein is referred to as ORF5a and is expressed in infected cells. Pigs infected also express ORF5a antibodies. It is found in all PRRSV subgenomic RNA5 genes as an alternative reading frame and in all other arteriviruses, which suggests that it may play an important role.[16]

There is evidence of recombination between vaccine and wild-type PRRSV strains.[17] There is an exceptional diversity in PRRSV strains in Eastern Europe,[18] which is developed from European viruses and the use of attenuated virus vaccines (containing North American viruses) and these are associated with new genetic subtypes.

GP4-specific neutralizing antibodies might be a driving force in PRRSV evolution.[19] Amino acid substitutions in the GP4 neutralizing epitope may abrogate antibody recognition and favor the development of neutralizing antibody-resistant variants.

The genetic and antigenic characterization of the complete genomes of type 1 PRRSV isolated in Denmark over a period of 10 years have been described. In Denmark, more than 50% of the herds are affected[20] by type 1 and/or type 2.[20] The study showed that there were two major clusters within the type 1 genotype. The differences from the original Lelystad virus varied from 84.9% to 98.8% for ORF5 and 90.7% to 100% for ORF7 for the nucleotide identities. The results strongly suggest that there have been at least two independent introductions of type 1 PRRSV into Denmark with significant drift in several regions of the virus. The genetic dissection of complete genomes of type 2 PRRS viruses isolated in Denmark over 15 years has been described.[21] The virus arrived at the same time as Ingelvac PRRS-MLV, and since then the viruses were found to be 94.0% to 99.8% identical to the vaccine strain. The nucleotide identity was 90.9% to 100% for ORF5 and 93.0% to 100% for ORF7. There was wide diversity in the nsp2 with some deletions in the NSP2 region. The analysis showed that all Danish isolates belonged to a single cluster (sublineage 5.1) resembling the type 2 prototype isolate VR-2332.

North American Viruses (Type 2 Porcine Reproductive and Respiratory Syndrome Virus)

The North American genotype PRRSVs in China have evolved independently from those in other countries, suggesting that geographic separation might be one factor influencing the molecular evolution of PRRSV.[22]

There is an exceptional diversity of North American type 1 PRRSV[4,5] in China.[23]

Korean strains have evolved from North American strains imported some years earlier and have evolved separately from other Asian countries, suggesting that geographic separation may influence the molecular evolution.[24]

European Viruses (Type 1 Porcine Reproductive and Respiratory Syndrome Virus)

In a study of over 100 new PRRSV UK isolates, all type 1, in the period from 2003 to 2007, it was found that some strains were

similar to those found in the early 1990s.[25] It was also found that the diversity is greater now than it was then.[26]

The evolution of Spanish strains of PRRSV from 1991 to 2005 has been studied from 1991 to 2005 using the ORF5 of the virus.[27,28]

The emergence of PRRSV in Sweden was described in 2007 when it was detected through a national surveillance program.[29]

In a study in Thailand, European isolates seem to have evolved from the Lelystad virus, whereas the Thai U.S. isolate may have come from vaccine strains that were not available in Thailand so they may have been imported in pigs or semen and later spread.[30]

High Pathogenicity Viruses

In June 2006, an unknown disease characterized by high fever, high morbidity, and high mortality was seen in many areas of China. It was highly pathogenic and characterized by a unique discontinuous deletion of 30 amino acids in the nsp2 protein with extensive substitutions in the GP5 sequence from the ORF5 gene. This epidemic has affected over 2 million pigs in China, with over 400,000 deaths.[31] A further 140,000+ pigs with 40,000 deaths occurred between January and July 2007.[32] A similar outbreak in Vietnam caused huge losses in 2007.[33,34] The genetic variation and pathogenicity of a highly virulent PRRSV has been described.[35] The genomic diversity of Chinese PRRSV isolates from 1996 to 2009 has been described.[36] They are divided into four highly diverse groups, and it is suggested that they developed from the domestic Chinese viruses by gradual variation and evolution. A new variant has since been described.[37] The complete genome sequences of two other variant PRRSVs isolated from vaccinated pigs have been described.[38] The high pathogenicity (HP)-PRRSV strain has become the predominant strain in China.[39] This virus has undergone rapid evolution and can circumvent immune responses induced by currently used vaccines.

A 1-year study of dynamics and evolution of type 1 and 2 PRRSV in a swine farm in Korea[40] showed the farm was first infected with a type 2 virus and then with a type 1 virus of unknown etiology. The type 1 virus has undergone further change.[41]

The magnitude of differentially expressed gene profiles in HP-PRRSV–infected pigs compared with the original VR-2332–infected pigs is consistent with the increased pathogenicity of HP-PRRSV in vivo.[42]

Spread of High Pathogenicity Porcine Reproductive and Respiratory Syndrome

Highly pathogenic strains of PRRSV have been identified within both genotypes[43-45] and have been isolated in China and Southeast Asia.[31,34,45] A 59 amino acid discontinuous deletion has been found in an HP-PRRSV Chinese virus,[46] whereas most of the previous HP-PRRSVs have had a 30 amino acid deletion.[47] Six different subgenotypic isolates have been found in pigs in China from 2006 to 2008.[48] New genomic characteristics of HP-PRRSV do not lead to significant changes in pathogenicity.[49] High pathogenicity strains have been described in Vietnam.[50] These have been found to be different from the Chinese strains and cause different pathogenic outcomes in American high-health swine.[51]

A highly pathogenic PRRSV virus isolated from a piglet stool in North America was sequenced.[52]

Experimental Infections With High Pathogenicity Porcine Reproductive and Respiratory Syndrome

Experimental infection with a Chinese HP-PRRSV in American swine showed that it replicated in swine with at least a 100-fold increased kinetic activity over VR-2332, which is the American reference strain. It caused significant weight loss, exacerbated disease because of bacterial sepsis, and had more severe histologic lesions. It rapidly transmitted between animals. It also greatly increased serum cytokine levels associated with innate (IFN-α and IFN-β; TNF-α;, and IL-1β, IL-6, and IL-8) and adaptive immunity (IL-2, IL-4, IL-10, IL-12, and IFN-γ) in bronchoalveolar lavage fluid and most of them in serum and tracheobronchial lymph node homogenates.[53] These included IFN-α, IL-1β, IL-2, IL-10, and IFN-γ. In addition, IL-12 was also elevated 11 days postexposure. None of the pigs inoculated with VR-2332 had significant elevations in the serum levels of the 10 cytokines. It may be that this represents a severe cytokine release syndrome or cytokine storm, as has been suggested for humans.[54-56] These conditions share many features including massive inflammatory responses, elevated serum cytokine levels, and multiorgan disease often with death.[57]

ECONOMIC LOSS

A recent study of economic loss caused by PRRSV was made in the Netherlands, and it was found that the loss varied between €59 and €379 per sow per 18-week period of the outbreak. The mean loss was €126. The costs after the outbreak varied from €3 to €160 per sow.[58]

EPIDEMIOLOGY

Occurrence

PRRSV was first reported as a new disease in swine-raising areas in North America in 1986 to 1987, and in 1991 it was recognized in, and spread rapidly across, Western Europe. The disease was first recognized in Germany in 1990 and in the Netherlands in 1991 and then spread rapidly. Based on serologic surveys, there is no evidence of infection in swine herds in Switzerland[59] and Australia.

The introduction of legislation in some countries to restrict the movement of pigs from affected farms slowed the spread of the disease, but the rapid spread of the disease initially to the southwest of Europe and then to the north, paralleled the direction of the wind. Airborne spread was also suspected because even well-managed and isolated herds became infected, but airborne spread over distances of a few kilometers continued to occur, particularly in areas with high pig population density.

The terms ***mystery pig disease*** and ***blue-eared pig disease*** were used because the etiology was unknown and the skin of the ears of affected pigs commonly appeared blue. The disease affects pregnant gilts and sows, unweaned and recently weaned pigs, and growing–finishing pigs. Outbreaks of late-term abortions, high numbers of stillbirths and mummified or weak newborn piglets, and respiratory disease in young unweaned and weaned pigs are common. After 10 or more years of acceptance and relief that the European virus was not as pathogenic as the North American virus, it is now accepted in Europe that the recent evolution of the virus may now be causing as many problems as the North American virus always has done.

A high seroprevalence of PRRSV and SIV on Spanish farms was found to be over 85% for sows, around 80% for finishers, and around 50% for boar studs.[60]

It has also been studied in wild boars in Germany and 15.9% of 531 examined were found to be positive.[61] The genetic diversity of PRRSV in selected herds in the pig-dense region of northwestern Germany showed that of 65 samples tested 5 were the North American type and 60 were the European type 1.[62] Of the original 18 herds visited only 2 reported clinical signs 2 years later.

Both the European genotype (type 1) and the North American (type 2) genotype now circulate globally.[63]

Strains within the genotype may differ by as much as 20% with the GP5 protein showing the widest heterogeneity with 50% to 55% difference between types 1 and 2.[5]

The distribution of genotypes of PRRSV in Ontario from 2004 to 2007 and the association between genotype and clinical signs of disease have been described,[64] and four RFLP types were recognized. In a further study, it was suggested that the diversity in Canada is not described adequately by RFLP typing.[65]

Prevalence of Infection

In endemic herds 30% to 70% of pigs may be seropositive to the virus and about 60% of herds have some seropositive pigs. Although the seroprevalence may be high in herds in some regions, the incidence of clinical disease is lower and variable. Although the number of herds with the acute form of the disease has been decreasing, the infection is now **endemic** in many herds, characterized

by increased mortality and suboptimal performance in nursery pigs, with active spreading of the virus mainly in nurseries. In endemically infected herds, **subpopulations of infected animals** may exist consisting of a low prevalence (<10%) of seropositive animals in the breeding animals and a high prevalence (>50%) of seropositive nursery piglets. The elimination of these susceptible subpopulations, by exposing all members of a population to the virus, is used as a control strategy in large herds in which there may be subpopulations of highly susceptible breeding females. The virus can persist in nonpregnant sows and be transmitted to naive in-contact sows. A PRRSV strain may persist in a herd for up to 3.5 years displaying as little as 2% variation in ORF5 during this period. In 78% of herds with multiple submissions, genetically different strains were identified within 1 year of the original identification. Virulent PRRSV isolates exhibit longer viremias but not more elevated levels; they induce higher death rates and cause more severe clinical signs in a respiratory disease model. More virulent strains grew to significantly higher levels in pigs than did cell culture–adapted isolates. Pathogenic consequences and immunologic responses of pigs to PRRSV are closely related to viral load in acute infections as reflected in viral titers in blood.

On some farms in Great Britain, PRRSV fails to persist indefinitely on some infected farms, with fade-out more likely in smaller herds with little or no reintroduction of infectious stock. Persistence of infection may be associated with large herds in pig-dense regions with repeated introductions.[66]

In a study of 33 sites established as PRRSV free, it was found that 40% became positive within 1 year of establishment.[67]

Morbidity and Mortality

The morbidity rate in young pigs may be up to 50%, and mortality in nursery piglets can reach 25%. Death is usually associated with secondary bacterial infections such as *Salmonella* Choleraesuis, *Streptococcus suis, Actinobacillus pleuropneumoniae,* and *Haemophilus parasuis.* Major losses occur in reproductive failure, but figures for the magnitude of reproductive losses during an outbreak are not readily available. Generally, the reproductive performance of positive herds is significantly lower than negative herds.

Risk Factors

The severity and duration of outbreaks following infection are variable. Some herds may be devastated by high production losses, whereas other herds may have almost no losses. Differences in morbidity and mortality may be caused by dose of virus at exposure, differences in host susceptibility, differences in strain virulence, environmental or housing differences, or the production practices in the herd.

A study of risk factors in Quebec[68] suggested that the transmission of PRRSV is likely to occur through the sites belonging to the same owner or through a 5-km area.

In a study of risk factors for PRRSV infection, it was found that there was a higher proportion of infected farms in areas of high pig density (more than 15,000 pigs within a 10-km radius from the farm), if they used live virus vaccines, if they were located in a high-density pig area, or if dead pigs had been collected. Farms weaning at 28 days or more had lower odds of being PRRSV positive compared with those weaning at 21 to 27 days.[69]

Animal Risk Factors

Nursing piglets lacking maternal immunity, young growing and finishing pigs, and sows lacking acquired immunity from natural infection or vaccination are highly susceptible to infection and clinical disease. Severe disease appears to be more likely in large herds that have a large turnover of pigs, purchase replacements from other herds, and do not use a quarantine system. Introduction of the virus to previously virus-naive herds may cause severe economic losses. In the recent outbreaks in Denmark, the study of 107 herds showed that a variety of hazards were identified including close neighboring herds, increasing herd size, and purchase of semen from infected studs used for artificial insemination.

There is a within-breed genetic variation for commercially relevant traits that could be exploited in future breeding programs.[70] A significant line difference in growth in two genetically diverse commercial pig lines was seen during infection with PRRSV.[71]

One study has shown that the number of piglets per litter infected by PRRSV was lower for the Landrace breed than for Large White, Duroc, and Pietrain breeds.[72]

In a study of 316 herds in Canada, it was found that the three most important factors for the spread of PRRSV RFLP 1-18-4 were sharing the same herd ownership, gilt source, and market trucks.[73] Spatial proximity could not be identified as a contributor to the spread.

Environmental and Management Risk Factors

Housing of all age groups in one building, introduction of new animals, housing on slatted floors, storage of slurry under floors, exposure to transport vehicles, and lack of disinfection procedures have been suggested as factors that increase the probability of herd infection. Lack of quarantine facilities for recently imported pigs is a major risk factor. There appears to be infrequent spread during warm weather compared with cold weather.

Pathogen Risk Factors

PPRS virus strains have many identical properties with some antigenic differences. Strains of the virus from the United States and Canada are antigenically similar but different from the European Lelystad virus isolate. All the strains appear to be highly infectious. There are serologic differences between the European and American strains, and the antigenic and genomic differences between the North American and European isolates suggest the existence of two genotypes. There are different genotypes and at least three minor genotypes within the major U.S. genotype. The simultaneous coexistence of the strains has been shown, but the significance of the observation is not understood. Genetic variations exist not only between European and U.S. strains but also among the U.S. isolates, indicating the heterogeneous nature of the virus. Antigenic variation may affect the accuracy of diagnostic tests and the efficacy of vaccines. The North American strains have been called type 2 virus, and they are continually varying. The European type 1 virus was thought to be less virulent and less likely to change, but this may not be so because recent isolations show that it is also continuing to change.

Infection with the virus does not always result in clinical disease, and the detection of high levels of serum antibody in many herds without history of clinical disease suggests that the consequences of natural and experimental infection depend on a complex of factors associated with host susceptibility and virus virulence. From 2000 to 2001, there were severe outbreaks in the United States associated with new isolates. There are now both European and U.S. strains originating from viral vaccines in Poland. The effects of the virus on reproductive performance are strain dependent. Strains of the virus cross the placenta when given to pregnant sows, and most sows will remain clinically normal and farrow normally. However, depending on the strain used, the number of late-term dead fetuses from gilts infected experimentally at 90 days' gestation may vary widely, and all gilts become viremic and develop antibodies. There are also marked differences in pathogenicity for the respiratory tract between U.S. strains of the virus compared with the Lelystad virus when inoculated experimentally into 4-week-old cesarean-derived colostrum-deprived pigs. Some strains cause severe lesions of the lymphoid and respiratory systems, which appear to be the major sites of viral replication. The difference in pathogenicity may explain the variation in severity of clinical disease observed in field outbreaks.

Field observations have suggested that the presence of the virus in a herd may increase the susceptibility of animals to other infections. However, studies with sequential infection of the virus followed by experimental inoculation with *H. parasuis, Pasteurella multocida,* or *A. pleuropneumoniae* have failed to demonstrate increased severity of disease. There is, however, strong evidence to

say that PRRSV predisposes to *S. suis.* It may also predispose to *Salmonella* Choleraesuis, *Bordetella bronchiseptica,* or *M. hyopneumoniae.* This view is not universal in that infection with the virus did not increase the severity of experimental *M. hyopneumoniae* (MH) infection in young piglets. However, in the laboratory investigation of PRDC the most potent combination of agents is PRRSV and MH. A model of the dual infection has recently been described in which MH was shown to predispose to PRRSV infection. Based on diagnostic submissions, however, concurrent pulmonary bacterial infections may occur in up to 58% of cases in which the virus was also isolated.

There is also the possibility that many strains may be found in the same herd, e.g., three strains were found in one herd. Several viruses have been found in the same pig, and one great authority has expressed the view that each virus in each pig may be different from every other virus.

A syndrome was described in neonatal pigs marked by neurovirulence. Replication in the brain was verified by IHC in brain sections. Meningoencephalitis induced by the virus was unusually severe.

Methods of Transmission

Virus is produced rapidly after infection, probably within 12 hours. The virus was shown to evolve continuously in infected pigs with different genes of the viral genome undergoing various degrees of change.

There are unlikely to be any wildlife reservoirs (except for feral and wild pigs), although infected mallard can still excrete the virus 39 days later. Most pigs clear PRRSV within 3 to 4 months but some may remain persistently infected for several months. The antibody response does not reflect the carrier status. It is possible that cytokines can switch the balance from a subclinical infection to disease manifestation. There is no evidence that PRRSV is found in the tonsils as a representative tissue.

The virus spreads rapidly within herds when infected pigs are housed in confinement. Up to 90% of sows may seroconvert within 3 months of the virus being introduced into a closed breeding herd. The mode of spread is presumed to be by direct contact, probably nose to nose. The virus generally requires close pig-to-pig contact to achieve an exposure dose.

Presence Within the Herd

The virus is present in a variety of biologic fluids; nasal discharge (positive 21 days later); oropharyngeal scrapings (158 days later); possibly mammary secretions, although this is probably uncommon as previous vaccination does appear to prevent shedding; urine (28 days) and feces (28 days); and intranasal inoculation has been used to reproduce the disease experimentally. The feces may be an intermittent source, a usual source, or not a source. The virus is present in saliva and, considered in the context of the **social behavior** of pigs, may play an important role in transmission.

The virus may persist in, and circulate between, different age groups and locations in a herd for several months despite the absence of clinical disease and may be transmitted by contact to replacement animals or to uninfected farms. Infected pigs may remain carriers of the virus for up to 15 weeks. Persistent and contact infection can be maintained in a nursery if uninfected pigs are continuously exposed to infected pigs. Pigs in the nursery become infected through contact with older infected pigs and not by in utero or postpartum exposure to infected sows. Long-term surveys of farrow-finish herds reveal that isolation rates of the virus reach highest level of 70% to 100% of pigs, 6 to 8 weeks of age, which coincided with the lowest level of maternal immunity. If you rely on infected nursery pigs to transmit infection to incoming gilts in acclimatization studies, then nursery pigs may only be viremic for a maximum of 60 days. There is no association between lymphadenopathy and PRRSV viremia in nursery pigs 4 and 6 weeks postweaning. Viremia cannot be predicted solely on the basis of clinical signs. Large finishing enterprises purchasing pigs of variable infection and immune status provide ideal conditions for persistent virus circulation. Breeding herd subpopulations of infected pigs may exist and perpetuate and enhance the infection in a herd. The inability to control such subpopulations may reduce opportunities for successfully controlling the disease.

Infection may **persist** for an extended period of time because of the following:

- Incomplete infection of the susceptible population during the acute phase
- Introduction of susceptible breeding stock
- A persistent viral infection in individual pigs with the potential of shedding virus under certain conditions, such as grouping for weaning or farrowing
- A rapid decline in passive immunity in young pigs and variable periods of active immunity

Genetic randomness of isolates does not correlate with geographic distance. Movement on to the farm of PRRSV does not generally occur by distance-limited processes, such as the usual wildlife vectors, but more typically occurs because of long-distance transport of animals or semen.

It is likely that piglets born with infection from in utero exposure probably may remain viremic for life, even in the face of antibody formation. Neonatal infection is probably cleared slowly, but infection in the older animal may be cleared much more quickly.

Experimental infection suggests that PRRSV infection is eventually cleared from the host and persistent infection rarely lasts more than 200 days.

In a study in France, a semiquantitative real time RT-PCR was developed to assess the evolution of the viral genome in blood and nasal swabs from inoculated and contact pigs with time. Viral genome was detected from 7 to 77 days postinfection (DPI), whereas viral genome shedding was detectable from nasal swabs from 2 to 48 DPI. The infections increased from 7 to 14 days and then decreased slowly to 42 DPI. The evolution of infectiousness was mainly correlated with the time course of viral genome in the blood, whereas the decrease of infectiousness was strongly related to the increase in total antibodies.[74]

A mathematical model of within-herd transmission dynamics of PRRSV, fade-out and persistence, has been described.[75] It was found that fade-out was likely to occur when breeding females failed to pass the virus on to the piglets. Persistence was more likely to occur once infection was present in piglets, which in turn infected rearing pigs. The probability of persistence was higher in large herds, increased contact between different age groups, and increased reintroduction of infectious gilts.

Possible routes of transmission include introduction of vaccinated animals, use of semen from vaccinated artificial insemination boars, and aerosol spread.

Introduction of Vaccinated Animals

The disease has occurred in PRRSV-seropositive herds in Denmark with no previous clinical evidence of PRRSV. These herds were then vaccinated with a modified live virus vaccine licensed for use in pigs 3 to 18 weeks of age. Boars entering artificial insemination units were also vaccinated. Following vaccination, a large number of herds experienced an increased number of abortions and stillborn piglets and an increasing mortality in the nursing period. The problems occurred mainly in herds without clinical signs among sows and with sows with low antibody titers in the period immediately before vaccination. The PRRSV was isolated from fetuses and identified as the vaccine virus. The evidence suggested that the vaccine virus had spread to nonvaccinated sows followed by transplacental infection of the fetuses. Spread of the vaccine virus was also demonstrated in a nonvaccinated and previously virus-free breeding herd. There are three main methods of spread:

1. Introduction of infected animals: Spread between herds is associated with the introduction of infected carrier pigs.
2. Use of semen from vaccinated or infected boars: Infected boars may shed the virus in their semen for up to 40 days after experimental infection. In boars, the virus can be found in semen by PCR for much longer periods than

can be found in the blood by virus isolation or antigen detection, and the likelihood is that monocytes enter the bulbourethral glands, which then contaminate the semen. Following experimental infection of sexually mature boars the virus was present in the semen 3 to 5 days after infection and on days 13, 25, 27, and 43. Using a PCR assay the virus can be detected in semen for up to 92 days after experimental infection. The insemination of gilts with semen from experimentally infected boars resulted in clinical signs of disease and failure to conceive. Following artificial insemination of gilts with semen from experimentally infected boars, the gilts will seroconvert. The use of the modified-live PRRS virus vaccine in boars is controversial because some boars may still shed wild-type virus in semen after challenge exposure 50 days after vaccination. The inoculation of PRRSV-negative replacement gilts with serum from nursery pigs presumed to be viremic resulted in seroconversion of all 50 gilts tested.

Exposure of pregnant gilts to either attenuated (vaccine) or virulent (field) strains of the virus can result in congenital infection. Congenitally infected pigs can support virus replication for a long period of time during which the viral replication is confined to the tonsils and lymph nodes. After 260 days there were no serum antibodies, and between 63 and 132 days there was no evidence of virus in the lung. Vaccine and field strains can be transmitted postnatally from infected to noninfected littermates. Pigs infected with field strains have an inferior rate of survival and growth than do noninfected pigs. This suggests that use of attenuated virus vaccine during gestation is questionable.

3. Aerosol spread: Airborne spread across regions and between countries was suspected in Europe during the winter of 1990 to 1991. The infection appeared to spread by the airborne route from Germany, across the Netherlands, and into Belgium. Low temperatures, low sunlight, and high humidity may have facilitated airborne spread. Airborne spread up to 20 km has been suggested, but most airborne spread is probably limited to less than 2 km. Usually it is difficult to transmit the agent 1 m. Although an experiment failed to transmit infection from pig to pig in a trailer parked 30 m away, there is a suggestion that it is transmitted for a short distance, but this possibly only occurs intermittently. Aerosol infection might be responsible in the absence of any other means of spread.[76, 77] The effect of temperature and relative humidity on an aerosol of PRRSV has been calculated, and it is more stable at lower temperature and/or lower humidity.[78]

In a study of aerial transmission,[79] it was found that 21/21 aerial samples were positive from exhaust gases from an experimentally infected pig group. Five of 114 long-distance samples were positive and were collected 2.3, 4.6, 6.6, and 9.1 km from the experimentally infected herd. Interestingly, only PRRSV variant 1-8-4 was detected, whereas 1-18-2 and 1-26-4, the other two strains given to the source pigs, were not detected. A production region model to assess the airborne spread of PRRSV has been produced by the same team.[80] Animal age, MH coinfection, and PRRSV isolate pathogenicity did not significantly influence the concentration of aerosol shedding. The shedding of PRRSV in aerosols may be isolate dependent.[81]

A production region model has been used to assess the airborne spread of PRRSV.[82]

The median infectious dose of PRRSV via aerosol exposure has been described.[83] The MN-184 isolate was far more infectious than the VR-2332 isolate.

Long-distance transmission of PRRSV was confirmed in a study where 1.3% of 306 samples were positive. These samples were positive 4.7 km away from the source population.[84]

Other Sources

Fomites

Fomites and infected personnel were shown to be capable of transmitting the virus following contact with infected material. Infected hands, boots, and protective clothing can transmit it.[85] Needles will transmit the virus. People do not usually act as vectors.

Meat

Pig meat does not retain detectable amounts of the virus, and it is unlikely that the transmission through meat occurs. PRRSV can survive in fresh pork at refrigerator temperatures, and the moving of meat juice may increase the risk of viral spread from personnel to pigs.[86] In a study of PRRSV in muscle it was found that 13/89 samples between 28 and 202 days after inoculation were found to be positive by quantitative RT-PCR, but if fed to pigs there was no evidence of infection, suggesting that the test detected noninfectious PRRSV in pig meat.[87]

Insects

Mosquitoes were not seen in one study to be a likely vector for PRRSV. Houseflies may pose some level of risk for the transport and transmission of PRRSV between pig populations under field conditions.[88] The intestinal tract of houseflies will support infectious PRRSV for up to 12 hours following feeding on an infected pig but only for a short period of time on the surface of the fly.

Virus Survival

The PRRS virus is fairly labile and does not survive for more than 1 day on solid fomites, but does survive for several days in well and city water. It may survive for several years in deep frozen tissues, but only 1 month at 4° C (39° F), 48 hours at 37° C (99° F), and less than 45 minutes at 56° C (133° F). There appears to be a low risk from contaminated lagoon water, and the viability of PRRSV in swine effluent is relatively short (18 days), although this is very temperature dependent.

Economic Importance

The export market for pork from a country can be seriously affected when a disease such as PRRSV occurs. When the disease was recognized in the United States, countries such as Mexico, Japan, Canada, and South Korea banned the importation of pork from the United States or required certification that the swine originated from premises where, within the 30 days before the issuance of the health certificate, no swine were introduced from a municipality in which a premises infected with the virus is located.

The economic losses may be very high because of stillbirths, abortions, small litter sizes, and birth of weak pigs, which increases preweaning mortality and increased nonproductive days. In weaned pigs, losses are associated with respiratory disease. In addition, there are the costs of control, which may be high, dependent on the control strategies undertaken. Typically, about 20% loss in annual production can be expected from a severe outbreak.

Negative weaned pigs had an increased margin per pig of $2.12 over the pigs minimally affected by PRRSV in the nursery but which seroconverted in the finishing herd and $7.07 over the pigs with persistently circulating PRRSV in the nursery.

PATHOGENESIS

Effects on Macrophages and Dendritic Cells

PRRSV has a very restricted tropism for porcine alveolar macrophages (PAMs) and some peripheral blood monocytes.[63]

Replication of PRRS in the PAMs directly impairs their basic functions including phagocytosis, antigen presentation, and production of cytokines.[89,90] The virus undergoes a productive replication in these cells leading to cell death via both apoptosis and necrosis mechanisms. In addition, there is also a reduced expression of major histocompatibility complex (MHC) class I and MHC class II, CD14, and CD11 cells.

PRRSV also induces necrosis or apoptosis of macrophages and lymphocytes in the lung and lymphoid organs, impairing the host response.[91]

In eukaryocytes, autophagy is a widely found mechanism that transports damaged cell organelles and long-lived proteins to lysosomes for degradation.[92] The autophagy induced by PRRSV infection plays a part in sustaining replication in host cells.[93]

PRRSV causes a massive increase in the number of B cells, resulting in lymphoid hyperplasia, hypergammaglobulinemia, and autoimmunity in neonatal piglets. There is a preferential expansion of certain clones bearing certain H chain third complementary lengths. The same dominant B-cell type clones occur throughout the body. The authors believed that hypergammaglobulinemia results from the products of these cells.[94]

Many piglets are probably infected in utero. PRRSV infection modulates the leukocyte subpopulations in peripheral blood and bronchoalveolar fluids. Following infection the number of CD8+ cells increased in systemic lymphoid tissue, whereas numbers of B cells increased in mucosal associated lymphoid tissue. Virus infection induces a simultaneous polyclonal activation of B cells mainly in the tonsils and an exaggerated and prolonged specific humoral immune response caused by persistent viral infection in lymphoid organs. Piglets surviving in utero infections have a high count of CD8+, CD2 +, CD4+, and SLA-class II cells in the peripheral blood.

Persistent infection occurs in these pigs. Virus appears to persist in the lymphatic organs and particularly the tonsils and the lungs. Lymphoid tissue tropism occurs during persistent infection when the piglets have been exposed in utero.

Neonatal or nursery infection is probably through the virus reaching the nasopharyngeal epithelium following inhalation from the nose-to-nose contact with other pigs. It is then probably removed to the tonsils in which they are internalized into cells of the macrophage/monocyte series.

Initially, a viremia occurs, with subsequent distribution and multiplication of the virus in multiple body systems and organs causing interstitial pneumonia, vasculitis, lymphadenopathy, myocarditis, and encephalitis. Alveolar macrophages are primary targets for virus multiplication, but this does not fully explain the pathogenesis. Multiple glycoproteins appear to be involved in infection of pulmonary alveolar macrophages. Possibly up to 40% of alveolar macrophages are destroyed. Whether it is a particular group that is damaged or all the alveolar macrophages are damaged is not known, but after about 28 days there is a resumption of normal alveolar macrophage function. PRRSV causes the apoptosis of alveolar macrophages and pulmonary intravascular macrophages. The increase in IFN-γ–positive cells correlated well with the severity of the lung lesions, which may be because of the presence of PRRSV in the lung. IFN-γ markedly inhibits the replication of PRRSV in macrophages.

RECEPTORS

As few as 10 or even fewer virus particles inoculated into the nose or given IM will infect a pig. The virus may enter the cell through an endocytic pathway or through a virus receptor. A third possibility is that the virus may enter the cell through an antibody-dependent enhancement with virus–antibody complexes entering the cell through Fc receptors on the cell surface.

There may be a PRRSV ligand for a cell-surface heparin-like receptor on pulmonary alveolar macrophages.

Several receptors have been described including heparin sulfate, sialoadhesin,[95] and vimentin.[96] The interaction of PRRSV with sialoadhesin inhibits alveolar macrophage phagocytosis.[97] Recently, CD163, a molecule that is expressed solely on the monocytic lineage,[98] has been identified as a possible cellular receptor for PRRSV.[99] This is a receptor that allows previously nonpermissible cells to become susceptible to PRRSV. It is a hapten/hemoglobin scavenger in the scavenger receptor cysteine-rich superfamily. Other factors also appear to be necessary for PRRSV permissiveness.[100] The initial step in infection involves heparin sulfate glycosaminoglycans as an initial attachment receptor and subsequent engagement of the Siglec sialoadhesin resulting in a virus internalization via clathrin-mediated endocytosis. The viral membrane M and the M/GP5 complex were identified as ligands for the initial attachment receptor. Sialic acids present on the surface of the PRRS virions have been shown to play an essential role in PRRSV infection. Recently CD163 was identified as a key receptor and involved in the entry into macrophages.[101] In a recent study,[102] it was suggested that expression of CD163 on macrophages in different microenvironments in vivo possibly may determine the replication levels of PRRSV and the virus pathogenicity.

VIRUS ENTRY

For productive infection, viruses need to enter the target cell and release their genome.[103,104] It has been shown that PRRSV entry into the alveolar macrophage involves attachment to a specific virus receptor followed by a process of endocytosis by which virions are taken into the cell within vesicles by a clathrin-dependent pathway.

It has recently been shown that PRRSV enters early endosomes after internalization but does not continue through the endocytic pathway to late endosomes. It colocalizes with its internalization receptor sialoadhesin on the cell surface and beneath the plasma membrane.[105] Sialoadhesin downregulates phagocytosis in PAMs (not CD163).[97]

There is a significant role for IL-10 in the CD163 and PRRSV susceptibility during the differentiation of macrophages. Possibly the internalization of PRRSV via CD163 in the target cells may induce the expression of IL-10, which in turn induces the expression of CD163 on neighboring cells.

Virus entry into the porcine macrophage has been reviewed[106] as has the virus structural and nonstructural proteins in viral pathogenesis.[107]

REPLICATION

The primary targets for replication are alveolar macrophages of the lung and other cells of the monocyte/macrophage lineage including pulmonary intravascular macrophages, subsets of macrophages in lymph nodes, and spleen and intravascular macrophages of the placenta and umbilical cord. A highly pathogenic strain may possess an expanded tropism to include epithelial cells.

The virus can persist in the pig for up to 132 days after birth in tonsil and lymph nodes infected in utero and from 105 to 157 days from pigs infected in postnatal infection. Over time the initial levels of viral load may decrease 10,000-fold in the tonsil or lymph nodes. The wild-type virus is capable of inducing a higher level of viral load than the mutations.[108-111]

The high pathogenicity strains from China in 2006 contain a unique 30 amino acid deletion in the nsp2 coding region, but this is not associated with virulence of these strains but nsp2 can attenuate replication and virulence.[112] The virus can cross the placenta at about 90 days' gestation and infect the fetus and can use the thymus as the principal site of replication and induce antiviral cytokines.[113]

Macrophages are activated by endogenous danger signals.[114]

There are mitogen-activated protein kinase cascade pathways, which are essential building blocks in the intracellular signaling systems. There are four of these pathways that have been identified, and one of these is the extracellular signal-regulated kinase (ERK) signaling pathway. This has been shown to play an important role in the postentry steps of PRRSV replication cycle and contributes to viral infection.[115]

PRRSV E protein is likely to be an ion-channel protein embedded in the viral envelope and facilitates uncoating of the virus and release of the genome in the cytoplasm.[116] This E protein is probably nonessential for virus infectivity but promotes growth of the virus.[117]

PRRSV can infect and replicate in monocyte and bone marrow–derived dendritic cells.[90,118,119] The exposure of bone marrow–derived immature dendritic cells to PRRSV produced a downregulated expression of MHC class I.

The monocytes and macrophages are the main cellular target for PRRSV replication, particularly the alveolar macrophages. It also replicates in vitro in dendritic cells and bone

marrow–derived monocytes.[118-121] It has a higher predilection for PAMs than septal macrophages.[122] PAMs phagocytose whereas septal cells may modulate immune responses. There is a complex viral replication mechanism in immune cells such as alveolar macrophages for PRRSV.[123]

General Effects of Porcine Reproductive and Respiratory Syndrome Virus on the Immune System

Generally, both innate and adaptive immune responses to PRRSV are suppressed. It produces modest levels of IFN-α and proinflammatory cytokines.[120] In addition, the response is weak and slow. Neutralizing antibodies are slow to be produced. Cell-mediated responses in the form of IFN-γ producing cells can take 4 to 8 weeks to develop. The virus produces an increase in IL-10, which is possibly immunosuppressive because it suppresses antigen-presenting cell activities such as processing and presenting antigen and expression of IL-1, IL-12, IL-18, TNF-α, and type I IFN expression.[119]

Macrophage Damage

The PRRSV nucleocapsid protein regulates alveolar macrophages and, in a study of infected macrophages, 23 protein spots were found that were differentially expressed. Of these, 15 had a statistically significant alteration including 4 upregulated and 11 downregulated[124] spots. Individual mature nsps are found in virus-infected cells.[125]

The alveolar macrophages when infected round up, show bleb formation, and eventually rupture. TNF-α released from damaged macrophages after PRRSV infection may induce apoptosis in uninfected lymphoid cells. In a study of cells in the lungs, it was found in both noninfected and infected cells. The majority of the apoptotic cells were noninfected. The peak of apoptosis was at 14 days and was preceded by a peak of IL-1 and IL-10 production at 9 DPI. The PRRSV infection directly interferes with type I IFN transcriptional activation.

Toll-Like Receptors

PRRSV inhibits TLR expressions in PAMs at 6 hours postinfection and it is then restored at 24 DPI when the cells showed upregulated IL-12.[126]

The possibility of increased expression of TLR mRNA and cytokines in pigs with PRRSV has been shown.[127] In these experiments there was an upregulation of TLR 2, 3, 4, 7, and 8 in at least one of the lymphoid tissues and cells.

Modulation of Immune Responses

Cellular Changes (Natural Killer, T-Regulatory, etc.)

The original VR-2332 prototype North American strain of the virus induces immune modulatory changes at mucosal tissues. Peak antibody response and cytokine IFN-γ were detected at PID30 with increased TGF-β until PID60. Populations of CD4+, CD8+, CD4+ CD8+ T cells, natural killer (NK) cells, and γδ T cells in the lungs and lymphoid tissues were significantly modulated favoring PRRSV persistence. The NK-cell–mediated cytotoxicity was significantly reduced in infected pigs. In addition, increased populations of immunosuppressive T-regulatory cells (T-regs) and associated cytokines were also observed in infected pigs.[128] These results suggest that both innate (γδ T cells and NK cells) and adaptive immune cell subsets were modulated in mucosal tissues in which the virus persists for a long time. IL-10 and TGF-β are immunosuppressive in nature produced by T-regs and are upregulated in PRRSV-infected pigs.[129] Although wild-type parenteral strain VR-2332 is avirulent it dampens the most essential immune components at the site of replication, which are the lung parenchyma and lymphoid tissue, resulting in weak and delayed anti-PRRSV immunity.

NK cells are only a small fraction of circulating lymphocytes that are not B or T cells. Cytokines IL-2 and IFN-α are activators of T cells.[130] PRRSV is a poor inducer of IFN-α. These cells early in infections kill infected cells and produce cytokines.[131] PRRSV-infected macrophages are less susceptible to NK cells. This reduced activity begins at 6 hours postinfection and coincides with the detection of observable PRRSV structural proteins.[132] It is likely that the transcription of viral genes and proteins also contributes to the resistance of PRRSV-infected macrophages toward NK cells. PRRSV infection inhibits both NK and cytotoxic T-cell activity via a common mechanism.[133] It might be that during PRRSV infection the virus may modulate the ligands for the NK receptors on the surface of pulmonary alveolar macrophages, leading to insufficient NK cytotoxicity.

The PRRSV has a suppressive effect on the NK cells, which are part of the innate immune response. They are usually activated by IL-2, IL-12, IL-15, IL-18, and IFN-α and by the interaction between NK activating receptors and their ligands on target cells.[134] One of the components of reduced NK cell activity is the possibility that there is incomplete activation of NK cells by a lower level of activating cytokines.[135] PRRSV-infected pulmonary alveolar macrophages showed a reduced susceptibility toward NK cytotoxicity, and this may represent one of the multiple evasion strategies of PRRSV.[133]

Replicating PRRSV in both infected and contact pigs was responsible for rapid modulation in NK cell–mediated cytotoxicity and alteration in the production of important immune cytokines. These changes produce a delay in adaptive immunity. At 2 DPI 50% of viremic pigs had a greater than 50% reduction in NK cell–mediated cytotoxicity, and nearly a onefold increase in IFN-α was found in the blood of some pigs. Enhanced secretion of IL-4 was found in 90% of pigs and IL-10 and IL-12 in a few pigs. IFN-γ was not enhanced. There was a reduced frequency of myeloid cells, CD4+ CD8+ T cells, and CD4– CD8+ T cells, and upregulated frequency of lymphocytes bearing natural T-reg cell phenotype were detected in viremic pigs.[136]

This is associated with a decrease in cytotoxicity but not the number of NK cells (increased IL-4, IL-10, and IL-12).[136] Regulatory T cells (induced by type 2 but not type 1 PRRS) also impair the host.[137-140]

There is a decrease in the number of antigen-presenting cells and T cells in the tonsil and lymph nodes of PRRSV-infected pigs, suggesting a modulation of the host immune response.[141]

CD14 + monocytes may also infiltrate the interstitial tissue in the lung and develop into interstitial macrophages. The early development of subneutralizing or nonneutralizing antibody may have a significant effect on the development of PRRS by antibody-dependent enhancement, which can facilitate the attachment and internalization of the virus onto host cells through Fc receptor-mediated endocytosis.[142]

A higher expression of proinflammatory cytokines is also expressed in septal macrophages in pigs.[122,143] T-regs[143] control the immune response and maintain homeostasis and are natural or induced. Induction of T-regs during the early stage of PRRSV infection is one of the ways pathogens escape the immune response.[138,139,144-147]

Cytokines

Many cytokines influence the immune response to PRRSV infection (Table 18-3). TNF-α may act as an antiviral cytokine protecting cells from infection by an IFN-independent mechanism, and several strains of PRRSV have a low ability to induce the expression.

The cytokines IL-10 and IL-12 are expressed in inflammatory lesions in the lung and play an important role in the defense against PRRSV. In some PRRSV infections, there was no change in the levels of IL-10, IL-12, and IFN-γ in PRRSV infections. It also induces minimal levels of T-helper-1 (Th1) cytokines (IL-12 and IFN-γ).[90]

In utero–infected pigs showed significantly increased IL-6, IL-10, and IFN-γ mRNA expression (IL-2, IL-4, and IL-12 remained the same) and this was concurrent with a significant decrease in the number of CD4+ CD8+ T cells. The cell-mediated and cytokine message profiles returned to normal.

The increased expression of IL-1α, IL-6, and TNF-α in the lungs of pigs with PRRSV is correlated with the development of interstitial pneumonia.[122] Different isolates induce

Table 18-3 Cytokines and porcine reproductive and respiratory syndrome[139]

Cytokine	Function
IL-1	Attracts macrophages, monocytes, polymorphs
IL-6	Induces acute phase proteins Upregulates CD163 receptor
IL-10	Upregulates CD163 receptor Upregulates in the lung
TNF-α	Inhibits replication of PRRSV Induces acute phase proteins Downregulated in PRRSV-infected macrophages Downregulates CD163 receptor
IFN-α	Interferes with replication of PRRSV Downregulated in PRRSV-infected macrophages
IFN-γ	Inhibits replication of PRRSV Enhanced by vaccination with IL-12/IFN-α Downregulates CD163 receptor
IL-10	Correlates with expression in the lung Inhibits IFN-γ in the lung
TGF-β	Induces T-regs after PRRSV infection Correlates with expression in the lung Downregulates CD163 receptor

IFN, *interferon;* PRSSV, *porcine reproductive and respiratory syndrome virus.*

different patterns of IL-10 and TNF-α. Four possible phenotypes were identified, but different cells had different capabilities. In addition, cytokine-release profiles on antigen-presenting cells could induce different expressions of cell markers.[121]

Certain regions of nsp2 also downregulate IL-1β and TNF-α.[148] The inhibition of early cytokine production contributes to the weak innate immune response, delayed neutralizing antibody, slow IFN-γ response, and a depressed cytotoxic T-cell response.[149]

In PRRSV infections, the production of proinflammatory cytokines is limited.[150] The nsps may downregulate TNF-α.[151,152]

IL-10 inhibits the synthesis of proinflammatory cytokines as well as inhibiting the production of IFN-α, and may also suppress the proinflammatory response to PRRSV-infected pigs. There is a significant correlation between the response to PRRSV antigen expression and the expression of regulatory cytokines, such as IL-10 and TGF-β in the lungs but not in the lymph nodes.[153,154]

There may be, as a result of the cytokine expression, a reduction of the infiltration and proliferation of inflammatory cells.[155] IL-10 is expressed mainly by septal macrophages and TGF-β mainly by PAMs. There may be different expressions of different cytokines by different subsets of the lung cells. TGF-β production may be dependent on the PRRSV strain.[156] CD163 is one component of a complex of receptors required for entry of PRRSV entry into the cell including heparin sulfate and sialoadhesin. It is upregulated by IL-10 and IL-6 promoting PRRSV entry into the cell and replication but downregulated by TNF-α, TGF-β, and IFN-γ.[102,157]

The induction of the IL-10 response may be one of the strategies used by PRRSV to modulate the host immune responses.[158] Increases in IL-4, IFN-γ, and TNF-α were found in the lymphocytes of infected piglets, but IL-8 showed a decrease. Other authors have the opposite view, which suggests that T cells showed an increase in CD8+ CD4+ and CD4– CD8+ subsets within activated cells, whereas CD4+ CD8– cells decreased with time. T cells responding to the virus showed a Th1 type cytokine production pattern. These authors[158] also reported a decrease in TNF-α and a decrease of IL-1α and macrophage inflammatory protein.

Perhaps this is the key to PRRSV infections in that all pigs may respond differently. There may be either depressive or stimulatory effects. The imbalance of IL-12 and IL-10 produced in PRRSV-infected pigs may favor the humoral responses and suppress cell-mediated immune responses for the first 2 weeks of life.

PRRSV was detected in the cytoplasm of macrophages at two peaks, 3 to 7 DPI and second at 14 DPI. IFN-α increased at 3 DPI, and IFN-γ and IL-12 were increased at 3 to 7 DPI and 14 to 17 DPI, but IL-10 was lower than the others suggesting that other factors also play a part.[153]

Interferons

PRRSV is able to downregulate the production of inflammatory cytokines such as type I interferons (IFN-α, IFN-β, TNF-α, and IL-1). Pigs that can clear PRRSV early have early expression of these cytokines.[160] Five of the 13 nsps were found to inhibit IFN-β promoter activation, particularly nsp1β[161] as well as TNF-α promoter activity.[162] One of the mechanisms to suppress the immune response would be to suppress several key immune regulatory cytokines, such as type IFN, IL-1, TNF-α, IL-12, and IL-6, and upregulate to aberrant levels the antiinflammatory cytokines IL-10.[162]

IFN-α is an early response to PRRSV, but the virus circumvents the host innate response with an inadequate production of type I IFNs, resulting in a delayed IFN-γ production, cellular immunity, and neutralizing antibodies and a delayed viral clearance.[163]

PRRSV is able to suppress the transcription of key antiviral genes, TNF-α and IFN-β, when infection was antibody-dependent enhanced. This pathway of infection allows PRRSV to specifically target antiviral genes and alters the innate intracellular immune responses in macrophages.[164]

The proposed model of how PRRSV nsp1 negatively regulates IFN-β has been shown.[165] Plasmacytoid dendritic cells are not present in large numbers in blood but, when exposed to viruses, usually morph into dendritic cells but not when exposed to PRRSV and may help in the persistence of the virus.[166]

In the bone marrow–derived monocyte cells, there was also a significantly increased secretion of IL-1, IL-6, IL-8, IL-10, and IFN-γ but not IL-12 or TNF-α.[167]

Infection with PRRSV increased serum levels of IL-1β, IL-6, TNF-α and IFN-γ. It also increased mRNA for the proinflammatory cytokines as well as the mRNA for TLR3, LR4, and TLR7 in the tracheobronchial tree. Most of the proinflammatory genes were also upregulated in the discrete brain areas.[168]

PRRSV does not elicit a specific IFN-γ response in nonadult animals, and IFN-γ cells may be present in similar numbers in both infected and control animals.[169] PRRSV suppresses T-cell recognition of infected macrophages.[170]

The ORF1a and ORF1b are translated to generate polyproteins, which are processed by viral proteases to form 14 different nsps.[7] Several of the nsps have been identified as integral members of viral replication and transcription machinery, whereas others might be involved in these processes through their interaction with host cell factors.[7,171] The nsps are also likely to regulate viral pathogenesis through their involvement in modulation of host innate immune responses. The nsp1β–mediated subversion of the host innate response plays an important role in PRRSV pathogenesis.[172]

The type I IFNs constitute a major player of the host innate immune system Viral replication intermediates like double-stranded RNA (dsRNA) are sensed by cytoplasmic (RIG-1–like helicases) as well as endosomal (TLR3) sensors, which trigger a complex signaling cascade.[173,174] These signaling events result in an activation of several transcription factors including interferon regulatory factor 3 (IRF3), nuclear factor kappa B ($NF\text{-}_{\kappa\beta}$) and activating transcription factor-2. These factors drive expression of type I IFN genes. Once secreted, they bind to receptors on the cell surface, which ultimately leads to the synthesis of IFN-stimulated genes.[175] Viruses have produced several measures to counteract the IFN production,[176] and PRRSV infection results in poor type I IFN production. The nsps of PRRSV inhibit IFN-dependent transcription. The nsp1α and nsp1β proteins suppress both IRF3 and $NF\text{-}_{\kappa\beta}$–mediated IFN gene induction.[148,177,178] The nsp1β also interferes with IFN signaling.[148,179] The nsp2 is likely to play an important role in the subversion of innate antiviral defenses and provides a basis for elucidating the mechanisms underlying

PRRSV pathogenesis.[180] The PRRSV nsp2 has a cysteine protease domain at its N terminal, which belongs to the ovarian tumor protease family and which appears to antagonize the type I IFN induction.[181]

It also interferes with the activation and signaling pathway of type I IFNs by blocking nuclear translocation.[179] Certain regions of nsp2 are nonessential for PRRSV replication but may play an important part in modulation of the host immunity.[148] PRRSV nsp2 interferes with NF-κβ signaling, which is important for its activation.[181] The virus lasts up to 5 months after infection in some lymphoid tissues. The levels of proinflammatory cytokines are also low, and the development of other effector components is slow (neutralizing antibodies and antigen-specific T cells). Therefore there is an inappropriate suboptimal initial innate response to PRRSV.[182] A nonsuppressive PRRSV virus could therefore be expected to stimulate a strong adaptive immune response.[183] The IFN inhibitory nature of PRRSV nsp1 in the context of virus infection was confirmed.[175,184,185] The nsp1 is cleaved into nsp1α and nsp1β, and the nsp1β has the ability to inhibit IFN synthesis and signaling.[186]

Type I IFNs (IFN-α and IFN-β) promote production of antiviral mediators and elicit NK-cell activity for killing viral-infected cells. They also induce the maturation of dendritic cells into antigen presenting cells, macrophage development, and maturation and together with IL-6 convert B cells into plasma cells.[187] How this might be achieved by the PRRSV has been suggested.[178,181,188] Increased levels of IFN-α at the time of challenge delays PRRSV viremia[189] and lessens the severity of the disease. That the presence of IFN-α at the time of infection can alter the innate and adaptive immune responses was confirmed.[190]

PRRSV encodes viral products that are able to suppress type I IFN production in different ways by interfering with the various transcription factors in the regulation of IFN expression.[172,180,181,190-192] The impairment of type I IFN induction seems to be linked to a weak adaptive immunity, which includes a delayed or slow development of humoral and cellular immunity responses leading to viral persistence in infected pigs.[193,194] Pigs infected with PRRSV had moderate interstitial pneumonia, and the virus was found in all tested tissues. Peak antibody response and IFN-γ occurred at 30 DPI with increased TGF-β until 60 DPI.[128]

The nsp2 inhibits the antiviral function of IFN-stimulated gene (ISG) 15.[195] IFN-stimulated genes are the ISGs of which ISG15 is one of the most highly expressed proteins that functions as an effector molecule in the host cell response to viral infection.

The induction of the IL-10 response may be one of the strategies used by PRRSV to modulate the host immune responses.[158] Increases in IL-4, IFN-γ, and TNF-α were found in the lymphocytes of infected piglets, but IL-8 showed a decrease. It has been shown that T cells show an increase in CD8+ CD4+ and CD4– CD8+ subsets within activated cells, whereas CD4+ CD8– cells decreased with time. T cells responding to the virus showed a Th1-type cytokine production pattern. There is also a reported decrease in TNF-α and a decrease of IL-1α and macrophage inflammatory protein. Perhaps this is the key to PRRSV infections in that all pigs may respond differently. There may be either depressive or stimulatory effects. The imbalance of IL-12 and IL-10 produced in PRRSV-infected pigs may favor the humoral responses and suppress cell-mediated immune responses for the first 2 weeks of life.

DIFFERENTIAL EFFECTS IN DIFFERENT PARTS OF THE BODY

The differential expression of proinflammatory cytokines in the lymphoid organs of PRRSV-infected pigs has been described.[196] The expression was different in the different body compartments. IL-1α and TNF-α were the most highly expressed in the mediastinal lymph nodes. IL-6 was most expressed in the retropharyngeal lymph nodes, but none was expressed in the tonsil. Proinflammatory cytokines are able to modulate the expression of CD163, a hemoglobin scavenger receptor that also acts as a PRRSV receptor and is involved in viral uncoating.[197] Whereas IL-6 can upregulate this receptor expression, TNF-α can downregulate it, inhibiting PRRSV replication. The imbalance in cytokines may play a role in the susceptibility to PRRSV replication.

Recombinant porcine IFN-α given to cells before infection reduced the cytopathogenicity of PRRSV, and viral propagation and antibody responses were delayed. It might be that the IFN alleviated damage to the immune system or enhanced the propagation of host cytotoxic T lymphocytes.[198]

Cytokine expression by macrophages in the lungs of pigs infected with PRRSV has been described.[122] Expression of IL-1α, IL6, and TNF-α correlated with the severity of pulmonary pathology and the numbers of pulmonary macrophages. Significant correlations were found between PRRSV infection and the expression of IL-12p40 and IFN-γ and between the expression of TNF-α and IFN-γ. These findings suggest that PRRSV modulates the immune response by the upregulation of IL-10, which may in turn reduce the expression of cytokines involved in viral clearance (IFN-α, IFN-γ, IL-12p40, and TNF-α). The results also suggest that expression of IFN-γ is stimulated by IL-12p40 and TNF-α but not IFN-α. All of these cytokines were expressed mainly by septal macrophages with weaker expression by alveolar macrophages, lymphocytes, and neutrophils. There appears to be a differential activation of septal and alveolar macrophages in PRRSV infection, with septal macrophages as the major source of cytokines.

There is probably a regulatory role of PRSSV ORF1A on porcine alveolar gene expression.[199]

The expression of PRRSV antigens is correlated with the expression of regulatory cytokines such as IL-10 and TGF-β in the lungs of pigs.[122,154] There are no substantial changes in the level of serum proinflammatory cytokines. Expression of proinflammatory cytokines were increased in mediastinal lymph nodes, but there was little increase in the tonsils and retroperitoneal lymph node.[196]

DIFFERENTIAL EFFECTS OF DIFFERENT STRAINS

There is a differential expression of cytokines by different PRRSV isolates[121] and within different lymphoid organs.[196]

The virulence of these strains may be caused by the impairment of TNF-α by inhibiting the ERK signaling pathway.[200] The limited expression of TNF-α with some strains of PRRSV may be a mechanism in which some are able to impair the host immune response and prevent viral clearance. These downregulations have been associated with nsps1α and 1β and 2.[148]

TGF-β and IL-10 are immunomodulatory cytokines that are able to downregulate the host response. An increased mRNA and protein expression of TGF-β has been observed in PRRSV infection with the North American type II PRRSV.[122,138,201] There is an enhanced expression of TGF-β protein in lymphoid organs and the lung following PRRSV, and this may be important because it is an immunomodulatory cytokine.[202]

In some cases new strains can induce a preferential cytokine profile,[203] and the experimental results show a defective pattern of both innate and adaptive immunity that underlies the long-term persistence of PRRS-infected pigs. Both serum-neutralizing antibody and IFN-γ secreting cells were defective in experimental infections.[204]

On the other hand, in the field, there are complex interactions of virus/host further complicated by interactions with bacterial agonists such as LPS. Under field conditions there was poor or no development of a specific IFN-γ response rather than a delayed one.[170,205] Type 2 isolates are more pneumovirulent than type 1 isolates as seen by clinical signs and macroscopic and microscopic lesions.[206]

Genotype 2 strains of PRRS are more efficient at escaping the intrinsic antiviral activity induced by type I and type II IFNs. Monocyte-derived macrophages can be used by the virus instead of alveolar macrophages.[207]

In a study comparing 39 isolates, there were different effects depending on the strain and the host cell infected.[121] All strains produced high levels of IL-1 and IL-8 in

macrophage cultures but could be differentiated in their responses with IL-10 and TNF-α.

STRAIN VARIATIONS

A comparative analysis of the immune response in experimental infections with three strains of PRRSV showed that although the outcome of infection was similar with clearance at 33 DPI, there were differences in the immune response to the viruses. The "Lena" strain produced fever and clinical signs, whereas the Lelystad virus and Belgium strain A did not. It also resulted in high virus titers in serum, low numbers of IFN-γ secreting cells, a change in leukocyte populations, and a delayed antibody response to immunization with Aujeszky's disease virus. Levels of IL-1β, IFN-α, IL-10, IL-12, TNF-α, and IFN-γ mRNA of the Lena-infected pigs were also increased but not in the other two infections.[208]

The phenotypic modulation and cytokine profiles of antigen presenting cells infected with European type subtype 1 and 3 PRRSV strains in vitro and in vivo was described.[209] The subtype 3 strains (largely Eastern European, e.g., the Lena strain) are more virulent than the type 1 strains. Bone marrow–derived dendritic cells and alveolar macrophages were infected. The Lena strain caused more apoptosis and a higher level of infectivity and some downregulation of the cell-surface molecules. These facts may have explained the increased pathogenicity of the Lena strain and have dampened the specific immune responses. This could explain the delayed and decreased adaptive immune responses observed after infections with this strain.

The effect of genotypic and biotypic differences among PRRS viruses on the serologic assessment of pigs for virus infection has shown that[210] all of the pigs inoculated with field virus became seropositive (indirect fluorescent antibody [IFA] and ELISA). There was a great deal of variation in the onset and level of serum virus neutralization antibody in individual pigs and with each virus. The authors concluded that biotype differences may affect the kinetics of humoral immune response.

Recent studies have suggested that the new strain (Lena) replicates more efficiently than the old Lelystad virus in nasal mucosal explants. This is probably caused by the use of a broader population of entry receptor cells.[211]

HIGH PATHOGENICITY PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME PATHOGENESIS

Classical PRRS produces apoptosis in a variety of organs including lungs, testes, lymph nodes, and thymus. Apoptotic changes in peripheral immune organs and lungs, following experimental infection of piglets with highly pathogenic and classical PRRS, have been described.[212] Previous reports have suggested that HP-PRRS induces thymic atrophy with related thymocyte apoptosis, but there have been no reports in other tissues. The HP-PRRS exhibited much greater cell tropism than the usual PRRS and led to serious injury in tonsil, spleen, and lymph nodes. There were large numbers of apoptotic cells in the organs examined. In HP-PRRS alone, in comparison with vaccinated pigs receiving HP-PRRS, piglets showed thymus atrophy, decreased serum levels of IL-4, and increased serum levels of IL-10 and IFN-γ. The results suggested that elevated IL-10 levels at the early stage of infection may enhance viral survival and delay the onset of protective immunity.[213] The HP-PRRSV affects all stages of production. Pregnant sows manifest abortion and give birth to weak and stillborn piglets, and there are morbidity and mortality rates of 50% to 100%.

DEVELOPMENT OF LESIONS

There was also a temporary immunosuppression in piglets at about 4 weeks postinfection. Vascular lesions associated with PRRSV infection are analogous to those observed in horses with equine arteritis virus, which is also a member of the Arteriviridae family, and the renal lesions of equine viral arteritis infection correspond to those of PRRSV. Inflammatory infiltrates are seen at the junction of the renal cortex and medulla, with vascular changes associated with the muscular tunics of small arterioles.

The characteristic lesions can be reproduced in conventional pigs at 1 week, 4 weeks, or 10 weeks of age, and the variation in severity of clinical disease can be attributed to differences in strain virulence. The effects of the virus on reproductive performance are also strain dependent. There is no evidence that virus will grow in the ovarian tissues but may be taken into them by circulating macrophages. PRRSV can replicate in the testicular germ cells, but there is no evidence that there is any PRRSV in ova, indicating that the female gonad is resistant to persistent infection. Some strains are of low pathogenicity, whereas others are highly pathogenic. The reproductive disease has been reproduced experimentally, and the effects on the fetus are dependent on the stage of gestation. Aerosol exposure of nonimmune pregnant gilts to the Lelystad virus in late gestations (84 days) results in clinical disease. After an incubation period of 4 to 7 days, all sows are inappetent and listless for 6 to 9 days. Some sows develop blue-colored ears accompanied by abdominal respirations. Sows may farrow at days 116 and 117 of gestation, giving birth to dead, mummified, and live piglets. Many of the live-born piglets are pale, listless, and weak, and some are in respiratory distress and exhibit varying degrees of splayleg or muscular tremors. The virus may be isolated from stillborn piglets or those born alive. Antibody is present in precolostral serum samples or ascitic fluids of piglets, which demonstrates transplacental passage of the virus.

The gross and microscopic lesions in the fetuses from sows experimentally infected oronasally with the virus at 90 days' gestation consist of hemorrhage of the umbilicus and necrotizing umbilical arteritis with periarterial hemorrhage. Severe pulmonary lesions are present in fetuses inoculated in utero with the virus between 45 and 49 days' gestation. Even the lowest PRRSV exposure dose caused reproductive failure in naive, unvaccinated animals. When sows are inoculated oronasally with the virus in midgestation, the virus does not readily cross the placenta but replicates in fetuses that are inoculated directly in midgestation. It is suggested in prenatal piglets that PRRS replicates primarily in lymphoid tissues, having gained access to them from the placenta via the bloodstream. Thus the fetuses are more susceptible in late gestation than earlier in midgestation, or there is greater likelihood of transplacental infection during late gestation. Experimentally, the intrauterine inoculation of the virus into gilts on the day after natural breeding may have little or no effect on their reproductive performance. There appears to be no direct or indirect effect on luteal function contributing to PRRSV-induced abortion. The virus may cause cell death directly, such as the alveolar macrophages, or in lymphoid tissues. PRRSV affects Marc 145 cells, which undergo necrosis at a much higher rate than apoptosis, and increases with virus levels used to infect the cells. Apoptosis does occur in PRRSV-infected cells, but it is a late event during PRRSV replication and rapidly results in a necrotic-like death. Lesions have been seen in the placenta and in the vessels of the umbilical cord, but these are rarely reported with European strains, although they may be more common with the North American strains.

The original descriptions of porcine necrotizing pneumonia (PNP) were associated with swine influenza, but more recent research has shown that PRRSV is consistently and predominantly associated with PNP and should be considered the key etiologic agent for PNP together with PCV2.

The pathogenesis of a Korean type 1 PRRSV in experimentally infected pigs has been described.[214] Infected pigs developed multifocal, tan-mottled areas of lung. Microscopic lesions were multifocal, mild to moderate, and generally most extensive at 5 to 7 DPI and were nearly resolved by 28 DPI. PRRSV nucleic acid was detected in cytoplasm of macrophages and type 1 and II pneumonocytes.

HIGH PATHOGENECITY PROCINE REPRODUCTIVE AND RESPIRATORY SYNDROME RESPONSES

HP-PRRSV infection could impair TNF-α production by inhibiting the ERK signaling pathway.[215]

In HP-PRRS the marked inappetence and severe respiratory signs are related to the severe interstitial pneumonia and high levels of expression of IL-1α in the lungs compared with other PRRSV strains.[215]

High pathogenicity PRRSV displays an expanded tissue tropism in vivo suggesting that this may contribute to its high pathogenicity. Positivity was recorded in macrophages in lymphoid organs but also in the epithelium including gastric mucous membrane and mucous glands.[216]

The HP-PRRS epidemic in China, the so-called high fever disease with nervous signs, has been on the increase in China since 2009. There was a nonsuppurative encephalitis with lymphohistiocytic perivascular cuffing and infiltration of leukocytes into the neuropil. The electron microscope showed that the virus that infected the endothelial cells crossed the blood-brain barrier into the central nervous system (CNS) and then induced cellular damage to the neurons and neuroglial cells.[217]

An HP-PRRSV strain (HuN4) was shown to produce a loss of appetite, decrease in BW, raised body temperature, and respiratory signs. Lesions were of multifocal interstitial pneumonia with macrophage infiltration. The lesions in the lymph nodes were characterized by collapsed follicles, depletion of germinal centers, and reduction in lymphocytes. Perivascular cuffing and glial nodules were observed in some brains. PRRSV was detected in macrophages, alveolar epithelial cells, and vascular endothelial cells in the tonsil and lymph nodes. It is more pathogenic than some strains because of its higher replication rate.[218]

Chinese and Vietnamese strains of HP-PRRSV cause different outcomes in U.S. swine.[53,218] The Vietnamese virus replicated in an approximately 10-fold lower level in serum than did the Chinese virus. It also produced a lower temperature response and resulted in a lower mortality. The cytokine responses in a 9-plex panel varied between the strains, between the tissues examined, and by the inoculum dose. In this study, also using the U.S. prototype strain VR-2332, all three produced detectable levels of TNF-α, IL-1β, IFN-γ, IL-10, and IL-12p70, but the levels and the kinetics also differed. There was also a high sustained level of IL-10 and IFN-γ, and these might impair effective immune clearance.[53,219,220] Polyclonal B-cell activation can result in IL-10 producing B cells.[221] PRRSV produces a polyclonal activation of B cells accompanied by a hypergammaglobulinemia.[222-224] This leads to deregulated cytokine production.

IMMUNOLOGY

The immune responses generated by PRRSV and control of the disease by immune mechanisms are not yet completely understood.

There are highly conserved T-cell epitopes on nsps9 and 10 of type 2 PRRSV,[225] and these may be important in the formulation of immunogens to provide broad cross-protection against diverse strains of PRRSV.

Inoculation with different PRRSV strains results in different virologic and immunologic outcomes and in different degrees of homologous and heterologous protection.[156] The core effect of the virus is to infect and cause abnormalities in the macrophages. Disturbed macrophages may fail to present antigen successfully. More important, whatever cytokines are present in the pig or are induced by the PRRSV in that particular host may determine the outcome. It was shown that PRRSV is slow to produce both neutralizing antibodies and cell-mediated immunity, but it does produce an IFN response in PRRSV-infected lymphoid tissue.

Following natural infection, most pigs are resistant to subsequent infection, but the mechanisms of protective immunity are not understood. It has been suggested that the immune response to PRRSV has some degree of strain specificity. Indeed, it has also been suggested that the ability to cross the placenta is also strain specific and that although maternal immunity may not prevent transplacental infection, it may exert additional selection pressure. Circulating antibodies to the virus are detectable within 14 and 21 DPI based on indirect immunofluorescence test or ELISA, and 15-kDa protein is the most immunogenic of the viral proteins and may provide the antigenic basis for the development of improved diagnostic tests. However, this response is not of neutralizing antibodies. These may take a long time to develop. At the same time the occurrence of IFN-γ–producing cells is initially weak, but this becomes much stronger from 3 to 6 months after infection. This response may be enhanced by the use of IL-12. Several structural, functionally distinct, and specific antibodies to the virus are generated following infection or vaccination. Cell-mediated immune responses specific to the virus also occur. The relative role of humoral and cell-mediated immunity in providing protection against disease is unknown.

A unique feature of infection is that viremia and circulating antibodies may exist together; the antibodies protect pigs from reinfection and reduce or eliminate shedding of the virus in the semen of boars. Sows are immune to further disease associated with the virus following recovery from acute infection. Following an outbreak of reproductive disease the level of performance will return to normal, suggesting that immunity develops following natural exposure. Protection against subsequent reproductive losses is of long duration in individual animals. However, cross-protection to different strains may not occur. Experimentally infected sows are protected against reproductive losses when challenged with homologous virus over 300 days after initial exposure. Extended studies against homologous infection found that the duration of protection was at least 604 days, which is essentially lifelong protection. Protective immunity was based on two criteria: the absence of transplacental transfer of challenge virus and the apparent lack of virus replication in the dam 21 days following inoculation.

Piglets born from seropositive sows acquire **colostral antibodies** that decline at highly variable rates from 3 to 8 weeks after birth. Passive immunity provides effective immunity for the piglets, but loss of passive immunity at various ages results in susceptible pigs and infection that results in persistence of the virus in pigs 6 to 9 weeks of age, which are considered as the major reservoir of the virus in farrow-finish herds. In the absence of natural infection, maternal antibodies become undetectable between 6 and 10 weeks of age. Some litters do not have maternal antibodies and may not have detectable antibodies until 4 weeks of age, and clinical disease may occur at 2 weeks of age. By 8 weeks of age, antibodies are usually detectable in all pigs and they persist for several months. However, there may be a large variation in the levels of antibodies in piglets at 10 to 12 weeks of age when they are moved to the finishing units. In longitudinal surveys, the seroprevalence of the virus in the 4- to 5-week-old pigs was higher than in the 8- to 9-week-old pigs, and most pigs were negative when they entered the finishing units. In herds where the virus persists, sows did not suffer repeated reproductive losses, indicating that some form of protective immunity develops.

The virus has a predilection for immune cells, and disease manifestations can be linked directly to changes in the immune system. The replication of the virus in the cells of the immune lineage, especially macrophages, may lead to immunosuppression and predispose to secondary infections. Thus immunity to the virus may be a double-edged sword; the virus attacks the immune system, which may cause immunosuppression, while inducing protective antibodies.

Antibody-dependent enhancement of infection may also occur, because low levels of antibody enhance the ability of the virus to enter the pulmonary alveolar macrophage cells and replicate and destroy the cells. This may be important in sucking and nursery pigs exposed to the virus during a period of declining maternal antibody.

PRRSV complicates the ability of the host to respond to infection through several immune evasion mechanisms.[63,226] PRRSV infection is characterized by a delayed appearance of neutralizing antibodies (3–4 months) and a slow development of virus-specific IFN responses. PRRSV nsp2 is increasingly emerging as a multifunctional protein possibly with a profound impact on PRRSV replication and viral pathogenesis.[227] Acquired immunity has been reviewed.[63,184,204,226] After

infection, most antibodies are nonneutralizing and are principally targeted to N and nsp2 proteins. Neutralizing antibodies appear from 2 to 4 weeks but do not peak until several weeks to months later. Virus persists in the presence of neutralizing antibody. It is possible that PRRSV produces "decoy" epitopes that produce nonneutralizing antibodies.[228]

The T-cell responses to PRRSV are induced 2 to 8 weeks postinfection and are detected against all structural proteins encoded by ORFs 2 to 7 but are considered to be weak, transient, and highly variable.

GP5 and M are the major proteins of the envelope of PRRSV, and the GP5/M ectodomain peptide epitopes are available for host antibody recognition but are not associated with antibody-mediated virus neutralization.[229]

CLINICAL FINDINGS

The main feature of clinical disease associated with this virus was the extreme variability of the clinical signs. Generally, signs associated with PRRSV appear to result from a combination of genetic factors and herd management characteristics. The relative influences of these two factors differ depending on the specific clinical signs in question. These may vary from inapparent infection to sudden death and abortion storms (the sow abortion and mortality syndrome).

The condition continues to evolve from the first descriptions of mystery swine disease in the United States and Canada and blue-eared pig disease in Europe. The swine mortality and abortion syndrome was then described in the United States. Then, there have been the high pathogenicity cases in China ("high fever disease") characterized with greater than 20% mortality[230-232] and the highly virulent 1-18-2 strain that occurred in the north central United States in 2007.[233]

Concurrent Infections

The increased secondary bacterial infection has been linked to an upregulation of CD14 and LPS-binding protein in PAMs.[234] The effects of the virus on the immune system may explain the suspected immunosuppression and secondary infections, which are recognized clinically but have not been reproduced experimentally.

Its synergism with PCV2 is in doubt. It does not seem to be potentiated by the other great pig pathogen PCV2 virus, but it has been proposed that it may increase the severity of PRRSV-induced interstitial pneumonia. PRRSV infection may enhance PCV2 replication. It is predisposed by MH, and this can be reduced by vaccination for MH. In turn, PRRSV predisposes to *B. bronchiseptica*. Both may interact to reduce the efficiency of lung defense mechanisms and facilitate infection with *P. multocida*. There is little effect on *H. parasuis* secondary infection with a slight increase in macrophage uptake of *H. parasuis* during the early infection, which is reduced after 7 days. There is evidence that concurrent infection with transmissible gastroenteritis virus and PRRSV is likely to have little or no effect on subsequent shedding or persistence of infection. Infection with PRRSV is common in pigs with postweaning multisystemic wasting syndrome (PMWS), but there is no evidence that PRRS is necessary for the development of it. PRRSV has been seen in a swine herd with porcine cytomegalovirus. Synergism between PRRSV and *S.* Choleraesuis has been described with unthriftiness, rough hair coats, dyspnea, and diarrhea. Pigs that received dexamethasone were the most severely affected and half died, but they also shed significantly more organisms in feces and also had significantly higher PRRSV titers. Simultaneous infection between PRRSV and *S. suis* is much more severe than with either agent on its own. PRRSV-induced suppression of pulmonary intravascular macrophage function may in part explain PRRSV associated susceptibility to *S. suis* infection.

There is also a clear synergism between PRRSV and LPS in the exhibition of respiratory signs in conventional pigs. In these infections with the virus and bacteria, the rise in TNF-α, IL-1, and IL-6 was 10 to 100 times higher than in the single infections. Reproductive failure and respiratory disease are the major clinical findings that are also highly variable between herds. All age groups in a herd may be affected within a short period of time.

Pigs infected with both PRRSV and MH had a greater percentage of pneumonic lung, increased clinical disease, and lower viral clearance than pigs with single infections. There were also increased levels of IL-β, IL-8, IL-10, and TNF-α in lung lavage fluid, and this may be the way that the combined infection increases the pulmonary response.

Clinical disease is often more severe when accompanied by infection with PCV2[1] and is associated with other conditions in the field that often appear as indicators of the underlying PRRSV infection.[135,235] These are mainly caused by the pneumovirulence of the virus and its persistence in lymphoid organs. There is a decrease in NK cell cell–mediated activity caused by a decreased expression of IFN. The adaptive immune response is also impaired, leading to an increased apoptosis of PAMs caused by increased IL-6 and IL-10.[135,236]

Pigs with PRRSV and subsequently exposed to porcine respiratory coronavirus (PRCV) had reduced weight gains, higher incidence of fever, and more severe pneumonia compared with either single infection.[236] This was caused by reduced IFN-α in the lungs and reduced NK cells, and it coincided with the pneumonia. The subsequent PRCV enhanced the level of PRRSV replication in the lung and a tendency to increased serum Th1 activity (IFN-γ) but decreased type II activity (IL-4) responses, further exacerbating the PRRSV pneumonia. More severe alveolar macrophage apoptosis then occurred.

Pulmonary function has been studied in PPRRS-affected pigs.[237] Infected pigs developed fever, reduced appetite, respiratory distress, and dullness within 9 DPI. The noninvasive pulmonary tests revealed airway obstruction, reduced lung compliance, and reduced lung gas transfer. The effects were worst at 9 to 18 DPI in which they were accompanied by an increased respiratory rate and decreased tidal volume. Expiration was affected more than inspiration, and this is caused by airflow limitation predominantly in the peripheral airways. Pigs have both obstructive and restrictive disorders and have shorter breathing cycles and shallower respiration. The energy requirement for breathing increases because of the increased effort.

Infection with the European PRRSV causes CNS disorders in the suckling pig.[238] PRRSV was detected in the macrophages in the cerebrum by IHC.

Reproductive Failure

If 90-day gestational gilts are given vaccine or field strains of PRRSV then some pigs are born dead, most pigs survive, and some pigs are infected in utero. Vaccine strains did not affect postnatal growth, but field strains reduced growth. It may be that the virus entered the reproductive tract through the viremia and then the seeded tissues may release the virus back into the serum at low levels.

The infection of fetuses with an attenuated virus shows the same immune dysfunction as in wild-type infections in piglets kept in isolators.[224]

All sows given IM injections of a mild strain of PRRSV at 90 days' gestation showed transmission of the virus in utero. The proportion of virus-positive pigs and their level of viremia were higher at 4 days of age than at birth or weaning. The findings suggest that monitoring piglets in late lactation will enable assessment of the shedding of the virus from sows.[239]

Landrace gilts when given PRRSV had a significantly reduced number of fetuses but a similar effect in crossbred pigs was not found. The Landrace had less weight loss during pregnancy, suggesting greater tolerance of PRRSV infection. Breeds do differ in phenotypic impacts of PRRSV.[240]

Anorexia, lethargy, depression, and mild fever in pregnant gilts and sows are common initial clinical findings affecting 5% to 50% of animals. This is commonly followed by a sudden increase in early farrowings at 108 to 112 days' gestation, late-term abortions, stillborn and mummified fetuses, partially autolyzed fetuses, weak neonates with high mortality within a few hours or days after

birth, late returns to estrus, and repeat breeders. This is generally followed by midgestation abortions and marked increases in the percentage of mummified fetuses, early embryonic death, and infertility. In large herds, successive groups of 10% to 20% of gilts and sows may become anorexic over a period of 2 to 3 weeks. Cyanosis of ears, tails, vulvas, abdomens, and snouts may occur in a small number of sows, which is more common in European outbreaks and uncommon in North America. Following the initial outbreak, a storm of reproductive failure may occur consisting of premature farrowings, late-term abortions, an increase in stillbirths, mummified fetuses, and weak neonates. This second phase of reproductive failure may last 8 to 12 weeks. Stillbirths may reach 35% to 40%. Weak-born piglets die within 1 week and contribute to a high preweaning mortality.

The interaction between PRRSV and the late gestation pig fetus has been described.[113] The major site of replication was the thymus. There were elevated IFN-γ and TNF-α in tissues from infected piglets. The hyperplastic fetal lymph nodes had large numbers of B cells. Fetal infection can alter the selection of PRRSV variants and may represent a source of PRRSV genetic diversity.

The pathogenesis of PRRSV in experimentally infected pregnant gilts has been described.[241] There was a significant increase in apoptotic cells in lung, heart, thymus, liver, adrenal gland, and spleen of stillborn fetuses compared with live-born piglets. The majority of cells were either full of PRRSV or apoptotic but not both. Apoptotic cells outnumbered PRRSV cells. PRRSV may replicate in the fetal implantation sites and cause apoptosis of infected macrophages and the surrounding cells.[242] In a review of the pathogenesis and prevention of placental and transplacental PRRSV infection, it was found that the virus replicates in the endometrium and placenta in late gestation, and this is responsible for the range of PRRSV-related reproductive problems.[243]

PRRSV is shed in the milk of infected sows, and the antigen is present in the in the mammary glands of experimentally infected sows.[244]

Today with the original European strains there may be just outbreaks of rolling inappetence or occasional early farrowings. However, there are serious clinical outbreaks in Italy, Poland, and the UK associated with new variants.

Reproductive disease may be preceded by, or follow, respiratory disease in the breeding herd, finishing pigs, or younger pigs. The reproductive aspect of the disease typically lasts from 4 to 5 months, occupying an entire reproductive cycle within a herd. This is followed by a return to normal performance. Repeated incidents of reproductive failure in individual gilts and sows are unusual, but recurrent episodes may occur in herds purchasing replacement gilts that do not have sufficient immunity.

Vaccinating sows with the North American PRRSV-based modified live vaccine does not prevent reproductive failure after insemination with European PRRSV-spiked semen.[245]

Outbreaks of the disease are characterized by a period of severe reproductive problems in the breeding herd, followed by a return to near normal reproductive performance, punctuated by recurrent episodes of reproductive failure. Most herds eventually return to preoutbreak levels of reproductive performance, but some herds never achieve preoutbreak performance levels.

Boars may also be affected with anorexia, fever, coughing, lack of libido, and temporary reduction in semen quality. PRRSV infection affects seminal quality for a limited period only. The virus can be transmitted to sows through insemination.[238]

Boars naturally coinfected with PRRSV and PCV2 can be found, and at least two different strains of virus from serum and semen can be detected.[246] A group of spontaneously infected boars seroconverted 4 weeks postinfection. There was an increase in the acrosome-defective spermatozoa and sperm motion patterns.[247]

Respiratory Disease

The most important problem facing many of the larger pig industries in the world is PRDC. The most important contributor to this syndrome is PRRSV. The generation of immunity capable of protecting pigs by mediating virus inhibition through virus-neutralizing antibodies or IFN takes time.

Disease occurs in pigs of any age, but especially in nursing and weaned pigs, and is characterized by anorexia, fever, dyspnea, polypnea, coughing, and subnormal growth rates. A bluish discoloration of the ears, abdomen, or vulva may also occur (blue-eared disease). Death may occur in the acute phase. In some herds, up to 50% of pigs are anorexic, up to 10% may have a fever, up to 5% are cyanotic, and up to 30% have respiratory distress. In weanling pigs, the morbidity may be as high as 30%, with a mortality of 5% to 10%. Nursery pigs exhibit respiratory distress and growth retardation. Conjunctivitis, sneezing, and diarrhea are common. All of these signs may appear to move through the various age groups in the herd over several days and a few weeks. The course of the disease in a herd may last 6 to 12 weeks. In gilts and sows of any parity, anorexia and fever, lasting for several days, are noted initially. The acute-phase respiratory disease may last several months but is often followed by a long period of postweaning respiratory disease, which may last up to 2 years. This long course is often accompanied by secondary infections in successive batches of weaned pigs. Unthriftiness may persist throughout the finishing period with an ineffective response to antibiotics and vaccines.

Preweaning morbidity and mortality is a major feature of the disease. Litters are often unthrifty, and many deaths occur within the first week of age.

In a study of a newly established farrow-to-finish farm in Poland that was negative for PRRS on establishment but positive for PCV2, it was found that the conception rate dropped from 89% to 51% and the abortion rate increased from 0.5% to 11.0% with the onset of PRRS infection. Then the mortality was elevated, and clinical disease typical of PMWS occurred. The abortion level returned to normal 4 months later, and the conception rate returned to normal 4 months after that.[248]

CLINICAL SIGNS IN HIGH PATHOGENICITY PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

Infection with these signs is associated with severe clinical signs, pulmonary lesions, and aberrant host responses.[249,250]

CLINICAL PATHOLOGY

Acute Phase Proteins

Acute phase proteins (APPs) are synthesized by the liver hepatocytes in response to proinflammatory cytokines. They induce inflammatory reactions and fever, but overproduction may produce an antiinflammatory state. PRRSV may not produce an APP response caused by a poor preinflammatory cytokine response. There is an early expression of haptoglobin (modulates immune response and interacts with CD163), which is the receptor for PRRSV, increasing expression of IL-10 (antiinflammatory), and pig major acute protein, but the response of C-reactive protein (CRP; activates complement and opsonization) and serum amyloid A (chemoattractant for monocytes, T cells, and polymorphs) is delayed and variable.[201] The haptoglobin may modulate the immune response and induce the antiinflammatory IL-10.

The CD163 removes the hemoglobin–haptoglobin complexes circulating in the blood and decreases the amount of iron available for bacteria and reduces oxidative stress.

Haptoglobin levels and pig major acute proteins were increased at 10 DPI, but CRP and serum amyloid showed a delayed and highly variable increase. All three proinflammatory cytokines (IL-1β, IL-6, and TNF-α) were poorly expressed, and only a mild increase in IL-1β was observed at 7 DPI. The increased expression of haptoglobin coincided with the light enhancement observed in both IL-6 and TNF-α and might be related with an increased expression of IL-10. The low expression of TNF-α may point to a possible mechanism of viral evasion of the host immune response.[201]

An 8-plex Luminex assay has been developed to detect swine cytokines after vaccination. It will detect innate (IL-1β, IL-8, IFN-α, TNF-α, and IL-12), regulatory (IL-10), Th1 (IL-4), and Th2 (IL-4) cytokines.[251]

PRRSV infection significantly increases the number of alveolar macrophages in bronchoalveolar lavage fluid approximately 10-fold between day 10 and day 21 of infection. Approximately 63% of the cells were cytotoxic T cells (CTLs) and NK cells. Serum haptoglobin levels were increased from 7 to 21 DPI.

Piglets also become anemic in PRRSV infections, and the most highly pneumovirulent strains induced the most severe anemia. This is probably caused by a direct or indirect effect on the erythroid precursor cells of the bone marrow.

A definitive diagnosis requires detection of virus in infected animals and detection of antibodies in fetal fluid or in precolostral blood of stillborn and weak-born piglets. Detection of antibodies in sera of groups of pigs of different ages is also necessary. The most suitable body fluid and tissue samples and diagnostic tests for the etiologic diagnosis of PRRS are dependent on several variables including:

- Age of pigs from which samples are collected
- Stage of infection (acute or persistent)
- Available complement of diagnostic reagents
- Urgency of obtaining results

When congenitally or neonatal pigs are affected, both serum and alveolar macrophages are reliable samples. For older pigs, alveolar macrophages are more reliable than serum.

Detection or Isolation of Virus

The gold standard is the isolation of the virus. A PAM cell line has been developed for the growth of PRRSV.[252]

In an interlaboratory ring trial in Europe to test the real-time RT-PCR tests it was found that there were great differences in the qualitative diagnostics as well as analytical sensitivity. False negatives were a problem, and to achieve maximum safety in the results it was suggested that different assays or kits should be used.[253]

Boars

Serum is the best method to detect PRRSV during an acute infection in boars.[254] Semen samples failed to detect the virus in most cases. Pooling of samples resulted in a decline of sensitivity.

In a study of commercial tests (RT-PCRs) for diverse strains of PRRSV in boars, in serum, semen blood swabs, and oral fluids[255] from experimentally infected animals, it was found that serum and blood swabs had the best performance and highest detection rates. These were at their highest between 3 and 5 DPI. Oral fluids had the lowest detection rates. The virus can be demonstrated by isolation using cell cultures, by direct detection of viral antigen in tissue sections, or by the detection of virus-specific RNA. Two commercial ELISAs and an in-house florescent microbead immunoassay were tested to detect IgG antibodies in serum and oral fluids for both type 1 and type 2 virus. The tests were similar in sensitivity and specificity but the commercial test kit IDEXX Se detected positive animals earlier than the test kit HIPRA Se. The oral fluid and serum had similar detection rates.[256]

Samples used for virus isolation include serum, thoracic fluid, spleen, and lung. Porcine pulmonary alveolar macrophages are used for isolation of virus. Alveolar macrophages using immunofluorescence microscopy can be used for detection of virus during acute infections. The PCR assay is a reliable, sensitive, and rapid test for the detection of virus in boar semen. It can also be used to determine whether suckling piglets are infected with PRRSV before vaccination and for determining the relationship between parity and shedding of virus. It can also be used to obtain PRRSV piglets. PCR followed by RFLP analysis using several restriction enzymes provides a good genetic estimate for isolate differentiation. A reverse transcription and PCR, coupled with a microplate colormetric assay, is an automated system that is a reliable and easy test for the routine detection of the virus in semen samples from seropositive boars. Multiplex RT-nested PCR can be applied to formalin-fixed tissues.

A nested PCR has been described that is 100 to 1000 times more sensitive than the usual PCR. An assessment of the viral load can possibly be made by using the quantitative competitive RT-PCR. A quantitative TaqMan RT-PCR is time-saving, easy to handle, less likely to be cross-contaminated, and highly sensitive and specific. Immunohistochemical techniques are available for the detection of virus in formalin-fixed tissues. The virus was detected in 11% to 23% of animals with interstitial pneumonia. It was found in 21% to 31% of animals less than 3 months of age but in only 6% to 17% of those more than 4 months of age. The immunogold silver staining i s superior to the immunoperoxidase staining systems for detection of virus in formalin-fixed tissues. RT-PCR is also available and can distinguish between North American and European strains.

A double in situ hybridization (ISH) technique has been developed that can show both PRRSV and PCV2 and a small number of alveolar macrophages stain for both antigens.

A rapid detection method using RT-loop mediated isothermal amplification assay has been described.[257,258]

RT-PCRs have been developed for the detection and differentiation of European and U.S. PRRSV.[259,260] These cannot differentiate U.S. and HP-PRRS, but the duplex real-time RT-PCR test developed[261] will do this. The test was also compared with standard single PCRs, and the results were found to be in 98.7% agreement.

A method using phages harboring specific peptides that recognize the N protein of PRRSV has been used to distinguish it from other viruses.[262]

Serology

A recent study has described the production of GP3, GP5, and N-specific hybridomas and an extensive collection of monoclonal antibodies that may help in diagnosis because they reacted with a range of genetically different PRRS viruses.[263] ELISAs differ in their sensitivity, and those that showed higher sensitivity could be used for early detection in individual pigs, especially in PRRSV-free herds.[264]

In a study of the humoral responses in boars measured in serum samples and oral fluid specimens, it was found that IgM, IgA, and IgG were first detected in serum samples collected on DPI 1, 7, and 10, respectively, and in oral fluids from 3 to 7 DPI for IgM, 7 to 10 DPI for IgA, and 8 to 14 DPI for IgG, respectively.[265]

Serologic tests have good sensitivity and specificity for diagnosis on a herd level but less so on the individual animal. The tests in common usage are described below. One of the problems is that the serologic response to a nonvirulent strain is the same as it is to a virulent strain. It is also important to realize that although a positive result for antibody indicates exposure to virus, a negative test does not necessarily mean that the pig is free from PRRSV or has not been in contact with the virus.

Immunoperoxidase Monolayer Assay Test

The immunoperoxidase monolayer assay (IPMA) is often the first test used. Approximately 75% of sows infected with the virus seroconvert to the Lelystad virus. However, the IPMA does not allow for large-scale surveys.

Indirect Enzyme-Linked Immunosorbent Assay (iELISA)

The iELISA is used for the routine serodiagnosis; it is simple, inexpensive, effective, and a better alternative to the indirect immunofluorescent assay or the immunoperoxidase assay. It is suitable for the screening of large numbers of samples and is best used as a herd test. Because of marked differences between and within North American and European virus isolates, serologic tests using only one antigenic type of the virus may potentially yield false-negative results with antisera against diverse antigenic types of the virus. A mixture of ELISA antigens from North American and European strains gives

superior results when both types of viruses are known to exist.

A meat juice ELISA has been developed that gives complete agreement with the serum ELISAs.

Unexpected positives have been shown following the use of commercial ELISA testing kits, and these results can be improved by using competitive and blocking ELISA.[266]

A multiplex method for simultaneous serologic detection of PRRS and PCV2 has been described.[267]

Indirect Florescent Antibody Assay (IFAT)

The IFAT is a highly sensitive test. Antibody titers are detectable in infected pigs 8 days after inoculation. The IgM IFAT is also a rapid and simple test for diagnosing recent infection as early as 5 to 28 DPI in 3-week-old piglets, and 7 to 21 DPI in sows.

Modified Serum Neutralization Test

This test is useful for the detection of later and higher levels of antibody when the conventional methods cannot detect antibody. The test can differentiate between strains. The serum neutralization test is not used for routine diagnosis because neutralizing antibodies do not appear early in the infection.

Herd Diagnosis

The serologic diagnosis must be used and applied on a herd basis and acute and convalescent sera submitted for optimal results. A baseline herd sampling is necessary to evaluate the status of a herd and to determine whether and in which groups the virus is circulating. In large herds of over 500 sows, samples are taken from 30 animals in each breeding, gestation, and farrowing group, with representation from all parties. In addition, 10 nursery pigs (5 weeks old), 10 pigs at the end of the nursery period, and 10 pigs in the late finishing stage constitute a **herd profile**. Thus serologic monitoring can be used to monitor the circulation of virus within a closed herd and to determine infection status of breeding animals that are to be introduced into seronegative herds. Results from the sow sera indicate whether the sow herd is virus negative, stable, or has an active virus circulation. Comparison of the early and late nursery pigs indicates if the virus is circulating in the nursery. Comparing the nursery results with the end of the finishing period indicates if the virus is circulating in the finishing groups of pigs. IFAT titers in pigs range from 1:256 to 1:1024 by 2 to 3 weeks after infection. Titers decline over 3 to 4 months unless reintroduced by exposure to circulating virus. Uninfected nursing pigs are negative or have maternal antibody. Seropositive 9- to 10-week-old pigs leaving the nursery indicate virus circulation in the nursery. If pigs leaving the nursery are negative and positive later in the finishing unit, virus circulation is occurring in the finishing unit.

Sera from outbreaks of the disease in the United States, Canada, and Europe have been compared, and although the isolates from both continents are closely related, the strains isolated in the United States and Canada are more closely related serologically than they are to the European strains.

Oral Fluids

Saliva has also been used for haptoglobin and CRP estimations in PRRS-affected pigs under field conditions.[268,269] The values were higher in a conventional herd with chronic PRRS than a specific pathogen-free herd. Increases were also found independently with age. The use of preweaning oral fluid samples detects the circulation of wild-type PRRSV.[270] Overall, preweaning litter oral fluid samples could provide a sensitive approach to surveillance for PRRSV in infected, vaccinated, or presumed negative pig breeding herds.

Antigen Detection

PCR reactions were partially inhibited in the oral fluid matrix compared with RNA extraction, and it should not be assumed that methods designed for use in serum would perform as well in oral fluid.[271-275] Oral fluid testing was found to be useful for virus detection[276] and superior to serum for the detection of PRRSV using PCR over the 21-day observation period of their study. Individually penned oral-fluid sampling could be an efficient, cost-effective way to maintain surveillance in a boar stud.

Serology

An assay was developed and validated for use in oral fluids.[277] A titer of 1:8 in oral-fluid samples was considered to be virus specific and could be detected 28 days after vaccination or infection. It had 94.3% specificity and 90.5% repeatability. The levels were correlated with serum levels.

The IgG oral fluid ELISA can provide efficient, cost-effective PRRSV monitoring in commercial herds and be used in elimination programs.[278] In a study of 100 oral-fluid samples from pens containing positive pig at five levels of PRRSV prevalence tested at six laboratories, it was found that the mean positivity for PRRSV RNA was 62% and for antibodies it was 61%. The study supported the use of pen-based oral-fluid sampling for PRRSV surveillance.[279] An oral fluid assay was ring tested in the United States [280] in 12 laboratories and was found to be highly repeatable and reproducible.

NECROPSY FINDINGS

There is a high level of viremia for 102 weeks, then a lower level for another 2 to 3 weeks, and subsequently low levels of virus may persist for several months, but finally PRRSV is eliminated after 2 to 4 months. PRRSV-specific nonneutralizing antibodies arise quickly from 7 DPI, but low titers of neutralizing antibody are only detected from 25 to 35 DPI. In some pigs, both low levels of replicating virus are found in the presence of neutralizing antibodies. The adaptive cell-mediated immune response is exerted by CTLs and Th cell lymphocytes in cooperation with Th1-activated NK and macrophages. The CTLs may reduce viral replication in the lungs and lymphoid tissue after 2 weeks DPI and in the complete clearance of virus in 2 to 4 months. It was shown that peripheral blood monocytes fail to exert CTL activity toward PRRSV-infected macrophages.[281]

Type 2 PRRSV is more virulent than type 1in the experimental setup with higher mean viral titers and greater macroscopic and microscopic lesions at the same points on a timescale similar to a type 1 virus. Mean numbers of PRRSV-positive cells in lungs and lymph nodes were also higher for the type 2 virus.[282]

Type 2 PRRSV infection mediates apoptosis in B- and T-cell areas in lymphoid organs of experimentally infected pigs, and the increased apoptosis may play a part in the impairment of the host immune response during PRRSV infection.[283]

In a study of three European viruses it was shown that a Belgian strain was more highly pathogenic than the Lelystad virus and a British field strain, not because of increased viral load and better replication but because of an enhanced inflammatory immune response.[284]

A series of postmortem examinations of different aged pigs from different stages of production will reveal what is going on over time. A series of such examinations will probably show more than any other investigations.

No characteristic gross lesions are present in sows, aborted fetuses, or stillborn piglets. Microscopic lesions that may be present in aborted fetuses include vasculitis of the umbilical cord (not recorded in European strain infections) and other large arteries, myocarditis, and encephalitis. Unfortunately, none of these changes is present consistently, and the majority of fetuses and placentas are histologically normal. These lesions are all more common in the North American virus infections.

In suckling and grower pigs, infection with the PRRSV is usually characterized by an interstitial pneumonia. The PRRSV affects both pulmonary intravascular macrophages, which may be important as a replication site, and alveolar macrophages. Loss of bactericidal function in pulmonary intravascular macrophages may facilitate hematogenous bacterial infections. When Danish isolates were injected into piglets, PRRSV was isolated from the lungs and/or tonsillar tissues from both dead and culled piglets under 14 days of age. Tracheobronchial and

mediastinal lymph nodes are usually enlarged and firm. The gross pulmonary changes vary from lungs that appear normal but fail to collapse, to lungs that are diffusely red, meaty, and edematous. Porcine proliferative and necrotizing pneumonia has been linked to infection with PRRSV, although the involvement of an unidentified copathogen cannot yet be discounted. Grossly, this form of pneumonia appears as confluent consolidation of the cranial, middle, and accessory lobes, together with the lower half of the caudal lobe. Affected lobes are red-gray, moist, and firm (meaty) in consistency. On cross-section, the affected lobes are bulging and dry, and the pulmonary parenchyma appears similar to thymic tissue.

Generally, histologic lesions in piglets are focal nonsuppurative inflammatory conditions particularly in the lung and heart. Most of the cells undergoing apoptosis do not have markers for PRRSV, which suggests that there is an indirect mechanism for the induction of apoptosis.

Multifocal areas of interstitial pneumonia (more extensive at 10 DPI rather than 21 DPI) were regarded as the structural basis for reduced lung compliance and gas exchange disturbances.[237] There was a cough that the authors interpreted as caused by bronchospasm because there was no evidence of tracheitis, bronchiolitis, or airway mucus, and this was supported by the presence of peripheral airway obstruction. Cell death occurs through both apoptosis and necrosis.[285]

Histologically, in addition to marked proliferation of type II pneumocytes in alveoli, there is severe necrosis of bronchiolar epithelium, with necrotic cellular debris plugging the airway lumina.

In pigs infected with HP-PRRSV, there was a distinct thymus atrophy. The lesions in the thymus were found to have severe cortical depletion of thymocytes. There was a 40-fold increase in apoptosis of thymocytes compared with piglets infected with non–HP-PRRSV at 7 DPI.[286]

In the less severe and more common forms of PRRSV pneumonia, the alveoli contain protein-rich fluid and large macrophages, some of which may appear degenerate. There is patchy thickening of the alveolar septa caused by infiltrating mononuclear leukocytes and mild, type II pneumocyte hyperplasia. Lymphoplasmacytic cuffing of arterioles is common, and syncytial cells are occasionally seen. In field outbreaks, it is usual for the lung pathology to be complicated by concurrent respiratory pathogens.

Microscopic lesions may be found in many other tissues and include multinucleate cell formation within lymph nodes; infiltrates of lymphocytes and plasma cells in the heart, the brain, and the turbinates; and a lymphocytic perivasculitis in various sites. Thymic lesions include severe cortical depletion of thymocytes. An ISH technique is a rapid, highly specific, and sensitive detection method for the diagnosis of PRRS virus in routinely fixed and processed tissues. Immunohistochemical techniques can also be used to detect the virus in neurovascular lesions. PRRSV and reovirus 2 have been found in brain, lung, and tonsil by inoculation into Marc 145 and CPK cells. IHC on one section would give a positive in 48% of cases, but if five sections were studied then there are positives in >90% of PRRSV-infected pigs. If the animals are vaccinated then the positives fall to 14%.

PNP is a common finding in Spain and is characterized by hypertrophy and proliferation of type 2 pneumonocytes and the presence of necrotic cells in the alveolar lumina. PCV2 was found in 85.1% of the cases by ISH and IHC and PRRSV was found in 44.6% of the cases; 39.1% had PCV2 as the sole agent and only 4.1% had PRRSV as the sole agent.[287]

Samples for Confirmation of Diagnosis

Lung appears to be the best tissue for identification of the virus in various ages of the pig and at various times following infection. Thymus is probably the best choice for aborted fetuses.

- **Histology:** lung, tonsil thymus, thoracic lymph node, brain, kidney, heart, (umbilicus from fetus) (light microscopy, immunohistochemistry (IHC)); a monoclonal antibody-based IHC method for the detection of European and U.S. PRRSV was shown to be useful in detecting both types.[288]
- **Virology:** lung, thoracic lymph node, tonsil (virus isolation, fluorescent antibody test (FAT), PCR).

DIFFERENTIAL DIAGNOSIS

Respiratory disease must be differentiated from the following:

- Swine influenza
- Porcine respiratory coronavirus
- Enzootic pneumonia (*Mycoplasma hyopneumoniae*)
- *Actinobacillus pleuropneumoniae*
- *Pasteurella multocida*
- Glasser's disease (*Haemophilus parasuis*)
- *Streptococcus suis.*

Reproductive disease must be differentiated from other causes of abortion, stillbirths, and weak neonates in pigs:

- Leptospirosis
- Encephalomyocarditis virus
- Hog cholera virus
- Pseudorabies virus
- Parvovirus
- Fumonisin, which is a recently identified mycotoxin produced by *Fusarium moniliforme,* has been associated with the appearance of PRRS in swine herds in the United States

A definitive diagnosis requires a detailed epidemiologic investigation of the epidemic including a detailed analysis of the breeding and production records for the previous several months, and the submission of tissue and serum samples for laboratory investigation.

TREATMENT

There is no specific treatment against the virus. In outbreaks of respiratory disease, mortality can be reduced by ensuring that the environmental conditions in the barns and pens are adequate, the stocking density is kept low, and the feeds and feeding programs are monitored. Routine procedures such as tail docking, iron injections, castrations, teeth clipping, and cross-fostering should be delayed or not done during the acute phase of the disease. Supplemental heat for neonatal pigs should be provided if necessary. Sows that have aborted their litters should not be bred until the normal time of weaning. This will reduce the incidence of infertility common at the first estrus after the abortion or premature farrowing. Culling of sows should be minimized and weekly breedings increased by 10% to 15%. Replacement gilts may be introduced into the premises for exposure to infection before breeding. The consequences of boar infertility and low libido may be minimized by use of artificial insemination or by using multiple sires on each sow. Recurrent illness and secondary infections in weaner and growing pigs can be continuing problems for a few months after an acute outbreak. Reducing the stocking density and an all-in/all-out strategy have been successful in reducing the chronic problem. If there is the possibility of treating secondary infections, then this should be undertaken. Serum inoculation of naive gilts has been described, and this was shown to be capable of stabilizing sow herds, as shown by the production of negative weaned pigs.

Tylvalosin, a macrolide antibiotic, and to some extent tilmicosin inhibit the in vitro replication of European and American PRRSV possibly by raising the endosomal pH (PRRSV requires a low endosomal pH).[289]

A report has suggested that *N*-acetylpenicillamine will inhibit PRRSV replication.[290]

CONTROL

It is the stealthy nature of PRRSV infection and its efficient transmission that has prevented elimination.[291] The challenges of control have resulted in the development of regional control systems.[292,293] These involve cooperation in a region, new technologies, and the demonstration that PRRSV has been eliminated.

The potential role of noncommercial swine populations in the United States in the spread of PRRSV have been highlighted.[294] They comment on the lack of knowledge of biosecurity in this group of swine herders,

the practice of showing pigs at many events, evidence that exposure to PRRS is very frequent, and close interactions with commercial herds and that these facts make it necessary to involve these groups in regional control.

Control of PRRSV is difficult, unreliable, and frustrating because of the complexity of the disease; the uncertainty of some aspects such as immunity, persistence, diagnosis, and the lack of published information based on control programs have been evaluated under naturally occurring field conditions. Much of the information available on control is anecdotal and not based on well-designed control programs that can be compared and evaluated. A major problem is the difficulty of obtaining a definitive etiologic diagnosis when presented with young growing pigs with respiratory disease and the possibility that other pathogens could be involved. The diagnosis of reproductive failure in gilts and sows is also commonly uncertain.

Some characteristics of the disease are important in planning control programs for individual herds:

- Infection is highly contagious and is transmitted by direct contact. Nonimmune pregnant gilts and sows and young pigs are highly susceptible to infection, resulting in large economic losses.
- Infection of breeding stock results in immunity. The efficacy of vaccination is not well established.
- Maternal immunity is present in piglets born from seropositive sows.
- Infection can persist for many weeks and months in individuals and in subpopulations of animals.
- Infections are usually introduced into a herd by the introduction of infected pigs.

There are two main options for control: eradication of the virus from individual swine herds and controlling the disease in individual herds to create a stable positive system that allows to live with the disease. Controlling the disease requires developing strategies to make pigs immune to the infection by controlling infection pressure in the herd and inducing naturally acquired immunity in the herd or inducing acquired immunity through vaccination. The recommendations for control set out here are guidelines that can be applied and modified to meet different circumstances.

Dietary plant extracts (capsicum, garlic, and turmeric) improve immune responses and growth efficiency of pigs experimentally infected with PRRSV.[295]

FILTRATION SYSTEMS

A production region model was used to assess the spread of PRRSV[296] and showed the importance of aerosol spread. More than 30 swine systems in the Midwest have remained free from PRRSV for 2 to 3 years following implementation of an air-filtration system using MERV 16 filters, and this system should be regarded as the gold standard.[297-299]

Retrograde air movement is a real risk for PRRSV introduction into filtered airspaces in animal houses, and different treatments have been investigated.[300]

In a study of before and after filtration it was found that outbreaks occurred at a rate of 0.5 outbreaks a year before filtration, but after the risk was reduced by introducing air filtration the outbreaks were reduced to 0.06 to 0.22 outbreaks a year.[301]

The financial implications of air-filtration systems have been studied.[302] Model outputs suggested that the filtered farm produced 5927 more pigs on a 3000-sow farm and paid for the installations within 5.35 to 7.13 years, depending on the sow herd productivity. If there was a premium of $5 per PRRS-negative piglet, then the payback period was reduced to 2.1 to 2.8 years.

Eradication of the Virus From the Herd

Depopulation and Repopulation

Eradication of the virus from the herd by depopulation of the entire herd followed by repopulation with virus-free breeding stock is biologically possible, but in most cases it is impractical and too expensive. Obtaining virus-free breeding stock is usually not possible and, if possible, the herd is highly susceptible to accidental reinfection.

Control in Infected Herds

Nursery Depopulation

Control within a breeding herd is based on the observation that pigs commonly seroconvert to the virus during the nursery period. Pigs are seronegative shortly after weaning, but 80% to 100% are seropositive at 8 to 10 weeks of age. A control program based on **nursery depopulation** consists of emptying the nurseries and moving **all of the pigs** to off-site finishing facilities or selling them as feeder pigs. Test and removal has been described. This is combined with batch farrowing and weaning at intervals of at least 3 weeks. The nurseries are completely emptied, cleaned three times with hot water and disinfectant, the slurry pits are pumped out after each cleaning, and the facilities are kept empty for 14 days, during which time all pigs weaned are moved to off-site nurseries and after which the conventional flow of pigs into the cleaned facilities is resumed. The control program can result in significant improvements in both average daily gain and percentage mortality, but it will not eliminate the virus from the herd. Using a partial budget model to measure the profitability of nursery depopulation, the financial consequences indicate that it is a profitable strategy to improve pig performance in herds affected with the virus. Additional income is generated by the increased number and weight of marketable pigs, as a result of their increased growth rate and decreased mortality. Lower treatment costs reduce overall expenses, but there are additional costs because of the extra feed necessary to raise the additional pigs and the costs required to house the depopulated pigs. However, it is possible that the economic benefits are from the control of other pathogens and not merely the PRRS virus.

Table 18-4 Nursey depopulation and cleanup protocol for elimination of PRRS

Day	Procedure
1	Empty all nurseries, off-site wearing, pump out slurry pits, clean and wash rooms with hot water (>95°C, 203°F), and disinfect with formaldehyde-based product; allow disinfectant water to remain in pits overnight
2	Pump out pits, repeat washing procedure, and disinfect in phenol-based product; allow disinfectant to remain in pits
311	Allow facility to remain vacant
12	Pump out slurry pits, repeat washing procedure, and disinfect with formaldehyde-based product
13	Allow facility to remain vacant
14	Resume conventional flow of pigs into clean nurseries

The details for nursery depopulation and cleanup protocol for the elimination of the virus are shown in Table 18-4.

In an experimental infection with PRRSV, it was found that the infected pigs had greater serum concentrations of IL-1β, TNF-α, IL-12, IFN-γ, IL-10, and haptoglobin than sham controls. The results indicated that PRRSV-stimulated secretion of cytokines involved in innate, Th1, and T-reg immune responses. Mannan oligosaccharides regulated the expression of nonimmune and immune genes in pig leukocytes[303] and were able to enhance the immune response without overstimulation. Mannan oligosaccharide-containing compounds were found to decrease the levels of the serum TNF-α. The levels of IL-1β and IL-12 may help to promote innate and T-cell immune functions.[304]

Management of the Gilt Pool

Management of the gilt pool is the single most important strategy for long-term effective control. Controlling the infection in the breeding herd is a prerequisite to controlling infection in the nursery and finishing pig groups. Strategies like partial depopulation and piglet vaccination are ineffective unless the breeding herd is first stabilized, preventing piglets from becoming infected

before weaning. Replacements are a major source of introduction of the virus and activating existing virus in the breeding herd. They also initiate the formation and maintenance of breeding herd replacements.

Subpopulations are subsets of naive or recently infected gilts or sows that coexist within chronically infected herds. These subpopulations perpetuate viral transmission in the breeding herd and farrowing units, which ultimately produces successions of infected piglets before weaning. Modifications in gilt management that may minimize subpopulations include ceasing introduction of replacement animals for a 4-month period, beginning to select replacements from the finishing unit, or introducing a 4-month allotment of gilts at one time.

Exposure to the virus in the breeding herd can be controlled by managing the gilt pool using two strategies. In one strategy, herds may be closed to outside replacements, and replacement males and females are raised on the farm. In the other strategy, replacement gilts are held in an off-site holding facility from 9 to 12 weeks of age until breeding age at 7 to 7.5 months, or even much earlier. This is combined with nursery depopulation as described earlier. Before entry of the gilts into the herd, they are serologically tested for evidence of seronegativity or a declining titer, which is required for entry into the herd. The gilts are isolated and quarantined for acclimatization for 45 to 60 days. This may be combined with two vaccinations, 30 days apart, after entering quarantine. This method reduces the risk of introducing potentially viremic animals into the existing population. The method selected will depend on the production system, management capabilities, and facilities available on each farm. The introduction of younger gilts, in larger groups, less frequently throughout the year, is being recognized as the most effective method for introducing replacement stock to virus-infected herds and long-term control of the disease.

Controlled Infection of Breeding Herd

The presence of subpopulations of highly susceptible breeding animals in the herd can be a major risk factor for maintaining viral transmission within problem herds and may explain recurrent outbreaks of reproductive failure. By intentionally exposing all members of a population to the virus, it may be possible to eliminate subpopulations and produce consistent herd immunity. In endemic herds, exposure of gilts to the virus before breeding is critical for prevention of reproductive failure. Seronegative replacement gilts can be introduced into seropositive herds at 3 to 4 months of age to allow for viral exposure before breeding. If the status is uncertain, quarantine and exposure to nursery pigs of the importing unit is a suitable policy if replacement gilts are bought in before they are bred. It is possible to convert a PRRS-positive unit to a negative herd by managing the gilt pool and regulating the pig flow. It appears that PRRSV infection eventually either disappears or becomes inactive in the donor gilt population. Similarly, serum from nursery pigs (thought to be PRRSV viremic) given to negative replacement gilts resulted in seroconversion of all 50 gilts receiving the serum.

Control of Secondary Infections

When outbreaks of the disease occur in nursing piglets, and virus circulation is occurring continuously in the farrowing facility, the following are recommended:

- Cross-foster piglets only during the first 24 hours of life
- Prevent movement of pigs and sows between rooms
- Eliminate the use of nurse sows
- Euthanize piglets with low viability
- Minimize injections of suckling pigs
- Stop all feedback of pig and placental tissues
- Follow strict all-in/all-out pig flow in the farrowing and nursery rooms.

These are similar to the system developed in the United States called the McRebel system. This was a method of control showing that cross-fostering of piglets should be minimal within the first 24 hours and banned after this time.

Feedback has been tried, although there are a lot of reasons not to do so. Minced whole piglets were fed to sows and the herd then closed for 23 weeks. No clinical signs were observed. One-third of the sows present at the time of the outbreak were still seropositive 20 months after the deliberate infection. Disinfection at cold temperatures was described.

Biosecurity

Standard methods, such as quarantining and serologic screening of imported breeding stock and restrictions on visitors, are recommended to keep units free of infection. Control of infection between herds depends on restricting the movement of pigs from infected herds to uninfected herds. If pigs have to be bought in, then seropositive animals should be imported into seropositive herds. Only seronegative boars should be allowed entry into artificial insemination units.

Biosecurity practices regarding PRRSV have been investigated in Quebec in two areas of different swine density. A questionnaire was sent to 125 breeding sites and 120 growing sites. The frequency of biosecurity practices ranged from 0% to 2% for a barrier at the site entrance, 0% to 19% for showering, 20% to 25% for truck washing between loads, 51% to 57% for absence of rendering or rendering without access to the site, and 26% to 51% for absence of gilt purchase or purchase with quarantine. Better practices were found in the breeding herds. In the high-density area, there was a lower level of biosecurity on the growing sites. There were two patterns of biosecurity, a low one and a high one. For the breeding sites the higher pattern was observed when the site was away from other pig sites, more than 300 m from a public road, with a higher number of sows or being part of integrated production.[305] In a second part of the study, on prevalence and risk factors, it was found that the overall prevalence of PRRS was 74.0%. Four main factors were associated with PRRS positivity, and these were large pig inventory, proximity to closest site (16%), absence of shower (27%), and free access to the site by the rendering truck (10%).[305] Boar studs that are free should only import boars that are certified free from tested herds. The status of the boar stud should be tested every 2 weeks with a combination of ELISA and PCR.

Testing protocols that used PCR on serum detected the PRRSV introduction earlier than the protocols that used PCR on semen, and these were earlier than those that used ELISA on serum. The most intensive protocol (testing 60 boars three times a week by PCR on serum) would need 13 days to detect 95% of the PRRSV introductions.[306]

A vaccination study using a modified live PRRSV vaccine on European and North American PRRSV shedding from boars showed that boar vaccination decreased the shedding of U.S. PRRSV but not the European strain.[307]

Vaccine and Vaccination

The inefficiency of current vaccines to cross-protect against all strains of PRRSV may be caused by variability within GP5.[2]

Adjuvants for use in PRRSV vaccines have been reviewed.[308] Of 11 adjuvants tested 5 enhanced cell-mediated immunity to PRRSV. In particular, IL-12 and CpG ODN significantly enhanced the protective efficacy of PRRSV vaccines in challenge models. The immunostimulatory oligodeoxynucleotides have been used previously.[309]

TLR ligands enhance the protective effects of vaccination against PRRS syndrome in swine using killed vaccines.[310]

Vaccination with a combined PRRSV/MH vaccine did not differ in protective efficacy compared with the protective efficacy of the two single vaccines. This indicates that neither vaccine interfered with each other.[311]

Vaccine efficacy of PRRSV chimeras has been described,[312] and the study suggested that only specific chimeras can attenuate clinical signs in swine and that attenuation cannot be directly linked to primary virus replication.

Pigs infected with PRRSV at the time of vaccination for swine influenza had an increased level of macroscopic and microscopic pneumonia, suggesting that there was

a reduced SIV vaccine efficiency.[313] In addition, there was also increased clinical disease and shedding of SIV during the acute phase of SIV infection.

Immunologic solutions for the treatment and prevention of PRRSV have been reviewed.[314] No differences were found between intradermal and IM vaccinated pigs and those subsequently exposed to a heterologous Italian strain.[315]

The antibody response and the maternal immunity when PRRSV-immune sows were boosted with experimental farm-specific and commercial PRRSV vaccines has been described.[316] The study was designed to boost PRRS-immune sows against circulating viruses. Three PRRSV isolates were taken. Booster vaccinations used either commercial vaccines or inactivated farm-specific isolate vaccines. A boost was found in all three farm-specific vaccinations. The commercial attenuated vaccine boostered immunity in 2/3 herds but the commercial nonattenuated dead vaccine did not affect the immunity on any of the three farms. In a second part of the study, similar vaccines were given at 60 days' gestation. The farm-specific vaccines produced a significant increase in farm-specific neutralizing antibodies in all sows. Virus-neutralizing antibodies were also transferred to the piglets via colostrum and were detectable in the serum of these animals until 5 weeks after parturition. Not all sows vaccinated with the commercial attenuated vaccine showed an increase in the farm-specific virus-neutralizing antibodies, and the piglets in this group received a lower level of colostral antibodies. The number of viremic animals was significantly lower in the piglets of both groups of vaccinated animals than among mock vaccinated animals until at least 9 weeks of age.

Vaccination of Gilts

The two commercial modified live virus vaccines against PRRSV in pregnant gilts were shown to replicate in pregnant gilts and to cross the placenta.[317] It was concluded that the vaccines had no marked detrimental effects in pregnant gilts but that they could cross the placenta and lead to the birth of congenitally affected piglets.

Intranasal delivery of PRRS-MLV with a potent adjuvant (from *M. tuberculosis* whole-cell lysate) to elicit cross-protective immunity to a heterologous strain of PRRSV generated effective cross-immunity. There was reduced lung pathology, enhanced neutralizing antibodies, and reduced viremia. There was a reduced secretion of immunosuppressive cytokines (IL-10 and TGF-β) and an upregulation of the Th-1 cytokine IFN-γ in blood and lungs.[318]

The ORF5a antibody response is neither neutralizing nor protective.[319]

Vaccination is an aid to management in developing effective immunity. The goal is to produce a constant level of immunity across a defined population. This effectively immunizes the entire population and eliminates the nonimmune, susceptible subpopulations. Vaccination is most effective when used in replacement gilts combined with adequate isolation and acclimatization and in sows after farrowing and prebreeding. The routine vaccination of sows is not economically viable in herds affected with PRRSV. The vaccine is best suited for stabilizing the herd and is a necessity before nursery depopulation or commingling segregated early weaning piglets from virus-positive herds. Vaccination is also intended to produce protective immunity in weaned and growing pigs. The PRRS virus exists in many forms and therefore the closer the genetic makeup between the immunizing virus and the challenge virus the better.

Both inactivated and modified live virus vaccines are available.

Previous vaccination with a live attenuated strain produced an increase in proinflammatory cytokines and proimmune cytokine gene expression. In addition, a higher level of cortisol production suggested that there was an activation of the hypothalamus-pituitary-adrenal axis response. Vaccination produces an early immune response in pigs and a more efficient control of inflammation.[320]

Inactivated Vaccines

Immunization of pigs with a genotype I attenuated vaccine provided partial protection against challenge with a highly virulent genotype II strain. There was a lower mortality, fewer days of fever, lower frequency of catarrhal bronchopneumonia, higher weight gains and lower viremia compared with unvaccinated control pigs.[321]

Killed vaccines that are inactivated using methods that preserve the PRRSV entry-associated domains are most useful for the development of effective inactivated vaccines because they facilitate internalization into macrophages.[322] An experimental inactivated PRRSV vaccine that induces virus-neutralizing antibodies has been described.[323] The vaccine uses an optimized inactivation procedure and a suitable adjuvant, and by using these methods it was shown that inactivated PRRS vaccines can be developed that induce virus-neutralizing antibodies and offer partial protection on challenge.

Killed vaccines may not produce a measurable antibody response stimulation, but activation of lymphocytes does occur and any subsequent exposure with vaccine or field virus increases that response. There is no possibility of producing a viremia and no chance of producing shedding, and there are no detrimental effects on the host. However, there is no evidence that killed vaccines protect against heterologous challenge.

A killed, oil-adjuvanted vaccine based on a Spanish isolate of the virus is intended for protection against reproductive disease in gilts and sows. Initial vaccination involves 2 vaccinations, 21 days apart, with the second vaccination at least 3 weeks before breeding and with booster vaccinations recommended during subsequent lactations. Experimental challenge provides 70% protection based on pigs born alive and surviving to 7 days.

An autogenous inactivated vaccine was compared with commercial vaccines against homologous and heterologous challenge.[324] In this study the experimental inactivated homologous vaccines shortened the viremia on challenge, but the experimental heterologous and commercial inactivated vaccine had no or only a limited effect on the viremia.

Live Vaccines

A study in China[325] on farms with a complex microbial ecology showed that mass vaccination with an attenuated virus vaccine can improve health status and production performance of sows and their offspring.

Modified live vaccines do give a safe and efficacious protection against a wide variety of heterologous challenge strains. The vaccine virus can be transmitted from vaccinated to naive pigs and to naive herds. Vaccination of boars causes the virus to be shed, but if they have been previously exposed and then are vaccinated then there is no release of virus.

The live vaccine given to finishing pigs will protect against respiratory infections. A modified live virus vaccine given once is safe for use in pregnant sows, and vaccine virus is not transmitted to susceptible contact pigs. In growing pigs vaccinated at 3 to 18 weeks of age, the vaccine elicits protective immunity within 7 days and lasts 16 weeks. Compared with controls, vaccinated animals have a reduced level of viremia, their growth rates are superior, and they have a reduced number of lung lesions. Field trials suggest that the vaccine provides protection to nursery pigs in units with endemic infection. Live viral vaccines in sows may or may not be a good idea because they demonstrated that reduced numbers of pigs were born alive and there were increased numbers of stillborn piglets to vaccinated sows irrespective of the stage of vaccination. Both single-strain and multi-strain vaccines can be attenuated and be useful immunogens, but additional studies are needed to make sure that the multistrain vaccines can be recommended for routine field use.

In Denmark in 1996, the use of a modified live virus vaccine licensed for use in pigs 3 to 18 weeks of age was used in a large number of PRRSV seropositive herds. Following vaccination, a large number of herds experienced an increased incidence of abortions, stillbirths, and poor performance during the nursery period. The vaccine virus was isolated from fetuses, and it was concluded that the virus was transmitted to seronegative nonvaccinated pregnant gilts and sows (see the section Methods of

Transmission). The viruses were collected and sequenced and shown to have only a 60% homology to Lelystad virus, the European type strain, but a 98.5% homology to strain ATC-2332, which is the North American reference strain. It was therefore thought that the vaccine viruses were reverting to their natural antecedents and their virulence. Describing the vaccine virus it was shown that given to piglets it could infect nonvaccinated sows. Given to sows it can produce congenital infection, fetal death, and an increased preweaning mortality.

The vaccine virus can be maintained in the population where it may undergo considerable genetic change and then lead to the establishment of new variants. Vaccination with the U.S. type vaccine produces little effect on viremia with EU PRRSV. Vaccination with EU type vaccines produced complete suppression of EU PRRSV isolates.

A modified live virus vaccine has been evaluated in pigs vaccinated at 3 weeks of age and challenged at 7 weeks of age. Efficacy was evaluated using homologous and heterologous strains of virus known to cause respiratory and reproductive disease. The vaccine controlled respiratory disease but did not prevent infection and viremia. There are no published reports of randomized clinical trials evaluating the vaccines under naturally occurring conditions. In many cases of PRDC, vaccination fails simply because it was given too late or because there was no cross-protection to heterologous strains.

DNA vaccination is said to produce both humoral and cellular responses and neutralization epitopes on the viral envelope glycoproteins encoded by ORF4. Possibly recombinants can be used as vaccines.

In a survey in Germany, 18.5% of the samples were positive for the EU wild-type virus, EU genotype vaccine virus was detected in 1.3%, and the North American genotype vaccine virus was found in 8.9% of all samples. North American vaccine virus was frequently detected in nonvaccinated animals.[326]

The first modified-live vaccine was first released in 1994 and since then a number of other modified live and killed-virus vaccines have been developed. Vaccines should induce rapid immunity, have no adverse reactions, and be able to differentiate vaccinated from naturally infected animals (DIVA vaccine).[308,327,328]

Mass vaccination using modified live virus against homologous infection was shown to be effective in reducing economic losses from PRRSV. It did not eliminate the virus but it did reduce viral shedding 97 DPI.[329] Two vaccines were compared (one inactivated and one modified live), and the modified live virus was the only type of vaccine capable of establishing protective immunity as measured by viral load in blood and tissues. The inactivated vaccine evoked no measurable protective immunity. The modified live vaccine seemed to be based on cell-mediated immunity.[330]

A modified live vaccine partially protected a group of pigs given a heterologous virus vaccine; intervention reduced the duration of shedding but did not reduce the viral load in tissues or the proportion of persistently infected pigs. When the pigs were subsequently given the highly virulent virus, infection and shedding were not prevented.[331]

The modified live vaccines for PRRSV have been reviewed.[332] None of the vaccines studied (Ingelvac PRRS MLV, Amervac PRRS, Pyrsvac-183, and Porcilis PRRS by the IM route) caused detectable clinical signs in vaccinated pigs, although lung lesions were found. Neither Pyrsivac-183 nor Porcilis PRRS could be detected in the pulmonary alveolar macrophages or in lung sections by IHC, suggesting that these viruses may have lost their ability to replicate in PAM. In these pigs, there was also a lower transmission rate and a delay in the onset of viremia, which may be explained by the lack of infection and therefore replication in the alveolar macrophage.

Novel strategies for the next generation of vaccines have been described[333] and stress the future importance of reverse genetics system-based vaccine development. Serologic marker candidates have been identified.[334,335] Vectored vaccines may have a place in the future.[336-338]

Recombinant fowlpox virus-based virus with coexpression of GP5/GP3 proteins of PRRSV and swine IL-18 has been described[339] as potential vaccines.

The fusion of the heat shock protein (HSP70) of *H. parasuis* with GP3 and GP5 of PRRSV enhanced the immune responses and protective efficacy of a vaccine.[340] The strategy of coexpressing GPGP-linked GP5 and M fusion protein may be a promising approach for future PRRSV vaccine development.[341] A canine adenovirus has also been used as a vehicle.[342]

Overattenuation of an HP-PRRSV (over 100 passages) was used to produce a possible vaccine[343] suggesting that loss of pathogenicity has to be balanced with loss of antigenicity.

Vaccination against PRRSV resulted in significantly lower viral loads of PCV2 in animals over 13 weeks compared with nonvaccinated animals but it had no effect on quantitative PCR results for PRRSV in 4- to 12-week-old pigs. PRRS vaccinates had significantly lower levels of PCV2 viral loads when peak wasting disease was seen.[344]

Concurrent PRRSV and PCV2 vaccination produced no interference with the development of the specific humoral and cell-mediated immunity and is associated with clinical protection on natural challenge.[345] PRRSV vaccine induced a low but significant virus-specific response IFN-γ secreting cell response on stimulation with both the vaccine strain and two heterologous PRRSV isolates.[346]

An isolate of PRRSV has been shown to produce IFNs and may be useful for the development of vaccines.[347]

Vaccination Against High Pathogenicity Porcine Reproductive and Respiratory Syndrome

A live attenuated vaccine was successfully produced from an HP-PRRSV strain TJ and the attenuation produced a further 120 amino acid deletion as well as the 30 amino acid deletion found in these HP-PRRSV strains.[348] The pigs were protected from the lethal challenge and did not develop fever and clinical disease. The vaccinated pigs also gained more weight and had milder pathologic lesions. The effective protection lasted at least 4 months.

A live attenuated vaccine has been used against HP-PRRSV.[349]

Vaccination of Boars

The use of an attenuated virus vaccine in boars resulted in a marked reduction in viremia and shedding of the virus in semen compared with nonvaccinated control animals. Introducing a vaccination program using the live virus vaccine may be considered as a potential method to reduce the risk of transmission of virus by artificial insemination. In contrast, no changes in onset, level, and duration of viremia, or shedding of virus in semen, were observed using the inactivated virus vaccine.

FURTHER READING

Dee S, et al. Use of a production region model to assess the efficacy of various air filtration systems for preventing airborne transmission of PRRS and *M. hyopneumoniae:* Results from a 2 year study. *Virus Res.* 2010;154:177-184.

Dokland T. The structural biology of PRRSV. *Virus Res.* 2010;154:86-97.

Frossard J-P. Porcine reproductive and respiratory syndrome virus evolution and its effect on control strategies. *Pig J.* 2013;68:20-25.

Gomez-Laguna J, et al. Immunopathogenesis of PRRSV in the respiratory tract of pigs. *Vet J.* 2013;195:148.

Karniychuk UU, Nauwynck HJ. Pathogenesis and prevention of placental and transplacental porcine reproductive and respiratory syndrome virus infection. *Vet Res.* 2013;44:95.

Murtaugh MP, Genzow M. Immunological solutions for treatment and prevention of PRRS. *Vaccine.* 2011;29:8192-8204.

Murtaugh MP, et al. The ever-expanding diversity of PRRSV. *Virus Res.* 2010;154:18-30.

Nauwynck HJ, et al. Microdissecting the pathogenesis and immune response of PRRSV infection paves the way for more efficient PRRSV vaccines. *Transboundary Emerg Dis.* 2012;59(suppl 1):50-54.

Sang Y, et al. Interaction between innate immunity and PRRSV. *Anim Health Res Rev.* 2011;12:149-167.

Shi M, et al. Molecular epidemiology of PRRSV: A phylogenetic perspective. *Virus Res.* 2010;154:7-17.

Thanawongnuwech R, Suradhat S. Taming PRRSV: Revisiting the control strategies and vaccine design. *Virus Res.* 2010;154:133-140.

Yoo D, et al. Modulation of host cell responses and evasion strategies for PRRSV. *Virus Res*. 2010;154:48-60.
Zhou L, Yang H. PRRSV in China. *Virus Res*. 2010;154:31-37.

REFERENCES

1. Opriessnig T, et al. *Anim Health Res Rev*. 2011;12:133.
2. Murtaugh MP, et al. *Virus Res*. 2010;154:18.
3. Gorbalenya AE, et al. *Virus Res*. 2006;117:17.
4. Fang Y, et al. *Arch Virol*. 2007;152:1009.
5. Shi M, et al. *Virus Res*. 2010;154:7.
6. Martinez-Lobo FJ, et al. *Vaccine*. 2011;29:6928.
7. Fang Y, Snijder EJ. *Virus Res*. 2010;154:61.
8. Firth AE, et al. *J Gen Virol*. 2011;92:1097.
9. Johnson CR, et al. *J Gen Virol*. 2011;92:1107.
10. Wei Z, et al. *J Virol*. 2012;86:9941.
11. Xia Pa, et al. *Vet Microbiol*. 2009;138:297.
12. Lee C, Yoo D. *Virology*. 2006;346:238.
13. Kim W-I, et al. *Vet Microbiol*. 2013;162:10.
14. de Lima M, et al. *Virology*. 2009;390:31.
15. Brockmeier S, et al. *Virus Res*. 2012;169:212.
16. Johnston CR, et al. *J Gen Virol*. 2011;92:1107.
17. Wenhui L, et al. *J Virol*. 2012;86:9543.
18. Stadejek T, et al. *J Gen Virol*. 2006;87:1835.
19. Costers S, et al. *Virus Res*. 2010;154:104.
20. Kvisvgaard LK, et al. *Virus Res*. 2013;178:197.
21. Kvivsgaard LK, et al. *Vet Microbiol*. 2013;167:334.
22. Zhu L, et al. *Vet Microbiol*. 2011;147:274.
23. Li Y, et al. *Vet Microbiol*. 2009;138:150.
24. Cha S-H, et al. *Vet Microbiol*. 2006;117:248.
25. Frossard J-P, et al. *Vet Microbiol*. 2012;158:308.
26. Frossard J-P, et al. *Vet Microbiol*. 2013;162:507.
27. Mateu E, et al. *Virus Res*. 2006;115:198.
28. Prieto C, et al. *Vet J*. 2009;180:363.
29. Carlsson U, et al. *Transbound Emerg Dis*. 2009;56:121.
30. Amonsin A, et al. *Virol J*. 2009;6:143.
31. Tian K, et al. *PLoS ONE*. 2007;2:3526.
32. Xiao XL, et al. *J Virol Methods*. 2008;149:49.
33. Normile D. *Science*. 2007;317:1017.
34. Feng Y, et al. *Emerg Infect Dis*. 2008;14:1774.
35. Wu J, et al. *Arch Virol*. 2009;154:1589.
36. Li B, et al. *Vet Microbiol*. 2010;146:226.
37. Wang L, et al. *J Virol*. 2012;86:13121.
38. Zhang G, et al. *J Virol*. 2012;86:11396.
39. Li B, et al. *J Clin Microbiol*. 2011;49:3175.
40. Kim HK, et al. *Vet Microbiol*. 2011;150:230.
41. Kim S-H, et al. *Vet Microbiol*. 2010;143:394.
42. Miller LC, et al. *Vet Res*. 2012;8:208.
43. Xiao S, et al. *BMC Genomics*. 2010;11:544.
44. Hu SP, et al. *Transbound Emerg Dis*. 2012;60:351.
45. Lv J, et al. *J Gen Virol*. 2008;89:2075.
46. Shen J, et al. *Genome Announc*. 2013;1:e00486-13.
47. Zhou L, et al. *Virus Res*. 2009;145:97.
48. Zhou Y-J, et al. *Virus Res*. 2009;144:136.
49. Yu X, et al. *Vet Microbiol*. 2012;158:291.
50. Metwally S, et al. *Transbound Emerg Dis*. 2010;57:315.
51. Guo B, et al. *Virology*. 2013;446:238.
52. Song T, et al. *J Virol*. 2012;86:4040.
53. Guo B, et al. *Virology*. 2013;435:372.
54. Descotes J, Gourand A. *Expert Opin Drug Metab Toxicol*. 2008;4:1537.
55. Tarrant JM. *Toxicol Sci*. 2010;117:4.
56. Sun Y, et al. *Viruses*. 2012;4:424.
57. Behrens EM, et al. *J Clin Invest*. 2011;121:2264.
58. Nieuwehhuis N, et al. *Vet Rec*. 2012;170:225.
59. Corbellini LG, et al. *Vet Microbiol*. 2006;118:267.
60. Lopez-Soria S, et al. *Transbound Emerg Dis*. 2010;57:171.
61. Reiner G, et al. *Vet Microbiol*. 2009;136:250.
62. Greiser-Wilke I, et al. *Vet Microbiol*. 2010;143:213.
63. Kinman TG, et al. *Vaccine*. 2009;8:2704.
64. Rosendal T, et al. *Can J Vet Res*. 2010;74:118.
65. Brar MS, et al. *J Gen Virol*. 2011;92:1391.
66. Evans CM, et al. *Vet Res*. 2008;4:48.
67. Holtkamp DJ, et al. *Prev Vet Med*. 2010;96:186.
68. Lambert M-E, et al. *Vet Res*. 2012;8:76.
69. Velasova M, et al. *Vet Res*. 2012;8:184.
70. Lewis CRG, et al. *J Anim Sci*. 2009;87:876.
71. Doeschl-Wilson AB, et al. *J Anim Sci*. 2009;87:1638.
72. Badaoui B, et al. *BMC Vet Res*. 2013;9:58.
73. Kwong GPS, et al. *Prev Vet Med*. 2013;110:405.
74. Charpin C, et al. *Vet Res*. 2012;43:69.
75. Evans CM, et al. *Prev Vet Med*. 2010;93:248.
76. Dee S, et al. *Can J Vet Res*. 2006;69:64.
77. Pitkin A, et al. *Vet Microbiol*. 2009;136:1.
78. Hermann J, et al. *Vet Res*. 2007;38:81.
79. Otake S, et al. *Vet Microbiol*. 2010;145:198.
80. Pitkin A, et al. *Vet Microbiol*. 2009;136:1.
81. Cho JG, et al. *Can J Vet Res*. 2006;70:297.
82. Pitkin A, et al. *Vet Microbiol*. 2009;136:1.
83. Cutler TD, et al. *Vet Microbiol*. 2011;151:229.
84. Dee S, et al. *Vet Res*. 2009;40:39.
85. Pitkin A, et al. *Can J Vet Res*. 2009;73:298.
86. Cano JP, et al. *Vet Rec*. 2007;160:907.
87. Molina RM, et al. *Transbound Emerg Dis*. 2008;56:1.
88. Pitkin A, et al. *Can J Vet Res*. 2009;73:91.
89. De Baere MI, et al. *Vet Res*. 2012;43:47.
90. Wang X, et al. *Arch Virol*. 2007;152:289.
91. Gomez-Laguna J, et al. *Transbound Emerg Dis*. 2012;10:1865.
92. Klionsky DJ. *Nat Rev Mol Cell Biol*. 2007;8:931.
93. Liu Q, et al. *Virology*. 2012;429:136.
94. Butler JE, et al. *J Immunol*. 2007;178:6320.
95. An T-Q, et al. *Vet Microbiol*. 2010;143:371.
96. Kim JK, et al. *J Virol*. 2006;80:689.
97. De Baere MI, et al. *Vet Res*. 2012;43:47.
98. Welch S-K, Calvert JG. *Virus Res*. 2010;154:98.
99. Calvert JG, et al. *J Virol*. 2007;81:7371.
100. Cafruny WA, et al. *Virol J*. 2007;3:90.
101. Lee YJ, Lee C. *Vet Immunol Immunopathol*. 2012;150:213.
102. Patton JB, et al. *Virus Res*. 2009;140:161.
103. Gruenberg J, van der Goot FG. *Nat Rev Mol Cell Biol*. 2006;7:495.
104. Misinzo GM, et al. *Vet Res*. 2008;39:55.
105. Van Gortp H, et al. *Arch Virol*. 2009;154:1939.
106. Van Breedam W, et al. *J Gen Virol*. 2010;91:1659.
107. Music N, Gagnon CA. *Anim Health Res Rev*. 2010;11:135.
108. Lee C, et al. *Virology*. 2006;346:238.
109. Pei Y, et al. *Virus Res*. 2008;135:107.
110. Kwon B, et al. *Virology*. 2008;380:371.
111. Wang C, et al. *Vet Microbiol*. 2008;131:339.
112. Faaberg KS, et al. *Virus Res*. 2010;154:77.
113. Rowland RR. *Virus Res*. 2010;154:1.
114. Zhang X, Moser DM. *J Pathol*. 2008;214:161.
115. Lee YJ, Lee C. *Virus Res*. 2010;152:50.
116. Lee C, Yoo D. *Virology*. 2006;355:30.
117. Du Y, et al. *Virus Res*. 2010;147:294.
118. Chang HC, et al. *Vet Microbiol*. 2008;129:281.
119. Flores-Mendoza L, et al. *Clin Vaccine Immunol*. 2008;15:720.
120. Loving CI, et al. *Immunology*. 2007;120:217.
121. Gimeno M, et al. *Vet Res*. 2011;42:9.
122. Gomez-Laguna J, et al. *J Comp Pathol*. 2010;142:51.
123. Zhang Y, et al. *Vet Microbiol*. 2012;160:473.
124. Sagong M, Lee C. *Virus Res*. 2010;151:88.
125. Li Y, et al. *J Gen Virol*. 2012;93:829.
126. Chaung H-C, et al. *Comp Immunol Microbiol Infect Dis*. 2010;33:197.
127. Liu C-H, et al. *Vet Microbiol*. 2009;136:266.
128. Manickam C, et al. *Vet Microbiol*. 2013;162:68.
129. Silva-Campo E, et al. *Virology*. 2012;430:73.
130. Pintaric M, et al. *Vet Immunol Immunopathol*. 2008;121:61.
131. Lodoen MB, Lainier LL. *Curr Opin Immunol*. 2006;18:391.
132. Costers S, et al. *Arch Virol*. 2008;153:1453.
133. Cao J, et al. *Vet Microbiol*. 2013;164:261.
134. Caliguri MA. *Blood*. 2008;112:461.
135. Renukaradhya GJ, et al. *Viral Immunol*. 2010;23:457.
136. Dvivedi V, et al. *Virol J*. 2012;9:45.
137. Cecere TE, et al. *Vet Microbiol*. 2012;160:233.
138. Silva-Campo E, et al. *Virology*. 2009;387:373.
139. Silva-Campo E, et al. *Virology*. 2010;396:264.
140. Silva-Campo E, et al. *Virology*. 2012;85:23.
141. Rodriguez-Gomez IM, et al. *Transbound Emerg Dis*. 2013;60:425.
142. Halstead SB, et al. *Lancet Infect Dis*. 2010;10:712.
143. Belkaid Y. *Nat Rev Immunol*. 2007;7:875.
144. Dwivedi V, et al. *Vaccine*. 2011;29:4067.
145. LeRoith T, et al. *Vet Immunol Immunopathol*. 2011;140:312.
146. Wongyanin P, et al. *Vet Immunol Immunopathol*. 2010;132:170.
147. Silva-Campo E, et al. *Virology*. 2012;430:73.
148. Chen Z, et al. *J Gen Virol*. 2010;91:1047.
149. Costers S, et al. *Vet Res*. 2009;40:46.
150. Gomez-Laguna J, et al. *Comp Immunol Microbiol Infect Dis*. 2011;34:143.
151. Subramaniam S, et al. *Virology*. 2010;406:270.
152. Subramaniam S, et al. *Virology*. 2012;432:241.
153. Barranco I, et al. *Vet Immunol Immunopathol*. 2012;149:262.
154. Gomez-Laguna J. *Vet Microbiol*. 2012;158:187.
155. Backus GS, et al. *Environ Health Perspect*. 2010;118:1721.
156. Diaz I, et al. *Vet Res*. 2012;43:30.
157. Weaver LK, et al. *J Leucocyte Biol*. 2007;81:663.
158. Hou J, et al. *Virol J*. 2012;9:165.
159. Gomez-Laguna J, et al. *Vet J*. 2013;195:148.
160. Lunney JK, et al. *Virus Res*. 2010;154:185.
161. Beura LK, et al. *J Virol*. 2010;84:1574.
162. Subramaniam S, et al. Proc 90th Meet Conf Res Work Anim Dis, Chicago 2009; Abstr. 176.
163. Overend C, et al. *J Gen Virol*. 2007;88:925.
164. Bao D, et al. *Vet Immunol Immunopathol*. 2013;156:128.
165. Shi X, et al. *Virus Res*. 2010;153:151.
166. Calzada-Nova G, et al. *Vet Immunol Immunopathol*. 2010;135:20.
167. Peng Y-T, et al. *Vet Microbiol*. 2009;136:359.
168. Miguel JC, et al. *Vet Immunol Immunopathol*. 2010;135:314.
169. Klinge KL, et al. *Virol J*. 2009;6:177.
170. Dotti S, et al. *Res Vet Sci*. 2013;94:510.
171. Beura LK, et al. *J Virol*. 2011;85:12939.
172. Beura LK, et al. *J Virol*. 2010;84:1574.
173. Bowie AG, Unterholzer L. *Nat Rev Immunol*. 2008;8:911.
174. Kawai T, Akira S. *Int Immunol*. 2009;21:317.
175. Beura LK, et al. *Virology*. 2012;433:431.
176. Versteeg GA, GarciaSastre A. *Curr Opin Microbiol*. 2010;13:508.
177. Beura LK, et al. *J Virol*. 2010;84:1574.
178. Song C, et al. *Virology*. 2010;407:268.
179. Patel D, et al. *J Virol*. 2010;84:11405.
180. Li H, et al. *J Gen Virol*. 2010;81:2947.
181. Sun Z, et al. *J Virol*. 2010;84:7832.
182. Kimman TG, et al. *Vaccine*. 2009;27:315.
183. Nan Y, et al. *Virology*. 2012;43:261.
184. Yoo D, et al. *Virus Res*. 2010;154:48.
185. Zhou Y, et al. *Can J Vet Res*. 2012;76:255.
186. Chen Z, et al. *Virology*. 2010;198:87.
187. Huber JP, Farrar JD. *Immunology*. 2011;132:446.
188. Luo R, et al. *Mol Immunol*. 2008;45:2839.
189. Brockmeier SL, et al. *Viral Immunol*. 2009;22:173.
190. Brockmeier SL, et al. *Clin Vaccine Immunol*. 2012;19:508.
191. Kim O, et al. *Virology*. 2010;402:315.

192. Sagong M, Lee C. *Arch Virol*. 2011;156:2187.
193. Lee YJ, Lee C. *Virology*. 2012;427:80.
194. Ait-Ali T, et al. *Immunogenetics*. 2011;63:437.
195. Sun Z, et al. *J Virol*. 2012;86:3839.
196. Barranco I, et al. *Transbound Emerg Dis*. 2012;59:145.
197. Van Gorp H, et al. *J Gen Virol*. 2008;89:2943.
198. Dong S, et al. *Res Vet Sci*. 2012;93:1060.
199. Gudmundsdottir I, Risatti GR. *Virus Res*. 2009;145:145.
200. Hou J, et al. *Virus Res*. 2012;167:106.
201. Gomez-Laguna J, et al. *Comp Immunol Microbiol Infect Dis*. 2010;33:51.
202. Gomez-Laguna J, et al. *Vet Microbiol*. 2012;158:187.
203. Darwich L, et al. *Vet Microbiol*. 2011;150:49.
204. Mateu E, Diaz I. *Vet J*. 2008;177:345.
205. Dotti S, et al. *Res Vet Sci*. 2011;90:218.
206. Martinez-Lobo FJ, et al. *Vet Microbiol*. 2011;154:58.
207. Garcia-Nicolas O, et al. *Virus Res*. 2014;179:204.
208. Weesendorp E, et al. *Vet Microbiol*. 2013;163:1.
209. Weesendorp E, et al. *Vet Microbiol*. 2013;167:638.
210. Kim W-I, et al. *Vet Micrbiol*. 2007;123:10.
211. Frydas IS, et al. *Vet Res*. 2013;44:73.
212. Wang G, et al. *Virol J*. 2014;11:2.
213. Wang G, et al. *Vet Immunol Immunopathol*. 2011;142:170.
214. Han K, et al. *J Comp Pathol*. 2012;147:275.
215. Hou J, et al. *Virus Res*. 2012;167:10.
216. Li L, et al. *Virol J*. 2012;9:203.
217. Cao J, et al. *J Vet Diagn Invest*. 2012;24:767.
218. Guo B, et al. *Virology*. 2013;435:372.
219. Mege JL, et al. *Lancet Infect Dis*. 2006;6:557.
220. Borghetti P, et al. *Comp Immunol Microbiol Infect Dis*. 2011;34:143.
221. Parcina M, et al. *J Immunol*. 2013;190:1591.
222. Butler JE, et al. *J Immunol*. 2007;178:6329.
223. Butler JE, et al. *J Immunol*. 2008;180:2347.
224. Sun XZ, et al. *Vaccine*. 2012;30:3646.
225. Parida R, et al. *Virus Res*. 2012;169:13.
226. Diaz I, et al. *Virus Res*. 2010;154:61.
227. Han J, et al. *J Virol*. 2010;84:10102.
228. Kinman TG, et al. *Vaccine*. 2009;27:3704.
229. Li J, Murtaugh MP. *Virology*. 2012;433:367.
230. Tong GZ, et al. *Emerg Infect Dis*. 2007;13:1434.
231. An T-Q, et al. *Emerg Infect Dis*. 2010;16:365.
232. Li L, et al. *Virus Res*. 2010;154.
233. Murtaugh M Proc 40th Ann Meet Am Assoc Swine Vet, Dallas, TX, 459.
234. Qiao S, et al. *Vet Microbiol*. 2011;149:213.
235. Diaz I, et al. *Vet Res*. 2012;43:30.
236. Jung K, et al. *J Gen Virol*. 2009;90:2713.
237. Wagner J, et al. *Vet J*. 2011;187:310.
238. Beilage EG, et al. *Tierartzl Prax*. 2007;35:294.
239. Cano JP, et al. *Can J Vet Res*. 2009;73:303.
240. Lewis CRG, et al. *Anim Prod Sci*. 2010;50:890.
241. Han K, et al. *J Comp Pathol*. 2013;148:396.
242. Karniychuk U, et al. *Microb Pathog*. 2011;51:194.
243. Karniychuk U, Nauwynck HJ. *Vet Res*. 2013;44:95.
244. Kang I, et al. *Res Vet Sci*. 2010;88:304.
245. Han K, et al. *Clin Vaccine Immunol*. 2012;19:319.
246. Burgara-Estrella A, et al. *Transbound Emerg Dis*. 2012;59:532.
247. Schulze M, et al. *Acta Vet Scand*. 2013;55:16.
248. Stadejek T, et al. *Vet Rec*. 2011;169:441.
249. Xiao S, et al. *BMC Genomics*. 2010;11:544.
250. Hu SP, et al. *Transbound Emerg Dis*. 2012;10:1865.
251. Lawson S, et al. *Vaccine*. 2010;28:5356.
252. Lee YJ, et al. *J Virol Methods*. 2010;163:410.
253. Wernike K, et al. *J Vet Diagn Invest*. 2012;24:855.
254. Rovira A, et al. *J Vet Diagn Invest*. 2007;19:502.
255. Gerber PF, et al. *J Clin Microbiol*. 2013;51:547.
256. Gerber PF, et al. *J Virol Methods*. 2014;197:63.
257. Li Q, et al. *J Virol Methods*. 2009;155:55.
258. Rovira A, et al. *J Vet Diagn Invest*. 2009;21:350.
259. Lurchachaiwong W, et al. *Lett Appl Microbiol*. 2008;46:55.
260. Balka G, et al. *J Virol Methods*. 2009;115:1.
261. Chen N-H, et al. *J Virol Methods*. 2009;161:192.
262. Ren X, et al. *J Clin Microbiol*. 2010;48:1875.
263. Van Breedam W, et al. *Vet Immunol Immunopathol*. 2011;141:246.
264. Diaz I, et al. *J Vet Diagn Invest*. 2012;24:344.
265. Kittawornrat A, et al. *Vet Res*. 2013;9:61.
266. Okinaga T, et al. *Vet Rec*. 2009;164:455.
267. Lin K, et al. *J Clin Microbiol*. 2011;49:3184.
268. Gutierrez AM, et al. *Vet Immunol Immunopathol*. 2009;132:218.
269. Gomez-Laguna J, et al. *Vet J*. 2010;85:83.
270. Kittawornrat A, et al. *Vet Microbiol*. 2014;168:331.
271. Chittick WA, et al. *J Vet Diagn Invest*. 2011;23:248.
272. Prickett JR, et al. *J Swine Health Prod*. 2008;16:86.
273. Prickett JR, et al. *J Vet Diagn Invest*. 2008;20:156.
274. Prickett JR, Zimmerman JJ. *Anim Health Res Rev*. 2010;10:1.
275. Ramirez A, et al. *Prev Vet Med*. 2012;104:292.
276. Kittawornrat A, et al. *Virus Res*. 2010;154:1700.
277. Ouyang K, et al. *Clin Vaccine Immunol*. 2013;20:1305.
278. Kittawornrat A, et al. *J Vet Diagn Invest*. 2012;24:262.
279. Olsen C, et al. *J Vet Diagn Invest*. 2013;25:328.
280. Kittawornrat A, et al. *J Vet Diagn Invest*. 2012;24:1057.
281. Costers S, et al. *Vet Res*. 2009;40:46.
282. Han K, et al. *Vet J*. 2013;195:313.
283. Gomez-Laguna J, et al. *Transbound Emerg Dis*. 2013;60:273.
284. Morgan SB, et al. *Vet Microbiol*. 2013;163:13.
285. Lee S-M, Kleiboeker SB. *Virology*. 2007;365:419.
286. He Y, et al. *Vet Microbiol*. 2012;160:455.
287. Grau-Roma L, Segales J. *Vet Microbiol*. 2007;119:144.
288. Han K, et al. *J Vet Diagn Invest*. 2012;24:719.
289. Stuart AD, et al. *Pig J*. 2008;61:42.
290. Jiang Y, et al. *Vet Res Commun*. 2010;34:607.
291. Rowland RR. *Sci Direct*. 2007;174:451.
292. Rowland RR, Morrison RB. *Transbound Emerg Dis*. 2012;59(suppl 1):55.
293. Mondaca-Fernandez E, Morrison RB. *Vet Rec*. 2007;161:137.
294. Wayne SR, et al. *J Am Vet Med Assoc*. 2012;240:876.
295. Liu Y, et al. *J Anim Sci*. 2013;91:5668.
296. Pitkin A, et al. *Vet Microbiol*. 2009;136:1.
297. Dee S, et al. *Vet Microbiol*. 2009;138:106.
298. Dee S, et al. *Vet Rec*. 2010;167:976.
299. Spronk G, et al. *Vet Rec*. 2010;166:758.
300. Alonso C, et al. *Vet Microbiol*. 2012;157:304.
301. Alonso C, et al. *Prev Vet Med*. 2013;112:109.
302. Alonso C, et al. *Prev Vet Med*. 2013;111:268.
303. Che TM, et al. *J Anim Sci*. 2011;89:3016.
304. Che TM, et al. *J Anim Sci*. 2012;90:2784.
305. Lambert M-E, et al. *Prev Vet Med*. 2012;104:74.
306. Rovira A, et al. *J Vet Diagn Invest*. 2007;19:492.
307. Han K, et al. *Clin Vaccine Immunol*. 2011;18:1600.
308. Charerntantanakul W. *Vet Immunol Immunopathol*. 2009;129:1.
309. Linghua Z, et al. *Vaccine*. 2007;25:1735.
310. Zhang L, et al. *Vet Microbiol*. 2013;164:253.
311. Drexler CS, et al. *Vet Rec*. 2010;166:70.
312. Thanawongnuwech R, Suradhat S. *Virus Res*. 2010;154:133.
313. Kitikoon P, et al. *Vet Microbiol*. 2009;139:235.
314. Murtaugh MP, Genzow M. *Vaccine*. 2011;29:8192.
315. Martelli P, et al. *Vaccine*. 2007;25:3400.
316. Geldhof MF, et al. *Vet Microbiol*. 2013;167:260.
317. Scortti M, et al. *Vet J*. 2006;172:506.
318. Dwivedi V, et al. *Vaccine*. 2011;29:4058.
319. Robinson SR, et al. *Vet Microbiol*. 2013;164:281.
320. Borghetti P, et al. *Comp Immunol Microbiol Inf Dis*. 2011;34:143.
321. Roca M, et al. *Vet J*. 2012;193:92.
322. Delrue I, et al. *Vet Res*. 2009;40:62.
323. Vanhee M, et al. *Vet Res*. 2009;40:63.
324. Geldof MF, et al. *BMC Vet Res*. 2012;8:182.
325. Zhao Z, et al. *Vet Microbiol*. 2012;155:247.
326. Beilage EG, et al. *Prev Vet Med*. 2009;92:31.
327. de Lima J, et al. *Vaccine*. 2008;26:3594.
328. Fang Y, et al. *J Gen Virol*. 2008;89:3086.
329. Cano JP, et al. *Am J Vet Res*. 2007;68:565.
330. Zuckerman FA, et al. *Vet Microbiol*. 2007;123:69.
331. Cano JP, et al. *Vaccine*. 2007;25:4382.
332. Martinez-Lobo F, et al. *Vet Res*. 2013;44:115.
333. Huang YW, Meng XJ. *Virus Res*. 2010;154:141.
334. de Lima M, et al. *Virology*. 2006;353:410.
335. Vu HLX, et al. *Vaccine*. 2013;31:4330.
336. Cruz JLG, et al. *Virus Res*. 2010;154:150.
337. Pei Y, et al. *Virology*. 2009;389:91.
338. Huang Q, et al. *J Virol Methods*. 2009;160:22.
339. Guoshan S, et al. *Vaccine*. 2007;25:4193.
340. Li J, et al. *Vaccine*. 2009;27:825.
341. Chia M-Y, et al. *Vet Microbiol*. 2010;146:189.
342. Cai J, et al. *J Vet Med Sci*. 2010;72:1035.
343. Yu X, et al. *Clin Vaccine Immunol*. 2013;20:613.
344. Genzow M, et al. *Can J Vet Res*. 2009;73:87.
345. Martelli P, et al. *Vet Microbiol*. 2013;162:358.
346. Ferrari L, et al. *Vet Immunol Immunopathol*. 2013;151:193.
347. Nan Y, et al. *Virology*. 2012;432:261.
348. Leng X, et al. *Clin Vaccine Immunol*. 2012;19:1199.
349. Tian Z-J, et al. *Vet Microbiol*. 2009;138:34.
350. Hu SP, et al. *Transbound Emerg Dis*. 2013;60:351.

MENANGLE

This causative virus was first identified in a three-farm disease outbreak in New South Wales in 1997. It causes reproductive problems in pigs and congenital defects and has the fruit bat as an asymptomatic reservoir. It can cause a flu-like disease in man. Only one outbreak has been described. It normally lives asymptomatically in fruit bats.

ETIOLOGY

The causative agent is an RNA virus in the family Paramyxoviridae in the genus *Rubulavirus*. It is closely related to Tioman virus found in fruit bats on Tioman Island, Malaysia.

EPIDEMIOLOGY

A variety of fruit bats are seropositive, including the gray-headed flying fox, black fruit bat, and spectacled fruit bat, but the virus has not been isolated from them. These fruit bats have been found in other areas of Australia as well as the original area around Menangle, New South Wales.

Bat feces and urine are probably the source of infection. Transmission from pig to pig is slow and probably requires close contact. In one building, it took a long time for the sows to become affected. It probably spreads from farm to farm via infected animals. There is no sign of persistent infection and no evidence of long-term virus shedding. Present evidence suggests that

virus survival in the environment is short because sentinel pigs placed in an uncleaned area did not seroconvert.

CLINICAL SIGNS

There is no knowledge of the incubation period as yet. In the initial outbreak, clinical signs were seen only on the farrow-to-finish farm but infected pigs were found in all three farms.

The disease was an outbreak of reproductive disease with fetal death; fetal abnormalities including congenital defects, such as skeletal and neurologic defects[1]; mummified fetuses; stillborn fetuses; smaller litters with fewer live piglets; and a reduced farrowing rate. The farrowing rate fell from over 80% to around a low of 38% reaching an average of 60%. Many sows returned to estrus 28 days after mating, which suggests that there has been an early death of the litter. Some sows remain in pseudopregnancy for more than 60 days. It probably crosses the placenta and spreads fetus to fetus. Once the infection became endemic in the farrow-to-finish herd the reproductive failures ceased.

PATHOLOGY

The mummified fetuses vary in size and are 30 days or older. The virus causes the degeneration of brain and spinal cord. In particular, the cerebral hemispheres and cerebellum are smaller. Occasionally there may be effusions and pulmonary hypoplasia. Eosinophilic inclusions are found in the neurons of the cerebrum and spinal cord. Sometimes there is a nonsuppurative meningitis, myocarditis, and hepatitis. Experimental infections show shedding 2 to 3 days after infection in nasal and oral secretions. A tropism for secondary lymphoid tissues and intestinal epithelium has been demonstrated.[2] No lesions have been seen in piglets born alive or other postnatal pigs.

DIAGNOSIS

The diagnosis is suspected when the reproductive parameters change very suddenly, as shown earlier.

Diagnosis is confirmed by virus culture, and electron microscopy and virus neutralisation tests confirm the identity of the virus. Serologic tests include ELISAs, and the best way to test the herd is to use this for the sows for antibody.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis includes porcine parvo virus (PPV), classical swine fever (CSF), porcine reproductive and respiratory syndrome (PRRS), encephalomyocarditis virus (EMCV), pseudorabies virus (PRV), Japanese encephalitis, swine influenza virus (SIV), and blue eye. Noninfectious causes such as toxins or nutritional deficiencies should also be considered.

TREATMENT

It seems likely that young pigs are infected by the virus when the maternal antibody concentration declines at 14 to 16 weeks of age. By the time they enter the breeding herd their immunity is quite strong.

CONTROL

The best advice is to avoid contact with all fruit bats.

FURTHER READING

Philbey AW, et al. An apparently new virus (family *Paramyxoviridae*) infection for pigs, humans and fruit bats. *Emerg Infect Dis*. 1998;4:269.

REFERENCES

1. Philbey AW, et al. *Aust Vet J*. 2007;85:134.
2. Bowden TR, et al. *J Gen Virol*. 2012;93:1007.

JAPANESE ENCEPHALITIS (JE; JAPANESE B ENCEPHALITIS)

Japanese encephalitis is an infectious disease primarily affecting horses and to a lesser extent pigs, with important zoonotic potential. It causes in excess of 50,000 human cases a year, with a case mortality rate of 25%. The condition in equides is associated with encephalitis and is covered in detail in Chapter 14 under Japanese encephalitis. In pigs the condition is associated with reproductive failure, whch is covered hereunder.

ETIOLOGY

The causative agent is the Japanese encephalitis virus of the family Flavivirdae, genus *flavivirus*. Based on the phylogenetic analysis of the viral envelope "E" gene, 5 different genotypes have been identified.

EPIDEMIOLOGY

The natural distribution range of the virus is southeast Asia and Australasia. The vectors are *Culex* spp and in particular *C. tritaeniorhynchus*. The virus activity is naturally maintained through bird–mosquito cycles with the heron family in particular. The night herons, little egrets and plumed egrets are particularly active as a reservoir. Pigs are important "amplifying hosts." Pigs and these birds may allow the overwintering of the virus when mosquitoes are absent.

PATHOGENESIS

Viremia results from the mosquito bite and usually nothing is seen. Occasionally there may be a mild fever. but quite often the virus goes straight to the testicles and causes an orchitis.

CLINICIAL SIGNS

Fetal death is common with mummified fetuses as well as stillborn and weak pigs. Boars undergo reproductive failure.

PATHOLOGY

Largely related to the abnormal fetuses.

DIAGNOSIS

RT-PCR and nested RT-PCR can be used to detect the virus when virus isolation is negative. Antibody can be detected by haemagglutination inhibiton, ELISAs (IgM capture ELISA), and latex agglutination tests.

CONTROL

Live attenuated vaccines should be given to breeding stock 2 to 3 weeks before the start of the mosquito season. Attenuated and adjuvanted vaccines are also available.

FURTHER READING

Mackenzie JS, Williams DT. The zoonotic flaviviruses of southern, southeastern and eastern Asia and Australasia: The potential for emergent viruses. *Zoonoses Public Health*. 2009;56:338.

NEOSPOROSIS

SYNOPSIS

Etiology The protozoan parasite *Neospora caninum;* the dog is identified as the definitive host of *N. caninum,* but the main route of infection in cattle appears to be by vertical transmission.

Epidemiology An infection of cattle worldwide and associated with epidemic and endemic abortion. Point source and congenital infections occur.

Clinical findings Abortion in cows and perinatal mortality and encephalomyelitis in congenitally infected calves.

Clinical pathology Serologic testing of maternal serum and fetal fluids.

Necropsy findings Fetal lesions of multifocal nonsuppurative encephalitis, myocarditis, and/or periportal hepatitis. Infection confirmed by immunohistochemistry or polymerase chain reaction-based tools.

Diagnostic confirmation A presumptive diagnosis can be based on the fetal histologic lesions and seropositivity of the dam, but the definitive diagnosis requires the demonstration of the parasite in fetal tissues by immunohistochemical labeling, coupled with serologic examinations.

Control Feed hygiene and calving hygiene. Cull congenitally infected cattle.

ETIOLOGY

Neospora caninum is a cyst-forming coccidial (apicomplexan) parasite with an indirect life cycle.[1-9] *N. caninum* primarily infects dogs and cattle; however, it has a **wide host range** and infects all major domestic livestock species as well as companion animals and some wildlife animals. Dogs are the definitive host and cattle the major intermediate host. Natural infection is infrequently reported in sheep, goats, and deer.[1-3] *N. caninum* is a sporadic cause of

encephalomyelitis and myocarditis in several species, but its principal importance is its association with **endemic and epidemic abortion in cattle.** It is now the most common diagnosis for abortion in cattle in most countries.

EPIDEMIOLOGY

Occurrence

N. caninum was initially associated with abortion in the early 1990s in pastured cattle in Australia and New Zealand and as a major cause of abortion in dairies in the United States. Since then, abortion associated with *N. caninum* has been reported in many countries in cattle under varying management conditions and has a **worldwide occurrence.**[2,3]

Abortion may be **epizootic or sporadic**. In epizootic abortion, the number of cows aborting varies. It is usually between 5% and 10%, but up to 45% of cows may abort within a short period. The period of abortion may be a few weeks to a few months. There is no major seasonal occurrence, and abortion occurs in both beef and dairy cows. Sporadic abortions occur mainly in cows that have been infected congenitally, and seropositive cows have greater risk for repeat abortions. Seropositivity in herds can be high but varies considerably. Seropositive dams have a 3- to 7-fold greater risk of abortion than seronegative dams.

Methods of Transmission

There are two routes of infection of cattle. The dog is the definitive host of *N. caninum*. Infection of cattle can occur via the ingestion of oocytes from dog feces contaminating feed or water. However, vertical (i.e., congenital) transmission occurs in both cattle and dogs, and vertical transmission appears the major route for infection in most cattle.[1-3] Live-born calves from congenitally infected cows are themselves congenitally infected; the infection is thought to be **persistent and lifelong**. A study conducted on two dairies found 81% of seropositive cows gave birth to congenitally infected calves.[1] Seroprevalence did not increase with cow age and was stable through the study period. The probability of a calf being congenitally infected was not associated with dam age, dam lactation number, dam history of abortion, calf gender, or length of gestation. Other studies have shown that this route of transmission is highly efficient, resulting in infection of 50% to 95% of the progeny of seropositive dams.

Congenital infection can result in abortion or the birth of a "normal," infected calf, and an infected cow can give birth to a clinically normal, infected calf at one pregnancy and abort in the subsequent pregnancy.[2,3] The occurrence of infection in some herds can be associated with specific family lines.

Although vertical transmission is the major route of infection that leads to sporadic abortions in cattle associated with *N. caninum,* epidemiologic evidence suggests that postnatal (point) infection is often the cause of outbreaks of abortion. Where dog feces are the source of infection, many cattle are often exposed, and this point source of infection commonly results in outbreaks of abortion. Farm dogs have been shown to have a higher seroprevalence to *N. caninum* than urban dogs, suggesting that neosporosis cycles between cattle and dogs in rural environments.[4]

The importance of postnatal infection versus vertical infection in the genesis of abortion may vary among countries, and be associated with differences in farm management systems.[4]

Experimental Studies

Abortion has been produced by experimental challenge of fetuses and pregnant cattle with culture-derived tachyzoites of *N. caninum.*[1] Fetal death and resorption or abortion has been reproduced in ewes challenged at 45, 65, and 90 days' gestation, but not 120 days, and lesions resemble those of ovine toxoplasmosis.[2] The disease has also been reproduced experimentally in goats,[1] but the importance and prevalence of this infection in naturally occurring abortions in small ruminants remains to be determined. Contaminated placenta, milk, and colostrum can result in infection of calves less than 1 week of age.

Risk Factors

Outbreaks of abortion often appear to be point source infections, but the risk factors, other than probable mass exposure to dog feces containing sporulated *N. caninum* oocysts, are not known. Neosporosis in dairy herds often occurs as an epizootic, with multiple abortions occurring in a 1- to 2-month period. Severely autolytic fetuses are aborted between 5 and 7 months of pregnancy in most reports, but earlier or later abortions can occur (range is between 3 and 8.5 months of pregnancy).

Endemic abortion is more likely associated with the presence of **congenitally infected** cattle in the herd, which are at **high risk of aborting**, particularly in the initial pregnancy and in the pregnancy during the first lactation.[2,3] Cows that have aborted have a higher risk for abortion in subsequent pregnancies, but this risk decreases with each subsequent pregnancy. It has been postulated that immunosuppression resulting from concurrent infection with other agents, such as bovine viral diarrhea virus (BVD) , may increase the risk for infection with *N. caninum* and precipitate abortion outbreaks.

Economic Importance

Economic losses relate to abortion and costs associated with establishing the diagnosis and rebreeding or replacement costs.[5] Seropositivity is also associated with increased risk of stillbirth and increased risk of retained placenta. Losses associated with epidemic abortion have been estimated at tens (20–85) of millions of dollars to the dairy or beef industries in Australasia and the United States.

Although seropositive heifers have been reported to produce less milk than seronegative herdmates, this difference in milk production between seropositive and seronegative animals is not necessarily apparent in herds unaffected by an abortion problem. Study of beef cattle has suggested that seropositivity might be associated with reduction in average daily weight gain, but production performance and carcass measures are not consistently reported to be affected.

PATHOGENESIS

N. caninum has a predilection for fetal chorionic epithelium and fetal placental blood vessels, producing a fetal vasculitis and inflammation and degeneration of the chorioallantois, and widespread necrosis in the placentome.[6] Tachyzoites penetrate host cells and are located in a parasitophorous vacuole. They can be found in macrophages, monocytes, vascular endothelial cells, fibroblasts, hepatocytes, renal tubular cells, and in the brain of infected animals. With neuromuscular disease, cranial and spinal neural cells are infected. Cell death is caused by the replication of tachyzoites (during endodyogeny).

CLINICAL FINDINGS

Abortion is the cardinal clinical sign observed in infected **cows**.[2,3] Fetuses may die in utero, or can be reabsorbed, mummified, stillborn, born alive but diseased, or born clinically normal but infected. Cows that are infected can have **decreased milk production** in the first lactation, producing approximately 1 L less of milk per cow per day than uninfected cows, are prone to abort, and have a higher risk of being culled from the herd at an early age.

In addition to the occurrence of early abortion, the disease in beef herds is associated with the birth of live-born, premature, **low birthweight** calves. Depending on the degree of prematurity, these calves can be kept alive with intensive care during the neonatal period.

Most congenitally infected calves are born alive without clinical signs. Occasionally, congenital infection can be manifest with ataxia, loss of conscious proprioception, paralysis, and/or other **neurologic deficits** in new-born calves,[2] but most congenitally infected calves appear as clinically normal and, surprisingly, some evidence suggests that congenital infection does not necessarily have a detrimental effect on calf health and survival.[3]

N. caninum infection has been demonstrated in the nervous system of a **horse** with progressive debilitation, followed by a sudden onset of neurologic disease with paraplegia. It appears to be a rare cause of

neurologic disease in horses, but should be considered in the differential diagnosis of equine protozoal myeloencephalitis.

CLINICAL PATHOLOGY

Serologic testing can be conducted using IFAT or ELISA, and there appears to be good agreement in results between the two tests. ELISA using recombinant protein appears to have a higher diagnostic specificity and sensitivity than using whole-tachyzoite lysates.[7] IFAT is commonly used and achieves a relatively high diagnostic specificity and sensitivity for the detection of maternal infection.[7] The persistence of serum antibody titers following infection is uncertain, and they might fluctuate during pregnancy. A positive titer in a cow that has aborted indicates exposure but not causality. IgG avidity patterns have been used to predict the duration of infection. Diagnosis can also be conducted by detecting anti-*N. caninum* antibody or genomic DNA of *N. caninum* in **fetal pleural fluid or sera**.[7]

NECROPSY FINDINGS

Gross findings are not specific and the fetus may be fresh, autolyzed, or in early stages of mummification; in the placenta, the cotyledons are usually necrotic.[10] The brain may be autolyzed, but should still be submitted for examination as well as the heart, liver, and placenta, if available. Histologic findings commonly relate to **multifocal nonsuppurative encephalitis, myocarditis hepatitis, and/or placentitis** Liver lesions may be more prominent in epizootic abortions. IHC or PCR can be used to detect tachyzoites or their DNA in tissues (particularly in the brain).[7] IHC can be specific, but insensitive for identifying *Neospora* in the placenta; therefore, maternal serology should be used in conjunction.

TREATMENT

There is no treatment that can be used to curtail an ongoing abortion epidemic. Possible drug therapies are generally not considered an option because of likely unacceptable milk and meat residues and withdrawal problems.

DIFFERENTIAL DIAGNOSIS

Serology and/or polymerase chain reaction can confirm infection in individual cows.

Because of the high prevalence of infection, and the occurrence of congenital infection, care must be taken in extrapolating the results of a single positive diagnosis to problems of abortion. The high rate of natural congenital infection means that evidence of infection in an aborted fetus is not proof of causation of abortion, and fetal examination should be coupled to serologic examination of aborting and nonaborting animals in the herd to assess statistical differences.

- Other causes of abortion in cattle
- Weak calf syndrome

CONTROL

All efforts should be made to exclude the possibility of dog **fecal contamination** of cattle feed and water and of the grazing environment.[4] **Placentas, aborted fetuses, and dead calves** should be removed immediately and disposed of so that the definitive host and cattle cannot gain access to them.

Congenitally infected cows are at high risk of abortion, and abortion rates in infected herds can be substantially reduced by **culling** infected animals.[2-4] Congenitally infected calves can be identified by testing precolostral blood samples using a specific and sensitive serologic test and culled at a young age. If precolostral blood sampling is not feasible, examination of sera at 6 months of age should identify infected calves, with positive titers indicating either congenital infection or postnatal infection. Calves introduced into a herd should be seronegative.

It is possible that strategic therapy of pregnant cows with an appropriate antiprotozoal drug could abort the infection. This could be effective in beef cattle, but would probably not be legal or appropriate in lactating dairy cattle.

Although evidence for increased risk for *Neospora* abortion caused by immunosuppression resulting from concurrent infection with BVD virus is equivocal, control of BVD infections should be a component of anti-neosporosis control.

There has been a considerable effort to develop vaccines against neosporosis.[8,9] An inactivated tachyzoite vaccine was approved in the United States for use in pregnant cows. There are no controlled studies on its efficacy in mitigating the effects of bovine neosporosis in dairy cattle. Vaccination of dairy cattle may interfere with a herd test and cull policy.

FURTHER READING

Goodswen SJ, Kennedy PJ, Ellis JT. A review of the infection, genetics, and evolution of Neospora caninum: from the past to the present. *Infect Genet Evol.* 2003;13:133-150.

Gondim LF. *Neospora caninum* in wildlife. *Trends Parasitol.* 2006;22:247-252.

Hemphill A, Vonlaufen N, Naguleswaran A. Cellular and immunological basis of the host-parasite relationship during infection with *Neospora caninum*. *Parasitology.* 2006;133:261-278.

Innes EA. The host-parasite relationship in pregnant cattle infected with *Neospora caninum*. *Parasitology.* 2007;134:1903-1910.

Innes EA, Bartley PM, Maley SW, Wright SE, Buxton D. Comparative host-parasite relationships in ovine toxoplasmosis and bovine neosporosis and strategies for vaccination. *Vaccine.* 2007;25:5495-5503.

Williams DJ, Hartley CS, Björkman C, Trees AJ. Endogenous and exogenous transplacental transmission of *Neospora caninum*—how the route of transmission impacts on epidemiology and control of disease. *Parasitology.* 2009;136:1895-1900.

REFERENCES

1. Radostits O, et al. Diseases associated with protozoa. In: *Veterinary Medicine: A Textbook of the Disease of Cattle, Horses, Sheep, Goats and Pigs.* 10th ed. London: W.B. Saunders; 2007:1509.
2. Dubey JP, Lindsay DS. *Vet Clin North Am Food Anim Pract.* 2006;22:645.
3. Dubey JP, Schares G. *Vet Parasitol.* 2011;180:90.
4. Dubey JP, et al. *Clin Microbiol Rev.* 2007;20:323.
5. Reichel MP, et al. *Int J Parasitol.* 2013;43:133.
6. Dubey JP, et al. *J Comp Pathol.* 2006;134:267.
7. Dubey JP, Schares G. *Vet Parasitol.* 2006;140:1.
8. Innes EA, Vermeulen AN. *Parasitology.* 2006;133(suppl):S145.
9. Reichel MP, Ellis JY. *Int J Parasitol.* 2009;39:1173.
10. Schlafer DH, Miller RB, Maxie MG, eds. Female genital system. In: *Jubb, Kennedy and Palmer's Pathology of Domestic Animals.* Vol. 3. 5th ed. Edinburgh, UK: Saunders; 2007:429.

DOURINE (*MALADIE DU COIT*)

SYNOPSIS

Etiology *Trypanosoma equiperdum.*

Epidemiology Venereal disease of horses, mules, and donkeys, endemic in southern and northern Africa, Asia, and possibly South and Central America.

Clinical signs Primary genital signs, secondary cutaneous signs, and tertiary nervous signs and emaciation.

Lesions Edematous swelling and later, depigmentation of external genitalia, emaciation, anemia, and subcutaneous edema.

Differential diagnosis list

- Nagana
- Surra
- Coital exanthema
- Equine infectious anemia.
- Purulent endometritis

Treatment Chronic cases unresponsive to trypanocides and may become carriers. Treatment is thus not recommended.

Control Elimination of reactors, control of breeding and movement of animals in affected regions or countries.

ETIOLOGY

Trypanosoma equiperdum belongs to the *brucei* group, subgenus *Trypanozoon,* but occurs only as long, slender, and monomorphic form. It may be more appropriately referred to as *T. brucei equiperdum.* Unlike *T. brucei. brucei,* it has lost part of its kinetoplast DNA (hence dyskinetoplastic). The parasite is morphologically indistinguishable from *T. evansi* in blood smears. *T. equiperdum* is the only pathogenic trypanosome that does not require an arthropod vector for its transmission. It resides more in extra vascular tissue fluid than in blood.

EPIDEMIOLOGY

Occurrence

Dourine is endemic in Asia, Africa, southeastern Europe, and Central America. It has been eradicated from North America, and strict control measures have reduced the

incidence to a low level in most parts of Europe. It occurred in Italy in 2011.[1] The disease is endemic in parts of Ethiopia and Namibia and is rarely reported in other parts of sub-Saharan Africa. It has not been reported in Latin America for over 20 years. It is possible that lack of reporting in some countries may be caused by very strict international regulations that tend to discourage official notification of the disease. All Equidae are susceptible, and natural infection is known to occur only in horses, mules, and donkeys. In Ethiopia, the disease is more prevalent during the breeding season from June to September.[2]

Measures of Disease Occurrence

In most countries, dourine now occurs only sporadically; its prevalence has declined generally because the horse is no longer that important militarily, economically, and agriculturally, and because of strict control measures in many countries. A recent survey of 237 horses from an endemic area of Ethiopia showed that infection rates varied with the method of examination.[3] The rates were 4.6% based on standard parasitologic methods, 27.6% on serology, and up to 47.6% on DNA detection by PCR. This was the first time in more than 30 years that a fresh strain of *T. equiperdum* was isolated from clinical cases of dourine. Case mortality varies; in Europe, it may be as high as 50% to 70%, but it is much lower elsewhere, although many animals may have to be destroyed as a means of control.

Methods of Transmission

Natural transmission occurs only by coitus, but infection can also be acquired through intact oral, nasal, and conjunctival mucosae in foals at birth. The source of infection may be an infected stallion or mare actively discharging trypanosomes from the urethra or vagina, or an uninfected male acting as a physical carrier after serving an infected mare. The trypanosomes inhabit the urethra and vagina but disappear periodically so that only a proportion of potentially infective matings result in infection. Invasion occurs through intact mucosa, and no abrasion is necessary.

Risk Factors

T. equiperdum is incapable of surviving outside the host. Like other trypanosomes, it also dies quickly in cadavers. Some animals, especially donkeys and mules, may be clinically normal but act as carriers of the infection for many years. Because the disease does not require an arthropod vector for its transmission, and in view of the extensive movement of horses across continents that now takes place, the risk of infection, though small, is present in every country, as with any other venereal disease. Thoroughbred horses are more susceptible than indigenous horses, and donkeys tend to show more chronic signs.

Immune Mechanisms

Infected animals produce antibodies to successive antigenic variants, as in *T. brucei*. Recovered animals often become carriers. Blood from infected horses is rarely infective to other horses, and the disease is not easily transmitted to ruminants under experimental conditions. Humans are not affected.

Biosecurity Concerns

There are none except when animals have to be moved internationally.

PATHOGENESIS

T. equiperdum shows a remarkable tropism for the mucosa of genital organs, the subcutaneous tissues, and the peripheral and CNSs. Trypanosomes deposited during coitus penetrate the intact genital mucosa, multiply locally in the extracellular tissue space, and produce an edematous swelling that may later undergo fibrosis. Subsequent systemic invasion occurs, and localization in other tissues causes vascular injury and edema, manifested clinically by subcutaneous edema. Invasion of the peripheral nervous system and the spinal cord leads to incoordination and paralysis.

CLINICAL FINDINGS

The severity of the clinical syndrome varies depending on the strain of the trypanosome and the general health of the horse population. The disease in Africa and Asia is much more chronic than in South America or Europe and may persist for many years, often without clinical signs, although these may develop when the animals' resistance is lowered by other disease or malnutrition.

The incubation period varies between 1 and 4 weeks, but could extend to more than 3 months in some animals. Initial signs may not be recognized until the breeding season. The ensuing disease will manifest genital signs in the primary stage, cutaneous signs in the secondary stage, and nervous signs in the tertiary stage.

In stallions, the **initial signs** are swelling and edema of the penis, scrotum, prepuce, and surrounding skin, extending as far forward as the chest. Paraphimosis may occur, and inguinal lymph nodes are swollen. There is a moderate mucopurulent urethral discharge. In mares, the edema commences in the vulva and is accompanied by a profuse fluid discharge, hyperemia, and sometimes ulceration of the vaginal mucosa. The edema spreads to the perineum, udder, and abdominal floor. In Europe, the disease is more severe; genital tract involvement is often accompanied by sexual excitement and more severe swelling.

In the **secondary stage**, cutaneous urticaria-like plaques, 2 to 5 cm in diameter, develop on the body and neck and disappear within a few hours up to a few days. These so-called silver dollar spots are pathognomonic for dourine but are not always present and are uncommon in endemic areas. Succeeding crops of plaques may result in persistence of the cutaneous involvement for several weeks.

Progressive anemia, emaciation, weakness, and nervous signs that appear at a variable time after genital involvement characterize the **tertiary stage**. Stiffness and weakness of the limbs are evident and incoordination develops, progressing terminally to ataxia and paralysis. Marked atrophy of the hindquarters is common, and in all animals there is loss of condition, in some to the point where extreme emaciation necessitates destruction. Lack of coordination of the hind legs, swelling of the external genitalia, and emaciation were the most common clinical signs in horses suspected to have dourine in Ethiopia.

CLINICAL PATHOLOGY

Trypanosome detection is difficult, but should be attempted in edema fluid, subcutaneous plaques, and vaginal or urethral washings or blood in early stages. Inoculation of blood into laboratory rodents is not as helpful as with other members of the *brucei* group.

An efficient CFT is available and was the basis for a successful eradication program in Canada. However, the test does not distinguish between members of the *brucei* group. Other serologic tests that can be used include the IFAT, the capillary agglutination test for trypanosomes, and the ELISA, but the CFT remains the most reliable. Serologic tests do not distinguish between members of the *brucei* group; hence they are of limited value in areas where *T. brucei* or *T. evansi* is endemic, even when monoclonal antibodies are used. In recent interlaboratory ring trials to evaluate serologic methods for dourine diagnosis, 9 out of 22 laboratories observed a false-positive result with a known *T. evansi*-positive serum, whether by CFT or IFAT.[4] However, diagnosis can be made based on serologic tests and characteristic clinical signs under the right epidemiologic setting.[2]

PCR has been used to detect trypanosome DNA and is an indication of an active infection, unlike serologic tests that detect past and current infections. Still, the PCR test cannot yet distinguish *T. equiperdum* from *T. evansi* or *T. brucei*.[5,6]

With the recent isolation of new strains of *T. equiperdum* from clinical cases in Ethiopia,[3] the first in 4 decades worldwide, there is hope that new internationally recognized tests for the diagnosis of dourine will be developed soon.

NECROPSY FINDINGS

Emaciation, anemia, and subcutaneous edema are always present, and edema of the external genitalia may be evident or the external genitalia may have healed, leaving the characteristic depigmented scars of permanent leukodermic patches. Lymph nodes

are enlarged, and there is softening of the spinal cord in the lumbosacral region.

Histologic lesions consist of lymphoplasmacytic infiltration in the spinal nerves, ganglia, and meninges of the lumbar and sacral regions and in affected skin and mucosa. Trypanosomes can be found in sections of the skin and genital mucosa during the primary and secondary phases of the infection. Affected lymph nodes show nonspecific lymphoid hyperplasia.

DIFFERENTIAL DIAGNOSIS

The full clinical syndrome is diagnostic, when present, because no other disease has the clinical and epizootiologic characteristics of dourine. However, when the full clinical picture is not developed, other diseases like nagana, surra, coital exanthema, equine infectious anemia, and purulent endometritis should be considered. With one exception, all recent reports of the disease have been based on clinical signs, serology, and detection of trypanosome DNA, but not on parasitologic detection.

TREATMENT

TREATMENT AND CONTROL

None is recommended.

Many trypanocidal drugs have been used in the treatment of dourine, but results are variable, chronic cases in particular are unresponsive to treatment. The main drawback is that treated animals may remain inapparent carriers and could continue to spread the disease or complicate serologic tests. Nevertheless, in Ethiopia, treatment of experimentally infected horses with Cymelarsan at 0.25 mg/kg BW was found to be effective for both acute and chronic cases.[7]

Berenil (diminazene) at 7 mg/kg BW as a 5% solution injected IM, with a second injection of half the dose 24 hours later, or suramin (10 mg/kg IV for two to three treatments at weekly intervals), or quinapyramine sulfate (3–5 mg/kg in divided doses injected subcutaneously) have been tried in the past.

CONTROL

In dourine-free countries, an embargo should be placed on the importation of horses from countries in which the disease is endemic, unless the animals have been properly tested and found negative. Eradication on an area or herd basis is by the application of the CFT, along with strict control of breeding and movement of horses. Positive reactors are disposed of, and two negative tests not less than a month apart can be accepted as evidence that the disease is no longer present. Castration or neutering of infected animals is not adequate because mating can still occur.

FURTHER READING

Abebe G. Trypanosomosis in Ethiopia. *Ethiopia J Biol Sci*. 2005;4:75-121.

Barrowman P, et al. Dourine. In: Coetzer JAW, Thomson GR, Tustin RC, eds. *Infectious Diseases of Livestock With Special Reference to Southern Africa*. Vol. 1. Cape Town: Oxford University Press; 1994:206-212.

Desquesnes M. *Livestock Trypanosomoses and Their Vectors in Latin America*. Paris: OIE (World Organization for Animal Health); 2004.

Hunter AG, Luckins AG, Trypanosomosis. In: Sewell MMH, Brocklesby DW, eds. *Handbook on Animal Diseases in the Tropics*. 4th ed. London: Bailliére Tindall; 1990:204-226.

Luckins AG, et al. Dourine. In: Coetzer JAW, Tustin RC, eds. *Infectious Diseases of Livestock*. Vol. 1. 2nd ed. Cape Town: Oxford University Press; 2004:297-304.

OIE. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. Vol. 2. 6th ed. 2008:845-851.

Stephen LE. *Trypanosomiasis: A Veterinary Perspective*. Oxford: Pergamon Press; 1986.

REFERENCES

1. Pascucci I, et al. *Vet Parasitol*. 2013;193:30.
2. Hagos A, et al. *Proceedings of ISCTRC*. Kampala, Uganda: 2009:317.
3. Gari FR, et al. *Trop Anim Health Prod*. 2010;42:1649.
4. Cauchard J, et al. *Vet Parasitol*. 2014;205:70.
5. Li FJ, et al. *Mol Cell Probes*. 2007;21:1.
6. Tran T, et al. *Parasitology*. 2006;133:613.
7. Hagos A, et al. *Vet Parasitol*. 2010;171:200.

Toxic Agents Primarily Affecting the Reproductive System

ESTROGENIC SUBSTANCES

ETIOLOGY

Poisoning occurs either accidentally or intentionally from administration of a number of different products. Supplementation may be by addition to the feed, but is usually by subcutaneous implants. Many of them are used as growth promotants to increase weight gain and feed efficiency in animals.[1] Estrogen in some form can be found in the following four categories of growth promotants:

- Endogenous hormones (estradiol-17-β, progesterone, testosterone)[1,2]
- Synthetic hormones (ethinylestradiol, others)[1]
- Xenobiotics (zearalenone [α-zearalanol; zeranol], trenbolone)[1,3]
- Miscellaneous (diethylstilbestrol and related compounds such as hexestrol and dienestrol)[1]

EPIDEMIOLOGY

Occurrence

Poisoning by estrogenic substances occurs in the following circumstances:

- Natural substances such as genistein present in plants and as zearalenone in fungi[1,3]
- Dietary supplements for fattening cattle[1]
- Overdosage of medications used in clinical infertility cases
- Pigs fed hexestrol implants in capon necks
- Cattle fed on chicken litter from farms on which estrogens are used as supplements.

Risk Factors

Animal Risk Factors

Steers implanted with an estrogen at a standard dose rate may respond in an exaggerated manner and show signs of toxicity. Estradiol implants are reputed to be associated with more of these problems than zeranol.

Environmental Risk Factors

Estrogens from treated animals are found in the environment in water and animal manure and may act as endocrine disrupters. Water treatment plants are able to remove most of the estrogens, but animal manure is not regulated in the many parts of the world unless it is discharged into a water supply.[4-6]

Farm Risk Factors

Pasture may be contaminated by manure from cattle treated orally or by subcutaneous implants with estrogenic substances that pass significant amounts in the feces.[2,6] Ensilage made from the pasture may also be contaminated.

Human Risk Factors

Estrogenic substance administration as a management tool is regarded unfavorably in many countries because of the risk of intoxication occurring in humans eating contaminated meat. Their use is banned in some and strictly controlled in others. In one small study, a palpable mammary tumor was observed in a rat implanted with a 12-mg zeranol pellet.[3] The presence of environmental zearalenone has been proposed as a link to early puberty and anabolic growth effects in young girls.[7]

PATHOGENESIS

Signs and lesions are the direct result of amplification of the pharmacologic effects of the substances.

CLINICAL SIGNS

Idiopathic Female Estrogenism

In addition to the toxic effects associated with estrogens in specific plants, increased estrogenic activity is also encountered in mixed pasture, generally only at certain times and on particular fields. Clinically the effects are those of sterility, some abortions, swelling of the udder and vulva in pregnant animals and virgin heifers, and endometritis with a slimy, purulent vaginal discharge in some animals. Estrous cycles are irregular. In milking cows, there is depression of the milk yield, reduction in appetite, and an increase in the cell count of the milk.

Male Estrogenism

Steers in feedlots may exhibit excessive mounting by other steers, sometimes to the point of causing death. Head injuries caused by head-to-head butting, frequent bawling, stampedes, and pawing the ground to the point of hole-digging are other reported signs. These problems tend to pass off after a short time. Preputial prolapse may be a problem in *Bos indicus* cattle. Experimental feeding of zeranol to young bulls is associated with retardation of testicular and epididymal development.

Nymphomania in Cows

Larger doses of stilbestrol, usually administered accidentally to cows, may be associated with prolapse of the rectum and vagina and elevation of the tail head caused by relaxation of the pelvic ligaments. Susceptibility to fracture of the pelvic bones and dislocation of the hip are common sequelae. Nymphomaniac behavior in such animals results in other skeletal injuries, especially fracture of the wing of the ilium.

Swine Estrogenism

Common clinical signs include weight loss, decreased feed efficiency, straining, prolapse of the rectum, incontinence of urine, anuria, and death.[8] Estrogens such as zearalenone ingested by sows after day 11 to 13 of the estrous cycle can be associated with retention of corpora lutea and a syndrome of anestrus or pseudopregnancy, which typically persists for 45 to 60 days postestrus. This effect may occur at zearalenone concentrations of 3 to 10 ppm in the diet. Pregnant sows given zearalenone postbreeding may have failure of implantation and early fetal abortion.

Urethral Obstruction

Heavy mortalities have occurred in feeder lambs after the use of implants of estrogens as a result of prolapse of the rectum, vagina, and uterus, together with urethral obstruction by calculi. The calculi consist largely of desquamated epithelial and inflammatory cells that form a nidus for the deposition of mineral; the desquamation is probably stimulated by the estrogen. Also, urethral narrowing caused by the estrogen facilitates complete obstruction by the calculi.

CLINICAL PATHOLOGY

High blood levels of estrogens are characteristic. In swine, the syndrome of anestrus associated with zearalenone will be accompanied by elevated progesterone concentrations caused by the retention of corpora lutea.

NECROPSY FINDINGS

Enlargement and vascular engorgement of accessory sex organs, especially in neutered animals, are characteristic. Uterine enlargement and keratinization of vaginal epithelium may be detected, and in mature female swine there may be persistent multiple retained corpora lutea. Swine also show inflammation and necrosis of the rectal wall, enlargement of the kidneys, thickening of the ureters and distension of the bladder, and gross enlargement of the prostate and seminal vesicles. Histopathology on jejunum obtained from pigs treated with low doses of zearalenone and T-2 toxin showed normal crypts and villi but decreased numbers of goblet cells and acidophilic granulocytes in the mucous membrane and numerous plasma cells in the intestinal epithelium.[8]

FURTHER READING

Adams NR. Detection of the effects of phytoestrogens on sheep and cattle. *J Anim Sci*. 1995;73:1509-1515.

Burnison BK, Hartman A, Lister A, et al. A toxicity identification evaluation approach to studying estrogenic substances in hog manure and agricultural runoff. *Environ Toxicol Chem*. 2003;22:2243-2250.

Leffers H, Naesby M, Vendelbo B, et al. OEstrogenic potencies of zeranol, oestradiol, diethylstiboestrol, bisphenol-A and genistein; implications for exposure assessment of potential endocrine disruptors. *Hum Reprod*. 2001;16:1037-1045.

Soto AN, Calabro JM, Prechtl NV, et al. Androgenic and estrogenic activity in water bodies receiving cattle feedlot effluent in Eastern Nebraska, USA. *Environ Health Persp*. 2004;112:346-352.

REFERENCES

1. Biswas S, et al. *J Soil Water Con*. 2013;66:325.
2. Khanal SK, et al. *Environ Sci Technol*. 2006;40:6537.
3. Zhong S, et al. *Anticancer Res*. 2011;31:1659.
4. Chen TS, et al. *Sci Total Environ*. 2010;408:3223.
5. Alvarez DA, et al. *Water Res*. 2013;47:3347.
6. Gadd JB, et al. *Environ Pollut*. 2010;158:730.
7. Massart F, et al. *J Pediatr*. 2008;152:690.
8. Andretta I, et al. *Arch Zootech*. 2010;59:123.

PHYTOESTROGEN TOXICOSIS

SYNOPSIS

Etiology Ingestion of plants that produce estrogen (phytoestrogens) resulting in a number of reproductive problems.

Epidemiology Pastures dominated by specific strains of legumes, in lush growth mode, or hay or silage made from such pasture, are associated with problems if exposure is prolonged. Sheep are much more susceptible than cattle.

Clinical pathology Positive estrogen assay in blood.

Lesions

Live animals: Severe flock infertility in sheep; prolongation of estrus periods, interestrus periods shortened.

Postmortem: Ewes show cystic endometrial degeneration.

Diagnosis confirmation Laboratory assay of feed, blood, and tissue; the appearance of genital pathology at necropsy, or with a uterine biopsy or laparoscopy.

Treatment None.

Control Grazing management, use of low-phytoestrogen cultivars.

ETIOLOGY

Important estrogenic substances found in plants and fungi include the following:

- Plants
  - Coumestans (coumestrol, 4-methoxycoumestrol, repensol, trifoliol)[1]
  - Isoflavones (daidzein, formononetin, genistein, biochanin A, glycitein)[2,3]
  - Isoflavan (equol, a metabolite of daidzein)[3]
- Fungi (resorcylic acid lactones [zearalenone])[4]

Compared with pharmaceutical agents, these substances have low estrogenic activity, but they are associated with serious clinical effects because of the high concentrations they reach in some plants and daily intake over long periods. The coumestans are most common in plants of the *Medicago* genus; isoflavones are most common in the *Trifolium, Baptisia,* and *Cytisus* genera. Only *Medicago* and *Trifolium* spp. are of any importance to animals. Those likely to contain sufficient amounts to be associated with disease are

Fusarium (variety of species); contains zearalenone[4]
Glycine max (soybean; contains coumestans and isoflavones; affects pigs)
Medicago sativa (alfalfa, lucerne; contains coumestans; affects cattle, sheep)
Trifolium alexandrinum (isoflavones)
T. alpestre (alpestrine clover; contains isoflavones)
T. pratense (red clover; contains isoflavones; affects sheep)[1]
T. repens (white clover, Ladino clover; contains coumestans)[1]
T. subterraneum (subterranean clover; contains isoflavones; affects sheep).

EPIDEMIOLOGY

Occurrence

Animals on pasture are at the greatest risk, but poisoning can also occur on diets containing prepared feeds such as soybean (*Glycine max*) meal, or moldy feed containing *Fusarium* fungi.

Risk Factors

Animal Factors

Phytoestrogen toxicosis is clinically important only in sheep. Cattle are generally considered to be less sensitive than sheep.[1,5,6] For example, cows can ingest large amounts of estrogens (over 40 g per day per cow) in red clover without showing any reduction in reproductive efficiency. Horses usually graze the toxic pasture without ill effects.

Massive reproductive wastage has been experienced in sheep on pastures dominated by such plants as *Trifolium subterraneum,* and the death rate from dystocia and prolapse of the uterus can also be high. The most common abnormality is a failure to conceive, even with multiple matings, and the flock breeding status worsens progressively, with the lambing percentage falling from a normal 80% down to 30%. Sheep eating a lot of estrogenic clover in the spring can become temporarily infertile, but are normally fertile again by the usual breeding season in the autumn. However, ingestion of the plant in several successive years is associated with "permanent clover disease"—infertility from which ewes do not recover. Under these conditions sheep farming becomes unprofitable, and large areas of country have been made unsuitable for sheep raising because of this disease.

Human Factors

Various phytoestrogens have been found in foods of animal origin (eggs, milk, meat, fish, and seafood). Equol was found in several foods, including eggs, milk, and meat.[7] Not all phytoestrogens are harmful and many of them are have known human health benefits.[8] Many, however, are endocrine disruptors, which means that they can produce adverse health effects as well.

Plant Factors

The estrogenic activity of pastures depends on the degree of domination of the pasture by the toxic plants, the variety of the plant species, and the duration of the animal's exposure to them. Newly sown pastures are usually most toxic because of domination by the sown legume. Pastures deficient in phosphorus are also likely to be clover dominant. High nitrogen fertilizer applications reduce phytoestrogen content. Varieties of *Trifolium subterraneum,* e.g., Yarloop, Dwalganup, Dinninup, and Geraldton, are much more toxic than Bacchus Marsh and Daliak. Pastures containing more than 30% of the first four varieties are likely to be unsafe. In some clovers, e.g., red clover, the estrogen content varies with the season, and is high in early spring, low in midsummer, and high again in the autumn after the hay has been taken off. Insect damage to pasture can increase the estrogen content 10-fold, and bacterial infection (e.g., by *Pseudopezzia medicaginnis,* a leaf-spotting organism on alfalfa) and fungal infection by 100-fold. Plants that have matured in the field and set seed have no estrogenic potency, but the making of potent fodder into hay causes little depression of estrogen content. Clover ensilage can contain high levels of estrogens, and the ensiling process is considered to increase the estrogenic effect of clover 3- to 5-fold.

Trifolium repens (white clover, in contradistinction to Ladino clover), does not have a high content of estrogens.[1] However, when heavily infested with fungi it can contain significant amounts. It is thought that the production of estrogens is a byproduct of the plant's mechanism of resistance to the fungal infection. Ladino clover, a large-growing variety of white clover, may contain large quantities of a highly active estrogen (coumestrol), and when it dominates a pasture and is grazed when the pasture is lush, it may be associated with the cornification of vaginal epithelium and functional infertility in ewes. Three estrogenic compounds have been isolated from *T. pratense* (red clover), and where this plant dominates the pasture a clinical syndrome similar to that associated with subterranean clover may be observed. Ewes grazing on red clover pasture, especially a toxic cultivar of the plant, may have their conception rate at the first mating cycle reduced from 75% to as low as 25%.

PATHOGENESIS

Much of the metabolism of phytoestrogens in ruminants occurs in the rumen as well as in the liver.[1] The differences between sheep and cattle in the ruminal metabolism of these compounds are thought to be the reason for the comparative freedom of cattle from the clinical disease.

The amount of phytoestrogen ingested by a ewe on a highly poisonous pasture may equal her daily estrogen secretion at the peak of her estrous cycle. The effect of the phytoestrogens is exerted mainly on the uterus and ovaries. Structurally, there is hyperplasia and hypertrophy of the epithelium of the uterus, vagina, and cervix, and dysplasia of the granulosa cells of the ovary, with a consequent reduction in secretion of estradiol. Increases in teat size and milk secretion are additional, secondary effects.

The functional abnormality is not one of estrus; in sheep the demonstration and duration of estrus may be normal or depressed, and the defect is one of sperm transport because of changes in the composition of cervical mucus and the structure of cervical glands. The change is to more watery mucus, and this is the basis of a test in affected sheep in which the watery mucus is more readily absorbed by a cottonwood plug inserted in the vagina. The increased weight of the plug is a positive test.

It is possible that a good deal of the infertility seen in ewes on improved clover pasture may be associated with its high estrogen content, in spite of the absence of the more dramatic evidence of hyperestrogenism described earlier. Because it is necessity to use this pasture, a great deal more needs to be known about the seasonal occurrence of the estrogenic substances and the management of sheep grazing the pasture so that the effects of the disease can be minimized.

CLINICAL FINDINGS

Ewes

Clover disease, the severe clinical manifestation of phytoestrogen poisoning, and rarely seen today, includes dystocia, prolapse of the uterus or vagina, severe infertility, and death. The more common and less severe field expression of phytoestrogen poisoning is a significant decrease in fertility rate. It may be temporary with normal reproductive efficiency returning soon after the ewes are moved to clover-free pasture. In ewes exposed to a low level intake of estrogens over a long period, e.g., in excess of two grazing seasons, a process of irreversible "defeminization" may occur. This is a state of permanent subfertility. The estrous cycle is normal, but an abnormally large number of ewes fail to conceive. In affected flocks, there may also be a high incidence of maternal dystocia caused by uterine inertia, or failure of the cervix or vagina to dilate. Affected ewes show little evidence of impending parturition and many full-term fetuses are born dead.

Male Castrates

Wethers may secrete milk, and metaplasia of the prostate and bulbourethral glands is evident. These can be detected at an early stage of development by digital rectal palpation. Continuing hyperplasia and cystic dilatation of these glands is associated with their prolapse in a subanal position, followed by rapid weight loss and fatal rupture of the bladder. Rams usually show no clinical abnormality, and their fertility is not impaired.

Cattle exhibit clinical signs less often than sheep, with experimental reports of decreases in conception and fertilization caused by prolongation of oocyte maturation and decreased sensitivity of the corpus luteum to luteolytic agents.[5,6] Temporary infertility; discharge of cervical mucus; and swelling of the mammary gland, vulva, and uterus have all been recorded in cattle.

Gilts exposed to genistein may develop structural changes and abnormalities in the cervix and uterus.[9]

CLINICAL PATHOLOGY

Laboratory assays are available and essential to diagnosis and monitoring of feed contents of phytoestrogens.[7] Chemical assays are not as sensitive as biologic assessments based on increased size of genitalia in subject animals.

NECROPSY FINDINGS

Severe cystic degeneration of the endometrium is present in the most severe cases. Similar clinical and histopathologic changes have been produced by the daily injection of 0.03 mg of diethylstilbestrol per ewe for a period of 6 months. There is also a long-term change in the cervix with an increased incidence of cervicitis and a histologically observable transformation to a uterine-like

appearance. In ewes on a long-term intake of toxic pasture, the lesions include elevation of the tail head, partial fusion of the vulvar labia, and clitoral hypertrophy.

Diagnostic confirmation of phytoestrogen poisoning requires laboratory assay of feed, blood, and tissue, and the appearance of genital pathology at necropsy, or with a uterine biopsy, or laparoscopy.

DIFFERENTIAL DIAGNOSIS

Differential diagnosis list

- Overdose of pharmaceutical preparation as part of a program to improve fertility in a herd.
- Overdose of an implant or feed additive with a growth stimulant that has estrogenic capability.

TREATMENT

Administration of testosterone is a logical response to poisoning but appears to be an unlikely commercial proposition.

CONTROL

Avoidance of high estrogenic activity strains of the respective plants, grazing management to avoid dangerous pasture at the most toxic part of the season, and dilution of the estrogen intake by providing additional and alternative feeds, are all used to control the disease. Prevention of clover disease can only be achieved by proper management of sheep and pasture to avoid ingestion of excessive amounts of estrogens. Vaccination with a phytoestrogen-immunogenic protein conjugate has produced good levels of antibodies, but has not been successful in preventing the problem. Careful management of flocks on estrogenic pasture can significantly improve reproductive output.

FURTHER READING

Adams NR. Detection of the effects of phytoestrogens on sheep and cattle. *J Anim Sci*. 1995;73:1509-1515.

Hughes CL. Phytochemical mimicry of reproductive hormones and modulation of herbivore fertility by phytoestrogens. *Environ Health Persp*. 1988;78:171.

Kuiper G, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. *Endocrinology*. 1998;139:4252-4263.

Radostits O, et al. Phytoestrogen poisoning. In: *Veterinary Medicine: A Textbook of the Disease of Cattle, Horses, Sheep, Goats and Pigs*. 10th ed. London: W.B. Saunders; 2007:1873.

REFERENCES

1. Steinshamn H, et al. *J Dairy Sci*. 2008;91:2715.
2. Hoikkala A, et al. *Mol Nutr Food Res*. 2007;51:782.
3. Jackman KA, et al. *Curr Med Chem*. 2007;14:2824.
4. Zinedine A, et al. *Food Chem Toxicol*. 2007;45:1.
5. Borzym E, et al. *Med Weter*. 2008;64:1107.
6. Piotrowska KK, et al. *J Reprod Dev*. 2006;52:33.
7. Kuhnle GGC, et al. *J Agric Food Chem*. 2008;56:10099.
8. Patisaul HB, et al. *Front Neuroendocrinol*. 2010;31:400.
9. Ford JA, et al. *J Anim Sci*. 2006;84:834.

ZEARALENONE TOXICOSIS

SYNOPSIS

Etiology Zearalenone is an estrogenic mycotoxin produced primarily by fungus in the genus *Fusarium,* which is the causative agent. *F. graminearum* is the species most responsible for animal reproductive problems, but *F. cerealis, F. culmorum, F. cookwellense, F. equiseti,* and *F. semitectum* are contaminants of moldy maize, wheat, oats, and barley grain and cause issues as well.

Epidemiology Global issue with zearalenone found in a variety of cereals and foodstuffs in many countries.

Clinical pathology None in particular; progesterone levels may be decreased.

Lesions Associated with hyperestrogenism and include abortions, stillbirths, mammary gland enlargement and secretions, vulvar edema, and vaginitis in females as well as testicular atrophy and mammary gland enlargement in males.

Diagnostic confirmation Presence of zearalenone and/or metabolites in feces, urine, and serum; presence in feedstuffs.

Treatment Remove animals from contaminated feed and correct prolapses.

Control Keep moisture content of stored grain below 15%–16%; feed contaminated grains to less susceptible animals.

ETIOLOGY

Zearalenone is a nonsteroidal estrogenic mycotoxin produced primarily by fungi in the genus *Fusarium. F. graminearum* is the species most responsible for animals' reproductive problems, but *F. cerealis, F. culmorum, F. cookwellense, F. equiseti,* and *F. semitectum* are contaminants of moldy maize, wheat, oats, and barley grain and are associated with toxicosis.[1,2] Swine are most commonly affected, but cases have occurred in sheep and cattle[3,4] and more rarely in horses.[5]

EPIDEMIOLOGY

Occurrence

The fungi that produce zearalenone primarily colonize corn, but they also infect other cereal grains such as barley, wheat, and oats.[1,2] Zearalenone has also been detected in a number of other plants including rice, sorghum, millet, and soybeans. Most typically, contamination occurs from high moisture during storage; field contamination has been reported but occurs less often. Zearalenone has been detected in pastures in New Zealand, which has been associated with infertility in ewes.[6] Contamination of food and animals is considered a global problem because zearalenone has been found in Africa, Asia, Australia, Europe, North America, and South America.[2]

Risk Factors

Animal Risk Factors

Swine of all ages, but especially prepubertal gilt, are the most sensitive to the effects of zearalenone. The primary effects are reproductive and depend on the dose and time of administration in relationship to the animal's estrous cycle.[5,6]

Farm Risk Factors

Elevated levels of zearalenone in the feed are primarily associated with improper storage and not contamination in the field.[2]

Human Risk Factors

There is considerable concern that humans, especially young girls, will be adversely affected by zearalenone in cereal products, milk and milk-based products, and meats. In Europe, 32% of mixed cereal samples from nine countries were found to be contaminated with zearalenone. Zearalenone is excreted in milk and present in some concentration in meats in animals with high intake, but currently the risk to humans is thought to be low.[2]

PATHOGENESIS

Zearalenone is rapidly absorbed following an oral exposure, with an estimated uptake of 80% to 85%.[1,2] In swine, it can be detected in the serum within about 30 minutes after ingestion.[2] Distribution is primarily to the adipose tissue and the ovary and uterus. The liver is the main site of metabolism, but other tissues such as the intestine, kidney, ovary, and testis are metabolic sites.[1] Two different biotransformation pathways have been proposed and likely play a role in the susceptibility `of different species.[1,5] Zearalenone is either conjugated with glucuronic acid or hydroxylated to α- and β-zearalenol.[1,5] In swine, the preferred route is conjugation with conversion to primarily α-zearalenol.[1,4,5] Sheep are similar to swine but cattle convert to β-zearalenol, a less estrogenic metabolite.[4] Excretion is biliary in most species with significant enterohepatic recirculation occurring.[1]

Zearalenone crosses cell membranes and binds to cytosolic 17β-estradiol receptors. Once this occurs, it is translocated into the nucleus where it binds to estrogen-responsive elements and stimulates mRNA synthesis resulting in estrogen-like effects.[1,3]

CLINICAL FINDINGS

Swine

Pigs of all ages are affected, including piglets nursing on sows, which themselves show no signs of estrogenism. The most significantly affected are the 6 to 7-month-old gilts. Vulvovaginitis, including swelling of the vulva to three to four times normal size, enlargement of mammary glands, a thin catarrhal exudate from the vulva, and increased size and weight of the ovaries and uterus, is the severest form

of the poisoning.[3,6] Prolapse of the vagina is common (up to 30% of affected pigs) and there is prolapse of the rectum in some pigs (5%–10%). The toxin reduces serum progesterone levels in sows, but the administration of progesterone to affected gilts does not counteract the estrogenic effects. The syndrome is indistinguishable from that produced by long-term overdosing with diethylstilbestrol. Signs appear 3 to 6 days after feeding of moldy grain commences and disappear soon after the feeding stops. The mortality rate is high because of the secondary development of cystitis, uremia, and septicemia.

The more important manifestation of the poisoning may be infertility, including absence of estrus, high levels of stillbirth, neonatal mortality, and reduced litter size. Small fetal size, fetal malformations, splayleg and hindlimb paresis, pseudopregnancy, and constant estrus are also recorded.[3]

Zearalenone in male pigs can induce feminizing characteristics; suppress libido; and decrease spermatogenesis, testicular weights, and serum testosterone concentrations.[2]

Ruminants

In cattle, the effect of zearalenone is largely on conception rate, and the rate of services per conception may rise, but the overall effect is less than in sows. Milk production may be decreased.[2] Behavioral estrus occurs at times unrelated to ovarian cycles and in late pregnant cows. There is idiopathic vaginitis. Symmetric enlargement of the mammary glands is recorded in prepubertal dairy heifers feeding on fungus-infected corn. Estrogenic disturbances are also suspected in sheep. Abortion is suspected to occur, and mild vulvovaginitis and hypertrophy of the uterus are recorded. Experimental feeding of zearalenone to lactating cows and ewes does result in minor contamination of their milk sufficient to produce hyperestrogenism in a lamb sucking a poisoned ewe.

Horses

Zearalenone toxicosis is rarely reported in horses.[1] A recent study using equine ovarian cultured granulosa cells demonstrated that zearalenone may play a role in some equine reproductive disorders.[5]

CLINICAL PATHOLOGY

Zearalenone and its metabolites can be identified in urine, plasma, and feces by high-performance liquid chromatography[7] and in feedstuffs by liquid chromatography mass spectrometry and a rapid immunoassay.[8,9] In 2003, 16 countries limited the amount of allowable zearalenone in maize and cereals; the allowable concentration varies from 50 to 1000 µg/kg depending on the country.[5]

NECROPSY FINDINGS

On necropsy, there are nonspecific findings other than expected changes associated with estrogen-related reproductive tract abnormalities. These include changes in ovarian weight with decreased numbers of corpora lutea, increased dead piglets, vaginal and rectal prolapses, vulvar edema and vaginitis in females, and testicular atrophy and mammary gland enlargement in males.[10]

DIFFERENTIAL DIAGNOSIS

Differential diagnosis list

- Accidental overdose of synthetic estrogen substances
- Estrogenic substances
- Phytoestrogens

TREATMENT

Complete recovery follows when the feeding of the affected grain is stopped and no treatment other than surgical repair of the prolapsed organs is attempted.

CONTROL

The moisture content of grains should be kept below 15% to 16% during storage. If contaminated feeds must be used, they should be fed to animals less susceptible to toxicosis. The 2006 EU guidelines for zearalenone in feeds recommend that piglets and gilts do not receive more than 0.1 mg zearalenone/kg BW; sows and fattening pigs no more than 0.25 mg zearalenone/kg BW; and sheep, goats, calves, and dairy cows no more than 0.5 mg zearalenone/kg BW.[10]

FURTHER READING

Etienne M, Jemmali M. Effects of zearalenone (F2) on estrous activity and reproduction in gilts. *J Anim Sci*. 1982;55:1-10.

Tanaka T, Hasegawa A, Yamamoto S, et al. Worldwide contamination of cereals by the *Fusarium* mycotoxins nivalenol, deoxynivalenol, and zearalenone. Survey of 19 countries. *J Agric Food Chem*. 1988;36:979-983.

Radostits O, et al. Zearalenone. In: *Veterinary Medicine: A Textbook of the Disease of Cattle, Horses, Sheep, Goats and Pigs*. 10th ed. London: W.B. Saunders; 2007:1911.

REFERENCES

1. Minervini F, et al. *Int J Mol Sci*. 2008;9:2570.
2. Zinedine A, et al. *Food Chem Toxicol*. 2007;45:1.
3. Kanora A, et al. *Vet Med-Czech*. 2009;12:565.
4. Malekineja HR, et al. *Vet J*. 2006;172:96.
5. Minervini F, et al. *Reprod Biol Endocrinol*. 2006;4:62.
6. Upadhaya SD, et al. *Asian-Aus J Anim Sci*. 2010;23:1250.
7. Songsermsakul P, et al. *J Chromatography B*. 2006;843:252.
8. Tanaka H, et al. *Rapid Commun Mass Spectrom*. 2006;20:1422.
9. Kolosova AY, et al. *Anal Bioanal Chem*. 2007;389:2103.
10. Tiemann U, et al. *Food Addit Contam*. 2007;24:306.

MARE REPRODUCTIVE LOSS SYNDROME (EARLY FETAL LOSS, LATE FETAL LOSS, FIBRINOUS PERICARDITIS, AND UNILATERAL UVEITIS)

SYNOPSIS

Etiology Exposure to Eastern tent caterpillars (ETCs; *Malacosoma americanum),* in particular during the spring when the caterpillars are most active.

Epidemiology Occurs primarily in the Ohio River valley, but reported in other states. Risk factors are the presence of black cherry trees on pasture, ETC, and feeding hay on the ground.

Clinical pathology Culture of fetal and placental tissue most commonly results in growth of non–β-hemolytic streptococci and/or *Actinobacillus.*

Lesions Inflammation of the intraamniotic umbilical cord (funisitis), premature placental separation, placental edema, placentitis, diffuse alveolitis, and hemorrhage in a variety of organs.

Diagnostic confirmation Based on the presence of appropriate clinical signs with a history of exposure of affected horses to ETCs.

Treatment Supportive care only.

Control Removal of cherry trees from pasture, spraying ETC nests and pastures with pyrethrin pesticides, keeping horses off pasture or muzzling mares on pasture during active ETC months.

ETIOLOGY

In 2001 an epidemic of early fetal loss (40–80 days; range 40–140 days) and late fetal loss (about 340 days) was recognized in north central Kentucky, southern Ohio, and Tennessee affecting over 3500 mares.[1,2] It occurred again in 2002 but far fewer horses were affected. The epidemic was termed mare reproductive loss syndrome (MRLS). At the same time there was also a marked increase in incidence of birth of weak foals and fibrinous pericarditis and unilateral uveitis in adult horses in the same region.[1-3] Research in horses and pigs confirmed the causative agent as *Malacosoma americanum,* the Eastern tent caterpillar (ETC). Similar episodes of equine abortions, now referred to as equine amnionitis and fetal loss (EAFL), occurred in Australia and have been associated with the *Ochrogaster lunifer,* the processionary caterpillar.[4,5]

EPIDEMIOLOGY

Historically, many epidemiologic studies were performed to determine the source of the epidemic. Several toxins such as fescue, nitrate/nitrite, phytoestrogens, and mycotoxins were examined and ruled out leaving a

strong association between the presence of ETCs (*M. americanum,* black cherry trees (*Prunus serotina*), and feeding horses hay off the ground. Black cherry trees were involved because they are the preferred host tree for ETC and may be a source of cyanide. Black cherry trees (i.e., cyanide) were ruled out as a cause of MRLS, and an association with ETC was examined experimentally. In several different experiments, pregnant horses (50 to 200 days' gestation) were exposed to various forms of ETC and only those mares exposed to live ETC larvae aborted. These were the first studies to reproduce MRLS and demonstrate that ETC could cause pregnancy loss in mares. Further studies demonstrated that the cuticle (setae; hairs) is the structure responsible for the abortigenic activity.[1,2] Culture of the placental fluid or fetal tissues in both early and late losses showed non–β-hemolytic streptococci and *Actinobacillus,* which are bacteria routinely found in the oral cavity of horses.[2,6] Finally, the syndrome was reproduced in pigs with abortions occurring 13 to 16 days after first ingestion.[1] More important, histopathologic examination showed ETC setae imbedded in the gastrointestinal mucosa that were surrounded by microgranulomatous lesions.[1,2,6] A similar pattern was subsequently confirmed in pregnant and nonpregnant mares.

Occurrence

The first well-studied and documented outbreak of abortions occurred from April 26 through mid-June of 2001, with a lower incidence of disease during the same months in 2002. An abortion storm, which may have been related, occurred in Kentucky in 1991 and 1982, but no epidemiologic studies were performed.[1] In 2006, a similar syndrome associated with large numbers of ETC was reported in Florida.[2]

The 2001 to 2002 outbreak caused early fetal loss in 25% to 63% of mares on one-third of farms, 14% to 24% on another third, and 2% to 13% on the remaining one-third. Approximately 21% of mares pregnant at 42 days' gestation were not pregnant when examined at 70 to 90 days' gestation. The expected pregnancy loss rate between 42 days and parturition is 12%. Over 3500 mares (3000 early fetal losses; 500 late fetal losses) aborted during the outbreak.[1,3] The economic losses incurred because of MRLS during 2001 and 2002 are estimated to be $500 million.[1]

Risk Factors

Animal Risk Factors

Risk factors for the disease are the presence of black cherry trees, exposure to ETC (especially the presence of large numbers of caterpillars on pasture), and pasturing or feeding hay to horses at pasture.

For late-term abortion the risk factors include increased amount of time at pasture, less time in stall, feeding concentrate on the ground, increased proportion of feed obtained from pasture, and being fed exclusively in pasture during the final 4 weeks of gestation. All of these factors favor exposure to ETC.

Risk factors for pericarditis include presence of mares or foals with MRLS on the farm, grazing, and exposure to ETC. Risk factors for uveitis have not been defined.

Farm Risk Factors

ETCs are endemic to the eastern United States including the Ohio River valley. Egg masses are laid on many trees in the Rosaceae family including black cherry trees, which are the preferred host. Eggs hatch in the early spring when the cherry trees bud. Local populations of the caterpillars fluctuate dramatically from year to year, but mares are likely exposed to small numbers of the caterpillars every spring. Climatic conditions that favor survival of ETC and synchronize their maturation result in simultaneous hatching of large numbers of eggs. The rapid emergence of large numbers of caterpillars results in abrupt and heavy exposure of horses and consequent development of MRLS. Weather conditions thought to contribute to the 2001 outbreak include a period of low temperatures in March, above normal temperatures in April, and a frost and freeze in late April immediately followed by several warm days.

PATHOGENESIS

The pathogenesis of the diseases associated with MRLS has not been well defined. Based on experimental studies and natural cases, ETC setae are likely involved in the pathophysiology. Two different hypotheses have been proposed:

- Setae lodged in the gastrointestinal submucosa causes inflammation, form microgranulomas, and disrupt the mucosal barrier. Resident bacteria such as *Actinobacillus* spp. penetrate the barrier, resulting in bacteremia and hematogenous spread to the placenta, fetus, pericardium, uvea, and meninges.[1,6]
- Setae or parts of the exoskeleton contain an as yet unidentified toxin that is toxic to the placenta and fetus.[1]

CLINICAL FINDINGS

Early Fetal Loss

This is detected by per rectum uterine examination, either manual or using ultrasonographic visualization of uterine contents, during early pregnancy. Fetal loss occurs after 35 days, conception not being affected, and affected mares do not come into estrus because of the presence of endometrial cups, which do not regress until 100 to 180 days after ovulation.[3]

Mares have no clinically detectable premonitory signs of fetal loss.[1,2] Ultrasonographic examination of the uterus of pregnant mares reveals that the allantoic fluid of fetuses <80 days of age has increased echogenicity on the day of fetal death. Allantoic fluid increases in echogenicity with increasing fetal age, and care should be taken when interpreting this observation.

Late Fetal Loss

Late fetal loss occurs as a late-term abortion (final several weeks of gestation), birth of a stillborn foal at full term, and the birth of a foal that is weak and of reduced viability. The birth of an affected foal is associated with premature placental separation ("red bag" deliveries), foaling while standing, and explosive expulsion of the fetus and placenta. Foals born alive are weak, have sunken eyes, progressive neurologic signs consistent with hypoxia, and have a high death rate (50%) despite intensive care. Severe leukopenia at birth often progresses to leukocytosis at 24 to 48 hours of age. Serum biochemical abnormalities include elevated serum creatinine concentrations, hypoglycemia, and increased serum creatine kinase activity. Bacteria isolated from stillborn foals at necropsy or on culture of blood samples from sick foals are nonspecific organisms, including nonhemolytic streptococci and *Actinobacillus* spp.

Fibrinous Pericarditis

Clinical signs in horses of both genders include tachycardia, pleural effusion, pericardial effusion, ascites, fever, abdominal pain, and sudden death.[1,2] Younger horses (<2 years of age) may be more susceptible to developing pericarditis. There is accumulation of large quantities of pericardial fluid and fibrin deposition on the parietal and visceral pericardial surfaces evident on ultrasonographic examination of the chest. The lungs have ultrasonographic evidence of consolidation consistent with pneumonia in approximately 50% of cases. Pericardiocentesis yields abundant fluid that is light yellow and has a low white blood cell count ($<5 \times 10^9$/L) characterized by well-preserved neutrophils. Horses with a prolonged course of the disease (>2 weeks) can have elevated white cell counts in pericardial fluid secondary to opportunistic infection, usually with *Actinobacillus* spp.[6] Hematologic abnormalities are minimal and characterized by a slight leukocytosis in approximately 50% of cases. Azotemia occurs in horses with severe cardiac tamponade.

Unilateral Uveitis

Clinical signs are acute and unilateral and include corneal edema, exudates in the anterior and posterior chambers, and iris hemorrhage.[1] Progression of the syndrome leads to blindness and global atrophy. There is no age predilection and no organisms have been found on culture.

CLINICAL PATHOLOGY

Culture of fetal and placental tissue most commonly results in growth of non–β-

hemolytic streptococci and/or *Actinobacillus*. *Actinobacillus* spp., along with several other bacteria, has been isolated from fibrinous pneumonia.

Diagnostic confirmation is based on the presence of appropriate clinical signs with a history of exposure of affected horses to ETCs.

DIFFERENTIAL DIAGNOSIS

Differential diagnosis list:
Cyanide toxicosis
Ergot/fescue
Infectious causes of placentitis
Mycotoxicosis
Nitrate toxicosis
Phytoestrogens

NECROPSY FINDINGS

Examination of the placenta, stillborn foals, and foals that die after birth reveals inflammation of the intraamniotic umbilical cord (funisitis), premature placental separation, placental edema, placentitis, diffuse alveolitis, and hemorrhage in a variety of organs. Horses with pericarditis have impressive accumulation of hairy fibrin in the pericardial space with marked thickening of the visceral and parietal pericardium (a hoary heart).

TREATMENT

Treatment of affected foals is primarily supportive in nature. Horses with pericarditis should have the fluid drained to relieve or prevent cardiac tamponade and to minimize the accumulation of fibrin. Pericardial fluid may need to be drained several times, and its accumulation should be monitored ultrasonographically. Administration of broad-spectrum antibiotics should be based on culture and sensitivity of pericardial fluid. Treatment for uveitis is standard and includes atropine, antiinflammatory agents, topical and systemic antibiotics (culture and sensitivity as indicated), and other agents such as cyclosporin or tissue plasminogen activator.

CONTROL

This is based on prevention of ingestion of ETC by horses. Preventing horses from ingesting caterpillars by minimizing access to pasture and feeding hay in stalls is likely to be beneficial.

Other control measures include removing wild or black cherry trees, the favored host species for ETCs, from pastures, hedges, and fence rows; applying pesticides to trees to kill over wintering eggs or, after hatching, caterpillars; installation of barriers to caterpillar migration onto pasture; manual removal of egg tents; installing pheromone traps; and restricting access of mares to pasture.[7,8]

Application of bifenthrin or permethrin, but not 3% horticultural oil, to egg masses (tents) during the winter prevents emergence of caterpillars in the spring. Insecticidal soap or oils sprayed on neonatal caterpillars is minimally effective. Bifenthrin or spinosad are effective against all instars for 7 days when sprayed on foliage. Injection of trunks of cherry trees with dicrotophos or emamectin is effective against all instars, but injection with milbemectin or avermectin is not effective. A spray of 50 mL of 39% permethrin diluted in 4 L of water and applied to a 2-m wide band of pasture outside the fence line kills migrating caterpillars and prevents them obtaining access to pasture. This solution can also be sprayed on the trunks of trees to kill caterpillars as they leave the tree.

FURTHER READING

Cohen ND, Donahue JG, Carey VJ, et al. Case-control study of late-term abortions associated with mare reproductive loss syndrome in central Kentucky. *J Am Vet Med Assoc*. 2003;222:199-209.

Cohen ND, Carey VJ, Donahue JG, et al. Descriptive epidemiology of late-term abortions associated with the mare reproductive loss syndrome in central Kentucky. *J Vet Diagn Invest*. 2003;15:295-297.

Dwyer RM, Garber LP, Traub-Dargatz JL, et al. Case-control study of factors associated with excessive proportions of early fetal losses associated with mare reproductive loss syndrome in central Kentucky during 2001. *J Am Vet Assoc*. 2003;222:613-619.

Sebastian MM, Gantz MG, Tobin T, et al. The mare reproductive loss syndrome and the eastern tent caterpillar: a toxicokinetic/statistical analysis with clinical, epidemiologic, and mechanistic implications. *Vet Ther*. 2003;4:324-339.

REFERENCES

1. Sebastian MM, et al. *Vet Pathol*. 2008;45:710.
2. McDowell KJ, et al. *J Anim Sci*. 2010;88:1379.
3. Volkmann D, et al. *Reprod Domest Anim*. 2008;43:578.
4. Perkins NR, et al. Pregnancy loss in mares associated with exposure to caterpillars in Kentucky and Australia. In: Panter KE, Wierenga TL, Pfister JA, eds. *Poisonous Plants: Global Research and Solutions*. Wallingford, UK: CAB International; 2007:165.
5. Cawdell-Smith AJ, et al. *Equine Vet J*. 2012;44:282.
6. Donahue JM, et al. *Am J Vet Res*. 2006;67:1426.
7. Townsend L, et al. *J Equine Vet Sci*. 2007;27:249.
8. Haynes KF, et al. *Envrion Entomol*. 2007;36:1199.

EQUINE AMNIONITIS AND FETAL LOSS

EAFL is the name given to a syndrome of abortions that occurred in horses in New South Wales between April and October 2004.[1] Mares from 4 months to term aborted fetuses with signs of inflammatory changes primarily involving the amnion (amnionitis) and amniotic portion of the umbilical cord (funisitis).[2,3] Clinical signs in mares before abortion were minimal.

The syndrome, while occurring several years after the epidemic of MRLS in the United States, had some similarities and caterpillars were looked at as a possible source of the problem. Several caterpillars were examined with the *O. lunifer* (the processionary caterpillar) ultimately causing abortion in two different experimental studies involving early pregnancy and midlate pregnancy.[3,4]

There are some differences between the two syndromes. An infectious agent has been identified in both EAFL and MRLS, but they are not the same bacteria. The predominant bacteria isolated from EAFL cases were environmental coryneforms and gram-negative rods, whereas *Actinobacillus* and non–β-hemolytic streptococci were common isolates from MRLS cases.[2,5] Fibrinous pericarditis and unilateral uveitis affected a number of horses in the MRLS epidemic but did not occur with EAFL.[1] Finally, although devastating, the number of horses involved in the 2004 EAFL outbreak was considerably less than the 2001 to 2002 MRLS epidemic.

REFERENCES

1. Todhunter KH, et al. *Aust Vet J*. 2009;87:35.
2. Cadwell-Smith AJ, et al. *Equine Vet J*. 2012;44:282.
3. Caldwell-Smith AJ, et al. *Proceedings Australian College of Veterinary Science Annual Conference*. 2009:31.
4. Cadwell-Smith AJ, et al. *J Equine Vet Sci*. 2013;33:321.
5. Todhunter K, et al. *Aust Vet J*. 2013;91:138.

PLANTS AND FUNGI (UNKNOWN TOXINS) AFFECTING THE REPRODUCTIVE SYSTEM

PLANTS

Plants Associated With Abortion

- *Iva angustifolia* (narrow-leafed sumpweed)
- *Salvia coccinea* (red salvia)
- *Tanacetum vulgare* (tansy)
- *Verbena bonariensis* (purple top)

Plants Associated With Prolonged Gestation

- *Lysichiton americanus* (skunk cabbage)
- *Salsola tuberculatiformis* (cauliflower saltwort; in ewes it is associated with atrophy of the pituitary, adrenal, and thymus glands of the fetus and prolongation of pregnancy to as long as 213 days).

Plants Associated With Congenital Defects

- *L. americanus* (skunk cabbage; is associated mostly with craniofacial deformity).

FUNGI

Fungi Associated With Reproductive Dysfunction

- *Penicillium roqueforti,* growing on moldy mixed grain and ensilage, is suspected of causing bovine abortion and retained placenta.

- *T. repens* (white clover) does not normally contain estrogens, but when heavily infested with fungi it may contain significant amounts.
- *Ustilago hordei* (barley smut) fungus is thought to be toxic to farm animals; feeding it to experimental animals has been associated with infertility and stillbirths.
- In southeastern Australia a common infertility syndrome, including abortion and fetal mummification, has been ascribed to an onion-like weed, *Romulea rosea.* There is a suspicion that the disease may be caused by a toxin produced by a fungus, *Helminthosporium biseptatum,* which grows on the weed.

Congenital and Inherited Diseases Primarily Affecting the Reproductive System

CHROMOSOMAL TRANSLOCATIONS IN CATTLE

A chromosomal translocation is a mutation occurring when two nonhomologous chromosomes exchange parts, which results in a chromosomal rearrangement. The most common type or translocation is the **reciprocal translocation** (RCP) in which a segment from one chromosome is exchanged with a segment of another nonhomologous chromosome, creating a pair of translocation chromosomes. A particular form of reciprocal translocation is the **Robertsonian translocation (ROB)**. During a ROB participating chromosomes break at their centromeres (center pieces) and the long arms of the two chromosomes merge to form a single chromosome with one centromere and two long arms. At the same time, a new chromosome containing both short arms is also created, which typically only contains nonessential genetic information and is lost during following cell divisions. Chromosomal translocations are identified by the chromosomal series involved. Thus a 1/29 translocation represents a fusion between a chromosome of each of the pairs numbered 1 and 29.

A number of chromosomal rearrangements have been identified in different livestock species over the years and have been associated with clinical conditions such as intersexuality, congenital malformations, and reproductive dysfunction.[1] Some of the translocations that occur endemically in certain regions have been associated with significant economic losses.[2] Several European countries have established cytogenic screening programs to monitor the occurrence of chromosomal translocations in the livestock population.[1] In Italy the incidence of RCP in cattle determined in an official cytogenic screening program was 0.3%, whereas 7.1% of studied animals were carriers of a ROB.[2] By far the most common ROB identified was the so-called translocation 1/29, which is endemic in the region, accounting for 99.6% of all ROBs.[2]

Translocation 1/29 has been identified in many breeds of cattle and has been associated with significant reductions in the fertility of cows bred by artificial insemination services. Early embryonic death occurs in embryos produced by fertilization of affected gametes or fertilization of normal gametes by spermatozoa carrying the 1/29 translocation. There is no abnormality of serving behavior or semen quality. The translocation has been shown to be inherited in most European beef breeds including the Blonde d'Aquitaine, Swedish Red and White, Charolais, Danish Limousin, British Friesian and Red Poll breeds, and in the wild British White cattle. In Bolivian Creole cattle breeds, in the Creole-like cattle, the average frequency was 10.42% with a variation from 0% to 28.2%. In contrast, Yacumeño and Creole-type cattle did not show the centric fusion. The highly significant differences between Creole cattle breeds in relation to the 1/29 translocation could be the consequence of factors such as founder group, genetic drift, and selection. The low frequency observed in the Saavendreño Creole dairy cattle might be caused by breeding under a more intensive system and selection according to milk yield and fertility traits. The frequency of affected animals in a breed may vary between 1% and 20%. Karyotyping and culling of abnormal bulls in most artificial breeding centers has reduced the impact of the defect.

Translocations 1/21, 2/4, 14/20, and **13/2** have also been identified in bulls, the 1/21 in Holstein Friesian cattle, and the latter two seem to be widespread in Simmental cattle. None of them has been linked with a disease, but it is becoming accepted practice not to use such animals for artificial insemination and in some countries to refuse their importation.

A cytogenetic survey of Holstein bulls at a commercial artificial insemination unit to determine the prevalence of bulls with centric fusion and chimeric anomalies found that chimeric fusion is extremely rare in Holstein bloodlines available by artificial insemination in the United States. However, chimeric bulls are more common and reportedly have decreased reproductive performance. Because of the possibility of de novo onset of chimeric fusion at any time, early cytogenetic screening should be encouraged for prospective bulls intended for artificial insemination programs.

Translocation 27/29 is suspected of being associated with reduced fertility in Guernsey cattle. These and other abnormalities of chromosomal structure were detected in an examination of a large number of infertile dairy heifers.

REFERENCES

1. Ducos A, et al. *Cytogenet Genome Res.* 2008;120:26.
2. DeLoreni L, et al. *J Anim Breed Genet.* 2012;129:409.

INHERITED PROLONGED GESTATION (ADENOHYPOPHYSEAL HYPOPLASIA)

Prolonged gestation occurs in cattle and sheep in several forms and is usually, although not always, inherited.[1]

The forms of the disease are prolonged gestation with fetal gigantism or prolonged gestation with deformed or normal or small size fetuses. Differential diagnoses include: mistaken breeding date, intrauterine death and fetal mummification, and pituitary abnormalities in the fetus caused by infection by BVD virus, Akabane virus or bluetongue virus, ingestion of *Veratrum californicum,* and genetic abnormalities.[1]

The disease is caused by lack of a functioning fetal hypothalamic-pituitary axis and consequent inability of the fetus to initiate parturition. The result is prolonged gestation and continued growth of the fetus. The hypothalamic-pituitary axis is also critical for survival of the newborn and affected animals are not viable.

PROLONGED GESTATION WITH FETAL GIGANTISM

The inherited disease is recorded in Holstein,[2] Ayrshire, and Swedish cattle with prolongation of pregnancy from 3 weeks to 5 months. The cows may show marked abdominal distension, but in most cases the abdomens are smaller than one would expect. Parturition, when it commences, is without preparation. Udder enlargement, relaxation of the pelvic ligaments, and loosening and swelling of the vulva do not occur, and there is also poor relaxation of the cervix and a deficiency of cervical mucus. Dystocia is usual and cesarean section is advisable in Holstein cattle, but the Ayrshire calves have all been reported as having been born without assistance. The calves are very large (48 to 80 kg BW) and show other evidence of postterm growth, with a luxuriant hair coat and large, well-erupted teeth that are loose in their alveoli, but the birthweight is not directly related to the length of the gestation period.

The calves exhibit a labored respiration with diaphragmatic movements more evident than movements of the chest wall. They invariably die within a few hours in a hypoglycemic coma. At necropsy there is adenohypophyseal hypoplasia and hypoplasia of the adrenal cortex and the thyroid gland. The progesterone level in the peripheral blood of cows bearing affected

calves does not fall before term as it does in normal cows.

PROLONGED GESTATION WITH CRANIOFACIAL DEFORMITY

This form of the disease has been observed in Guernsey, Jersey, and Ayrshire cattle. It differs from the previous form in that the fetuses are dead on delivery, show gross deformity of the head, and are smaller than the normal calves of these breeds born at term. In Guernsey cattle the defect has been shown to be inherited as a single recessive character, and it is probable that the same is true in Jersey cattle. The gestation period varies widely with a mean of 401 days.

Clinical examination of the dams carrying defective calves suggests that no development of the calf or placenta occurs after the seventh month of pregnancy. Death of the fetus is followed in 1 to 2 weeks by parturition unaccompanied by relaxation of the pelvic ligaments or vulva or by external signs of labor. The calf can usually be removed by forced traction because of its small size. Mammary gland enlargement does not occur until after parturition.

The calves are small and suffer varying degrees of hypotrichosis. There is hydrocephalus and in some cases distension of the gut and abdomen caused by atresia of the jejunum. The bones are immature and the limbs are short. Abnormalities of the face include cyclopian eyes, microphthalmia, absence of the maxilla, and the presence of only one nostril. At **necropsy** there is partial or complete aplasia of the adenohypophysis. The neural stalk is present and extends to below the diaphragm sellae. Brain abnormalities vary from fusion of the cerebral hemispheres to moderate hydrocephalus. The other endocrine glands are also small and hypoplastic.

The disease has been produced experimentally in ewes by severe ablation of the pituitary gland, or destruction of the hypothalamus, or section of the pituitary stalk in the fetus and by adrenalectomy of the lamb or kid. Infusion of adrenocorticotropic hormone into ewes with prolonged gestation caused by pituitary damage produces parturition but not if the ewes have been adrenalectomized beforehand.

PROLONGED GESTATION WITH ARTHROGRYPOSIS

A form of prolonged gestation, which occurs in Hereford cattle and is thought to be inherited, is accompanied by arthrogryposis, scoliosis, torticollis, kyphosis, and cleft palate.

Prolonged gestation is also reported in **Belgium Blue cattle** and appears to have a genetic component. Affected calves were not grossly abnormal.[1]

REFERENCES

1. Cornillie P, et al. *Vet Rec*. 2007;161:388.
2. Buczinski S, et al. *J Vet Med A*. 2007;54:624.

INHERITED INGUINAL HERNIA AND CRYPTORCHIDISM

Inguinal hernias and cryptorchidism in pigs have been considered to be inherited defects for many years, but the evidence is uncertain.

INGUINAL HERNIAS

Inguinal hernias of pigs have been shown to be inherited in some breeds (e.g., Duroc and Landrace), but not in others (e.g., Yorkshires). The genetic basis has been investigated in Large White pigs and Landrace pigs and the candidate genes narrowed to a region on SSC13 (Sus scrofa chromosome) between 34 and 37 Mb.[1] In Pietrain pigs, genes involved in collagen metabolism (homeobox A10 [HOXA10] and matrix metalloproteinases 2 [MMP2]) and one gene encoding zinc finger protein multitype 2 (ZFPM2; important in the development of diaphragmatic hernia) were significantly associated with hernias.[2]

Cryptorchidism

Evidence suggesting the inheritance of cryptorchidism in swine, sheep, horses, and Hereford cattle and hermaphroditism in swine is also available.

Cryptorchidism is a common congenital anomaly in pigs, and a genome-wide association study of Large White and Landrace pigs localizes the associated gene or genes to candidate genes to SSC8 (Sus scrofa chromosome) between 65 and 73 Mb.[1]

Cryptorchidism is common in equids, and there is concern that it might be hereditary.[3] Unilateral cryptorchidism is overrepresented in Percherons, American Saddle Horses, and American Quarter Horses among hospital admissions for cryptorchid castration and has an incidence of 15% among Friesian colt foals.[4] Approximately 9% of the ~600 Icelandic Horse yearling stallions did not have both testes in the scrotum.[5] The likelihood of cryptorchidism in yearlings was significantly influenced by farm and time period of birth. Heritability estimates for cryptorchidism ranged from 0.12 to 0.32 (standard error [SE] 0.08–0.12) on the observable scale, and from 0.35 to 0.96 (SE 0.24–0.40) when transformed to the underlying continuous scale.[5] Cryptorchidism in horses appears to be inherited with a polygenic pattern of transmission, although analysis of microsatellite markers of 24 affected horses did not reveal significant associations with allelic or genotypic frequencies.[6]

REFERENCES

1. Sevillano CA, et al. *Genet Sel Evol*. 2015;47:18.
2. Zhao X, et al. *Am J Vet Res*. 2009;70:1006.
3. Hartman R, et al. *J Am Vet Med Assoc*. 2015;246:777.
4. Stout TAE. *Equine Vet J*. 2013;45:531.
5. Eriksson S, et al. *Livestock Sci*. 2015;180:1.
6. Diribarne M, et al. *J Equine Vet Sci*. 2009;29:37.