

## **UC Davis**

### **UC Davis Previously Published Works**

**Title**

Diagnostics of Dairy and Beef Cattle Diarrhea

**Permalink**

<https://escholarship.org/uc/item/99h5c91c>

**Journal**

Veterinary Clinics of North America Food Animal Practice, 28(3)

**ISSN**

0749-0720

**Author**

Blanchard, Patricia Carey

**Publication Date**

2012-11-01

**DOI**

10.1016/j.cvfa.2012.07.002

Peer reviewed



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.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

# Diagnostics of Dairy and Beef Cattle Diarrhea

Patricia Carey Blanchard, DVM, PhD

## KEYWORDS

• Calf diarrhea • *Cryptosporidium* • Coronavirus • Rotavirus • *E coli* • *Salmonella*

## KEY POINTS

- Calf diarrhea is a multifactorial disease related to a combination of host and pathogen factors.
- The most common pathogens found in diarrheic calves are *Cryptosporidium*, rotavirus, coronavirus, *Salmonella*, attaching and effacing *Escherichia coli* (enterohemorrhagic *Escherichia coli* [EHEC], enteropathogenic *Escherichia coli* [EPEC], Shiga toxin-producing *Escherichia coli* [STEC]), and F5 (K99) *E coli*.
- Increased mortality and morbidity are often due to the presence of more than one pathogen.
- Identifying subsets of affected animals via farm interviews and record review may lead to key factors in which intervention could prevent future cases.

## INTRODUCTION

The clinical history can be an important tool in the diagnosis, prevention, and treatment of causes of calf diarrhea. The age of onset of diarrhea is an important factor in ruling in or out some agents. F5 (K99) *E coli* typically causes diarrhea in calves less than 6-days old, whereas *Cryptosporidium* will not be detectable in the feces before 3-days old and coccidia not before 15 to 21 days due to their prepatent periods. The presence of blood in the feces of calves less than 30-days old is most commonly associated with *Salmonella*, coronavirus, attaching and effacing *E coli*, and, rarely, *Cryptosporidium* and *Clostridium*.

Identifying subsets of affected animals via farm interviews and record review may lead to key factors in which intervention could prevent future cases. For instance, in a dairy situation, if affected calves are disproportionately born in the evening, weekends, or holidays, this might indicate inadequate training of some personnel responsible for ensuring calves receive colostrum and are moved to a clean, dry area (calf

---

The author has nothing to disclose.

University of California Animal Health and Food Safety Laboratory, School of Veterinary Medicine, University of California-Davis, 18830 Road 112, Tulare, CA 93274, USA

E-mail address: [pcbanchard@ucdavis.edu](mailto:pcbanchard@ucdavis.edu)

Vet Clin Food Anim 28 (2012) 443–464

<http://dx.doi.org/10.1016/j.cvfa.2012.07.002>

[vetfood.theclinics.com](http://vetfood.theclinics.com)

0749-0720/12/\$ – see front matter © 2012 Elsevier Inc. All rights reserved.

hutch, calf pen or clean cow-calf area). When most affected calves are the offspring of primiparous heifers and the dams' colostrum is fed to calves, this might indicate dry cow vaccines (rotavirus, coronavirus, and K99 *E coli*) that are used in multiparous cows have not been given to first-calving heifers. First-calf heifers may also have lower quality and quantity of colostrum. In beef cow-calf operations, use of pens for calving and nursing cows may lead to build up of pathogens shed by cows and their calves over the duration of the calving season, thereby exposing newborn calves to higher pathogen loads and increasing the likelihood of their developing diarrhea. The calving pen or calf housing may be a source of pathogens that persist from year to year in moist cool areas.

The source of milk may be a factor if hospital milk with antibiotics or unpasteurized milk with pathogens (ie, *Salmonella*) is fed to calves. The pasteurizer might be overheating and degrading the milk quality or not reaching sufficient time and temperature to kill pathogens. Diarrhea outbreaks following the purchase of a new lot, source, or type of calf milk replacer might indicate product quality problems.

Sentinel bull calves may be used when calf diarrhea results in high morbidity but low mortality and when the owner is unwilling to sacrifice a potential replacement heifer calf. Sentinel calves should have necropsies performed within 1 to 2 days after onset of diarrhea at the age when other calves on the premise are also affected. To improve detection of pathogens, these calves should not be treated with any therapeutics besides electrolytes and supportive therapy. Transport and comingling of calves from multiple premises may result in exposure to pathogens to which some calves may not have colostral immunity. Purchase of calves less than 30-days old can bring pathogens on to a farm and expose a naïve population of cows and calves.

Response to antibiotic treatment would support the presence of a bacterial component. Bacterial overgrowth secondary to undigested feed in the lower intestine may show some response to antibiotics, even though the primary cause is villus atrophy from a viral or *Cryptosporidium* infection. An antibiotic treatment failure does not rule out bacterial causes because the organism may be resistant or the antibiotic may not attain adequate distribution and therapeutic levels to kill bacteria in the intestinal lumen. Use of antibiotics on nonbacterial diarrheas may worsen the diarrhea by suppressing the normal flora, which could allow yeast, *Salmonella*, and attaching and effacing *E coli* to overgrow.

#### FIELD NECROPSY FOR CALF DIARRHEA

Calves should be assessed for presence of dehydration because dehydrated calves may no longer have evidence of the diarrhea. Other causes of dehydration, such as prolonged lack of fluid intake or renal disease, can be ruled out by the history and gross and histologic evaluation of kidneys. During the necropsy, note the presence or absence of fat stores around the kidney and size of the thymus. These observations give an indication of the length of time the animal has been ill. Decreased fat stores are more common in 10- to 21-day old calves, particularly in cold, wet weather.

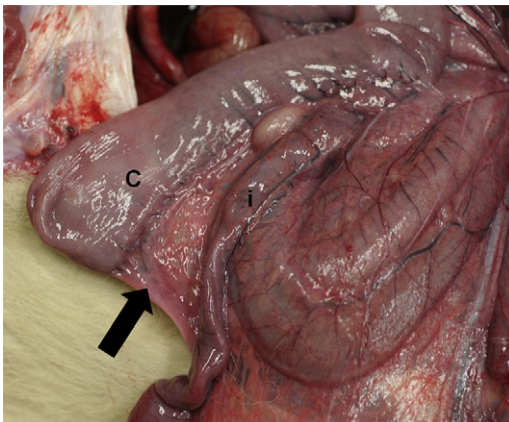
Examine the oral cavity and esophagus for evidence of erosion, proliferation (bovine papular stomatitis virus), and bruises. Examine the contents of the forestomachs. Unusual color (eg, pink, green) or texture (gelatinous) of contents may indicate the use of oral medications. White plaques progressing to curd like attachments on the rumen, reticulum, or omasum mucosa are commonly seen with yeast infections. Small, often red, raised foci may be present in the rumen mucosa and this is a nonspecific vesicular and suppurative change. Determine if there is fermenting milk or curd in the rumen because this can cause mucosal irritation. Assess the position, size, color,

thickness, and texture of the abomasum. Reddening and edema often indicate inflammation, black linear ulcers or round deep ulcers should be noted and collected for histopathology (see later discussion).

Examination of the small intestine should include spot checks of areas with different color, thickness, and contents. Place 1 cm, partially opened sections of four different areas of the small intestine into formalin. Include the following areas: one duodenum, one midjejunum, one between midjejunum and distal ileum, and, the last, and most important, the ileum at the ileocecal ligament (**Fig. 1**). Collect a second section of ileum to be submitted unfixed. Examine the cecum, which may have a full thickness infarct on the antimesenteric border or a circumferential infarct. These lesions are of undetermined cause. The cecum may have milder lesions similar to the spiral colon. Spiral colon sections should be submitted both fresh and fixed with two loops (2 cm) attached to each other to identify the tissue as spiral colon (see later discussion). The descending colon should also be examined and, if abnormal, a section submitted for histopathology. Collect 2 to 5 mL of feces in a tube or bag for fecal tests because the contents remaining in the spiral colon alone may be insufficient for all the testing needed. See **Table 1** for suggested samples to submit from field necropsies.

#### TECHNIQUES FOR DIAGNOSIS OF PATHOGENS

There is no one test that will detect a pathogen with 100% accuracy. **Table 2** lists a summary of diarrhea pathogens, specimen, and types of tests available. Each test method has limitations; the laboratory to which the veterinarian submits samples should be able to provide this information. Case-control studies and fecal surveys have detected all enteric pathogens associated with calf diarrhea in calves that do not have diarrhea. Both host and pathogen factors influence the severity and likelihood a calf will develop diarrhea. The major host factors include the general health, nutrition, immunity to specific pathogens, and age of exposure. The major pathogen factors include the number of infectious particles, number of concurrent diseases, and virulence of the agent. For this reason, it is incumbent on the clinician to interpret the detection of pathogens in the context of the signs and clinical history. Necropsy and histopathology of good quality tissues can aid in determining the role of an agent in causing diarrhea by the detection of lesions in the tissues associated with that agent.



**Fig. 1.** Ileocecal ligament (*arrow*) identifies the location of the cecum (c) and ileum (i).

Tissue	Size	Container	Use
Lung Liver	8 × 6 × 6 cm	Bag (separate)	Culture (for sepsis) Toxicology
Ileum (at ileocecal ligament)	2 cm long	Bag (separate)	Culture FA
Spiral colon (2 attached loops)	2 cm long	Bag (separate)	Culture FA
Feces	2–5 mL	Bag (separate)	Culture EM agELISA Lateral flow Etc
Ileum (at ileocecal ligament) Midjejunum and distal jejunum Duodenum (1each)	1 cm (part open)	10% formalin	Histopathology
Spiral colon (2 attached loops)	1 cm long (part open)	10% formalin	Histopathology
Lung Heart Liver kidney Thymus Spleen	1 × 1 × 1 cm	10% formalin	Histopathology
Lesion (fresh and fixed)	—	Fresh and 10% formalin	Testing as appropriate

Molecular diagnostics, most often polymerase chain reaction (PCR)-based tests, are becoming more common in diagnostic laboratories and present their own challenges in interpretation. PCR methods are usually more sensitive ( $\sim 10^3$  organisms/mL) than antigen-detection methods ( $10^4$ – $10^7$  organisms/mL), such as ELISA, fluorescent antibody (FA), agglutination, and chromatography (lateral flow, dipstick); and more sensitive than direct visualization ( $10^6$ – $10^7$  organisms/mL), such as electron microscopy (EM), fecal flotation, or stained fecal smears.<sup>1,2</sup> However, PCR methods may miss viruses or bacteria that have mutated or drifted from the sequence specific to the primers in use.<sup>1</sup> PCR methods may detect lower levels of pathogens that may not be causing disease due to age resistance (F5 [K99] *E coli*) or may detect the pathogen after the signs of illness have resolved.<sup>2</sup> PCR methods detect pathogens even in the presence of antibody-antigen complexes. However, if the agent is bound to antibody, it is less likely to be causing disease, so detection by PCR may overestimate its significance. PCR methods may fail to detect an agent due to inhibitory substances in the sample, which is more likely to occur in fecal samples than in lung or nasal swabs. Newer methods to extract nucleic acid have overcome many of the problems posed by testing of feces for organisms such as *Salmonella*, making PCR as sensitive as culture. However, the sensitivity of each method may vary with the species of *Salmonella* found in the sample.<sup>1,3</sup> Dead or stressed bacteria can be detected by PCR in samples in which they might not grow in cultures.<sup>3</sup> Sample quality, quantity, appropriate type, storage, and transport are all important issues to be aware of when submitting samples for PCR to ensure the pathogen of interest is not denatured or

<b>Table 2</b>		
<b>Detection methods and specimen type for the diagnosis of calf diarrhea agents</b>		
<b>Pathogen</b>	<b>Specimen</b>	<b>Test Methods</b>
<i>Clostridium perfringens</i> Type C	Small intestine contents (frozen)	Culture and genotyping, toxin: agELISA, PCR
<i>Clostridium difficile</i>	Feces (if alive) Intestine lesion (fixed) Colon contents or feces (fresh or frozen) Colon and ileum (fixed)	HP (tissue) Culture and toxin testing HP (tissue)
<i>E coli</i> , K99 (F5)	Feces and/or ileum content Ileum (fixed)	agELISA Culture IC, LA, SA, PCR HP
<i>E coli</i> , attaching and effacing	Ileum and colon (fresh and fixed)	HP and culture (typing, PCR eae gene and/or toxin detection)
<i>Salmonella</i> spp	Feces Intestine Tissues	Culture, IC, PCR
Coronavirus	Colon and ileum (fresh-FA) Feces	FA, HP agELISA, EM, IC, PCR
Rotavirus	Feces, small intestine (fresh- FA)	agELISA, EM, FA, IC, LA, PCR
<i>Cryptosporidium</i> spp	Feces	AF, agELISA, FA, Flotation, IC, PCR
Coccidia	Feces Colon	Flotation, McMaster's HP, scraping- direct (unfixed)
Nematodes	Feces Abomasum, intestine	Flotation, McMaster's Gross examination
Septicemia	Lung and/or liver (fresh)	Culture
Copper deficiency	Liver Serum	ICP-AES or AA

**Abbreviations:** AA, atomic absorption; AF, acid-fast stain on direct smear; agELISA, antigen ELISA; EM, electron microscopy; FA, fluorescent antibody test; HP, histopathology; IC, immunochromatography assay (lateral-flow agELISA); ICP, inductively coupled plasma atomic emission spectroscopy; LA, latex agglutination; PCR, polymerase chain reaction with nucleic acid probe; SA, slide agglutination.

destroyed. PCR methods will increase the detection of low levels of organisms that arise from cross-contamination from one calf to another when fecal samples are collected using the same instruments to open the intestinal tract or while wearing the same pair of examination gloves to collect feces from multiple animals. If the goal is simply to determine the presence of the agent in a group of animals, cross-contamination is less of a concern on the same premise than if the goal is to determine the prevalence of agents in clinically affected animals to prioritize prevention strategies. Cleaning instruments well between premises is vital to ensure agents are not detected on a premise where they do not exist due to use of contaminated instruments from another premise.

EM has been used for virus detection in feces for decades. This technique requires approximately  $10^6$  to  $10^7$  particles/g or ml of feces for detection, although this level can be reduced by the use of immunoelectron microscopy.<sup>4</sup> Particle integrity, the

likelihood to become damaged in processing or during storage so as to make it unidentifiable, or the presence of cell material that can look like the virus particle, can cause false negative or false positive results particularly for coronaviruses.<sup>1,4</sup> EM, like virus isolation, has the unique ability to detect novel viruses or viruses for which there is no diagnostic assay. The drawback is that EM does not always allow the determination of the type of virus, particularly when small round viruses are detected. These viruses may have no specific visual characteristic that differentiate them from among several types of viruses in the same size range.

Antigen-detection assays (ELISA, agglutination, immune-chromatography, immunohistochemistry [IHC], and FA) are based on detection of conserved antigens (often proteins) found among a heterologous agent population. Their advantage is they allow detection of a wide array of similar agents. For instance, group A rotavirus ELISA assays designed for human medicine will detect group A rotavirus in many species including calves.<sup>2</sup> A disadvantage is that the host or colostrum antibody binding to the antigen will inhibit detection. However, calves with antibody may not develop illness because their immune response is preventing or resolving the infection.

Culture methods on feces using enrichment broth for *Salmonella* may detect isolates missed by PCR and vice versa.<sup>1,3</sup> The precollection use of antibiotics and overgrowth of normal flora or altered flora, particularly in samples that are not freshly collected, may lead to failure to detect the pathogen by culture. Virus isolation is particularly difficult on feces due to the presence of bacteria and other agents that may be toxic to cell cultures.

### INFECTIOUS CAUSES OF DIARRHEA IN CALVES LESS THAN 3-MONTHS OLD

The most common causes of diarrhea reported in calves less than 30-days old are rotavirus and *Cryptosporidium*.<sup>5,6</sup> **Table 3** shows the agents in the frequency they

Pathogen	Positive (%)	Mean Age (Days)	Age Range (Days)
<i>Cryptosporidium</i> sp	37.2	13.0	3–33
Coronavirus	30.5	10.4	1–30
Rotavirus	26.6	10.5	1–32
<i>Salmonella</i> spp:	15.7	—	—
<i>Salmonella</i> group D1 ( <i>S dublin</i> )	5.8	17.1	2–30
<i>Salmonella</i> group C2 ( <i>S newport</i> )	4.2	8.7	1–30
<i>Salmonella</i> group B ( <i>S typhimurium</i> )	2.7	8.1	1–30
<i>Salmonella</i> group E	2.3	12.6	2–21
<i>Salmonella</i> C1 and not grouped	0.7	—	—
Attaching and effacing <i>E coli</i>	10.5	12.0	1–31
K99 <i>E coli</i>	4.5	2.3	1–7
Bovine viral diarrhea virus	1.3	16.1	2–30
Production type	%	—	—
Calf ranch	62	—	—
Dairy	33	—	—
Beef	2	—	—
Not reported/Other	3	—	—



were detected, mean age, age range, and production type from calves less than 35-days old submitted over a 4-year period (January 2008–January 2012) to the California Animal Health and Food Safety Laboratory (CAHFS) in Tulare. Only cases in which whole calves or tissues from calves including ileum and colon were submitted were included. These data show coronavirus as the second most common pathogen, unlike several other studies based on testing of feces from live animals. This may reflect a higher mortality due to coronavirus or, possibly, a more sensitive detection method using FA on colon tissue compared with methods used on feces only (ELISA, EM). Like other studies, most calves had more than one pathogen detected. More than 95% of these animals originated from calf ranches or dairies. Fecal only submissions were excluded because coronavirus and attaching and effacing *E coli* could not be evaluated in those animals.

After 30-days of age, *Salmonella dublin*, coccidia, and occasionally rumen acidosis are the more common causes of diarrhea. Bovine viral diarrhea virus (BVDv) and abomasal parasitism in pasture calves occurs most often after 3-months of age.

### **CLOSTRIDIUM PERFRINGENS TYPE C AND CLOSTRIDIUM DIFFICILE**

*Clostridium perfringens* type C is a rare cause of enteritis or diarrhea in calves less than 10-days old. The gross pathologic lesions in the small intestine are hemorrhagic and necrotizing. If diarrhea is present, there will be blood or a red color to the feces. The major differentials when performing a necropsy of a calf that has died of *C perfringens* type C is a mesenteric root torsion, segmental intestinal volvulus, intestinal entrapment, or a severe *Salmonella* infection. Confirmatory diagnosis of this infection requires the detection of beta and alpha toxin in intestinal contents. Intestine contents from dead animals or feces from live animal should be frozen as soon as possible to prevent the breakdown of the toxin. In some cases, only beta toxin will be detected and these should also be considered strong evidence of *C perfringens* type C presence. Isolation of *C perfringens* alone is not diagnostic unless genotyping reveals the organisms are type C. Normal flora includes *C perfringens* type A, which has also been reported to cause gastroenteritis in calves. Typical gross and histologic lesions of small intestine necrosis, hemorrhage, suppurative inflammation, and large numbers of large gram-positive rods provides a strong presumptive diagnosis of *C perfringens* type C.

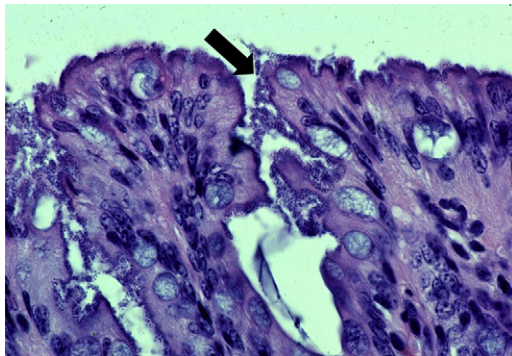
*Clostridium difficile* toxins have been shown experimentally and naturally to be associated with small intestinal villus tip degeneration and superficial colon erosion with fibrin and neutrophil and eosinophil exudate. Experimentally purified toxin B caused more severe small intestine and colon ulceration. Both the toxin and organism (by culture) have been found in diarrhea and nondiarrhea calves.<sup>7</sup>

### **E COLI, ATTACHING AND EFFACING**

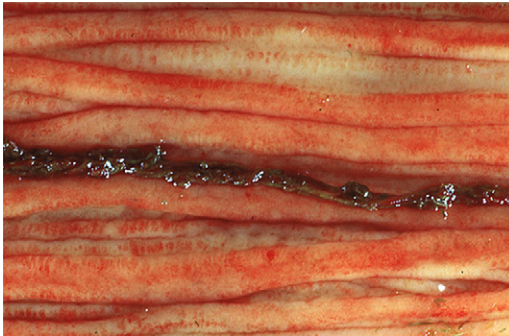
Moxley and Smith<sup>8</sup> wrote an excellent review of the literature of experimental and natural infection and epidemiologic studies related to finding attaching and effacing *E coli* (AEEC; with intimin [eae] genes) with or without Shiga toxin (also known as verotoxin), Stx1 or Stx2 genes, and Shiga toxin-producing *Escherichia coli* (STEC) without the eae genes. Using the human literature definitions, *E coli* with both Shiga toxin and eae genes are considered enterohemorrhagic *Escherichia coli* (EHEC) and include O157:H7, which can be found in cattle as carriers and has been shown to attach and cause diarrhea in some studies.<sup>8</sup> *E coli* with the eae genes but no Shiga toxin genes are referred to as enteropathogenic *Escherichia coli* (EPEC). All three groups (EHEC, EPEC, and STEC) have been found in diarrhea calves with natural

infections and have been used successfully to reproduce enteritis and/or colitis in some, but not all, inoculated calves. Several studies demonstrated that calves less than 24-hours old without colostral antibodies are more likely to develop diarrhea and intestinal lesions when inoculated with the organisms than are older calves or calves that have received colostrum.<sup>8</sup> Epidemiologic studies that are stratified by age also show as much as 10 to 12 times higher recovery of AEEC or STEC-type organisms in calves less than 30-days old with diarrhea compared with controls without diarrhea.<sup>8</sup> In calves over 30-days old or in reports in which calves of all ages were clustered, as well as in adult animals, there is either no difference or only an approximately twofold difference in fecal detection rates between diarrhea and nondiarrhea animals.<sup>8,9</sup> Based on histologic confirmation, AEEC organisms most often cause diarrhea in calves between 2- to 21-days old with a mean age of 10 to 12 days.<sup>8,10-12</sup> Occasional cases have been reported in calves up to 4-months old and adults.<sup>8,10-12</sup> Following experimental inoculation, onset of diarrhea ranged from 1 to 4 days and lasted for 1 to 7 days.<sup>8</sup> In some studies, calves had onset of a mild fever (up to 40°C) between 36 hours and 4 days postinoculation that lasted 1 to 3 days. However, experimentally, the onset and severity of clinical signs are influenced by specific type of *E coli*, dosage, age at exposure, and colostral immunity. The literature suggests the same is true of natural infections.

The confirmatory diagnosis of AEEC is by microscopic examination of the small intestine and colon. Janke and colleagues<sup>10,12</sup> observed attachment in the colon in 88.4% of cases, which included 31.7% of cases with both small intestine (ileum) and colon attachment, only 11.7% of cases had attachment in the small intestine alone. AEEC cause a distinct histologic appearance at the attachment sites where clusters of short gram-negative rods attach and form a scalloped appearance to the epithelial cells (**Fig. 2**).<sup>8,10,12</sup> These cells undergo damage to their microvillus surface, round up, degenerate, and slough from the lamina propria. When the small intestine is affected, villus atrophy results from loss of affected cells. Inflammation may be mild but varies with duration of the lesion.<sup>8</sup> On gross necropsy, the colon mucosa may be normal or have longitudinal reddening, roughening, and petechial hemorrhages (**Fig. 3**).<sup>8</sup> Blood flecks, attached clots, or frank hemorrhage, mucus, and fibrin are also seen in some calves.<sup>8</sup> Occasional cases may develop a pseudomembrane in the small intestine. The gross findings are not specific to AEEC and may be seen with coronavirus and *Salmonella* in calves less than 30-days old. Feces can vary in color and consistency. Some calves will have watery yellow feces, whereas others



**Fig. 2.** Attaching and effacing *E coli* bacteria attaching in piled up clumps (arrow) on surface epithelium resulting in scalloped irregular surface. H and E slide 1000 $\times$ .



**Fig. 3.** Attaching and effacing *E coli* resulting in hemorrhage and roughening of mucosa with fibrin in lumen of spiral colon. The same lesion could be seen with coronavirus or *Salmonella*.

may have blood in the feces, which is most common with EHEC strains.<sup>8,10,12</sup> In some calves, the feces may appear near normal, probably due to dehydration (Pat Blanchard, unpublished observation). The cecum and descending colon or rectum may have lesions when the spiral colon does not.<sup>8</sup>

Optimal diagnostic detection is gained by histopathology of the ileum near the ileocecal junction and at least two loops of spiral colon and, when gross lesions are noted, sections of cecum and descending colon or rectum. Samples submitted for histopathology should have minimal handling to prevent removal of the affected surface epithelium. Formalin-fixed sections should be partially opened to allow entry of formalin into the lumen. Do not tie off sections placed in formalin because this slows formalin fixation of the mucosa unless the ligated tissue has been filled with formalin.

AEEC has also been reported as the sole pathogen detected in 17% to 35% of cases in three reports.<sup>10-12</sup> These reports found 51% to 64% of isolates produced verotoxin (Shiga toxin) though higher percent of toxin-producing strains associated with diarrhea have been reported by others.<sup>8</sup> In one review, 27% of AEEC-affected calves were also septicemic.<sup>11</sup> Occasionally, veterinarians request an antimicrobial sensitivity of the attaching organism. To confirm an *E coli* isolated from the intestine is an attaching and effacing strain, PCR screening for the presence of the *eae* or toxin genes (*Stx1*, *Stx2*) is needed. A small in-house study at the authors' laboratory, found 85% of calves with moderate to large number of attaching organisms in the colon had the *eae* gene in 9 to 10 of 10 isolates selected from the 4+ growth zone of a colon swab plated on MacConkey agar; whereas only two of four calves had the *eae* gene in 1 to 2 of 10 isolates and the other two had no isolates with the *eae* gene when rare to small number of organisms were seen attaching (Pat Blanchard, unpublished observation). These findings indicate that random selection of an *E coli* from the 4+ growth zone of a colon mucosal swab is likely to yield an attaching and effacing strain when there are moderate to large number of organisms seen attaching histologically.

### **ENTEROTOXIGENIC *E COLI* (F5 [K99])**

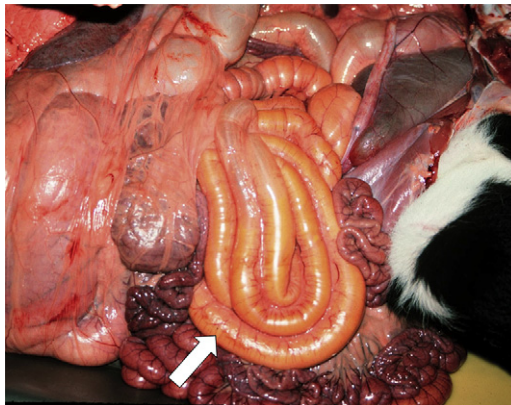
Enterotoxigenic *E coli* bacteria possess the fimbrial antigen F5 often referred to as K99. The fimbria allows the bacteria to attach to the villus epithelial cells of the small intestine and secrete a toxin. After attaching, the heat stable toxin (STa) acts on the epithelial cells to result in marked fluid efflux into the small intestine lumen.<sup>13</sup> The attachment factors are most commonly expressed on immature villus epithelial cells. Therefore, normal postnatal maturation limits the disease potential of this organism to

calves usually less than 6-days old.<sup>13,14</sup> There may be periods of increased susceptibility after 5-days old if calves experience villus atrophy from other causes (viruses and *Cryptosporidium*) resulting in immature cells migrating from the crypts to cover the villus tips.<sup>13</sup> In one study testing only feces from calves with diarrhea, 17 of 26 animals with F5 *E coli* isolated had concurrent infection with *Cryptosporidium*, rotavirus, and/or coronavirus.<sup>15</sup> F5 *E coli* were found in all ages up to 30 days, but the authors did not indicate if the animals with F5 *E coli* isolated after 5-days old were the same ones with concurrent other pathogens. Unfortunately, most published prevalence studies are based on testing feces from diarrhea and nondiarrhea calves; therefore, there is no histopathology to show the organism is attaching. In **Table 3**, the mean age of 2.5 days and age range of 1 to 7 days for K99 (F5) *E coli* is based on necropsies in which the organism was seen attaching by histopathology and confirmed by latex agglutination on feces or isolates.

The fluid loss into the intestine (**Fig. 4**) with this disease may be so severe that calves die of dehydration and electrolyte imbalance before diarrhea is detected. In some cases, the diarrhea may go unnoticed due to the very thin watery nature being mistaken for urine or seeping rapidly into the bedding. The feeding of colostrum from cows vaccinated with F5 (K99)-specific antigen is effective in preventing the disease. Once an outbreak occurs, if dry cow vaccines are not in use, oral products containing F5 (K99) specific antibodies can also be used to prevent attachment until colostral antibodies are available from cows vaccinated 10 days or more prepartum.

The presence of F5 (K99) *E coli* can be detected by isolation and typing, PCR, or antigen-detection methods (ELISA, immunochromatography, slide, or latex agglutination). Test methods with lower sensitivity are more likely to detect the organism when it is causing diarrhea because higher numbers will be present when it attaches to the epithelium. More sensitive methods, such as PCR and even culture, will find the organism in calves in which it may not be causing disease, but this provides information about the presence of the bacteria on the premise.

Other enterotoxigenic *E coli*, F41 and 987P, are occasionally reported in calves with diarrhea and when present will have a similar histologic lesion to F5 (K99) *E coli* but F5 (K99)-detection methods would be negative for these organisms. However, methods that detect presence of heat-stable toxin (STa) would identify the possible presence of these uncommon organisms. Histopathology of freshly collected and formalin-fixed sections of ileum will have typical attachment of medium-size gram-negative bacterial



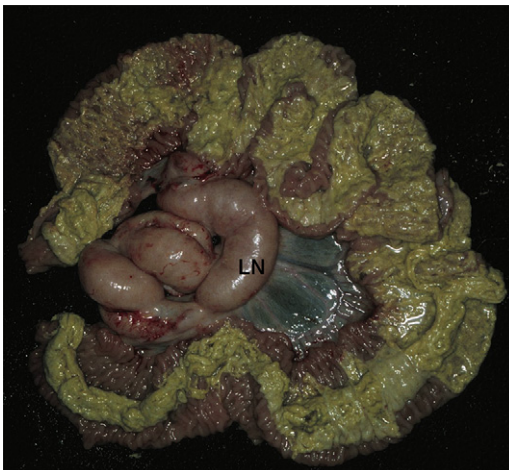
**Fig. 4.** F5 (K99) *E coli* resulting in marked spiral colon distention with watery yellow content (arrow).

rods inserting into the brush border of the villus epithelial cells with morphologically normal epithelial cells. Often there is very little inflammation.

### **SALMONELLA SP**

Enteric *Salmonella* infections can result in the full gamut of gross lesions ranging from watery yellow feces to hemorrhage, necrosis, fibrin casts, and pseudomembrane (Fig. 5) or lesions similar to those described earlier for attaching and effacing *E coli*. Abomasal reddening, roughening, and exudates may also be present, although these are nonspecific changes. Enlarged mesenteric lymph nodes are often seen (see Fig. 5). The most common causes of enteric *Salmonella* infections in calves less than 21-days old are *Salmonella typhimurium* (serogroup B) and *Salmonella newport* (serogroup C2)<sup>16</sup> (Pat Blanchard unpublished observation). However, disease with other serotypes have been described (Table 3). Both these agents are also common causes of diarrhea in cows in the first 5 days after parturition. *Salmonella* serogroups C1 and E may be found in calves the first few days of life and can be transient colonizers in calves without diarrhea.<sup>17</sup> These serogroups (C1 and E) and others (C3, F, G, K) less often associated with diarrhea may also be found on dairies in the environment, feed, water sources (eg, creeks, standing water, lagoons, troughs), and feces of nondiarrheic animals, rodents, and wildlife.<sup>18</sup> Pathogenic types like *S typhimurium*, *S newport*, and *S dublin* are also found at a lower rate.<sup>17,18</sup> Bacteremia and septicemia with *Salmonella* serogroups other than group D1 are usually late-onset events and probably result from invasion of the organism through necrotic areas of intestinal mucosa.

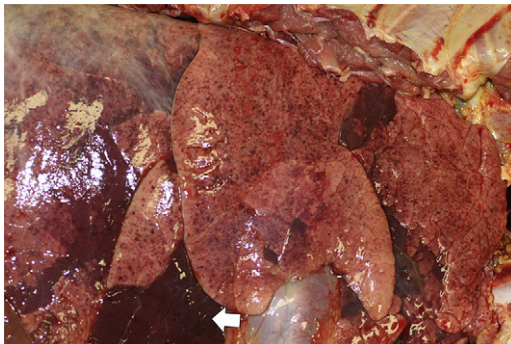
*S dublin* (serogroup D1), a cattle-adapted serotype, causes septicemia often as the primary and, sometimes, only disease condition. However, more often, some degree of enteritis is present histologically even in the absence of diarrhea. In some cases, the primary disease is enteric with diarrhea similar to *S newport* and *S typhimurium*. *S dublin* can cause diarrhea in calves as young as 5-days old but, more commonly, it occurs in calves from 1- to 4-months old.<sup>19</sup> A typical history is that calves show respiratory signs, diarrhea, illthrift, and have a poor response to antibiotics used for



**Fig. 5.** *Salmonella newport* or *S typhimurium* infection related pseudomembrane on mucosa of small intestine and enlarged mesenteric lymph nodes (LN).

common causes of bronchopneumonia. Outbreaks can occur with high morbidity and mortality. Lesions of *S dublin* septicemia commonly include splenomegaly; hepatomegaly with a bronze coloration to the liver, lungs that fail to collapse on opening the chest cavity with hundreds of petechial hemorrhages; or plum-colored, wet and heavy (meaty texture) lungs involving caudal lobes; one or more cranial lobes; or all lobes (Fig. 6). On occasion, lesions will be limited to the cranial lung but, histologically, these lesions, like the wet, heavy lung, are due to variable amounts of fibrin, some forming hyaline membranes, and interstitial pneumonia with neutrophils and histiocytes. A single plug (coagulum of fibrin and bile) may be found in the gall bladder of some calves (Fig. 7) and is pathognomonic for *Salmonella* septicemia. Some calves may be icteric or have petechial hemorrhages in the kidney and gastrointestinal serosa. A few calves will have meningitis or polyarthritis. A rare dry gangrene condition associated with *S dublin* may be seen in winter months. Dry gangrene develops in the tips of the ears, tail, and both rear legs. In the legs, a sharp line of demarcation may form across the midmetatarsus or the gangrene may be limited to the phalanges. The foot may fall off. Lesions are similar to those seen with ergot but are linked to vascular thrombosis due to *S dublin*. The organism can usually be isolated from the feces of some affected calves though classic septicemia lesions are not present. *S dublin* can be carried by healthy cows and shed sporadically in milk and feces.<sup>20</sup> It is possible that young calves harbor the organism in their intestine or mesenteric lymph nodes until colostral antibodies wane, allowing it to spread.

Detection of *Salmonella* sp may be done by culture of mesenteric lymph nodes, intestinal lesions or contents, feces, and affected parenchymal organs. *Salmonella* cultures often involve one or more enrichment steps followed by PCR, lateral-flow chromatography, or isolation on selective media. Laboratories performing nonisolation methods, such as PCR, may follow positive results with isolation by request—or this may be done routinely. False negative PCR or isolation may occur due to suppression of growth in enrichment broth by normal intestinal flora or use of antibiotics in the animal so the organism may be present in numbers too few to detect. PCR methods on intestine may be impacted by release of enzymes from other bacteria that damage the nucleic acid of *Salmonella* sp making them undetectable.<sup>1</sup> Rapid, accurate detection methods continue to be developed and improved, primarily driven by the food industry. However, veterinary diagnostics will benefit from spinoff use of some of this technology to provide cost-effective, sensitive, and rapid detection of *Salmonella*.



**Fig. 6.** *Salmonella dublin* infection causing swollen, edematous lung with petechial hemorrhages and localized areas of slightly firm (meaty) bronchointerstitial pneumonia (arrow).

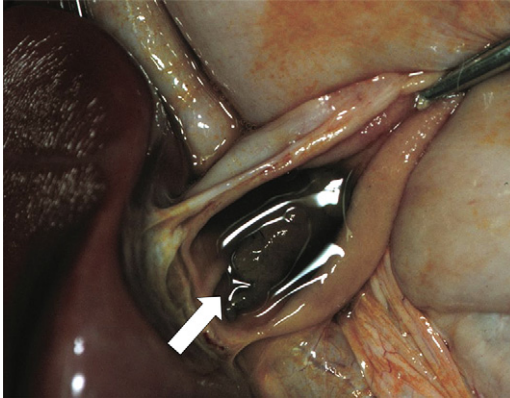


Fig. 7. *Salmonella dublin* infection a bile plug (fibrin) (arrow) in the edematous gall bladder.

In the face of a *Salmonella* outbreak in calves, all potential sources of spread should be considered and evaluated for possible sources of exposure. These include fomites on clothes, footwear, equipment, or feeding supplies (bottles, buckets, esophageal tubes—*Salmonella* can be found in saliva); as well as, water, colostrum, milk, conveyances (carts to remove dead calves and move newborn calves to hutches), wash water used in pens and under hutches, sprinklers, weigh scales, maternity pens, and sick cows.<sup>20</sup> Studies have shown increased fecal shedding in calves that did not receive antibiotic supplemented milk replacer,<sup>17,19</sup> on dairies where the maternity pen is used as a hospital pen,<sup>19</sup> when prophylactic antibiotics were given to newborn calves, and when the herd was not closed.<sup>17</sup> Reduced shedding and resistance to disease is seen with increasing age.<sup>17,20</sup>

### CRYPTOSPORIDIUM

*Cryptosporidium parvum*, *C. bovis* (formerly bovine B genotype),<sup>21</sup> *C. ryanae* (formerly deer-like genotype),<sup>22</sup> and *C. andersoni* have all been reported in calves.<sup>23,24</sup> Oocysts from *C. bovis* and *C. parvum* are indistinguishable based on size.<sup>21</sup> *C. ryanae* oocysts are slightly smaller<sup>22</sup> and *C. andersoni* are larger; the latter are primarily associated with subclinical disease from abomasal colonization in animals greater than 3-months old, usually adults. In a multistate study in the United States, *C. parvum* was the primary species (~90% of *Cryptosporidium*) colonizing calves up to 4-weeks old and the only species found in calves less than 3-weeks old.<sup>23</sup> The peak prevalence of calves shedding *Cryptosporidium* was 66.7% at 2-weeks old when only *C. parvum* was seen. *C. parvum* only accounted for 1% of the *Cryptosporidium* found after weaning. *C. bovis* and *C. ryanae* were the most common types seen postweaning with peak postweaning shedding prevalence of 30% at 6-months old. The predominant species varies by region. *C. bovis* was the most common type at all ages in Sweden where *C. parvum* was only found in calves before weaning with 73% of *C. parvum*-infected calves less than 2-weeks old.<sup>24</sup> Some reports in the literature show no statistical difference in shedding of *Cryptosporidium* between diarrhea and age-matched control calves; others report over 90% of calves shed the organism in the preweaning period.<sup>6,25</sup> Several surveys have found *Cryptosporidium* was the most common or second-most common pathogen in the feces of diarrhea calves. The authors' laboratory (see **Table 3**) and most surveys report over 35% of diarrheic calves less than 30-days old shedding this agent.<sup>5,6,23,25–27</sup> Like other enteric pathogens in calves,

*Cryptosporidium* has been associated with mixed infections; and a peak shedding prevalence at 7 to 14 days (53%–95%), both of which decline with increasing age.<sup>6,26,27</sup> Earlier surveillance studies did not determine the species of *Cryptosporidium* and often included calves older than 30-days old, which may explain the lack of correlation between shedding and diarrhea because some of the older calves may have been infected with *C. bovis*.

Histopathology and experimental studies with *C. parvum* demonstrate its attachment to the small intestine villus epithelial cells and resultant villus atrophy. In severe cases, the organism can be found in crypts in the colon accompanied by neutrophils. When the organism affects the colon it can lead to blood in the feces but this does not occur when found in the small intestine alone. The prepatent period for *C. parvum* is 3 to 4 days, though first shedding is more often detected at 6- to 7-days old and can last 4 to 18 days. Most affected calves are 6- to 25-days old. *C. bovis* has been identified in feces of calves as young as 7 days<sup>24</sup> and *C. ryanae* at 11- to 12-days old.<sup>21,24</sup> These two species are most often associated with subclinical infection.<sup>21,22,24</sup>

Antigen detection methods, such as ELISA and chromatographic methods, probably detect all species of *Cryptosporidium* (A. Broes, personal communication, 2012) and FA have been shown to detect all species.<sup>23</sup> Visualization of oocysts via fecal flotation, direct examination, and acid-fast stains also will not distinguish *C. parvum*, *C. bovis*, and, to a lesser extent, *C. ryanae* because they are all approximately the same size. PCR methods with 18S ribosomal RNA sequencing have been used to distinguish the four species of *Cryptosporidium* and subtypes of *C. parvum* that are associated with human infection.<sup>24</sup> PCR methods can detect lower number of organisms (as low as 50–100/g but, more consistently, 250 oocysts/g)<sup>24</sup> than antigen detection (FA, ELISA, chromatography) and fecal flotation or acid-fast stained direct smears, which have detection limits ranging from  $10^3$  to  $10^6$  oocysts/g of feces. The lower limits are more often associated with concentration of oocysts before detection. Quantitation of the organism by direct examination, including acid-fast stains and flotation may be misleading because low numbers may reflect the stage of infection (early or late) instead of the severity. However, one study found calves shedding more than  $2.2 \times 10^5$  oocysts/g of feces had a 6.1 times greater likelihood of having diarrhea than calves shedding lower numbers of organisms.<sup>27</sup>

*Cryptosporidium* are remarkably resistant to disinfectants, making them hard to kill and no effective therapeutics have been found. Five percent ammonia, 6% hydrogen peroxide, and 10% formalin are reported to kill the organism<sup>20</sup>; however, none of these are practical to use on farms. Increasing environmental temperatures from 4°C to 20°C<sup>28,29</sup> and the resultant higher temperatures found in fecal pats, which exceed 40°C, resulted in rapid inactivation of oocysts.<sup>30</sup> Likewise, reduced temperatures below –22°C also resulted in inactivation of oocysts.<sup>28</sup> Atwill and colleagues<sup>25</sup> found the organism could still be detected in scrapings from the walls and floor of wooden calf hutches after cleaning.

Szonyi and colleagues<sup>31</sup> determined calf age (less than 1-month old), housing calves in the cow barn, larger herd size (>200), use of hay bedding, and precipitation (100–150 mm) increased the risk of calves shedding *C. parvum*: whereas increased slope (5%–10%) in the housing area decreased likelihood of shedding. This organism has also been found on the inside of the nipple of bottles used to feed calves (ER Atwill, personal communication, 2007). This latter finding would justify keeping separate feeding supplies for calves less than 5-days old from those used for older calves to decrease exposure among young calves. The older the calf is at the time of exposure, the milder their infection will be in general. Dry, clean individual calf housing away from cows and free of moisture with good drainage can decrease exposure to



*Cryptosporidium*. Exposure of the organism to summer heat and winter cold should decrease the environmental burden of organisms on a farm.

*C. parvum* is the most common calf diarrhea pathogen to be associated with zoonotic disease, most often in veterinary students and those working with affected calves during necropsy, treatment, or clinical examination. Fomites on human clothing, hands, and footwear may transfer the parasite to other calves, pets, or family members when work clothing or footwear are worn home (Pat Blanchard, unpublished observation). Fortunately, most infections are self-limiting in immune competent humans and animals but they may take up to 15 days to resolve.

## COCCIDIA

Coccidia in cattle are in the genus *Eimeria* of which *E. bovis* and *E. zuernii* are the most common and pathogenic.<sup>20</sup> This organism is one of the most common causes of diarrhea in calves from 3- to 6-months old, but infections can be seen in pasture beef calves or group housed calves as young as 3-weeks old. The prepatent period is 15 to 21 days. *Eimeria* can persist in the environment for years so exposure of calves after return from a calf ranch to the home dairy in the absence of a coccidiostat can precipitate the disease. Coccidiosis has not been seen in calf hutch calves (Pat Blanchard, unpublished observation).

Though coccidia can cause severe bloody to watery diarrhea, ill thrift, and rough hair coats, up to 95% of infections are subclinical. Gross lesions may include frank thick blood in the spiral and/or descending colon, red watery contents, and roughened, granular, reddened colon mucosa with petechial hemorrhages and edema of the wall. The gamonts and oocysts of *E. bovis* and *E. zuernii* form in the crypt cells of the cecum and colon, which then undergo necrosis. Therefore, it is imperative to obtain content from the colon or rectum for parasite testing on necropsy cases because the small intestine content will not contain oocysts. Fresh and fixed cecum and colon sections are valuable because severe infections may have only small numbers of oocysts detectable in the feces but will have classic histologic lesions in the large intestine.

A quick field test for coccidia can be done at necropsy by scraping the abnormal-looking colon mucosa and mixing with a small drop of water on a slide and examining at 200× (combined ocular power of 10× and objective of 20×) magnification to detect oocysts. They will often be present in very large number. Standard detection methods include fecal flotation and McMaster's fecal examination. Feces from multiple animals should be submitted because, later in the infection cycle, very few oocysts may be found despite considerable damage to the large intestine. These animals may become chronic poor doers.

## OTHER PARASITES

Abomasal (*Haemonchus*, *Ostertagia*, *Trichostrongylus*) and intestinal (*Nematodirus*, *Trichuris*, and *Oesophagostomum*), parasites generally have prepatent periods greater than 5 weeks and are uncommon causes of diarrhea in calves less than 3-months old. The detection methods include fecal flotation and McMaster's examination.

*Giardia duodenalis* (also known as *G. lamblia* and *G. intestinalis*) has been reported to colonize 100% of dairy and beef calves in the first 5-months of life.<sup>32-34</sup> Peak infection rates (85%) and number of cysts shed<sup>34</sup> occur at 5-weeks old and the mean age of first shedding is 30-days old with a prepatent period of 7 to 8 days.<sup>33,34</sup> Because the

organism is commonly found in healthy calves and, less often, cows on pasture, particularly periparturient cows, its significance as a causative agent of diarrhea in calves is uncertain and infections are usually subclinical. The organism can colonize the small intestine by attachment to epithelial cells via an adhesive disk but this colonization is extremely rare to find on histopathology (Pat Blanchard, unpublished observation) in calves with diarrhea. Reportedly, it can cause villus atrophy, lymphocytic inflammation, and decreased digestion of nutrients.<sup>32</sup> The organism can remain viable for months in cool, moist environments. *Giardia* can be detected in feces by zinc sulfate or sugar centrifugation type of flotation methods, antigen ELISA, PCR, and FA techniques. Reinfection has been reported to occur rapidly after treatment.<sup>32</sup>

## ROTAVIRUS AND CORONAVIRUS

These viruses can be detected in calves as young as 1-day old and most often cause diarrhea in 2- to 24-day old calves, though older naïve calves can also be affected by either virus. Rotavirus has a prepatent period of 1 to 3 days and diarrhea lasts 2 to 5 days if uncomplicated.<sup>2,13</sup> Coronavirus has a prepatent period of 2 days and diarrhea lasts 3 to 6 days after onset.<sup>13</sup> Both viruses are ubiquitous and published reports have varied in whether there is a significant difference between diarrheic and age-matched controls for the presence of either virus. Rotavirus is the most common or second-most common enteric pathogen found in the feces of diarrheic calves, whereas coronavirus is the third-most commonly reported pathogen in numerous surveys.<sup>4-6,15</sup> When examining only calves from necropsies, the authors found coronavirus to be the second-most common pathogen and rotavirus the third (see **Table 3**). This probably reflects more severe disease caused by coronavirus resulting in death of calves compared with rotavirus infections; although multiple pathogens were commonly found with both agents.

Both rotavirus and coronavirus cause villus atrophy, which results in diarrhea due to maldigestion and malabsorption. Maldigestion results in undigested feed in the colon, which leads to bacterial overgrowth and osmotic pressure exacerbating the diarrhea.<sup>13,35</sup> Coronavirus affects a larger portion of the small intestinal villus epithelium and rarely the crypts and often causes colon crypt and adjacent lamina propria histiocyte necrosis; therefore, this virus is more likely than rotavirus to cause severe diarrhea.<sup>13,35</sup> Rotavirus usually affects the caudal small intestine but, in younger calves, has been reported to be more widespread in distribution.<sup>20</sup> Coronavirus affects the proximal small intestine initially, progressing distally and diarrhea onset begins when epithelial cells become infected before the onset of necrosis.<sup>13</sup> Gross lesions of coronavirus in the colon may vary from normal mucosa with diarrhea to lesions similar to those described earlier for attaching and effacing *E coli*. Gross lesions in the small intestine for both viruses are often not detected. Both viruses can be shed by cows in the periparturient period increasing potential exposure of their calves.<sup>20,35</sup> Coronavirus is also more likely to be shed by cows and survives better in the environment in winter months.<sup>35</sup>

Various detection methods exist for both viruses with wide variation in the level of agreement found between methods in several studies. Methods for both viruses include PCR, EM, ELISA, immune-chromatography, lateral-flow antigen capture, FA, isolation, immune-electron microscopy (IEM); and, for rotavirus alone, agglutination and electrophoresis (PAGE).

For rotavirus, agreement between ELISA and EM ranges from 85% to 96% on diagnostic samples<sup>1,4,36,37</sup> and 100% on samples from experimental challenge.<sup>37</sup> However, some commercial ELISA kits have shown a lower sensitivity (78%) and

specificity (68%) on diagnostic samples.<sup>2</sup> Agreement between reverse transcription PCR (RT-PCR) and ELISA and RT-PCR and lateral-flow antigen capture on experimental exposed calves was reported as 95% and 85%, respectively.<sup>2</sup> For coronavirus, ELISA methods compared with EM have been reported to have an overall agreement of 88% to 96% in diagnostic samples from diarrhea calves.<sup>4,37,38</sup>

PCR methods, which are increasingly offered by diagnostic laboratories, have a better sensitivity than currently available antigen detection or EM methods for both viruses.<sup>1</sup> Electron microscopy has been reported to detect oral vaccines up to 3 days for rotavirus and 7 days for coronavirus by PCR<sup>1</sup> but Theil and McCloskey<sup>39</sup> found rotavirus in only 1 of 41 serial fecal samples collected from three gnotobiotic calves following oral vaccination. The positive sample was found on day 3 after vaccination and no efforts were made to identify coronavirus in the study. Frequency and duration of PCR detection of oral vaccines has not yet been reported, but it is reasonable to assume that if they can be detected by EM, they would be detectable by PCR.

Some of the variation in agreement between methods is explained by ELISA methods, whether commercial or in-house developed, that may use different antibodies to the virus of interest, resulting in different sensitivity and specificity. Studies by Reynolds and colleagues<sup>37</sup> demonstrated a decreasing percent of agreement for coronavirus between EM and ELISA using samples taken from experimental calves (100%), field surveys (95%), and routine diagnostic submissions (82%). Athanassious and colleagues<sup>4</sup> demonstrated 95% to 96% agreement between rotavirus and coronavirus ELISA and EM methods in diarrhea calves. In their study, fecal samples were collected within 5 to 6 hours after onset of clinical signs and intestinal tissues within 2 days of onset and all samples were frozen at  $-70^{\circ}\text{C}$  until tested. Kapil and colleagues<sup>40</sup> reported a marked drop in virus shedding in calves 3-days postinfection with coronavirus. These three studies indicate timing of sampling after onset of diarrhea and quality and storage of samples have an impact on results.

In the Reynolds and colleagues study,<sup>37</sup> rotavirus had a lower agreement between EM and ELISA on field survey samples than diagnostic samples, which might reflect calves with group B rotavirus among survey samples. Group A rotaviruses cause approximately 95% of rotavirus infections in calves and are the predominant type seen in humans; therefore, diagnostic antigen-detection methods (ELISA, lateral-flow, and agglutination) are designed to only detect group A viruses.<sup>1,2</sup> However, group B rotavirus have been reported in calves with diarrhea<sup>41</sup> and can be detected by EM. By EM, these viruses are not distinguishable from group A rotavirus.

FA tests are not as reliable on small intestine for either virus because the infected villus tip cells undergo necrosis and slough. This, combined with postmortem loss of infected epithelial cells, leads to false negative results. One study<sup>36</sup> using small intestine for rotavirus FA noted a 33% agreement between FA and EM. For coronavirus, comparing FA and IHC to direct EM was reported to have a relative sensitivity of 63% to 80%<sup>38,42</sup> and 83%,<sup>42</sup> respectively. For coronavirus, the spiral colon is a more reliable site for FA because the infected cells are retained in the crypts, less susceptible to postmortem loss, and have been reported to persist in the colon longest.<sup>40</sup> Extensive FA positive staining may occur in the spiral colon in early infections because diarrhea onset precedes necrosis of colon cells. Later stage infections, when only rare necrotic crypts surrounded by fibrous tissue are present, will occasionally have focal positive staining. Detection of coronavirus by FA on frozen colon tissue has a good correlation with the presence of colon lesions by histopathology (Pat Blanchard, personal observation).

EM is more reliable for rotavirus, which is stable, shed in large amounts during diarrhea phase, and has a unique size and appearance compared with coronavirus, which

is less stable, often appears pleomorphic, and cell membranes and other cell substances may mimic the pleomorphic appearance, causing both false negative and false positive findings.<sup>4</sup> This problem is partially overcome by the experience of the electron microscopist and the storage, freshness, and preparation of the sample. Immunoelectron microscopy eliminates the false positive results but is rarely performed in most diagnostic laboratories. PAGE patterns of rotavirus isolates will allow determination of the group present.<sup>41</sup>

Colostrum antibodies help protect against infection following vaccination of dry cows. Oral vaccines with attenuated viruses are available for use in newborn calves. In some cases, the use of both dry cow vaccine and oral calf vaccine seems to negate the positive effect of either alone because the oral vaccine virus binds to colostrum antibody, reducing the antibody available for absorption or the virus available for local immunity stimulation.

### **BVDV**

Most BVDv infections in calves less than 30-days old are probably persistent infections. In this age group, BVDv causes a very mild neutrophilic exudate in the crypts of the ileum. This is a nonspecific lesion. BVDv fluorescent antibody testing often reveals widespread staining of smooth muscle and epithelium in the intestine of affected calves. The virus may contribute to diarrhea through nonspecific suppression of the immune system. At CAHFS-Tulare, over the past 10 years, 1.3% of calves submitted for calf diarrhea work up were infected with BVDv. BVDv persistently infected calves may have a higher death loss at a young age when challenged with other enteric pathogens.

The more typical lesions of BVDv, including oral and esophageal ulcers, necrosis of the rumen, and intestine crypt epithelium and Peyer patch lymphocytes are usually seen in cattle between 4- to 14-months old. Colostrum antibodies drop to undetectable levels between 4- to 8-months old, so calves become susceptible at that time. Most cattle will have asymptomatic exposures and develop antibodies. A few will become ill (anorexia, fever, diarrhea) but usually recover if no concurrent diseases occur.

### **OTHER VIRUSES**

*Torovirus* has been documented to be the sole pathogen or the one most consistently present in some calf diarrhea outbreaks in the Midwest.<sup>43</sup> Most positive animals were less than 3-weeks old. The organism can cause small intestine villus atrophy affecting the middle portion of the villi and crypts in the small intestine and colon. The incubation period is 1 to 3 days with diarrhea lasting from 3 to 5 days. *Torovirus* has been detected in cattle from a few days to 10-months old. The virus can be detected by EM but, like coronavirus, the amount of virus shed and a somewhat pleomorphic appearance limit the usefulness of this procedure. Methods to concentrate the virus for improved detection include use of IEM, PCR, and antigen-detection ELISA none of which are widely used outside of research facilities. Serologic surveys of cattle suggest that virus exposure is widespread.

### **ABOMASITIS AND ABOMASAL BLOAT**

*Clostridium* spp abomasitis occurs in calves less than 7-days old. Gross pathology findings include marked hemorrhage and emphysema of the abomasal wall with gas distention and bloody abomasal content.<sup>20,44</sup> Lesions may extend to involve the rumen, omasum, and reticulum.

Another cause of abomasal bloat, sometimes resulting in rupture, is a gas bloat condition associated with the presence of *Sarcina*, a gram-positive anaerobic cocci found in the soil. The abomasum wall is commonly emphysematous and may have patchy edema, congestion, hemorrhage, or brown to black discoloration from digested blood. *Sarcina* forms packets of 8 to 16 cocci detectable by histopathology in the abomasal lumen and sometimes the wall of affected calves.<sup>45</sup> Studies with this organism alone or in combination with *C perfringens* failed to reproduce the bloat condition (Ken Mills, PhD, Laramie, WY, personal communication, 2006). The appearance of the organism in the abomasum on histopathology is associated with bloat and has not been found in calves (Pat Blanchard, unpublished observation) or goats<sup>46</sup> that die from other causes. Affected calves are less than 30-days old and receive some type of milk product including whole milk or milk replacers and may even be nursing cows on pasture. Esophageal intubation is not effective in relieving the bloat because the tube does not reach the abomasum. Using a large-bore needle inserted in the right flank may relieve the gas. Neither of these abomasal bloat conditions is associated with diarrhea in calves.

## **NUTRITIONAL-ASSOCIATED CAUSE**

### ***Yeast or Fungi***

---

Heavy or prolonged use of antibiotics for treatment or as a component of medicated milk replacers can disrupt normal forestomach and intestinal flora allowing overgrowth of yeast and fungi causing white plaques or adherent curd-like lesions on the mucosa of the rumen, reticulum, and omasum, as well as inflammation in the abomasum. True fungi, such as zygomycetes, can also overgrow and invade the mucosa causing vascular thrombosis of the submucosa with round red-rimmed foci of necrosis in any of the forestomachs, but most often in the abomasum, of calves less than 30-days old. Yeast overgrowth may be profound enough to be found at all levels of the intestine and suggests dysbacteriosis, which may be associated with diarrhea.

### ***Milk Replacer Quality***

---

Osmotic diarrhea can result from poorly digestible milk replacers, which lead to an excess of undigested nutrients in the lower intestine. This can also provide a substrate for bacterial overgrowth and contribute to the diarrhea. Testing of milk replacers to validate the label claims may lead to detection of higher fiber than noted on the label, which would indicate poorly digestible plant proteins may have been substituted for casein, blood-based, or processed soy proteins.<sup>20</sup> The plant proteins, other than processed soy, are generally poorly digestible in nonruminating young calves.

### ***Rumen Acidosis***

---

Rumen acidosis is a common cause of diarrhea in transition cows but not commonly seen in calves less than 3-months old. However, it should be considered if there is evidence of a sudden change in the feed components or sudden access to a high carbohydrate source to which the calf is not conditioned. This can occur when unweaned beef calves are removed from pasture and given grain for the first time. Other events, such as keeping a pen of calves off feed for a prolonged period while processing or vaccinating them, then allowing free-choice access to a day's worth of grain, might lead to rapid consumption by aggressive eaters.

### ***Molybdenosis or Copper Deficiency***

---

This condition is more common in beef calves on pasture because most dairy cow diets are supplemented with copper when molybdenum is high in the diet. There

are no specific gross or histologic lesions in the intestine with this condition, but calves may be experiencing concurrent fading of coat color particularly around the eyes, ill-thrift, poor hair quality, and diarrhea. Serum can be tested for copper levels on live calves and cows or liver on dead animals.

## SUMMARY

Calf diarrhea is a multifactorial disease related to a combination of host and pathogen factors. The most common pathogens found in diarrheic calves are *Cryptosporidium*, rotavirus, coronavirus, *Salmonella*, attaching and effacing *E coli* (EHEC, EPEC, STEC), and F5 (K99) *E coli*. Increased mortality and morbidity are often due to the presence of more than one pathogen. This article includes a discussion of key information to obtain in a clinical history, the pathogens, pathology findings, and diagnostic methods.

## REFERENCES

1. Cho YI, Kim WI, Liu S, et al. Development of a panel of multiplex real-time polymerase chain reaction assays for simultaneous detection of major agents causing calf diarrhea in feces. *J Vet Diagn Invest* 2010;22:509–17.
2. Maes RK, Grooms DL, Wise AG, et al. Evaluation of a human group A rotavirus assay for on-site detection of bovine rotavirus. *J Clin Microbiol* 2003;41:290–4.
3. Eriksson E, Aspan A. Comparison of culture, ELISA and PCR techniques for *Salmonella* detection in faecal samples for cattle, pig and poultry. *BMC Vet Res* 2007;2:3–21.
4. Athanassios R, Marsolais G, Assaf R, et al. Detection of bovine coronavirus and type A rotavirus in neonatal calf diarrhea and winter dysentery of cattle in Quebec: evaluation of three diagnostic methods. *Can Vet J* 1994;35:163–9.
5. Izzo MM, Kirkland PD, Mohler VL. Prevalence of major enteric pathogens in Australian dairy calves with diarrhea. *Aust Vet J* 2011;89:167–73.
6. Naciri M, Lefay MP, Mancassola R, et al. Role of *Cryptosporidium parvum* as a pathogen in neonatal diarrhoea complex in suckling and dairy calves in France. *Vet Parasitol* 1999;85:245–57.
7. Hammitt MC, Bueschel DM, Keel MK, et al. A possible role of *Clostridium difficile* in the etiology of calf enteritis. *Vet Microbiol* 2008;127:343–52.
8. Moxley RA, Smith DJ. Attaching-effacing *E. coli* infections in cattle. *Vet Clin North Am Food Anim Pract* 2010;26(1):29–56.
9. Shaw DJ, Jenkins C, Pearce MC, et al. Shedding patterns of verocytotoxin-producing *Escherichia coli* strains in a cohort of calves and their dams on a Scottish beef farm. *Appl Environ Microbiol* 2004;70:7456–65.
10. Janke BH, Francis DH, Collins JE, et al. Attaching and effacing *Escherichia coli* infection as a cause of diarrhea in young calves. *J Am Vet Med Assoc* 1990;196:897–901.
11. Blanchard P, DeBey B, Akins J, et al. Attaching and effacing *E. coli* in calves [abstract 29]. In: Program abstracts of the 37th annual meeting of the American Association of Veterinary Laboratory Diagnosticians. Grand Rapids (MI): 1994, p. 29.
12. Janke BH, Francis DH, Collins JE, et al. Attaching and effacing *Escherichia coli* infections in calves, pigs, lambs, and dogs. *J Vet Diagn Invest* 1989;1:6–11.
13. Foster DM, Smith GW. Pathophysiology of diarrhea in calves. *Vet Clin North Am Food Anim Pract* 2009;25(1):13–36.

14. Runnels PL, Moon HW, Schneider RA. Development of resistance with host age to adhesion of K99 *Escherichia coli* to isolated intestinal epithelial cells. *Infect Immun* 1980;28:298–300.
15. de la Fuente R, Garcia A, Ruiz-Santa-Quiteria J, et al. Proportional morbidity rates of enteropathogens among diarrheic dairy calves in central Spain. *Prev Vet Med* 1998;36:142–5.
16. Cummings KJ, Warnick LD, Alexander KA, et al. The incidence of salmonellosis among dairy herds in the northeastern United States. *J Dairy Sci* 2009;92:3766–74.
17. Berge AC, Moore DA, Sischo WM. Prevalence and antimicrobial resistance patterns of *Salmonella enterica* in preweaned calves from dairies and calf ranches. *Am J Vet Res* 2006;67:1580–8.
18. Anderson RJ, House JK, Smith BP, et al. Epidemiologic and biologic characteristics of salmonellosis on three dairy farms. *J Am Vet Med Assoc* 2001;219:310–22.
19. Fossler CJ, Wells SW, Kaneene JB, et al. Herd-level factors associated with isolation of *Salmonella* in a multi-state study of conventional and organic dairy farms II. *Salmonella* shedding in calves. *Prev Vet Med* 2005;70:279–91.
20. Gunn AA, Naylor JA, House JK. Diarrhea. In manifestations and management of disease in neonatal ruminants. In: Smith BP, editor. *Large animal internal medicine*. 4th edition. St. Louis, MO: Mosby Elsevier; 2009. p. 340–62.
21. Fayer R, Santín M, Xiao L. *Cryptosporidium bovis* n. sp. (*Apicomplexa: Cryptosporidiidae*) in cattle (*Bos taurus*). *J Parasitol* 2005;91:624–9.
22. Fayer R, Santín M, Trout JM. *Cryptosporidium ryanae* n. sp. (*Apicomplexa: Cryptosporidiidae*) in cattle (*Bos taurus*). *Vet Parasitol* 2008;156:191–8.
23. Santin M, Trout JM, Xiao L, et al. Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. *Vet Parasitol* 2004;122:103–17.
24. Silverlas C, Naslund K, Bjorkman C, et al. Molecular characterisation of *Cryptosporidium* isolates from Swedish dairy cattle in relation to age, diarrhoea and region. *Vet Parasitol* 2010;169:289–95.
25. Atwill ER, Harp JA, Jones T, et al. Evaluation of periparturient dairy cows and contact surfaces as a reservoir of *Cryptosporidium parvum* for calfhoo infection. *Am J Vet Res* 1998;59:1116–21.
26. de la Fuente R, Luzon M, Ruiz-Santa-Quiteria JA, et al. *Cryptosporidium* and other concurrent major enteropathogens in 1 to 30-day-old diarrheic dairy calves in central Spain. *Vet Parasitol* 1999;80:179–85.
27. Trotz-Williams LA, Martin SW, Leslie KE, et al. Calf-level risk factors for neonatal diarrhea and shedding of *Cryptosporidium parvum* in Ontario dairy calves. *Prev Vet Med* 2007;82:12–28.
28. Peng X, Murphy T, Holden NM. Evaluation of the effect of temperature on the die-off rate for *Cryptosporidium parvum* oocysts in water, soils, and feces. *Appl Environ Microbiol* 2008;74:7101–7.
29. Jenkins MB, Bowman DD, Fogarty EA, et al. *Cryptosporidium parvum* oocyst inactivation in three soil types at various temperatures and water potentials. *Soil Biol Biochem* 2002;34:1101–9.
30. Li X, Atwill ER, Dunbar LA, et al. Seasonal temperature fluctuations induces rapid inactivation of *Cryptosporidium parvum*. *Environ Sci Technol* 2005;39:4484–9.
31. Szonyi B, Chang YF, Wade SE, et al. Evaluation of factors associated with the risk of infection with *Cryptosporidium parvum* in dairy calves. *Am J Vet Res* 2012;73:76–85.
32. O'Handley RM, Olson ME. Giardiasis and cryptosporidiosis in ruminant. *Vet Clin North Am Food Anim Pract* 2006;22:623–43.

33. O'Handley RM, Cockwill C, McAllister TA, et al. Duration of naturally acquired giardiasis and cryptosporidiosis in dairy calves and their association with diarrhea. *J Am Vet Med Assoc* 1999;214:391–6.
34. Ralston BJ, McAllister TA, Olson MA. Prevalence and infection pattern of naturally acquired giardiasis and cryptosporidiosis in range beef calves and their dams. *Vet Parasitol* 2003;114:113–22.
35. Boileau MJ, Kapil S. Bovine coronavirus associated syndromes. *Vet Clin North Am Food Anim Pract* 2010;26:123–46.
36. Benfield DA, Stotz IJ, Nelson EA, et al. Comparison of a commercial enzyme-linked immunosorbent assay with electron microscopy, fluorescent antibody, and virus isolation for the detection of bovine and porcine rotavirus. *Am J Vet Res* 1984;45:1998–2002.
37. Reynolds DJ, Chasey D, Scott AC, et al. Evaluation of ELISA and electron microscopy for the detection of coronavirus and rotavirus in bovine faeces. *Vet Rec* 1984;114:397–401.
38. Tahir RA, Pomeroy KA, Goyal SM. Evaluation of shell vial cell culture technique for the detection of bovine coronavirus. *J Vet Diagn Invest* 1995;7:301–4.
39. Theil KW, McCloskey CM. Rotavirus shedding in feces of gnotobiotic calves orally inoculated with a commercial rotavirus-coronavirus vaccine. *J Vet Diagn Invest* 1995;7:427–32.
40. Kapil S, Trent AM, Goyal SM. Excretion and persistence of bovine coronavirus in neonatal calves. *Arch Virol* 1990;115:127–32.
41. Chang KO, Parwani AV, Smith D, et al. Detection of group B rotaviruses in fecal samples from diarrheic calves and adult cows and characterization of their VP7 genes. *J Clin Microbiol* 1997;35:2107–10.
42. Dar AM, Kapil S, Goyal SM. Comparison of immunohistochemistry, electron microscopy, and direct fluorescent antibody test for the detection of bovine coronavirus. *J Vet Diagn Invest* 1998;10:152–7.
43. Hoet AE, Paul R, Nielsen PR, et al. Detection of bovine coronavirus and other enteric pathogens in feces from diarrhea cases in cattle. *J Vet Diagn Invest* 2003;15:205–12.
44. Van Kruiningen HJ, Nyaoke CA, Sidor IF, et al. Clostridial abomasal disease in Connecticut dairy calves. *Can Vet J* 2009;50:857–60.
45. Edwards GT, Woodger NG, Barlow AM, et al. *Sarcina*-like bacteria associated with bloat in young lambs and calves. *Vet Rec* 2008;163:391–3.
46. DeBey BM, Blanchard PC, Durfee PT. Abomasal bloat associated with *Sarcina*-like bacteria in goat kids. *J Am Vet Med Assoc* 1996;209:1468–9.