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.

Bovine Enteric Colibacillosis

*Dale L. Haggard, D.V.M., M.S.**

EPIDEMIOLOGY

Bovine enteric colibacillosis occurs wherever calves are maintained and is a significant cause of economic loss. The disease is most commonly seen in animals 2 to 10 days of age and may occur as early as 12 to 18 hours after birth.

There are many epidemiologic factors that influence the disease. Calves of first-calf heifers may be more susceptible, because such dams produce less colostrum with lower immunoglobulin levels than do older cows. A definite seasonal variation occurs in calf colibacillosis; the peak incidence of mortality is during the stabling period. Overcrowding and poor sanitation allow a build-up of the *E. coli* population within the pen in which the newborn is housed. Strains of *Escherichia coli* associated with an outbreak have been isolated in large numbers from pails and other equipment used for feeding. Reasons for decreased consumption of colostrum, which contributes to the occurrence, include maternal behavior, conformation of the udder, or shape of teats that makes it difficult for the calf to nurse.[7]

ETIOLOGY

The disease is caused by certain strains of *E. coli.* Such strains are described as being enteropathogenic *E. coli* (EPEC).

CLINICAL SIGNS

Enteric colibacillosis is characterized by diarrhea. Defecation is frequent and effortless. The rear quarters are pasted with fluid or semisolid feces. Feces are malodorous, may be fluid, containing chunks of partially digested

*Associate Professor, Department of Large Animal Clinical Sciences, University of Minnesota College of Veterinary Medicine, St. Paul, Minnesota

milk, or semisolid, and may be yellow-white or grey in color. Affected calves rapidly become dehydrated and lose weight. Depression, anorexia, and weakness follow. The rectal temperature is usually normal in the initial stages but becomes subnormal as the disease worsens. Affected calves die or recover in several days depending on the severity of the diarrhea and the therapy they receive. Death usually occurs in 3 to 5 days.[10]

Mild to moderately affected calves may be diarrheic for a few days and recover spontaneously with or without treatment. Those more severely affected become progressively worse over a period of several days, gradually become more weak, completely anorexic, and progressively more clinically dehydrated. The degree of dehydration will vary from 4 to 6 per cent of body weight (when it is just barely detectable clinically) to up to 10 to 16 per cent of body weight. Degree of hydration can be assessed by "tenting" the skin of the neck and measuring the time required for the skin fold to return to normal. In calves with 8 per cent dehydration, it will take 5 to 10 seconds for the skin fold to disappear; in calves with 10 to 12 per cent dehydration, it will take up to 30 seconds.[7]

MIXED INFECTIONS

Investigations of field cases of neonatal calf diarrhea have shown that infection with more than one enteropathogen occurring in different combinations is more common than infection with a single agent.[3,51] EPEC, rotavirus, coronavirus, and cryptosporidia are the enteropathogens most commonly encountered in field investigations.[76]

It has been shown that infection with both EPEC and rotavirus can precipitate a disease in circumstances in which each agent acting independently may not. It is likely that there are many other combinations that would induce a disease in calves, the severity of which may depend partly on noninfectious contributing factors and on the nature of the organisms involved.[5]

MORBIDITY AND MORTALITY

Morbidity in confined dairy calves may reach 75 per cent, but it is more commonly about 30 per cent. In beef calves, the morbidity will vary from 10 to 50 per cent in unvaccinated herds. The case fatality rate varies from 5 to 50 per cent, depending on management, observation, and therapy.[7]

DIAGNOSIS

One or more infectious agents are involved in nearly all cases of neonatal calf scours. Infection of the intestine by these agents causes abnormal intestinal function, resulting in diarrhea, dehydration, electrolyte imbalance, acidosis, starvation, and sometimes death.[6] The following procedures are used in differential diagnosis.

History

Calves affected with enteric colibacillosis are usually less than 1 week old. Rotavirus and coronavirus can affect calves from 1 day to 1 month of age. Cryptosporidia affects calves over 1 week of age.[6]

Specimens for Laboratory Examination

The ideal specimen for submission to a diagnostic laboratory is a live, acutely ill, and untreated calf. If only dead calves are offered, they should be submitted as soon after death as possible. Tissues should be removed from the animal as soon as possible in field necropsy and should include the following: (1) sections of duodenum, jejunum, ileum, and colon flushed and fixed with formalin for histologic examination; (2) unfixed impression smears of ileal mucosa for fluorescent antibody (FA) test; (3) cecal fluid fixed with formalin for electron microscopic examination for virus and flotation for protozoa; (4) portions of chilled jejunum, ileum, and colon for FA tests for rotavirus and coronavirus, bacterial culture, and virus isolation.

The importance of having a necropsy performed as soon after death as possible cannot be overemphasized, because postmortem autolysis of the gastrointestinal tract occurs very rapidly. It is important that none of the materials be frozen. Freezing greatly diminishes their diagnostic value.[17]

Histopathology

Rotavirus and coronavirus cause destruction of villous epithelial cells, which results in diarrhea because of malabsorption. Calves affected with enteric colibacillosis maintain the integrity of their intestinal mucosal structure; the diarrhea is caused by hypersecretion of intact intestinal crypt cells.[46]

Fluorescent Antibody Test

Enteropathogenic *E. coli* can be identified and enumerated by fluorescent antibody (FA) test specific for the K99 adherence pili antigen that is performed on sections of ileum or ileal impression smears.[17] Rotavirus and coronavirus can be demonstrated by FA test of fecal samples or intestinal sections of affected calves.[76]

Electron Microscopy

Rotavirus infection can be diagnosed by demonstration of virus particles in feces of affected calves using electron microscopy (EM). Using EM, coronavirus can be identified on fecal samples or intestinal sections of affected calves.[76] Enteropathogenic *E. coli* can be seen adhering to sections of intestinal mucosa using EM.[46]

Enzyme-Linked Immunosorbent Assay

Infection with rotavirus and coronavirus can be diagnosed by demonstration of the viruses in the feces of affected calves using enzyme-linked immunosorbent assay (ELISA).[14,15]

Fecal Flotation

Cryptosporidia oocysts can be detected by microscopic examination of fecal ova flotation samples. Oocysts can also be identified in Giemsa-stained fecal smears.[76]

Bacterial Culture

Fecal samples can be cultured to demonstrate the presence of *E. coli*. Identification of the K99 antigen by slide agglutination, enzyme-linked immunosorbent assay, electron microscopy, or fluorescent antibody testing is necessary to determine if the strain is enteropathogenic.

Other Procedures

Blood counts and measurement of serum immunoglobulins may assist in making a diagnosis.[6]

IDENTIFYING ENTEROPATHOGENIC STRAINS

The significance of *E. coli* in the etiology of bovine enteric colibacillosis was first recorded in 1893.[35] Because *E. coli* could be isolated from the feces of both normal and diarrheic calves, a primary objective of investigations of enteric colibacillosis was to determine which *E. coli* isolates were pathogens and involved in clinical disease.

Injured Intestinal Epithelium

In 1897, the view was expressed that injured intestinal epithelium allowed invasion by ordinary colon bacilli, which by a few hours after birth are already in the intestinal canal. By giving a newborn calf a small dose of creolin water or pykotanin solution, a severe enteritis was produced and colon bacteria occurred in the blood and organs.[34]

Colony Classification

Strains of *E. coli* produce two types of colonies. Low-convex mucoid colonies are formed by capsulated bacteria and grey, translucent colonies are formed by noncapsulated organisms. Mutation from mucoid to grey occurs readily, indicating that the presence or absence of a capsule did not indicate enteropathogenicity.[69]

Biochemical Tests

Isolates of enteropathogenic and nonenteropathogenic *E. coli* have been examined repeatedly over the decades using as many as 64 biochemical tests. These tests included fermentation of 30 different carbohydrates, hydrogen sulfide and indole production, methyl red and Voges-Proskauer reactions, litmus milk reaction, nitrate reduction, utilization of citrate and malonate, phenylalanine deamination, decarboxylation reactions on 15 amino acids, and production of amylase, catylase, lecithinase, lipase, protease, and urease. None of these tests were successful in differentiating pathogenic from nonpathogenic strains.[8]

Serological Typing

Strains of *E. coli* are serologically identified by the types of antigens they possess. The surface structures are expressed as O (somatic, cellwall) antigens, which are lipopolysaccharides; K (capsular) antigens, which are

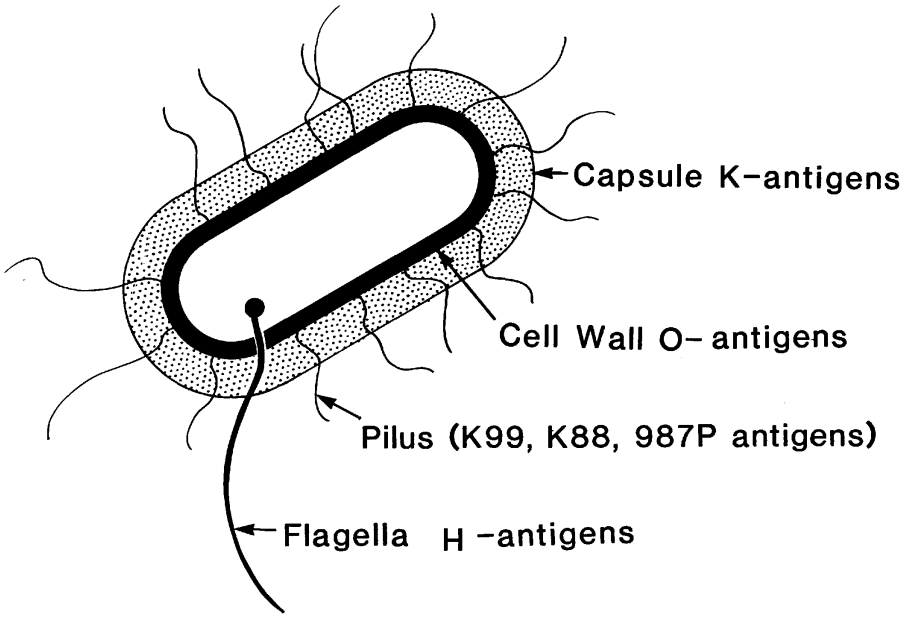

Figure 1. Schematic structure of antigenic components of *Escherichia coli.* (From Haggard, D.L.: Vaccine development in the prevention of bovine enteric colibacillosis. Compend. Contin. Educ. Pract. Vet., *6*(6):1984; with permission.)

polysaccharides; and H (flagellar) antigens, which are proteins (Fig. 1).[10,38] There are at least 150 recognized O antigens, 90 K antigens, and 50 H antigens. Each serotype is designated by the numbers of the antigens it carries—for example, 0139:K82:H2 (Table 1).[21]

Clinical investigations of outbreaks of calf enteric colibacillosis found that many different *E. coli* serotypes were capable of causing the disease.[23,51,64] Six serotypes were common in 90 per cent of the outbreaks of enteric colibacillosis in other studies.[53,58]

Enterotoxin Production

It was reported in 1976 that for a strain of *E. coli* to cause diarrhea in calves and lambs it must be able to produce heat-stable enterotoxin (ST) or heat-labile enterotoxin (LT), which stimulate secretion by intestinal cells.[24]

Table 1. *Location, Chemistry, and Function of* Escherichia coli *Antigens**

LOCATION	CHEMISTRY	PATHOGENIC FUNCTION
Somatic (O)	Lipopolysaccharide	Endotoxin production
Capsule (K)	Polysaccharide	Assists adhesion
Flagella (H)	Protein	Motility
Pilus (K99, K88, 987P)	Protein	Adhesion factor

*From Haggard, D.L.: Vaccine development in the prevention of bovine enteric colibacillosis. Compend. Contin. Educ. Pract., *6*(6):1984; with permission.)

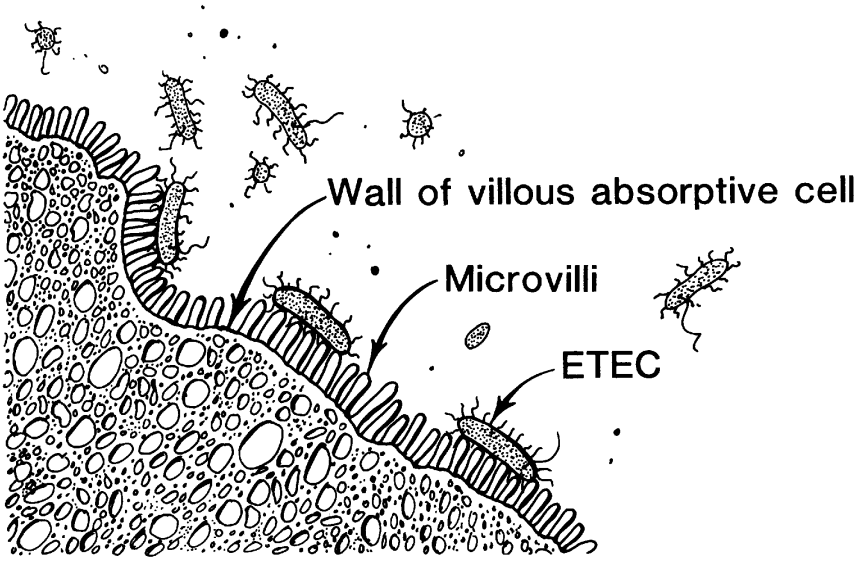

Figure 2. Drawing from electron micrograph of EPEC on surface of ileum villous absorptive cell. (From Haggard, D.L.: Vaccine development in the prevention of bovine enteric colibacillosis. Compend. Contin. Educ. Pract., 6(6):1984; with permission.)

Later it was determined that only ST is produced by such strains.[20] The ability to produce ST can be demonstrated by causing fluid distension following inoculation into a ligated segment of calf intestine[70] or by the infant mouse assay.[19] A strain of *E. coli* shown to produce enterotoxin is referred to as an enterotoxigenic *E. coli* (ETEC).

Adherence Factor

For an ETEC strain to be an enteropathogenic *E. coli* (EPEC) strain capable of causing clinical disease, it must also be capable of adhering to the intestinal mucosa of the small intestine and proliferating to a dense population (in excess of 10^8 per 5 cm segment of intestine). This results in sufficient enterotoxin to stimulate intestinal villous hypersecretion, causing diarrhea.[32]

Three adherence or colonizing factors have been identified on EPEC of calves, lambs, and pigs. They are pili (fimbriae), which are plasmid-mediated, hairlike projections on the surface of the bacteria (Figs. 1 and 2). These pili have been identified as the K88, K99, and 987P antigens. All three occur on EPEC of piglets, whereas only the K99 pilus has been commonly identified on calf and lamb EPEC.[62] The common adhesion factor of calf and lamb EPEC, which was first reported in 1971, was identified as a pilus and designated the K99 antigen in 1975.[13,62]

Pilus antigens behave serologically like capsular K polysaccharide antigens during serotyping, and some were designated as K antigens even though they were recognized as protein.[48] A new nomenclature has been

suggested to eliminate the confusion between capsular K and pilus K antigens. Pilus antigens are to be identified by the letter F (K99 is now F5).[50]

Capsule

A study in 1982 found that noncapsular mutants of an EPEC strain failed to colonize to large numbers in a calf's intestine and cause diarrhea.[24] These results were consistent with earlier work that found the capsule of some EPEC strains was required for intestinal adhesion, colonization, and full expression of pathogenicity.[61,71] It was thought that the K99 pili and capsule might act in concert to anchor the bacteria to intestinal villous epithelial cells.[24] Recently, it has been reported that most EPEC isolated from neonatal diarrheic calves had a type-A capsule, K99 pili, and produced ST. It was suggested that type-A capsular polysaccharide should also be considered a virulence factor of bovine EPEC.[65]

PATHOGENESIS

Categories in the pathogenesis of diarrhea have been described as (1) hypermotility, (2) increased intestinal permeability, (3) hypersecretion, and (4) malabsorption.[46] Enteric colibacillosis is an example of a diarrheal disease resulting from intestinal hypersecretion. In this disease, the intestinal villi, along with their digestive and absorptive capabilities, remain intact. The intestinal crypts also remain intact; however, their secretion is apparently increased beyond the absorptive capacity of the villi so that diarrhea occurs.[47]

Calves are most susceptible to intestinal colonization by EPEC immediately after birth. Age-related resistance to adhesion appears to develop in the first few days of the calf's life.[66] It has been demonstrated that prior or simultaneous rotavirus infection enables ETEC colonization of the intestine in older calves.[74] Adherence brings the bacteria into close association with the intestinal villous cells, allowing colonization, and facilitates the effect of enterotoxin on the secretory cells.[66]

Heat-stable enterotoxin initiates a buildup of cyclic guanosine 3′,5′monophosphate (cGMP) in gut secretory cells, which produces net secretion. Hypersecretion causes the loss of needed electrolytes, including bicarbonate, and fluid into diarrheic feces.[16,20] Dehydration and acidosis occur because of these losses, causing the clinical signs of enteric colibacillosis and possibly death.

LACTEAL IMMUNITY

The importance of colostrum consumption by neonatal calves for the prevention of scours was recognized as early as 1893.[44] Absorption of colostral antibodies occurs only during the first 24 hours of a calf's life. There is peak absorption at about 6 hours of age with a decrease to no absorptive capability by 24 hours of age.[4,5] Absorbed colostral antibodies are active systemically against septicemic colibacillosis. They also provide local immunity within

the intestinal lumen to protect against the scours of enteric colibacillosis.[42,43,45]

Colostral K99 antibodies are most protective if consumed previous to infection with EPEC.[41] Calves should consume about 50 ml per kg body weight of colostrum at the first feeding, and this should occur within 4 hours after birth for maximum absorption.[43] A second feeding of colostrum seems to increase the efficiency of antibody absorption from the first.[28] Consumption of colostral antibody should continue during the first 24 hours of life to maintain antibody with the intestine during the time that EPEC adhesion to the intestinal wall may occur.[36]

Colstral K99 antibodies have little effect once diarrhea has commenced; they are principally prophylactic in action. A recent study showed that K99 antibodies consumed up to 3 hours after oral challenge with EPEC were protective against enteric colibacillosis. These antibodies consumed 4 hours after challenge allowed disease to occur in most calves, with a 40 per cent mortality rate. After a 5-hour delay, there was no protective effect provided by the antibodies.[75]

VACCINE DEVELOPMENTS

Autogenous Bacterins

Before the pathogenesis of bovine enteric colibacillosis had been determined, there were many requests by veterinary practitioners for diagnostic laboratories to produce autogenous bacterins for use in problem cattle and swine herds. Efficacy reports on these products were variable. There were reports of protection in uncontrolled trials,[21,22,64] protection in partially controlled trials,[53,58] and no protection in controlled trials,[18,37,67]

O Antigen Vaccines

There was evidence that calves receiving colostral antibodies to certain strains of *E. coli* were protected against those strains.[9,30,31] It was determined, in 1961, that antibodies to the O antigens of a strain had little protective effect if that strain possessed a capsule.[13] A study in 1978 found that antibodies to the cell-wall antigen did not protect against diarrheic disease in the absence of antibodies to the capsular K antigen of the challenge strain.[54]

Capsular K Antigen Vaccines

Studies in 1961 reported that antibody to the polysaccharide capsular antigen was the main protective antibody against EPEC.[13] Many different capsular antigens were recognized on such strains of the bacteria. Colostral antibodies to the specific capsular antigen of the challenge strain have been shown to protect neonatal calves from enteric colibacillosis.[54,55,59]

Enterotoxin Vaccines

ETEC enterotoxin (ST) alone is not antigenic.[21] Studies using ST conjugated to porcine immunoglobulin (IgG) for immunization of rats demonstrated enterotoxin-neutralizing activity when ligated intestinal loops were challenged with enterotoxin.[39,40] In contrast, in an investigation in which

sows were vaccinated with ST-bovine IgG conjugate, the mortality of piglets suckled on immunized sows was not much different from that of piglets nursed on unimmunized sows.[49]

Common Capsular K Antigen Vaccines

In a 1975 Montana study, certain strains of *E. coli* were found to be prevalent in calves with diarrheic disease. In a controlled field trial using 23 herds (3508 cows) with histories of acute calf diarrhea, significantly fewer calves of cows vaccinated with the six-strain *E. coli* bacterin died of diarrheic disease than did calves of control cows. There was, however, marked interherd variation in efficacy.[57]

A four-strain *E. coli* bacterin was used in 1980 investigations to evaluate its ability to elicit passive immunity in calves of vaccinated cows. Strain B41 (0101:K-:K99) was used as the source of K99 antigen. Three other serogroups (0101:K30:K99, 09:K35:K99, 08:K85:K99) were also used because their capsular antigens were found on 90 per cent of the isolates of ETEC from diarrheic calves in Montana.[56] These same three capular antigens were also commonly associated with epizootics of neonatal calf enteric colibacillosis from other areas.[24,58] In a challenge study, all 21 calves nursing vaccinated dams were protected from acute enteric colibacillosis, whereas 14 of 20 calves nursing control dams developed the disease. In 12 privately owned herds with histories of naturally occurring diarrheic disease, significantly fewer calves nursing vaccinated dams died of enteric disease than did calves nursing control dams. There was considerable interherd variability in efficacy, which could be expected because of the variety of infectious agents, environmental conditions, and management factors that can contribute to occurrence of calf diarrhea.[56]

Pilus Antigen Vaccines

Because a variety of different capsular antigens have been shown to occur on EPEC affected calves, a vaccine based on these antigens would have to control multiple capsular antigens to provide protection against a wide variety of EPEC strains.[1] An alternate approach is to produce immunity to a common antigen possessed by many different EPEC strains. The plasmid-mediated K99 pilus antigen was the logical choice because it has been recognized as the common antigen on calf and lamb EPEC since its identification in 1975.[63] It also plays a vital role in the pathogenesis of bovine enteric colibacillosis by allowing EPEC to adhere to the intestinal mucosa and proliferate to abnormally high numbers.[58,63] Colostral antibodies from cows vaccinated with whole cell bacterins containing the K99 antigen[1,26] or purified K99 antigen[1,2,11,12,60,73] have been shown to protect calves from enteric colibacillosis via lacteal immunity. Recently, pili different from K99 have been reported to exist on calf and lamb EPEC.[12,29,52]

Specific Monoclonal Antibodies

With the use of cow-directed vaccines, precalving cows must be handled twice the first year and boostered once annually to obtain adequate levels of colostral immunity.[27] Also, the occurrence of enteric colibacillosis varies from herd to herd and year to year. This is because the epizootology of the

disease includes a variety of meteorologic, environmental, and management factors. These concerns have prompted investigations for techniques of direct oral, passive immunization of the calf against EPEC that can be used before or after the beginning of an outbreak of enteric colibacillosis.

Recently, a mouse hybridoma-derived monoclonal antibody (MCA) to the K99 antigen has been developed and evaluated in experimental challenge studies. When the MCA was administered orally to both colostrum-fed and colostrum-deprived neonatal calves, the mortality rate after challenge with virulent EPEC was significantly reduced when compared with control calves (p less than 0.001). Furthermore the severity of clinical disease was also significantly reduced in calves receiving oral K99 specific MCA.[68]

PREVENTION OF BOVINE ENTERIC COLIBACILLOSIS

Vaccines are only part of a total management program necessary for the prevention of enteric colibacillosis and other calf scours. Pregnant cows must be provided a proper level of nutrition to be in good condition at calving and to be capable of producing an adequate quantity of quality colostrum for their calves. The calving site should be clean, free of infection, and provide good footing and shelter during inclement weather. Crowding and stress should be avoided in the maternity area. If several calving pens are available, this will allow for vacating, cleaning, and disinfecting before another cow is put in the pen, especially if a calf has scoured in that pen. The pregnant cow should be observed closely to ensure that all goes well during the birth of her calf. Immediately after birth, the calf's navel should be dipped in iodine or teat-dip solution. At this time, the herdsman should ensure that the calf nurses and gets a good fill of colostrum. Monoclonal K99 antibody should be available for use if an outbreak occurs, especially with unvaccinated cow herds.

REFERENCES

1. Acres, S.D., Isaacson, R.E., Babiuk, L.A., et al.: Immunization of calves against enterotoxigenic colibacillosis by vaccinating dams with purified K99 antigen and whole cell bacterins. Infect. Immun., 25:121–126, 1979.
2. Acres, D.D., Isaacson, R.E., Khachatourians, G., et al.: Vaccination of cows with purified K99 antigen, K99+ anucleated live *E. coli*, and whole cell bacterins containing enterotoxigenic *E. coli* for prevention of enterotoxigenic colibacillosis of calves. *In* VIDO Proceedings of the International Symposium on Neonatal Diarrhea, 1978, pp. 443–456.
3. Acres, S.D., Laing, C.J., Saunders, J.R., et al.: Acute undifferentiated neonatal diarrhea in beef calves I. Occurrence and distribution of infectious agents. Can. J. Comp. Med., 39:116–132, 1975.
4. Balfour, W.E., and Comline, R.S.: Acceleration of the absorption of unchanged globulins in the newborn calf by factors in colostrum. J. Physiol., *147*:22–23P, 1959.
5. Balfour, W.E., and Comline, R.S.: The specificity of the intestinal absorption of large molecules by the newborn calf. J. Physiol., *148*:77–78P, 1959.
6. Bergeland, M.E., Johnson, D.D., and Shave, H.: Bovine cryptosporidiosis in the north central United States. *In* Annual Proceedings of the American Association of Veterinary Laboratory Diagnosticians, 1979, pp. 131–138.

7. Blood, D.C., Henderson, J.A., and Radostits, O.M.: Veterinary Medicine. Edition 5. Philadelphia, Lea & Febiger, 1979, pp. 461–471.
8. Bratten, B.A., and Myers, L.L.: Biochemical characteristics of enterotoxigenic and non-enterotoxigenic *Escherichia coli* isolated from calves with diarrhea. Am. J. Vet. Res., *38*:1989–1999, 1977.
9. Briggs, C., Lovell, R., Aschaffenberg, R., et al.: Nutritive value of colostrum for the calf. 7. Observations on the nature of the protective properties of colostrum. Br. J. Nutr., *5*:356–362, 1951.
10. Colibacillosis: Ciba Veterinary Monograph Series/Two 1967.
11. Contrepois, M., Girardeau, J.P., Doubourguier, H.C., et al.: Specific protection by colostrum from cows vaccinated with the K99 antigen in newborn calves experimentally infected with *E. coli* ent. K99. Ann. Rech. Vet., *9*(2):385–388, 1978.
12. Contrepois, M., Girardeau, J.P., Gouet, Ph., et al.: Vaccination contre l'antigene K99 et d'autres "adhesines" des *Escherichia coli* bovins. *In* Proceedings of the World Congress on Diseases of Cattle. Netherlands, 1982, pp. 332–337.
13. De Witt, C.W., and Allen, P.M.: The relationship of virulence and K antigen in *Escherichia coli*. *In* Bacteriological Proceedings of the American Society for Microbiology, 1961, p. M69.
14. Ellens, D.J., and DeLeeuw, P.W.: Enzyme-linked immunosorbent assay for diagnosis of rotavirus infections in calves. J. Clin. Microbiol., *6*:530–532, 1977.
15. Ellens, D.J., Van Balkan, J.A.M., and DeLeeuw, P.W.: Diagnosis of bovine coronavirus infections with hemadsorption-elution-hemagglutination assay. *In* VIDO Proceedings of the International Symposium on Neonatal Diarrhea, 1978, pp. 321–330.
16. Field, M., Graf, L.H., Laird, W.J., et al.: Heat-stable enterotoxin of *Escherichia coli:* In-vitro effects on guanylate cyclase activity, cyclic GMP concentration, and ion transport in small intestine. Proc. Natl. Acad. Sci. U.S.A., *75*:2800–2804, 1978.
17. Francis, D.H.: Diagnosis of colibacillosis. *In* Symposium on Neonatal Calf Scours. Western States Veterinary Conference, Las Vegas, Nevada, 1982, pp. 17–24.
18. Gay, C.C., McKay, K.A., and Barnum, D.A.: Studies on colibacillosis of calves. Can. Vet. J., *5*:297–308, 1964.
19. Gainella, R.A.: Suckling mouse model for detection of heat stable *Escherichia coli* enterotoxin: Characteristics of the model. Infect. Immun., *14*:95–99, 1976.
20. Gainnella, R.A., and Drake, K.W.: Effect of purified *Escherichia coli* heat-stable enterotoxin on intestinal cyclic nucleotide metabolism and fluid secretion. Infect. Immun., *24*:19–23, 1979.
21. Gillespie, J.H., and Timoney, J.F. (eds.): Hagan and Brunner's Infectious Diseases of Domestic Animals. Edition 7. Comstock Publishing Association, 1981, pp. 74–84.
22. Glantz, P.J., Rothenbacher, H., and Hokanson, J.F.: *Escherichia coli* isolated from dams and their calves: Bacteriologic and serologic test results. Am. J. Vet. Res., *29*:1561–1566, 1968.
23. Gossling, J., McKay, K.A., and Barnum, D.A.: Colibacillosis of calves in Ontario. II. Association of certain serotypes of *Escherichia coli* with calf scours. Can. Vet. J., *5*:9, 220–228, 1964.
24. Hadad, J.J., and Gyles, C.L.: Detection of bovine enteropathogenic *Escherichia coli* by direct fluorescent antibody technique. Am. J. Vet. Res., *39*:1651–1655, 1978.
25. Hadad, J.J., and Gyles, C.L.: The role of K antigens of enteropathogenic *Escherichia coli* in colonization of the small intestine of calves. Can. J. Comp. Med., *46*:21–26, 1982.
26. Haggard, D.L., Johnson, D.W., Springer, J.A., et al.: Evaluation of an *Escherichia coli* bacterin for preventing bovine neonatal enteric colibacillosis. Vet. Med. Small Anim. Clin., *77*(19):1391–1394, 1982.
27. Haggard, D.L., Springer, J.A., and Vosdingh, R.A.: Efficacy of a single annual booster inoculation of cows with *Escherichia coli* bacterin for preventing enteric colibacillosis in neonatal calves. Vet. Med. Small Anim. Clin., *78*(19):1525–1527, 1982.
28. Halliday, R., et al.: Passive immunity in the lamb. Effects of a second feed of colostrum on antibody absorption from the first feed. Res. Vet. Sci., *21*:173–175, 1976.
29. Huchappa, G.J., et al.: Controlling colibacillosis in neonatal calves. Vet. Med., *79*(3): 388–393, 1984.
30. Ingram, P.L., Lovell, R., Wood, P.C., et al.: *Bacterium coli* antibodies in colostrum and their relation to calf survival. J. Pathol. Bacteriol., *72*:561–568, 1956.

31. Ingram, P.L., Lovell, R., Wood, P.C., et al.: Further observations on the significance of colostrum for the calf. 11. The immunological importance of colostrum. *In* Proceedings of the International Dairy Congress, 1953, pp. 1369–1376.
32. Isaacson, R.E., Moon, H.W., and Schneider, B.S.: Distribution and virulence of *Escherichia coli* in the small intestine of calves with and without diarrhea. Am. J. Vet. Res., *39*:11, 1750–1755, 1978.
33. Isaacson, R.E., Schneider, R.A., and Moon, H.W.: *Escherichia coli* in the small intestines of calves with and without diarrhea: Identification of virulence factors. *In* VIDO Proceedings of the International Symposium on Neonatal Diarrhea, 1978, pp. 17–24.
34. Jensen, C.O.: Some observations on infection with *Bacterium coli* through the alimentary canal in newborn calves. Biological Society Meeting, 1897. Hospitalstidende, *40*:1158–1164, 1897.
35. Jensen, C.O.: Ueber die Kalberruhr und deren Aetiologie. Munchen. Prax. Tierheilkd., *4*:97–124, 1893.
36. Johnston, N.E., Estrella, R.A., and Oxender, W.D.: Resistance of neonatal calves given colostrum diet to oral challenge with a septicemia-producing *E. coli*. Am. J. Vet. Res., *38*:1323–1326, 1977.
37. Jones, J.E.T., Sellers, K.C., and Smith, H.W.: The evaluation of live and dead *Escherichia coli* vaccines administered to the pregnant female in the prevention of scouring (diarrhoea) in piglets. Vet. Rec., *74*:202–203, 1962.
38. Kauffman, F.: The serology of the *Coli* group. J. Immunol., *57*:71–100, 1947.
39. Klipstein, F.A., Engert, R.F., and Clements, J.D.: Protection in rats immunized with *Escherichia coli* heat stable enterotoxin. Infect. Immun., *34*:637–639, 1981.
40. Levine, M.M., Kaper, J.B., Black, R.E., et al.: New knowledge on pathogenesis of bacterial enteric infections as applied to vaccine development. Microbiol. Rev., *47*(4):1983, pp. 510–660.
41. Logan, E.F., Pearson, G.R., and McNulty, M.S.: Studies on the immunity of the calf to colibacillosis. VII. The experimental reproduction of enteric colibacillosis in colostrum-fed calves. Vet. Rec., *101*:443–446, 1977.
42. Logan, E.F., and Penhale, W.J.: Studies on the immunity of the calf to colibacillosis. II. Vet. Rec., *88*:222–228, 1971.
43. Logan, E.F., and Penhale, W.J.: Studies on immunity of the calf to colibacillosis. III. Vet. Rec., *88*:628–632, 1971.
44. Lovell, R.: Intestinal diseases of young calves with special reference to infection with *Bacterium coli*. Vet. Rev., *1*:1–32, 1955.
45. McEwan, A.D., Fisher, E.W., and Selman, I.W.: Observations on the immune globulin levels of neonatal calves and their relationship to diseases. J. Comp. Pathol., *80*:259–265, 1974.
46. Moon, H.W.: Mechanisms in the pathogenesis of diarrhea: A review. J. Am. Vet. Med. Assoc., *172*(4):443–448, 1978.
47. Moon, H.W.: Pathogenesis of enteric diseases caused by *Escherichia coli*. *In* Brandley, C.A., and Cornelius, C.E. (eds.): Advances in Veterinary Science and Comparative Medicine. New York, Academic Press, 1974, pp. 179–211.
48. Moon, H.W.: Pili as protective antigens in vaccines for the control of enterotoxigenic *Escherichia coli* infections. *In* VIDO Proceedings International Symposium on Neonatal Diarrhea, 1978.
49. Moon, H.W., Baetz, A.L., and Gianella, R.A.: Immunization of swine with heat-stable *Escherichia coli* enterotoxin coupled to carrier protein does not protect suckling pigs against an *Escherichia coli* strain that produces heat-stable enterotoxin. Infect. Immun., 39:990–992, 1983.
50. Moon, H.W., Kohler, E.M., Schneider, R.A., et al.: Prevalence of pilus antigens, enterotoxin types, and enteropathogenicity among K88-negative enterotoxigenic *Escherichia coli* from neonatal pigs. Infect. Immun., 27:222–230, 1980.
51. Morin, M., Lariviere, S., and Lallier, R.: Pathological and microbiological observations made on spontaneous cases of acute neonatal calf diarrhea. Can. J. Comp. Med., *40*:228–240, 1976.
52. Morris, J.A., Thorns, C., Scott, A.C., et al.: Adhesion in vitro and in vivo associated with an adhesive antigen (F41) produced by a K99 mutant of the reference strain *E. coli* B41. Infect. Immun., *36*:1146–1153, 1982.

53. Myers, L.L.: Characterization of *Escherichia coli* obtained from newborn calves with diarrhea. Infect. Immun., *11*:493–496, 1975.
54. Myers, L.L.: Enteric colibacillosis in calves: Immunogenicity and antigenicity of *Escherichia coli* antigens. Am. J. Vet. Res., *39*:761–765, 1978.
55. Myers, L.L.: Passive protection of calves against enteric colibacillosis by vaccination of their dams with capsular and fimbrial K antigens of *Escherichia coli*. *In* VIDO Proceedings International Symposium on Neonatal Diarrhea, 1978, pp. 427–433.
56. Myer, L.L.: Passive protection of calves against experimentally induced and naturally occurring enteric colibacillosis. Am. J. Vet. Res., *41*:12, 1952–56, 1980.
57. Myers, L.L.: Vaccination of cows with an *Escherichia coli* bacterin for the prevention of naturally occurring diarrheal disease. Am. J. Vet. Res., *37*(7):831–834, 1976.
58. Myers, L.L., and Guinee, P.A.M.: Occurrence and characteristics of enterotoxigenic *Escherichia coli* isolated from calves with diarrhea. Infect. Immun., *13*(4):1117–1119, 1976.
59. Myers, L.L., Newman, F.S., Wilson, R.A., et al.: Passive immunization of calves against experimentally induced enteric colibacillosis by vaccination of dams. Am. J. Vet. Res., *34*(1):29–33, 1973.
60. Nagy, B.: Vaccination of cows with a K99 extract to protect newborn calves against experimental enterotoxic colibacillosis. Infect. Immun., *27*:21–24, 1980.
61. Nagy, B., Moon, H.W., and Isaacson, R.E.: Colonization of porcine small intestine by *Escherichia coli* strains. Ileal colonization and adhesion by pig enteropathogens which lack K88 antigen and by some capsular mutants. Infect. Immun., *13*:1214–1220, 1976.
62. Orskov, I., and Orskov, F.: Serology of *Escherichia coli* fimbriae. Prog. Allergy., *33*:80–105, 1983.
63. Orskov, I., Orskov, F., Smith, H., et al.: The establishment of K99, a thermolabile, transmissible *Escherichia coli* K antigen, previously called "Kco," possessed by calf and lamb enteropathogenic strains. Acta Pathol. Microbiol. Scand., *83*:31–36, 1975.
64. Rees, R.A.: Studies on *Escherichia coli* of animal origin. I. *E. coli* from natural outbreaks of colibacillosis of calves. J. Comp. Pathol., *68*:388–396, 1958.
65. Renault, L., Contrepois, M., Dubourguier, H.D., et al.: Interest of slide agglutination test of A antigen for the detection of enteropathogenic strains of *Escherichia coli* in diarrheric calves. *In* Proceedings International Symposium of Veterinary Laboratory Diagnosticians, Lucerne, Switzerland, 1980, pp. 420–423.
66. Runnels, P.L., Moon, H.W., and Schneider, R.A.: Development of resistance with host age to adhesion of K99+ *Escherichia coli* to isolated intestinal epithelial cells. Infect. Immun., *28*:298–300, 1980.
67. Sellers, K.C., and Smith, H.W.: The evaluation of a dead *Escherichia coli* vaccine administered during pregnancy in the prevention of scouring (diarrhoea) in calves. Vet. Rec., *74*:7, 203–204, 1962.
68. Sherman, D.M., Acres, S.D., Sadowski, P.L., et al.: Protection of calves against fatal enteric colibacillosis by orally administered *Escherichia coli* K99 specific monoclonal antibody. Infect. Immun., *42*:653–658, 1983.
69. Smith, T., and Bryant, G.: Studies on pathogenic bacteria from bovine sources. II. Mutations and their immunological significance. J. Exp. Med., *46*:133–140, 1927.
70. Smith, H.W., and Halls, S.: Observations by the ligated intestinal segment and oral inoculation methods on *Escherichia coli* infection in pigs, calves, lambs, and rabbits. J. Pathol. Bacteriol., *93*:499–529, 1967.
71. Smith, H.W., and Huggins, M.B.: The influence of plasmid-determined and other characteristics of enteropathogenic *Escherichia coli* on their ability to proliferate in the elementary tracts of piglets, calves and lambs. J. Med. Microbiol., *11*:471–492, 1978.
72. Smith, H.W., and Linggood, M.A.: Further observations on *Escherichia coli* enterotoxins with particular regard to those produced by atypical piglet strains and by cow and lamb strains: The transmissible nature of these enterotoxins and of a K antigen possessed by calf and lamb strains. J. Med. Microbiol., *5*:243–250, 1971.
73. Snodgrass, D.R., Nagy, L.K., Sherwood, D., et al.: Passive immunity in calf diarrhea: Vaccination with K99 antigen of enterotoxigenic *Escherichia coli* and rotavirus. Infect. Immun., *37*:586–590, 1982.
74. Snodgrass, D.R., Smith, M.L., and Krautil, F.L.: Interaction of rotavirus and enterotox-

igenic *Escherichia coli* in conventionally reared dairy calves. Vet. Microbiol., 7:51–60, 1982.
75. Trainin, Z., Brenner, J., Meirom, R., et al.: Oral immunization of young calves against enteropathogenic *E. coli*. *In* Proceedings International Congress on Diseases of Cattle, Tel Aviv, 1980, p. 1313.
76. Tzipori, S.: The aetiology and diagnosis of calf diarrhea. Vet. Rec., *108*:510–514, 1981.

Department of Large Animal Clinical Sciences
College of Veterinary Medicine
University of Minnesota
St. Paul, Minnesota 55108