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Twenty-Five Years of Progress in Understanding Major Infectious Diseases of Dairy Cattle

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ABSTRACT

Disease, whether it occurs in cats, cattle, caribou, or other species, is the manifestation of insult to the structural or functional integrity of the living being. Infection may occur with or without evidence of disease. Disease may represent only the "tip of the iceberg" of infection.

Prevalence of disease is the proportion of a defined population that meets specific criteria at a point in time, and incidence refers to the proportion of a defined population in which the onset occurs during a specifiable interval. Risk is an instantaneous rate of incidence.

Prevalence, incidence, and risk in infectious diseases are quantitative inferences derived from assessment of implications — caveats derived from variables that often are elusive, such as antibiotic resistance to plasmid mediated determinants transferable between species of enteric bacteria, or such as increases in population density among insect vectors influenced by climatic conditions favorable to the insects. Carefully orchestrated manipulation of the etiologic agent or the host response is made possible by ever-expanding understanding of the molecular biology of pathogenicity on the one hand and cellular and humoral immunity on the other. Such understanding often leads to new caveats or destroys old ones based on incomplete information.

This paper deals with a selected limited number of infectious diseases of dairy cattle. Knowledge developed around the cited parameters in 13 infectious diseases during the past quarter century is brought into focus. Ten geographically dispersed specialists in infectious diseases of cattle were asked to identify priorities among the array of transmissible diseases important to North America. Except for the mastitis syndrome, which will be given special attention elsewhere in these anniversary papers, the diseases discussed are those wherein major advances in knowledge or major needs for advanced knowledge were of special importance.

INTRODUCTION

In the past 25 yr, there have been major contributions to knowledge about infectious processes and immune responses in dairy cattle, the most troublesome of which (exclusive of mastitis, which is given special attention elsewhere in this series of anniversary manuscripts) appear to be those afflicting mucous membranes of the respiratory, alimentary, and genital tracts. Many of them manifest similar signs of illness, so that differential diagnosis is difficult. Many are caused by viruses against which therapeutic agents are ineffective and against which immunoglobulin is not always available at the site of attack. Despite these deficiencies, unfolding knowledge about molecular characteristics of viruses and immune systems has clarified much of the mystery about infectious diseases and body responses to invading microorganisms.

This paper deals with selected infectious

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diseases that have tested the cutting edge of scientific inquiry during the past quarter century. They are arranged alphabetically to avoid illusion that one is more important than another; related diseases are introduced within the discussion of the major title. Omissions may concern readers who may feel that certain diseases have been overlooked. Those included were compiled after querying colleagues in the United States and requesting that they assign priority ratings to diseases they judged of major importance. Represented here, then, are those regarded as being contemporary problems with advanced, but incomplete, knowledge.

Bluetongue

In the past quarter century, bluetongue virus has emerged as a ubiquitous infectious agent of major importance in international commerce of the cattle industry and in commercial distribution of bovine semen. Bluetongue is a non-contagious, infectious viral disease of ruminants, especially sheep, but to less extent goats and cattle. The virus is transmitted by insect vectors, particularly gnats of the *Culicoides* genus.

In sheep, the disease is characterized by fever, emaciation, oral lesions, lameness, and a substantial death rate with heaviest losses in lambs.

In cattle, the infection usually is nonclinical, resulting in a persistent, silent infection and a virus-shedding carrier state. Occasionally cattle sicken, however, and develop erosions in the mouth, encrustations and excoriations on the muzzle, nasal discharge, laminitis, and acute dermatitis of a patchy nature involving the flanks, groin, perineum, udder, and teats. Necrosis of the skin in the interdigital spaces has been observed. These signs occur in a number of other diseases (the so-called mucosal diseases), and differential diagnosis may be difficult.

In utero transmission occurs in cattle and can result in abortion, hydranencephaly, congenital malformations, and immunotolerant calves. Further, the virus can produce pathologic changes in the reproductive tracts of bulls. Such changes include focal degeneration of the seminiferous tubules with inflammation and hemorrhage in the colliculus seminalis.

While of relatively minor importance to dairy cattle per se, fear of bluetongue infection

causes restrictions in international commerce. It has been suspected that cattle may be the reservoir of virus, serving as the source of infection for sheep, especially in areas where *Culicoides* gnats abound (24).

Twenty serotypes of bluetongue virus are known. In addition, cattle can be infected with several closely related viruses including the viruses of epizootic hemorrhagic disease of deer and Ibaraki disease, agents which cause similar disease in wild ruminants, resulting in diagnostic difficulties. Ibaraki virus was isolated first in the Ibaraki Prefecture, Japan, from the blood of a 10-yr-old cow. It is a bluetongue-like virus producing lesions in cattle that resemble bluetongue in sheep, but the virus has little pathogenicity for sheep.

Antibody against Ibaraki virus commonly is found in cattle in all regions of the United States except the northwestern states (23). There appears to be no antigenic relationship between bluetongue virus and Ibaraki virus, but Ibaraki virus and two types of epizootic hemorrhagic disease virus (EHDV) in deer are related serologically. A common complement-fixing antigen is shared by the two EHDV serotypes and bluetongue virus serotypes (42). The significance of Ibaraki virus disease of cattle in the United States is unknown, but judging from its ulcerative effects on Japanese cattle and the wide distribution of antibody against the virus in cattle in the United States, concern must be expressed.

Bovine Herpes Mammillitis

In the last quarter century, a new chapter has been added to the study of virus-induced lesions on the teats of dairy cattle. In 1966, Martin et al. (35) reported that bovine ulcerative mammillitis is caused by a herpesvirus. In 1970, Yedloutschnig et al. (59) identified the disease in the United States. It has been established recently that the disease has global distribution.

The disease is known as bovine ulcerative mammillitis as well as bovine herpes mammillitis. It can be manifested in two forms in a milking herd. In one form, from which the name of the disease was derived, ulcerative lesions appear on the teats and less frequently on the udders of affected milking cows. The virus usually causes gross swelling of the teat wall. Within 48 h, the skin over the affected areas becomes soft and

sloughs, revealing an irregularly shaped, painful, deeply ulcerated area which heals slowly, healing accompanied by the formation of brown scabs. Healing usually occurs within a week after skin lesions appear. The scabs begin to shed after about 2 wk. Lymph gland swelling occurs, and mastitis follows in approximately 22% of the cases. The causative herpesvirus probably is transmitted mechanically by milkers and milking machines or by biting flies (24). This form of the disease must be differentiated from paravaccinia or pseudocowpox, another affliction of the teats, and sometimes mouths of calves, which is a slow-healing nonulcerative virus disease, manifested by vesicle formation.

The second manifestation of this disease is the appearance of multiple nodules in the skin, and this is known as dermopathic bovine herpesvirus disease. The nodules appear as pronounced, circular, raised areas in the skin over the entire body. The nodules are deep and undergo tissue destruction with the formation of superficial scabs that ultimately fall off. Tufts of hair are cast off in the desquamation, leaving ulcerated lesions that resemble lumpy skin disease, a severe poxvirus infection indigenous to parts of Africa but currently exotic to the United States.

Bovine Viral Diarrhea and the Mucosal Disease Complex

In the past 25 yr a group of viral infections affecting the mucous membranes of the bovine alimentary tract has been studied extensively. These have gained a prominent place among the dairy cattle diseases because of their debilitating effects.

When necrosis, ulcers, or erosions are observed in the oral mucosa of cattle, the differential diagnosis should include bovine viral diarrhea-mucosal disease (BVD-MD), malignant catarrhal fever, papular stomatitis, rinderpest, bluetongue, the vesicular diseases, and ingestion of caustic substances.

Bovine Viral Diarrhea-Mucosal Disease. Bovine viral diarrhea-mucosal disease was described initially in 1946 (49) as an epizootic diarrhea of cattle. Today it is known that BVD-MD virus is a togavirus that causes multiple clinical and pathologic manifestations (29). The detection of serum antibody in much of the world's cattle

population suggests that inapparent infection is common. After natural infection, serum antibody frequently persists for the lifetime of the animal.

The classic clinical syndrome occurs sporadically and is characterized by elevated temperature, leukopenia, diarrhea, lacrimation, nasal discharge, and erosions of the oral mucous membranes. Most cases occur in cattle aged 6 to 24 mo, and many of these die. Survivors may develop a chronic debilitating disease characterized by weight loss, intermittent diarrhea, inefficient feed utilization, and erosions of the oral mucosa. Occasionally lameness occurs due to laminitis, necrotic interdigital dermatitis, or ulcerative coronitis. In some epizootics BVD-MD is regarded as a respiratory disease because nasal discharge, cough, and rapid respirations are prominent clinical signs (28).

Cattle infected during pregnancy can have normal pregnancies; however, abortions or malformed calves may be seen, even if the inciting infection is mild or inapparent.

Bovine malignant catarrhal fever (MCF) resembles BVD-MD in that crusting of the muzzle and oral erosion and necrosis occur. It usually can be differentiated by a severe panophthalmia with corneal opacities, prominent enlarged superficial lymph nodes, stertorous labored breathing and nervous behavioral changes, all of which are characteristic of MCF. When these signs are observed, they usually are associated with fatal encephalitis. The disease seems to occur when cattle contact sheep or wild ruminants. The nature of the virus causing MCF in Africa is known, but studies are still underway to determine the cause of the disease in North America.

Papular stomatitis, a mild poxvirus infection usually occurring in calves, causes raised or flat lesions in the oral mucosa. These lesions frequently contain characteristic streaks of various shades of brown and have irregular edges, which helps to distinguish them from BVD-MD or MCF erosions. Only rarely are papular stomatitis lesions manifested as punched-out depressions in the mucosa. Papular stomatitis infections usually are inapparent. The lesions are noticed only if the oral mucosa is examined. Occasionally, however, overwhelming infection is manifested by clinical signs.

Rinderpest, enzootic in Africa and in parts of Asia, is exotic to the United States. In this

acute, devastating disease of cattle and other ruminants, ulcerative lesions identical to BVD-MD are found, except that they usually are more severe. Introduction of rinderpest virus into the highly susceptible bovine populations in heretofore rinderpest-free areas presumably will result in high clinical attack rates, high case fatality rates, rapid transmissibility between animals and herds, and a disease problem likely to be devastatingly more drastic than that of enzootic BVD-MD. Rinderpest always must be considered when diagnosing BVD-MD because variations in virulence of rinderpest strains are known. A mild rinderpest strain easily can be misdiagnosed as BVD-MD, permitting considerable dissemination of the virus before the disease is diagnosed.

Vesicular stomatitis virus causes vesicles on the oral mucous membranes, teats, and the coronary bands in the feet of cattle. These are indistinguishable from the lesions of foot-and-mouth disease. The vesicles rupture rapidly, and as healing or secondary bacterial invasion occurs, the lesions may resemble the erosions of BVD-MD, especially if the epithelial flaps, the sole remnants of the vesicles, have been completely desquamated. Vesicular stomatitis virus is probably insect-borne. The disease is enzootic in the southern United States. Occasionally, however, it appears in the northern United States or Canada during summer months. Continued studies are needed to understand the epidemiological nuances of this disease, especially since it is caused by a rhabdovirus. Rhabdoviruses frequently have wide host spectra.

Bovine bluetongue infection occasionally results in a clinical syndrome similar to BVD-MD, with necrosis of the oral mucosa, crusting of the muzzle, and lameness. In clinical bovine bluetongue, diffuse necrosis of the oral mucosa, particularly the dental pad, can serve as a differentiating feature when contrasted with the usually discrete erosions of BVD-MD. Bluetongue occasionally is manifested also as drying, cracking, and exfoliation of the skin (pityriasis), suggestive of photosensitization — a finding rarely associated with BVD-MD (29). When skin lesions occur in BVD-MD, it is said that they resemble moist eczema.

Diarrhea, frequently a feature of BVD-MD, MCF, and rinderpest, is not always a consistent sign. Profuse intractable diarrhea in cattle also is seen in salmonellosis and Johne's disease.

Salmonellosis can be differentiated from mucosal disease by its lack of the latter's characteristic oral lesions and by isolation of *Salmonella* organisms from feces or tissues of infected animals. Acute arsenic poisoning and poisoning with organophosphate insecticides also cause diarrhea, and this must be noted in differential diagnosis.

When epizootics of BVD-MD sometimes are misdiagnosed as one of the more common respiratory diseases, such as shipping fever or infectious bovine rhinotracheitis (IBR), the diagnosis usually is based on elevated rectal temperature, nasal discharge, increased respiratory rate, and cough. The so-called respiratory form of BVD-MD usually is not accompanied by pneumonia, however, unless there is superimposed infection. When BVD-MD is manifested as a respiratory disorder, careful examination of the oral mucosa, gums, tongue, and hard palate of ill animals and apparently healthy herdmates sometimes reveals oral erosions. If oral erosions are found in cattle, and if there is a leukopenia, a diagnosis of BVD-MD is appropriate. In differential diagnosis, the nasal mucosa must be examined closely for evidence of white fibrinous plaques found in IBR.

There is a strong immunologic relationship between the hog cholera virus and the agent causing bovine virus diarrhea. Because of that, studies were instituted to determine if BVD-MD virus could be used to immunize swine against hog cholera. Such immunity could be induced if unattenuated live BVD-MD virus were used. However, it was learned subsequently that BVD-MD virus occurs naturally in some swine herds, and great concern has been expressed that such infected swine, if virus-shedders, would serve as sources of infection to susceptible cattle. That method of vaccinating hogs against cholera no longer is advised. A modified-live virus vaccine has been developed, however, and now is available from commercial firms for the vaccination of cattle against BVD-MD (23).

While it is recognized that BVD-MD modified living vaccines are quite effective and relatively safe for most cattle under field conditions, there have been reports indicating that in some cattle this vaccine may be a predisposing factor, or possibly the primary cause, of severe reactions in some animals. Such adverse reactions, which usually involve low morbidity and high

mortality, are not clearly understood. The use of vaccine in pregnant cows is ill-advised because the virus does cause abortions and is responsible for congenital abnormalities (23). In consideration of these reactions, BVD-MD vaccine is recommended only where previous or anticipated disease problems are of sufficient magnitude to warrant the risk.

Brucellosis

During the past 25 yr, it became painfully obvious that with the national trend toward larger dairy herds, with increases in cattle density, the probabilities of introducing brucellosis and the persistence of brucellosis were becoming greater. The trend also led to higher prevalence in some areas and, indeed, greater difficulty in eliminating the disease. Further, the trend has led to recognition of the probability that the disease might not be eliminated by conventional test and slaughter methods. This new awareness has reactivated an interest in brucellosis research. Renewed enthusiasm has been engendered for scientific explorations in a disease that has serious public health and economic importance to the cattle industries.

Bovine brucellosis is primarily a disease of sexually mature cattle in which the inciting organism, usually *Brucella abortus*, circulates systemically for variable periods of time after invading the body. It is believed that invasion is most commonly by ingestion and that the source of infection usually is in discharges of an infected cow (47). However, other methods of transmission have been reported, including inhalation. The organism localizes and multiplies in the reticuloendothelial tissues, such as the spleen and lymph nodes. Further, it has a predilection for reproductive organs including the udder, uterus, and testicle, epididymis and seminal vesicles and, less frequently, other structures, such as bones and joints. The most dramatic clinical manifestation of brucellosis and usually the only sign of infection is abortion resulting from placentitis (14).

Brucella abortus is an intracellular parasite. It multiplies especially abundantly in the placenta where there is a high concentration of erythritol. Placental lesions result in fetal anoxia and death, usually in the last trimester of pregnancy in initial infections. In the female, localization in the udder and

supramammary lymph nodes is most common and more permanent than other sites. After parturition or abortion, vaginal excretion of the organism continued for the first 15 days, then diminished and became intermittent (47).

The morphological features of the genus *Brucella* are remarkably consistent, but the organism shows limited activity in the conventional biochemical tests used for identification. It is recognized, however, that organisms identified as members of the genus *Brucella* possess a range of properties that can be clustered into species or biotypes, but clearly defined boundaries between species do not exist in all cases. For convenience of identification, it is recommended that strains should be classified into species by the preferred natural host, sensitivity to brucella phages, and oxidative metabolic profiles (44). Nearly all brucellosis in cattle is caused by ingestion of, and subsequent infection by, one of the biotypes of *Brucella abortus*.

Brucella endotoxins possess many structural and biological properties common to the lipopolysaccharide endotoxins of enterobacterial pathogens (33). The initial reaction to endotoxin shock is sensitized vascular damage. There is fever, release of histamine and catecholamines, and intravascular clotting. Repeated insults by endotoxin may result in tolerance to further vascular damage (32). However, exposure stimulates cell-mediated immunity in addition to humoral antibody production. Histiocytic granulomas appear to be manifestations of cell-mediated immunity in chronic brucellosis. Brucella agglutinins developed in response to endotoxin antigen will crossreact with some *Salmonella*, *Pasteurella*, *Yersinia*, and *Leptospira* genera (47), complicating diagnosis.

In an evaluation of brucellosis research by a committee serving the National Research Council, National Academy of Sciences, Sanford S. Elberg, Chairman, and six other preeminent committee members stated that there are few aspects of brucellosis so unexplored as its epidemiology:

When one views the volumes of literature which have been amassed on vaccination procedures, serologic tests, shedding of the organism and pathogenesis, it is appalling to see the limited number of studies relating to routes of transmission, relative importance of various sources,

survival of the organism, means of transmission within and between herds, the characteristics of high-risk herds, management practices to reduce transmission and the dynamics of herd immunity. The danger of accepting field observations as truths is well illustrated in brucellosis by the history of the questions concerning the role of the bull in the transmission of the etiologic agent. . . (18).

Nicoletti (47) has commented that it is accepted widely that sexually immature cattle are quite resistant to exposure to *B. abortus* and that susceptibility increases with sexual development and pregnancy. Calves may acquire infection in utero or by ingestion of contaminated milk. A small but important percentage of heifer calves infected in early life are negative to serologic tests and abort or have an infected calf during the first pregnancy. These are referred to as latent infections. Latent infections are difficult to diagnose early in the course of the disease and may present serious problems in the elimination of brucellosis from cattle herds (47).

In brucellosis, the term "incubation period" usually implies that period between exposure to infection and the time at which clinical or serological evidence indicates that infection has occurred. That period varies considerably and is affected by several factors such as gestation, exposure dose, age, vaccination, and other unknown host-resistant influences. The variable incubation period and the difficulties of diagnosing infection until after transmission are among the most serious technical problems in unraveling the epidemiologic patterns of brucellosis (47).

For many years, diagnosis was based upon isolating the organism from infected tissues or secretions or by employing agglutination tests on serums from cattle under surveillance. Serious limitations were found in the agglutination test. It was often ineffective in detecting chronic infections and could be influenced seriously by vaccines or other antigens. Subsequently, a battery of other serologic methods was developed including the ring test, card test, Rose Bengal test, complement-fixation, rivanol, and mercaptoethanol tests for humoral antibody. And recently the lymphocyte stimulation test has been evaluated for evidence of cell-mediated immunity (47). Varied sensitivity and

specificity among these have made it obvious that no single test is entirely reliable in the diagnosis of brucellosis, but in wisely selected combination, they are highly effective.

Strain 19 vaccine remains the most widely used immunogenic agent for the control of bovine brucellosis. In more than five decades of research on this strain, there has been no proved change in virulence or immunogenicity (47). It rarely causes permanent infection in vaccinated cattle, especially when administered in calfhood. There is no apparent pathogenicity for human beings except when it is injected. A reduced dose, proposed by Nicoletti (47) and administered subcutaneously, has enhanced the practicability of the use of the vaccine regardless of age of cattle.

Calf Diarrhea Caused by Viruses

Major progress in the understanding of calf diarrhea has resulted from the isolation and characterization in the late 1960's of a reo-like virus (now called rotavirus) and of a coronavirus in the early 1970's. These accomplishments resulted largely from the efforts of Mebus and coworkers at the Nebraska Agricultural Experiment station.

Rotavirus. In recent years, the importance of rotaviruses in the ubiquitous and troublesome diarrheal diseases in calves has become well established. And, as for some bacterial diarrheal diseases, rotaviruses have the propensity to infect more than one species of animal so that human, bovine, porcine, and equine strains possibly may produce diarrhea in neonatal calves (24). Although rotaviral infection may occur at all ages, including adult cattle, it is in neonates that the disease is often most severe and frequently fatal. The disease in calves usually becomes apparent between 3 days and 15 wk after birth.

It is likely that the disease heretofore known as pneumoenteritis in calves and piglets is primarily a rotaviral disease. In pneumoenteritis, diarrhea usually precedes pneumonia, occurring between 1 and 10 days of age.

In calves on a total milk diet, feces in rotavirus infection usually are brilliant yellow to white in color, not always putrid, and resemble those of classic milk scours. In other calves, however, feces may be watery, brown, gray, or light green and tinged with fresh blood

and mucus. The color appears to be dietary dependent. If the diarrhea is prolonged, dehydration becomes apparent, and the calf may die within 4 to 7 days after onset, showing a significant loss of body weight. Severely ill calves have recovered after administration of glucose and saline mixtures substituted for milk. Continued feeding of milk is harmful and probably accounts for the severity of epizootics in calves maintained in cow/calf operations. Inclement weather may complicate an outbreak. Many calves have developed severe pneumonia after the onset of diarrhea, especially when they were exposed to inclement weather. Severely affected animals may die 2 or 3 wk later (24).

The pathogenesis of rotavirus infection in calves is similar to that for transmissible gastroenteritis in pigs. The virus infects villous epithelium in the intestine. Immature enterocytes, which migrate from the crypts to the tip of the villus, fail to differentiate fully in their migration. This failure appears to be the major factor, which produces an ultimate electrolyte transport disorientation for sodium ions, resulting in fluid and electrolyte loss manifested by diarrhea (24).

Resistance to rotavirus disease appears to be mediated by local immunity at the epithelial surface of the small intestine (55). Unfortunately, the passive protection afforded by colostrum has limited value. Calves are protected when they are fed colostrum with rotavirus antibody of high content, but that colostrum must be fed continuously in the likelihood of constant exposure to virus (24).

The duration of immunity is not known, and the period of persistence of virus in the feces after illness also is unknown. The effectiveness of cell-mediated immunity apparently has an influence on the persistence of virus which is shed in the feces. It is likely that immunity is only partial and not long-lasting. Therefore, adults can become infected from infected neonates. Since the incidence of the disease is extremely high, and since reinfection may occur, it is likely that the disease is perpetuated through persistence of virus within an animal species. Also, there is a possibility that interspecies infection may be important in transmission of the infectious agent.

In control of this disease, despite the limitations cited, the feeding of colostrum to newborn

calves is extremely important. The colostrum must contain reasonably high antibodies to the rotavirus, and it must be fed every day during the crucial neonatal age period in which rotavirus infection is a threat if it is to be effective.

An attenuated bovine-derived strain of rotavirus has been used for production of a vaccine. The vaccine currently is given to day-old calves. Although there is some question about the efficacy of this new vaccine, it seems to show effectiveness when used in herds with serious rotavirus diarrheal problems (24).

Coronavirus. A second major virus, a coronavirus, also has been isolated from dairy calves with neonatal diarrhea (39). The coronavirus and the rotavirus both affect the absorptive epithelium of the gut. Both cause similar clinical syndromes and contribute significantly to calf morbidity and mortality. Both viruses are studied with similar technologic methods, and both are combined in a commercially available modified live virus vaccine. They are, therefore, frequently discussed together, even though they have slightly different pathogenic mechanisms and different virologic characteristics.

Coronavirus infection cannot be distinguished clinically from other causes of diarrhea in newborn calves. A laboratory diagnosis can be made by finding the virus in feces by immunofluorescent microscopy or electron microscopy.

Prevention of coronavirus-induced diarrhea is complex. It requires hygienic calving conditions, assurance that each calf receives colostrum, and sanitary rearing practices. Where a specific diagnosis has been established, use of the modified live virus in pregnant cattle and newborn calves may be of value.

Colibacillosis

The two most common bacterial enteric diseases of young cattle, especially calves, marked by severe diarrhea, dehydration, and high death loss, are colibacillosis and salmonellosis.

Colibacillosis is associated with infection by *Escherichia coli*, a ubiquitous organism found normally in the lower bowel of all warm-blooded animals. It is found abundantly in carnivora and omnivora but sparsely in horses and cattle and may or may not be pathogenic. Its pathogenic propensity is regulated by plasmid-mediated

determinants that, in addition to providing filamentous surface antigens on the organism that facilitate adhesion to the epithelium of the intestine and thence colonization and proliferation, cause the organism to produce a pathopotentiating diarrhea-inducing enterotoxin or sometimes a neurotoxin, which also is vasotoxic and produces enterotoxemic systemic disease (13). A third toxin, which is produced by all coliform bacteria, rarely causing diarrhea, is endotoxin.

E. coli endotoxin is responsible for systemic colibacillosis in immunoglobulin-deficient calves. This occurs even in calves that ingest but do not absorb protective quantities of colostral antibody (19, 41). Although the alimentary tract is usually the portal of entry, signs of nonenterotoxemic systemic colibacillosis in calves may or may not be accompanied by diarrhea or alimentary tract injury. It is important to emphasize that ordinarily endotoxin does not cause fluid loss from the intestine. Usually, manifestations of endotoxin injury are associated with hyperthermia, lethargy, complement activation, neutrophil degranulation, and various toxic effects on the vascular system that may culminate in irreversible, fatal shock. Signs include fever, depression, vomiting, trembling, edema, increased respiration, and some hemorrhage (41).

The vasotoxic neurotoxin elaborated by some varieties of *Escherichia coli* produces enterotoxemic colibacillosis, as in edema disease of swine. Brain hemorrhages in calves are believed to be induced by the same blood-vessel-damaging neurotoxin.

The most common and most troublesome of the toxins of *E. coli* is enterotoxin, and the disease it induces is enteropathic enterotoxic colibacillosis, so-called "white scours" or "calf scours". Enterotoxic colibacillosis is the only form of colibacillosis which is truly an enteric disease. It occurs in the young; adults are spared (41). Calves exposed to enteropathic enterotoxic *E. coli* during the 1st day of life most likely will develop disease whereas those exposed to the same organism 2 days later may not show signs of illness.

There is a gradient with the anterior small intestine being highly sensitive to enterotoxin and the posterior small intestine being relatively resistant. As with cholera in man, enterotoxin-induced secretion in the small intestine of

calves takes place across an intact, unaltered mucosa except for the discharge of goblet-cell mucus. Enterotoxic colibacillosis produces a profuse, watery, dehydrating diarrhea, not by hyperperistalsis (whip-lashing) of the gut but by biophysical directional alternation of water and electrolyte flow in the intestinal membranes.

It has been baffling that in enterotoxic colibacillosis the organism adheres to villus, not crypt, epithelium. Yet, it is the crypt epithelium that contains numerous secretory granules responsible for the diarrhea. It is believed that the enterotoxin mimics or stimulates the release of villus hormones, including prostaglandins, which regulate adenylcyclase and the intestinal secretory process and secretion of water and electrolytes (41).

When such secretion by the small intestine exceeds colonic absorption, diarrhea occurs. Losses of water, sodium, and carbonate result in dehydration, metabolic acidosis, and hyperkalemia. Acidosis leads to decreased intracellular potassium and then increased extracellular potassium. This effects cardiac arrhythmias and then myocardial failure, the immediate cause of death in most cases. Death frequently occurs in less than 24 h.

In the most acute form of white scours, the calf shows weakness, appears sleepy, and soon dies without other signs. In the usual form, however, the calf shows a severe diarrhea, the fecal material often being full of gas bubbles and whitish in color because of lack of bile. Some animals may die after a few days. In more protracted cases, lameness may appear because of acute inflammation of one or more joints. In such joints, the capsule is distended with a cloudy fluid in which there are myriads of colon bacilli. These animals almost always eventually die (24).

In controlling calf scours, it is essential to emphasize that colostrum and good management to prevent environmental stress are equally important. Colostrum, containing immunoglobulin, will interfere with the adherence and colonization of the enterotoxic coliforms on the intestinal villi.

The success of vaccination of cows to prevent diarrheal disease in calves appears to depend upon preparation of an antigen (vaccine) to stimulate formation of appropriate multivalent anticapsular immunoglobulins, not

antitoxin, because the common enterotoxin is not immunogenic (24).

Morgan et al. (43) vaccinated brood sows with purified somatic pili from clones of piliation phase enterotoxigenic *Escherichia coli* and protected neonatal suckling pigs against diarrheal disease caused by enterotoxigenic *E. coli* strains that possessed the same pili. The practicality of such a vaccine is diminished by the heterogeneity of pathogenic strains unless ways are devised to produce polyvalent immunoglobulin by genetic or hapten linkage of pilus immunogenic determinants. If that can be accomplished for pigs, it most likely will have benefit for calves.

Foot Rot or Infectious Pododermatitis

Foot rot remains a troublesome and enigmatic disease in cattle, although careful and difficult research continues to chip away at its elusive nature.

The anaerobic *Fusobacteria*, which produce copious amounts of butyric acid, and *Bacteroides* species, which produce copious volumes of isovaleric and isobutyric acids (24), have been isolated from cases of foot rot in cattle and studied extensively by Berg (4) and others, including Thorley et al. in Great Britain (56).

The disease in cattle frequently has been associated with *Fusobacterium necrophorum* but also associated with *Bacteroides melaninogenicus* (4) and *Bacteroides nodosus* (56).

These organisms have little or no capacity to invade normal epithelium, but under anaerobic conditions they readily enter and multiply in tissues damaged by injury or infection caused by other organisms (24). In all species of animals suffering from foot rot (or liver abscesses associated with *F. necrophorum* and pyogenic bacteria), the typical lesion is necrosis, and it has a putrid odor.

The genesis of the lesion in cattle is not understood completely; it is more thoroughly known in sheep. The early lesions of foot rot in cattle are similar to those of sheep; that is, swelling, and moistness in the interdigital cleft. In sheep, but not in cattle, inflammation of the sensitive laminae of the hoof occurs, often underrunning the sole.

The most important bacterium in foot rot of sheep is *Bacteroides nodosus*. The disease is initiated by the penetration of *B. nodosus* into

the epidermis damaged by injury (54). This is accompanied or followed by invasion of the same site by *F. necrophorum*, which extends the tissue damage, creating new invasion sites for *B. nodosus* (5). Perhaps the genesis of the foot lesion in cattle parallels that of foot rot in sheep. *Treponema penortha* may be a pathopotentiator. Pus-producing bacteria, like *Corynebacterium pyogenes*, are secondary invaders, but they operate in synergy with the primary invaders to produce tissue destruction and sometimes abscess formation. *F. necrophorum* facilitates the establishment and growth of *C. pyogenes* in the tissues by the phagocyte-inhibiting and leucocyte-destroying action of its exotoxin (48). *C. pyogenes* produces a macromolecular substance that stimulates the growth and invasiveness of *F. necrophorum*, particularly by removing oxygen from the tissue site under microbial attack. Whether a liberation of exotoxin is activated by phage (as for some species of *Corynebacteria*) is currently unknown (48).

Attempts to vaccinate animals with formalized bacterial cell preparations and with culture filtrates of *F. necrophorum* have not been successful, although it appears that most animals develop antibody as they age and are exposed to the organism (24). It is not clear that this antibody is protective against the disease, however.

Some prophylactic agents, like broad-spectrum antibiotics, are effective in preventing *F. necrophorum*-associated liver abscesses in cattle. Such agents may have potential in preventing or treating bovine infectious pododermatitis.

Infectious Bovine Rhinotracheitis

Twenty five years ago this disease had been newly described (8, 29) and during the intervening period, elaboration of the many facets of infectious bovine rhinotracheitis has resolved many perplexing questions.

Infectious bovine rhinotracheitis (IBR) is the name given to the respiratory form of a herpesvirus infection that also sometimes is manifested by a genital syndrome called infectious pustular vulvovaginitis (IPV) or coital exanthema. Close confinement of feedlot cattle and cattle in large dairy herds provides favorable conditions for the rapid transmission of the virus (51).

Sometimes the disease appears simply as a granular conjunctivitis, occasionally by meningoencephalitis, especially in calves under 6 mo of age. Frequently, the infection is manifested solely by abortions. Abortion can be a sequel either to respiratory infection by IBR virus or to injection of modified live IBR vaccine. Abortion usually is not a sequel to IPV.

The IBR virus, like other herpesviruses, may be latent, therefore being shed intermittently from infected animals for long periods following recovery from clinical disease. Latent infection was activated by the use of corticosteroids in studies reported by Sheffy and Rodman in 1973 (52).

The respiratory form of IBR infection probably is the most significant and economically important form of the disease in feedlots, but for dairy cattle abortion may be more costly. An outbreak may be mild with numerous subclinical cases, or it may be fulminating. It is not unusual to find cattle in an infected herd that appear bright and yet have markedly elevated body temperature (20). There may be noticeable decrease in feed consumption and signs of upper respiratory infection. The affected animals exhibit rapid rhythmic and sometimes dyspneic breathing, inappetence, elevated temperature, coughing, nasal discharge, foamy salivation, catarrhal conjunctivitis, and loss of weight. The course of disease usually runs between 7 and 10 days.

The muzzle and nasal mucosa become inflamed, giving rise to the term "red nose". Occasionally respiratory signs are not evident. Conjunctivitis, with ocular discharges which initially are clear but later infrequently become mucopurulent and sometimes with centripetally originating corneal opacities, may appear as principal manifestations of infection. Such infection may be misdiagnosed as pinkeye. A high percentage of cattle with classic pinkeye, caused not by a virus but by *Moraxella bovis* bacterium, have corneal opacities originating in the center of the cornea and spreading centrifugally. The diagnosis of IBR-associated conjunctivitis should be considered if opacities in a herd are few and appear to originate at the corneoscleral junction (29). Another disease wherein corneal opacities occur frequently and originate at the corneoscleral junction is malignant catarrhal fever (MCF). That disease, too, is

associated with dyspnea due to profuse accumulation of tenacious mucopurulent exudate in the nasal passages and, to some extent, the trachea and bronchi. Partial blockage of these airways may result in the forced open-mouthed breathing seen in IBR and MCF.

White pustules frequently appear and coalesce in the respiratory, ocular, and reproductive tract mucous membranes. When these lesions appear in all these loci, they are sufficiently distinctive for IBR to suggest that the disease in question is, in fact, IBR. In the nasal passages, however, similar lesions may occur in MCF.

Following natural IBR virus infection or vaccination with modified live virus IBR vaccines, cell-mediated and humoral components of the immune system are activated. The humoral response, usually measured by serum neutralization tests, has served traditionally as an indicator of prior infection and as an indirect measure of resistance. Evidence now suggests that a crucial role in resistance and recovery from early IBR involves locally deployed elements of the cell-mediated immune system. Thus, detection of neutralizing antibody in serum probably is not an accurate indicator of immunity but may be used until methods of measuring cell-mediated immunity to IBR come into common use (29).

The IBR can be controlled by maintaining protective antibody in a herd and by reducing the number of susceptible animals to a minimum. This can be accomplished by helping cattle acquire both passive and active immunity at an early age.

The calf receives IBR antibody from colostrum within the first 24 h after birth. The quantity that a calf receives depends upon the dam's serum antibody and the amount of colostrum ingested. Because the dam transfers IBR antibody to her offspring comparable to that in her own serum, some workers suggest maintaining a protective antibody titer in the brood stock. This can be accomplished by booster vaccinations 3 to 4 wk prior to breeding or by vaccinating in the last trimester of pregnancy with vaccines approved for pregnant cattle.

Colostrum antibody may persist as long as 4 mo and may prevent an antibody response to vaccine given during that period. Therefore, calves vaccinated before 6 mo of age should be

revaccinated after that age. Vaccination may provide immunity for longer than 2 yr (51). As an additional protective measure, all additions to a herd should be isolated and observed carefully for signs of illness every day for 30 days.

Today live modified combined vaccines against IBR, bovine virus diarrhea-mucosal disease, and parainfluenza-3 are available and are compatibly effective when administered at 5 to 7 mo of age to calves that have had colostrum immediately after birth and earlier to calves deprived of colostrum.

The first IBR vaccines were modified live virus products developed to be used intramuscularly. These had the disadvantage of causing abortion. In 1969, intranasal-administered, modified live-virus IBR vaccine was introduced and has gained widespread acceptance. Unlike vaccines for intramuscular injection, the intranasal vaccines usually are safe for use in pregnant cattle. Among the advantages ascribed to the intranasal vaccines are rapid protection attributed to interferon production and rapid induction of secretory antibody at mucosal surfaces (29).

Intranasal vaccines are believed to induce production of humoral antibody at titers comparable with those of intramuscular vaccines. The intranasal vaccine can be administered to cattle of the same ages as applied to use of the intramuscular vaccine. It has been suggested that intranasal-administered vaccine may override low colostrum-acquired maternal immunity, resulting in successful vaccination of some calves that do have colostrum-acquired antibody. If calves less than 6 mo old are vaccinated, booster doses are recommended for animals to be retained for long-term purposes, especially as breeding animals.

Inactivated IBR vaccine has been available from time to time in combination with bacterin containing organisms of the *Pasteurella* genus and inactivated myxovirus parainfluenza-3. The latter two agents have been associated with the shipping fever complex. There is controversy regarding the efficacy of inactivated vaccine for these respiratory illnesses, however. As in the case with many other inactivated vaccines, the initial vaccination procedure should be repeated, and annual booster doses are recommended. Advantages of inactivated vaccine are that it eliminates the risk of postvaccination abortion

associated with live-virus vaccine and avoids unfavorable postvaccination reactions except anaphylaxis. Also, with inactivated vaccine, there is no shedding of vaccine virus and seeding of populations otherwise not exposed. In addition, it eliminates concern that the vaccine virus itself may establish a latent infection that could be reactivated at a later date.

Although research on IBR continues, the past quarter century has seen it emerge from an undescribed disease to a well understood and well controlled infection.

Johne's Disease (Paratuberculosis)

Johne's disease is a chronic granulomatous enteritis of ruminants caused by an acid-fast bacterium, *Mycobacterium paratuberculosis*. Johne and Frothingham first described this disease in 1895 in Germany (23). They cited an unusual case of tuberculosis in a cow wherein they found acid-fast bacteria, which they thought to be either avian or bovine tubercle bacilli (9). In 1906, Bang in Denmark recognized it as an infection distinct from tuberculosis and suggested that it be named bovine pseudotuberculosis or paratuberculosis (9).

Paratuberculosis has been recognized in the United States for about 75 yr, and concern has been expressed that it is increasing in incidence. The disease is characterized by progressive emaciation and, in cattle, by a recurrent diarrhea. There is thickening, corrugation, and convolution of the mucosa of the distal end of the small intestine. The granulomatous enteritis that occurs is expressed by infection-damaged, malfunctioning short, club-shaped intestinal villi that assume wart-like configurations.

Diagnosis has been extremely difficult for a variety of reasons. Clinical disease usually occurs only in a small number of infected animals. Infected animals that shed the organisms can be inapparent (silent) carriers showing no evidence of disease. Fecal culture isolation techniques to recover the organism require 2 to 3 mo for completion, because the organism grows extremely slowly in laboratory media. Further, these techniques detect only a small number of infected animals. Heretofore, immunologic tests, including the complement-fixation test and the agar gel immunodiffusion test, used prior to the development of the

lymphocyte transformation test that employs phytohemagglutinin as mitogen and purified protein derivative from the organism as antigen, have lacked specificity or sensitivity (9). The difficulty in diagnosis has been compounded by the fact that humoral and cell-mediated immunologic status of infected animals varies with the stage of the disease. However, the availability and assessment of the lymphocyte transformation test, as described by Buergelt (9), offers encouraging potential for the diagnosis and ultimate eradication of this troublesome disease. The test provides an *in vitro* evaluation of cell-mediated immunity and offers a fairly sensitive method to assess the extent of disease in populations of cattle under surveillance.

To complicate the diagnosis, there is a close antigenic crossreactivity between *Mycobacterium avium*, the cause of avian tuberculosis, and *Mycobacterium paratuberculosis*. However, these two infections may be differentiated by the lymphocyte transformation test, provided appropriate purified protein-derivative antigens from each of these agents are used in parallel on a given serum specimen (9). Paradoxically, even with the hopefully effective lymphocyte transformation test, a humoral mitogen-suppressor substance has been found in serum fractions containing immunoglobulins IgG₁ and IgG₂. Notwithstanding the difficulties in serologic interpretations, the lymphocyte transformation test shows promise of being useful in the diagnosis of subclinical and clinical cases of Johne's disease and may provide an overall impression of the extent of exposure to *M. paratuberculosis* within a herd. Nonruminant hosts may serve as reservoirs from which the organism may be shed to infect cattle (9).

Although Johne's disease has been historically considered a disease that involves the lower end of the small intestine, the ileocaecal valve, and the adjacent lymph nodes, the organism has been isolated also from the liver, blood, udder, uterus, ovaries, milk, urine, feces, and fetus. Therefore, there is evidence that the disease is transmitted not only by fecal contamination but also by milk and by transplacental infection of the fetus (9).

A typical clinical case of Johne's disease is characterized by intermittent or continuous chronic diarrhea in young animals, especially in 2 or 3-yr-old lactating females. The disease most often occurs in the early part of the first

or second lactation. Feces are soft and watery and are passed without straining. Diarrhea may decrease in severity during late pregnancy, only to reappear more severely after parturition. Valuable high-producing animals may be affected suddenly. Milk production decreases, and the haircoat may become rough or loss of hair occurs. The appetite remains good. There is no fever. Usually only one or two animals show evidence of disease in a herd at the time of initial observation. After a course of weeks to months, with unresponsiveness to treatment, the disease terminates in death of those animals showing severe dehydration, emaciation, and weakness. Decreased absorption of amino acids associated with increased protein leakage results in a negative nitrogen balance with the clinical manifestations of a wasting disease.

Although *M. paratuberculosis* has been cultured from pharyngeal lymph nodes, tonsils, bronchial lymph nodes, spleen, lungs, kidney, reproductive tract, mammary gland, and placenta, gross and microscopic lesions usually are absent in these organs. Lymphatic and hematogenic spread through the thoracic and portal venous circulation, respectively, begins early in the disease process. However, the organisms do not seem to multiply to any extent or give rise to distinct lesions except in the intestine and its adjacent lymph nodes. The spread is assumed to occur passively by way of circulating macrophages. In the liver, these bacteria-laden macrophages may become trapped in sinusoids and, in conjunction with lymphocytes and possibly antibody, they may give rise to multiple small granulomas (9).

Merkal (40) introduced recent immunologic concepts to the understanding of the pathogenesis of Johne's disease. He postulated that an antigen-antibody reaction in the infected intestine causes the release of histamine in an immediate-type hypersensitivity reaction, resulting in mediation of diarrhea. Delayed hypersensitivity, also a phenomenon in this disease, involving specific antigens from the organisms and specifically sensitized lymphocytes, causes the release of cytotoxins and pyrogens that mediate emaciation and anemia.

Attention has been called in early literature to the similarity between leprosy and paratuberculosis (9). Both diseases are caused by acid-fast organisms that are difficult to culture and are characterized by long and slow

growth periods of organisms in culture. The granulomatous inflammatory response in both diseases is similar. Despite involvement of different organ systems, the microscopic lesions are dominated by epithelioid and Langhans' giant cells. In advanced leprosy the typical inflammatory cells have been termed "leprosy cells", enlarged macrophages with vacuolated, frothy cytoplasm resulting from the accumulation of lipid substances and bacillary debris. Although such cells are not identified clearly in Johne's disease, vacuolation of cytoplasm and lipid degeneration of epithelioid cells has been noticed (9), with clumping, distortion, and dissolution of bacilli within these cells. In the adjacent draining lymph nodes, large areas of lymphoid cells within the paracortical zone have been replaced by granulomatous inflammatory infiltrates in both diseases (9). The similarity in the histopathologic findings of leprosy and of paratuberculosis have attracted pathologists studying paratuberculosis to the adaptation of Ridley and Jopling's morphologic classification of leprosy for paratuberculosis, hence, the inclination among some pathologists to refer to Johne's disease on cellular morphologic grounds as bovine leprosy (9).

Vaccination of cattle with heat-killed or live, attenuated organisms has been used with some claims of success in France and England. Where vaccination is practiced, one dose for a 1-wk-old calf is recommended (9). But it generally is agreed that the vaccine does not confer absolute immunity, and some animals vaccinated with the live, attenuated cultures have excreted the organisms. A major problem in using vaccines of this nature against Johne's disease, in addition to their questionable reliability, is that vaccinated animals become sensitized and react to mammalian tuberculin when tested for tuberculosis. Further, the immunologic significance of antibody in Johne's disease and its relationship to the infectious agent remain unsolved.

Doubts have been raised about the protective nature of antibody in chronic, progressive, infectious diseases. In such diseases, it is thought that antibody may protect the etiologic agent rather than the host (9). There is evidence that such antibody abrogates effective cellular immunity and actually enhances dissemination of lesions in some chronic infectious diseases. The mechanism of enhancement of disease by antibody should be suspect when a disease

normally controlled by cellular immunity suddenly undergoes rapid dissemination of lesions, such as is seen classically in miliary tuberculosis (9). One must examine this question critically in Johne's disease. Does this occur in those clinical cases of Johne's disease resulting in diarrhea, emaciation, and death, as distinguished from the infected animals that show inapparent infection? If so, the value of vaccination needs critical reevaluation. In that evaluation, however, one must be sensitive to experience with other vaccines in similar modes. For example, the appearance of miliary tuberculosis in young children is rare after BCG vaccination (15). The BCG is a strain of originally virulent *M. bovis* attenuated by Calmette and Guerin by serial passage on inhibitory media. It is assumed that the effectiveness of the vaccine is dependent upon development of humoral antibody.

Due to the lack of adequate or effective vaccines or effective drugs for treatment, control of Johne's disease in infected herds is achieved best by strongly enforced management procedures, which include prompt isolation and culling of infected animals and their offspring as determined by serologic (9) or culturing (45) methods, segregating calf-rearing quarters, and good hygienic procedures.

Malignant Lymphoma of Cattle (Enzootic Bovine Leukemia)

The past quarter-century has brought remarkable progress in an understanding of bovine malignant lymphoma, particularly the identification of a virus as the cause of some such tumors in cattle. This progressive and ultimately fatal disease of cattle also is known as bovine lymphosarcoma, bovine lymphomatosis, or bovine leukosis. In clinically apparent cases, it is manifested by enlargement of lymph glands. The glandular enlargements in malignant lymphoma of cattle may be preceded by hematologic change, usually a persistent lymphocytosis. Many European workers believe that cattle may develop these hematologic changes without tumor formation (24).

Some cattle in herds afflicted with this disease show enlarged and firm superficial lymph nodes. Generally, the disease in these animals progresses rapidly. Emaciation develops, and death usually occurs in animals that show signs of illness.

Sporadic cases appear in calves, or endemics appear in adults, the incidence being highest in animals from 5 to 8 yr of age. Calves under 6 mo of age showing the sporadic form usually present generalized enlargement of lymph nodes and nodular or diffuse lesions of the spleen, liver, kidneys, or bone marrow. This is called the neonatal multicentric form.

Animals between 6 mo and 2 yr of age may show enlargement of limited groups of lymph nodes, usually with involvement of the thymus gland. Also, in that age group, the skin may show multiple tumors. These are referred to as the thymic and skin forms, respectively, and these conditions appear to be unrelated to bovine leukemia virus (24).

In animals over 2 yr of age, the multicentric form of malignant lymphoma usually is observed. Sometimes an eyeball may protrude because of tumor formation in the orbit. Chronic bloating occurs in some because of enlargement of thoracic nodes that interfere with normal eructation. Abomasal lesions may lead to digestive disturbances or abomasal ulceration. Lameness and paralysis may occur because of pressure on parts of the spinal cord or peripheral nerves from tumors. Lymphocytic infiltration of some of the internal organs may be seen in some cases with pronounced increase in the number of circulating lymphocytes, hence, the term lymphocytosis or leukemia (24).

There is evidence that B-lymphocytes are the target cells for bovine leukemia virus (BLV) infection. The B-cells are lymphocytes from which immunoglobulin-producing plasma cells are derived. Also, it is now clear that the agent is a C-type virus, an oncogenic RNA virus in the subfamily Oncornavirus. The C-type particles are budding forms at cell membranes, also observed as free particles in intercellular spaces. Particles with central nucleoids are designated C-type. The B-type particles, by way of contrast, have acentric nucleoids.

In laboratory diagnosis, phytomitogen is used in buffy coat cultures of leucocytes from cattle with persistent lymphocytosis or lymphosarcoma to enhance the appearance of C-type particles in the cell cultures. By this technique, C-type particles, heretofore demonstrated in cattle with persistent lymphocytosis and lymphosarcoma, also have been found in "normal" cattle (24). Perhaps this indicates

inapparent, silent infection which, in the absence of antibody, will skew the epidemiologic evidence of distribution of the infection in the national cattle population. Nonetheless, in the United States there is evidence that the disease is becoming more prevalent, with high and low incidence areas (23). Evidence is emerging that BLV is responsible for producing several forms of natural disease (24), but not all bovine lymphosarcomas have been associated with BLV.

Sero-epidemiologic evidence indicates that the virus in cattle is transmitted horizontally by contact as well as vertically, and it also is transmitted similarly in experiments with sheep and goats (24, 25). Further, BLV replicates in monolayers of bovine embryonic spleen cells, fetal lamb spleen, and human diploid embryonic cells with the production of syncytia (23). This observation, and the fact that BLV will produce lymphomatosis when inoculated into a variety of species, i.e., sheep, goats, and subhuman primates (25, 37), and the finding of BLV in cows' milk (17) have prompted careful studies on the human risk from exposure to BLV.

A seroepidemiologic study was conducted by Donham et al. (16) in an attempt to identify antibody against the bovine leukemia virus in people exposed to cattle with lymphosarcoma. Farm families, farm employees, and veterinarians in contact with dairy herds having documented cases of lymphosarcoma were tested for precipitating antibody to the BLV, using the agar-gel immunodiffusion test. The cattle also were tested by serologic procedures. Further, information was collected from the farm families regarding consumption of unpasteurized milk from their dairy herds. Twenty-one dairy herds with documented cases of lymphosarcoma were identified. A total of 846 cows from these herds were bled and 33% showed positive serologic tests.

No positive serums were found in the 45 dairy farmers, family members, and farm employees associated with the herds with lymphosarcoma. Consumption of raw milk was reported by 77% of the farm group.

In addition, 83 veterinarians, 30 leukemia patients, and 200 control human serums were tested and found negative for antibody to the BLV (16).

Although 77% of the farm people in this group consumed raw milk and showed no

seropositive evidence of infection, it is still advisable for people to drink pasteurized milk inasmuch as it has been demonstrated that flash pasteurization inactivates the virus in milk (23).

Special attention is called to the studies of 12 neonatal chimpanzees removed from their mothers at birth, nursed separately, and fed either unpasteurized BLV-containing milk or a prepared infant formula. Hematologic changes were noted at days 119 and 200 in two animals fed BLV-containing milk. The animals died at 33 and 46 wk, respectively. The bone marrow, peripheral blood, and other autopsy findings for both animals were indistinguishable from leukemia and were consistent with the Di-Guglielmo syndrome, erythroleukemia (37). However, neither the bovine lymphosarcoma virus nor BLV antibody was in these animals. Whether the disease developed as a direct result of BLV ingestion remains a moot question since none of the other chimpanzees developed illness nor antibody (1). In a symposium on bovine leukosis sponsored by the United States Department of Agriculture in May 1979, Glyn G. Caldwell reported the results of Public Health serologic studies and concluded that "adequate serologic tests which are sensitive and specific have failed to demonstrate infection in humans despite close contact or raw-milk ingestion from BLV-infected herds. Consequently, BLV does not appear to represent a public health hazard for humans" (10).

Further studies are needed before we understand fully the epidemiologic patterns of BLV and the details of its association with the variety of lymphoid tumors affecting dairy cattle.

Salmonellosis

Salmonella typhimurium and *S. dublin*. Unlike the emerging enteric viral diseases, salmonellosis has plagued dairymen for years. The past 25 yr, however, have brought considerable understanding to this ubiquitous enterobacterial disease.

Diarrhea, severe dehydration, prostration, and death characterize salmonellosis in young cattle, especially calves. Nevertheless, once established in young animals in a herd, adult animals tend to become infected. This appears more commonly today than 10 yr ago (20). At that time it was restricted primarily to young animals (20).

Salmonellosis is usually an enteric disease. Sometimes, though, it becomes systemic, involving organs and tissues other than the intestine, especially the joints. Most clinical cases in calves are due to *Salmonella typhimurium*. West of the Rocky Mountains, however, some cases in calves and adults are due to *Salmonella dublin*, (8) a common cause of bovine salmonellosis in Europe.

Salmonella dublin, first isolated in a maternity hospital in Dublin, Ireland, when found in domestic animals in the United States and elsewhere, occurs almost exclusively in cattle. However, in the western United States, it is the predominant species of *Salmonella* in wild foxes. Despite the wild fox problem, an outbreak of *S. dublin* infection in cattle is more likely to have originated from other domestic cattle (23).

Cattle that contract *S. dublin* infection continue, after recovery, to excrete some of the bacteria in their feces for many years; about 30 to 40% remain shedders during their lifetimes because the organism colonizes the gall bladder and liver. In contrast, in the more ubiquitous *S. typhimurium* infection of cattle, which is indistinguishable clinically from *S. dublin* disease, recovered cattle remain carriers and shedders for only a month or two because *S. typhimurium* does not colonize the gall bladder or liver in mammalian species, except perhaps rodents.

Notwithstanding these facts, it is possible to eliminate one of these infections from a herd while virtually impossible to eliminate the other. Elimination of host-adapted *S. dublin* can be accomplished by identifying and discarding reservoir carrier animals through culturing fecal specimens. This cannot be done in non-host-adapted *S. typhimurium* infection because of the ubiquitous distribution of that bacterium among many animal species which can contaminate the farm environment. Although outbreaks of *S. typhimurium* infection in cattle may originate from other cattle, there are usually opportunities for crossinfection from other species of livestock (especially swine), wild rodents, or poultry. However, in Great Britain there are specific phage types and clones peculiar to cattle alone (58). These can be eliminated from a herd. This emphasizes the importance of phage-typing all isolates of salmonellae from dairy herds.

The detection of salmonellae in a calf shipped from one locality to another does not necessarily indicate infection at the origin. Calves showing clinical evidence of salmonellosis are often those which have come from a clean source but which apparently have encountered the organism for the first time while in transit.

Acute salmonellosis in adult cattle, while by no means as common as in calves, occasionally can be very troublesome (30). Abortion is a common feature of adult infection (30). Diarrhea and dehydration in the adult usually are severe when they do occur.

Colonization of the latter part of the small intestine and colon is a necessary first step in pathogenesis of enteric salmonellosis. The normal intestinal flora may block access to attachment sites on the mucosal cells needed by the salmonellas. Also, indigenous fusiform bacteria that lie in the mucous layer coating the epithelium of the large intestine normally inhibit growth of salmonellas by producing volatile organic acids which are toxic for the salmonella organisms (24).

Factors such as antibiotic therapy and diet and water deprivation that disrupt the normal colonic flora, or otherwise stress cattle, greatly increase the host's susceptibility to enteric and septicemic salmonellosis (24). Clinical salmonellosis must be regarded as having a multifactorial cause.

After invading intestinal epithelial cells, salmonellas cause net secretion of water and electrolytes, possibly by means of lipopolysaccharide in the bacterial endotoxin, causing inflammatory release of prostaglandins that in turn activate adenylcyclase (24). This enzyme acts upon secretory granules in the crypt cells to stimulate water and electrolyte secretion.

Bacterial endotoxin also effects elevated body temperature, damage to capillary endothelium resulting in hemorrhage, thrombocytopenia, depletion of liver glycogen with prolonged hypoglycemia, and shock. Shock often induces severe circulatory collapse resulting in sudden death.

Relapses are certainly an important practical problem in salmonellosis, but a number of these are believed to be from physical tissue damage from treatment rather than from first-order resurgence of infection.

There is widespread agreement that cell-mediated immunity is more important than

humoral immunity in resistance to salmonellosis. Humoral antibody contributes primarily to bacterial clearance (24).

Chemically inactivated vaccines (bacterins) have been prepared for immunoprophylaxis against *Salmonella* species with varying and oftentimes questionable effectiveness. In assessing effectiveness, intracellularly-located organisms are not affected by humoral antibody. The body must depend upon effectiveness of cell-mediated immunity, the effectiveness of macrophages which are activated by sensitized lymphocytes, to phagocytize and kill the tissue-invading salmonellas. Live vaccines prepared from avirulent cultures of *S. typhimurium* and *S. dublin* are used widely in Europe. They promote cell-mediated immunity (58).

Macrophages also release a cloning-inhibiting factor, a lymphokine that like the secretory immunoglobulin IgA, blocks adherence and thus prevents colonization, multiplication, and cloning of the organisms on the intestinal epithelium.

Salmonella anatum. During the past decade, there has been a noticeable appearance and increase in incidence of *Salmonella anatum* infection in cattle (6, 7), vying only with *S. dublin* for second place in order of frequency (24). Next to *S. typhimurium*, it is the most widely distributed *Salmonella* type (24). The source of infection appears to be mostly wild birds that contaminate pastures and hayfields with droppings containing the organisms (37). However, the organism has a wide distribution among a great variety of birds and mammals, so feedstuff and water, in addition to pasture or hayfield, may be contaminated.

In infected lactating cows, there is a marked reduction in milk production associated with protracted illness (20, 37).

Salmonellosis and Antimicrobial Resistance. Another problem relating to enteric bacteria has appeared during the past decade. Increasing resistance among members of the *Enterobacteriaceae* to antimicrobial agents has been noted throughout the world (46). Particular interest has focused on *Salmonella* species (46). The antimicrobial resistance patterns and presence of plasmid-bearing resistance factors in isolates from human and animal sources have been studied.

Considerable anxiety has been expressed about the use of antibiotics in animal feeds as

growth promoters and prophylactics, as this practice has been suspected to be a major factor in the increased antimicrobial resistance. No effective method for assessing the magnitude of risk to the welfare of man has been devised. Opinions about the degree of risk are highly polarized.

The increase in resistance has not been sustained in at least one instance. An abrupt decline in ampicillin resistance of *Salmonella typhimurium* in humans was reported in New York in the late 1970's, in contrast to an increasing frequency of resistant isolates from upstate New York calves over a similar time (12). The sudden, unanticipated drop in antibiotic resistance, reported in this singular but noteworthy urban experience, introduces new and unanswered questions as to why a population of *S. typhimurium* suddenly should lose its ampicillin resistance (12). It is possible that plasmid coded protein on the *Salmonella* cell surface is antigenic to the host animal, thus resulting in immunoglobulin against the ampicillin-resistant strain and an emerging population immunity (58).

Genetic control mechanisms will continue to be studied. But the role of the gut flora in support of or antagonism toward survival of R-factor-bearing strains versus antibiotic-sensitive strains and clone-preference effectors needs review.

The life-span benefits of incorporating antibiotics in feeds as growth stimulants are not understood fully, because it is not known how the antibiotics act at the molecular level as growth promoters.

Considerable practical evidence of the prophylactic benefits of antibiotics in feed or water does exist. Nonetheless, it is not always certain that diseases against which protection is claimed actually exist in all areas where antibiotics are being used as prophylactic feed additives. Improved surveillance and monitoring methods are needed.

Further the need for better understanding of the ecologic impact of different R-factor incompatibility groups in enteric genera such as *Escherichia* and *Salmonella* (58) is of fundamental importance.

Siderophores. Since serum and tissue extracts contain all nutrients essential to bacterial growth, it is assumed that all materials required by the pathogen are available for its utilization.

This is not so. The potentiality of various pathogens to survive in an animal depends upon their ability to obtain sufficient iron for active metabolism (31).

All microorganisms, with the possible exception of lactic acid bacteria, require iron. Iron is used in the cytochrome system for energy production. Lactic acid bacilli do not use that system. They derive energy from the glycolytic system. The same is true for anaerobes, although anaerobes may utilize both pathways.

Although iron is one of the most prevalent of the earth's elements in aerobic environments the amount of free iron available for assimilation by microorganisms is restricted due to the proclivity of ferric iron to form large, insoluble aggregates at neutral or alkaline pH. To acquire necessary iron from these aggregates, most aerobic microorganisms have evolved specialized iron-solubilizing and transporting ligands, chelating agents, which have been termed siderophores.

A pathogen does not have to contend with the insolubility of ferric iron but must obtain iron from its host. Although the amount of iron in host fluids is more than adequate for microbial growth, the iron-binding proteins, transferrin and ferritin in the serum and lactoferrin in secretions, sequester essentially all the iron in these environments.

The most likely mechanism whereby a bacterial pathogen successfully competes with these iron-binding proteins is via siderophore production.

Enterochelin or enterobactin is a siderophore produced by *Salmonella typhimurium* and possibly other enteric bacteria. It antagonizes the iron-restricting mechanisms of the host and, therefore, is a virulence factor for *S. typhimurium* (60).

One of the most effective defense mechanisms a host might develop against iron-dependent parasites would be the production of antibody to siderophores. Perhaps anti-siderophore vaccines for animals might reduce the risk to man of antibiotic-resistant enterobacterial pathogens if the vaccinal antigens were rendered siderophore-specific for predetermined genera, not the entire aerobic microflora.

The ubiquity of salmonellas suggests that they will be a problem for dairymen for some time, in fact, as long as dairy cows and calves are subjected to stressful environments.

**Shipping Fever, "Hemorrhagic Septicemia",
Pasteurellosis**

Hemorrhage is not a prominent feature of the bovine respiratory disease, which has borne the name "hemorrhagic septicemia" or shipping fever for many years. The name evolved from habit rather than from a description of major lesions. All septicemias show some evidence of hemorrhagic lesions, many of which, however, are far less than those which characterize the extensive hemorrhagic diatheses.

In 1880, Louis Pasteur described the organism that caused cholera in fowls, a disease characterized by enteritis with submucous and subserous hemorrhages and vascular congestion — surely a hemorrhagic disease. Later, it was learned that Pasteur's fowl cholera bacillus was indistinguishable from the organism of rabbit septicemia and of swine plague, two other hemorrhagic diseases wherein isolations of this common generic organism had been made.

In 1886, Hueppa, in Germany, grouped the organism under one name, *Bacterium septicemiae hemorrhagicae*; the "hemorrhagic septicemia" bacillus. Even in that neophytic era of microbiology, it was reported that *Bacterium septicemiae hemorrhagicae* was isolated from cases of pneumonia (and sometimes septicemia) of cattle. While hemorrhage is a common lesion of septicemia, only occasionally has that lesion been identified with bovine pneumonia. In view of the primitive status of microbiology of that day, no assurance can be given that the hemorrhagic lesions were, in fact, attributable to the organism isolated.

Bacterium septicemiae hemorrhagicae subsequently was renamed. The genus *Pasteurella* was proposed, and the species name of the animal from which the organism was isolated was assigned according to a classification scheme proposed also in 1886 by another German scientist, Flügge.

Pasteurellosis was a name proposed shortly thereafter for a group of diseases in which *Pasteurella* species were associated. The fact that pasteurellosis in cattle is identified as a respiratory syndrome, particularly in groups of confined young cattle exposed to crowded stressful conditions associated with shipping and not a hemorrhagic diathesis like fowl cholera, was not sufficient cause to disassociate the firmly established synonym "hemorrhagic septicemia" from the disease, and so it has

remained, even to this day.

Pasteurellosis (shipping fever) in cattle attributed to *Pasteurella multocida* is characterized by a bronchopneumonia with thickened pulmonic septae and moderate amounts of fibrin on the lung surface. This is in contrast to the pneumonia attributed to *Pasteurella hemolytica*, where the amount of fibrin is much greater and the lesions represent a true fibrinous pleuropneumonia (24). Affected animals show elevated temperatures, difficulty in breathing, pulmonary râles, diarrhea, pulmonary and subcutaneous edema, and in advanced cases, cyanosis of mucous membranes due to oxygen deficit brought about by the diminished area of functional lung parenchyma.

It is likely that primary infections by viruses like parainfluenza-3 virus, IBR, respiratory syncytial viruses, BVD-MD virus, or others, or by mycoplasmas, can predispose to secondary invasion by pasteurellas through damaging the protective mucociliary clearance mechanisms in the trachea and bronchi and by impairing alveolar macrophage function. There seems to be a marked tendency toward seasonal incidence of pasteurellosis that corresponds to activity of respiratory virus and mycoplasma infections, particularly in the fall and winter.

The local inflammatory effect produced by viruses such as parainfluenza-3 or IBR leads to increased fluidity of the mucus coating with consequent sneezing and coughing. These inevitably form endogenous aerosols in the respiratory passages that can result in the downward vortical carriage of bacteria from upper parts of the tract during inspiration (24).

Researchers have experienced considerable difficulty in reproducing the disease in healthy cattle by inoculating pure cultures of *Pasteurella* isolates from infected cattle. Strains of *P. multocida* can be carried as commensals in the oropharynx of many animal species and cause disease only when predisposing factors enable them to multiply uncontrollably and penetrate the physical and immunological defenses of the respiratory tract.

Under conditions of physiological or immunological stress, including subclinical infection by immunosuppressive viruses, or by alveolar macrophage depressing viruses like parainfluenza-3, virulent clones of *Pasteurella* may evolve from populations of commensals.

sal *Pasteurella* in the oropharynx and cause opportunistic infection, particularly when lung clearance is inhibited. Frank (22) has reported that combination exposures with parainfluenza-3 or infectious rhinotracheitis virus and *P. hemolytica* via aerosol in experimental studies have resulted in a range of clinical responses from none to marked clinical signs of respiratory disease.

The riddles of pathogenesis and immunoprophylaxis remain elusive and, therefore, speculative. However, in many areas of the country practicing veterinarians are becoming increasingly alarmed at the frequency of intractable and often fatal "shipping fever" cases from which antibiotic-resistant pasteurillas are being recovered (20, 23). The pathopotentiators are unknown. A search for virulent clones among nonpathogenic oropharyngeal commensals continues.

Perhaps the behavior of another oropharyngeal commensal may shed some light on the mechanism of pathogenesis. *Corynebacterium diphtheriae*, a normal inhabitant of the oropharynx, is harmless until it is attacked by phage. Then it releases a deadly exotoxin which produces serious disease in its host.

If phage is not a likely incriminating pathopotentiator, perhaps the role of plasmids, extrachromosomal genetic elements bearing pathogenic potentiators, should be explored.

There appears to be some evidence that the number of *Pasteurellae* resistant to one or more antibiotics is increasing (48). Therefore, the search for an effective immunogen becomes increasingly important. Live-culture vaccines for cattle are under study following earlier experience with live *Pasteurella multocida* for use in poultry (24), the results of which have been encouraging but for relatively short duration. Studies of serological relationship between strains of *Pasteurella multocida* by Shigidi and Mustafa in Kartoum, Sudan (53), confirmed observations that there is considerable serologic heterogeneity of strains of *P. multocida*. Heterogeneity of somatic antigen may be responsible for irregular results in the current system of vaccination against pasteurellosis, even though indirect evidence indicates that immunogenicity of *P. multocida* might be primarily, but not completely, dependent upon the capsule antigen.

As long as cattle are exposed to stress,

shipping fever or pulmonic pasteurellosis will be a problem. Knowledge obtained in the last quarter century has brought us partially closer to an understanding of the disease.

Winter Dysentery

Winter dysentery is an acute, highly contagious catarrhal hemorrhagic enteritis of cattle characterized by a brief explosive attack of diarrhea. Occurrence of abdominal pain with the profuse, watery, sometimes bloody diarrhea identifies the disease as dysentery. In the northern United States it occurs usually in housed cattle during winter and results in a moderate to marked drop in milk production. In infected herds, the attack rate may reach 100%, but fatalities are rare. The causative agent is unknown but is suspected to be a virus (11, 34).

In 1931, Jones and Little (26) described the disease and attributed its cause to a new species of bacterium, *Vibrio jejuni*, subsequently renamed *Campylobacter jejuni*. In 1957, MacPherson in Canada challenged the vibronic cause when he reproduced the disease with filtered fecal material and suggested that it is caused by a virus (34).

There followed a period of some diagnostic confusion in differentiating winter dysentery, infectious bovine rhinotracheitis, and bovine viral diarrhea-mucosal disease in the 1950's until Roberts (50) characterized winter dysentery after a 3-yr study of 25 outbreaks, and Kahrs (27), employing serologic methods, compared winter dysentery, BVD-MD, and IBR. He found winter dysentery was unrelated to these other infections.

Roberts (50) reported that the incidence of the disease peaks every 10 yr, suggesting that immunity develops in herds in a given area, holding the disease in abeyance until susceptible herd replacements ultimately make up the milking herd, permitting the disease to cycle again.

Winter dysentery seems to affect young dairy cows most frequently. Those that are lactating, pregnant, or have recently calved usually are affected most severely. Milking cows often are first affected, followed several days later by younger stock (11).

Changes in feed and weather often are suspected as predisposing factors. Other stress

factors that might increase the incidence or severity of the disease include poor nutrition, parturition, stressful stabling, and respiratory disease in confined animals. The disease is not seen in cattle on pasture in the United States (11), but apparently it has been observed in cattle on pasture in Australia (23).

Winter dysentery is not manifested by a noteworthy change in body temperature. When elevated temperature does occur, it usually precedes the onset of diarrhea by 24 to 48 h (11) and is accompanied by cough. By the time diarrhea is apparent, body temperature usually is normal.

The onset of diarrhea may be preceded by dullness, depression, decreased milk production, slight to marked inappetance, extreme thirst, cough, nasolacrimal discharge, and excessive salivation with drooling. Abdominal pain is manifested by kicking at the abdomen and lying down and getting up at frequent intervals. Rumen atony is observed sometimes. Also there is loss of condition, dehydration, weakness, tottering gait, and occasionally recumbency with reluctance or inability to get up. Probably the two most common signs preceding the onset of diarrhea are a nasolacrimal discharge and a dry, harsh, hacking, or moist cough (11).

Diarrhea strikes suddenly, swiftly, and copiously but is of short duration. It often is characterized as explosive or projectile because of the considerable velocity with which intestinal contents are released. Another characteristic feature of this disease is a moderate to severe decrease in milk production. Animals that recently have calved seem to be most affected (11). In individual animals, winter dysentery may last for a few hours but more often it lasts for a few days but less than a week. In an infected herd the duration ranges between 3 and 4 wk, with 2 wk most commonly cited (11).

Whether substantial immunity develops to winter dysentery is not known. MacPherson hypothesized that young calves are not susceptible because of maternal antibody acquired in colostrum or perhaps freedom from stress (34). He felt susceptibility was greater in 1 to 3-yr-old animals when passive maternal antibody protection has disappeared and physiologic stresses are mounting.

The sudden onset of diarrhea in adult cattle merits consideration of several diseases in the

differential diagnosis. Among these are BVD-MD, dietetic gastroenteritis, coccidiosis, parasitosis, salmonellosis, Johne's disease, and rinderpest.

No effective vaccine is available for this disease, and it is likely that such will not be available until the precise cause is determined. It may be a distinct disease caused by a single infectious agent or a syndrome.

Many treatments have been advocated, but their merits have been questioned (50). Abundant drinking water should be available for affected animals. Fox has suggested intravenous blood transfusions for gaunt recumbent animals supported by gruels of high nutritional value given by mouth (21).

Winter dysentery has been known for 50 yr, but because its cause has remained elusive, it is still an enigma needing intensive study.

EPILOGUE

Opinions may vary about the relative economic importance of these diseases. Arguments point to the need for an effective, urgent system of reporting and analyzing morbidity and mortality data in dairy herds so that predictors and estimators can be provided on an immediate feedback, continuous-flow basis. In that way incidence and prevalence can be assessed in the population at risk, predisposing factors hopefully will be made visible, patterns of disease movement can be foretold and monitored, and management more wisely attained.

The technology of microbiology and immunology, if it advances as rapidly in the next quarter century as in the one past, undoubtedly will answer many questions raised in this chronicle.

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