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# Pathophysiology of Diarrhea in Calves

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## KEYWORDS

- Rotavirus • *Cryptosporidium* • Enterotoxigenic *Escherichia coli*
- Coronavirus • Torovirus

Infectious diarrhea remains one of the biggest health challenges in both the beef and dairy industries. More than 20% of beef cattle owners feel that calf diarrhea has a significant impact on their economic productivity,<sup>1</sup> and diarrhea accounts for more than half of all calf mortality on dairy farms.<sup>2</sup> Currently, enterotoxigenic *Escherichia coli* (ETEC), *Cryptosporidium parvum*, rotavirus, and coronavirus appear to be the most significant infectious causes of calf diarrhea. Research into the pathophysiology of these organisms may ultimately lead to more specific treatment and control recommendations.

## ENTEROTOXIGENIC *ESCHERICHIA COLI*

Epidemiologic studies of both beef and dairy calves have implicated ETEC as the major cause of neonatal diarrhea occurring in the first 4 days of life; however it rarely leads to diarrhea in older calves or adult cattle.<sup>3–6</sup> Immediately after birth, oral exposure to fecal coliforms leads to colonization of the gut with the normal commensal flora, and these organisms continue to move caudally through the gastrointestinal tract with ingesta.<sup>7,8</sup> If environmental contamination is high, ETEC organisms are ingested at this same time and are able to produce disease caused by the presence of two virulence factors, K99 fimbria and heat stable toxin. Because nonpathogenic *E coli* are extremely common, fecal cultures as a diagnostic test are of little value unless the presence of these two virulence factors can be demonstrated.

### ***Attachment of Escherichia coli to Intestinal Epithelium***

Attachment to the intestinal epithelium allows the bacteria to maintain residence in the small intestine and multiply instead of being passed through with the ingesta. Studies have shown that up to 80% of the organisms are attached in calves with ETEC diarrhea, instead of only 10% to 20% in normal calves.<sup>3,9,10</sup> This attachment is mediated by the presence of fimbrial antigens. The antigen most commonly associated with

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ETEC diarrhea in calves is K99, which is more appropriately referred to as F5.<sup>6,11</sup> The F41 and 987P antigens can also be found in calf ETEC isolates, often in conjunction with F5.<sup>12,13</sup> Because the K99 antigen is only expressed at an environmental pH level of less than 6.5, the distal small intestine is the initial site of colonization. This is because the pH level of the intestinal fluid increases as it moves caudally, and it only reaches this threshold at the ileum.<sup>14–18</sup> The ability of K99 ETEC to bind to the small intestinal epithelium is age dependent and gradually decreases from 12 hours of age to 2 weeks of age. However, there is not a precipitous drop in the binding ability that would explain the age resistance to ETEC.<sup>19</sup> The attachment of ETEC allows the bacteria to colonize the ileum, proliferate, and spread proximally through the small intestine.<sup>3,17,18,20</sup> Once established in the gut, ETEC produces heat stable toxin leading to secretory diarrhea.

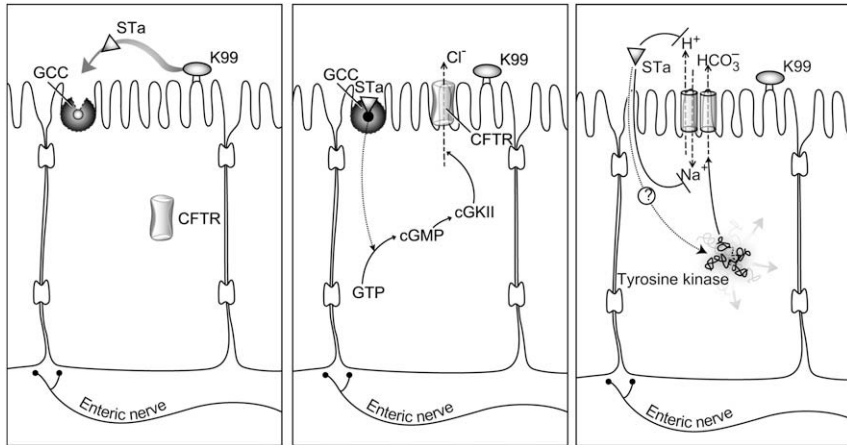
### ***Heat Stable Toxin–Mediated Secretory Diarrhea***

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Classically, mechanistic discussions of enterotoxin-mediated secretory diarrhea have focused on the cholera toxin of *Vibrio cholerae* and the heat labile toxin (LT) of *E. coli*. These are both significant causes of diarrhea in humans, and have a similar mechanism of action involving increases in intracellular cyclic adenosine monophosphate (cAMP), which activates the cystic fibrosis transmembrane conductance regulator (CFTR) and ultimately causes secretion of chloride. This movement of chloride ions osmotically draws water into the lumen of the intestine, leading to diarrhea.<sup>21,22</sup> These models of human diarrhea have less bearing on toxin-mediated secretory diarrhea in the calf because the heat stable toxin (STa) of ETEC is the primary mediator.<sup>11,23,24</sup> STa is an 18- or 19-amino-acid peptide that is secreted by many strains of ETEC; however, the production can vary up to 1,000 fold between strains when cultured under identical conditions.<sup>25,26</sup> After being secreted by *E. coli*, STa binds to guanylyl cyclase-C (GCC), a brush border membrane enzyme that is present throughout the villi and crypts.<sup>27,28</sup> The concentration of GCC appears to be highest in the lower villous,<sup>28</sup> but this may vary by species,<sup>27</sup> and its precise location on the villous has not yet been determined in the calf. In contrast to rodents and humans, in which concentrations of GCC decrease in the distal small intestine,<sup>28</sup> GCC is present throughout the gastrointestinal tract of calves and is concentrated in the ileum.<sup>29,30</sup> In both mice and humans, the density of this receptor declines after birth,<sup>26,31</sup> and it remains present in pigs until up to 7 weeks of age.<sup>32</sup> No specific research has been done detailing the expression of GCC at various ages of calves, however inoculation with STa induces diarrhea in animals up to 15 days of age.<sup>25</sup> This indicates that GCC is present until at least 2 weeks of age and down-regulation of the receptor is not the reason for age-dependent resistance to ETEC diarrhea.

Binding of STa to GCC leads to the production of intracellular cyclic guanylyl monophosphate (cGMP), which acts as a second messenger to activate cGMP-dependent protein kinase II (cGKII). This kinase phosphorylates CFTR, inducing movement of the protein to the cell surface and activation, which in turn leads to chloride secretion.<sup>33</sup> This up-regulation of chloride secretion osmotically pulls water into the intestinal lumen, which outweighs the absorptive ability of the villous (**Fig. 1**).<sup>21</sup> Blocking the CFTR dramatically decreases intestinal fluid secretion, indicating the importance of this protein in the pathogenesis of ETEC diarrhea. However, secretion is not completely prevented,<sup>34</sup> indicating that STa may have additional effects in the small intestine.

Further research has shown that STa can induce bicarbonate secretion through a tyrosine kinase that is independent of the GCC/cGMP/CFTR pathway, and this secreted bicarbonate can act as an osmotic agent to pull water into the lumen of the



**Fig. 1.** Frame 1: K99 ETEC binds to an intestinal epithelial cell, and heat stable toxin (STa) is secreted, which binds to the receptor GCC. The enteric nervous system becomes activated by the secretion of STa, but the mechanism of this activation is unclear. At this point, CFTR is not active. Frame 2: STa binds to GCC, which converts guanylyl triphosphate (GTP) to cGMP. cGMP activates cGKII to phosphorylate the CFTR, and the CFTR moves to the luminal surface and is activated, leading to chloride (Cl<sup>-</sup>) secretion. Frame 3: Secreted STa activates tyrosine kinase through an unknown pathway, which leads to bicarbonate (HCO<sub>3</sub><sup>-</sup>) secretion. STa also directly inhibits the sodium–hydrogen exchanger, decreasing the movement of sodium (Na) and hydrogen (H) across the membrane.

intestine.<sup>35,36</sup> The receptor and other messengers in this pathway have not been elucidated. An opposing model of STa-induced diarrhea has also been proposed that is not based on fluid secretion caused by movement of chloride or bicarbonate, but which instead is caused by decreased fluid absorption. In addition to activating the CFTR, STa inhibits the apical Na–H exchanger, leading to failure of sodium absorption. This failure of sodium absorption decreases fluid movement from the intestinal lumen to the interstitial space. The importance of this mechanism of ETEC diarrhea in calves is unknown because this has been most conclusively shown in the duodenum and proximal jejunum of rodent models.<sup>37,38</sup>

Similar to expression of K99, production of STa is pH dependent. When the environmental pH is less than 7.0, toxin production is severely limited.<sup>18,39</sup> Therefore, toxin production is maximized in the distal small intestine because the pH level is greatest in this segment. Although it has not been directly investigated, it can be theorized that STa-mediated secretion of bicarbonate and inhibition of the Na–H exchanger would have the net effect of alkalinizing the proximal small intestine. This would create an environment more hospitable to ETEC promoting its spread to the proximal small intestine.

The autonomic and enteric nervous systems are known to be involved in the secretory response to cholera toxin through the actions of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), 5-hydroxytryptamine (5-HT), and vasoactive intestinal polypeptide (VIP).<sup>40–42</sup> STa-mediated secretion may also involve local reflex arcs in the enteric nervous system (ENS); however, it does not involve the autonomic nervous system.<sup>43</sup> Most of the support for this idea comes from studies that inhibit the ENS and subsequently reduce the secretory effect of STa.<sup>44–46</sup> The neurotransmitters critical in these responses are nitric oxide (NO) and VIP,<sup>40,46</sup> whereas PGE<sub>2</sub> and 5-HT are not involved.<sup>41</sup> Furthermore, a well-defined example of the influence of the ENS in ETEC is its role in exacerbating

STa-mediated secretory diarrhea in states of malnutrition,<sup>47–49</sup> however the importance of these mechanisms in the calf is unknown because this was found in a rodent model of human disease.

Ultimately, the pathophysiology of ETEC is dependent on several factors. First is the exposure to and ingestion of the organism. Once ingested, ETEC must survive the acidic pH of the abomasum. This is facilitated in neonatal calves because the pH level of their abomasum ranges from approximately 6 to 7, which enables survival of ETEC. The pH of the abomasum decreases to less than 2 by 5 days of age, which is low enough to kill ETEC strains.<sup>18,50</sup> Once ETEC reaches the ileum, both the K99 antigen and STa are expressed as a result of the increased pH level, yet this may occur sooner because the pH level can be higher in the proximal GI tract of neonatal calves. K99 allows attachment of the organism, leading to colonization of the ileum. Production of STa induces secretion and may increase the luminal pH level, which would make the normally acidic proximal small intestine more hospitable for the organism. ETEC bacteria then move proximally toward the duodenum, and secretion dramatically increases, leading to diarrhea and dehydration.<sup>18,51</sup>

### ***Treatment of Escherichia coli Diarrhea***

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The focus of treatment for ETEC diarrhea should be to remove the organism from the gastrointestinal tract and combat dehydration until normal absorption is restored. Based on an extensive review on the topic by Constable in 2004, the only antibiotic with documented efficacy and legal use in food animals in the United States is amoxicillin trihydrate, which is recommended at a dose of 10 mg/kg, orally every 12 hours. Ideally, this would only be used in calves with signs of systemic illness caused by diarrhea.<sup>52</sup>

Oral electrolyte solutions remain the mainstay of on-farm fluid replacement therapy for most calves with ETEC diarrhea. Based on the pathophysiology of the organism, two characteristics of oral replacement fluids are critical. The first is to maximize sodium absorption through means other than the Na-H exchanger, because this may be inhibited by STa. Most oral electrolyte solutions take advantage of the sodium-glucose cotransporters to improve sodium absorption, which bypasses the inhibited Na-H exchanger. Although this will not reduce the secretory response (and diarrhea), it will improve the hydration status of the calf.<sup>21</sup>

Second, increasing the pH of the abomasum and proximal small intestine favors the survival of ETEC, as discussed above. Hence, oral replacement fluids with bicarbonate as the alkalinizing agent may favor the proliferation of ETEC, expression of the K99 antigen, and secretion of STa.<sup>18</sup> If secretion of bicarbonate caused by STa is a significant component of the disease in calves, as it appears to be in some models, the additional bicarbonate load from an oral electrolyte solution could even exacerbate the secretory response. Because of the potential harm of bicarbonate, oral electrolyte solutions containing acetate are recommended for treatment of ETEC diarrhea. Additional approaches for increasing the abomasal pH are discussed in the article by Marshall found elsewhere in this issue.

### ***CRYPTOSPOIDIUM PARVUM***

*C parvum* is one of the most commonly isolated gastrointestinal pathogens from dairy calves and immunosuppressed humans<sup>53</sup> and is a significant cause of waterborne diarrhea outbreaks.<sup>54</sup> Infection occurs when oocysts are ingested from the environment. Once in the host, the organism goes through a complicated life cycle that involves multiple stages. The cycle starts with exposure to gastric acid and bile salts,

leading to excystation of the oocyst to the first life stage, the sporozoite. The sporozoites invade the intestinal epithelial cells of the ileum, where the infection is typically concentrated, but they can infect the gastrointestinal tract anywhere from the abomasum to the colon. The sporozoites create an invagination of the luminal membrane, allowing them to maintain an extracytoplasmic but intracellular location known as a parasitophorous vacuole. From this location, the sporozoites transform into trophozoites. At this stage, asexual reproduction occurs and Type I meronts are formed. Merozoites are then released into the lumen. These organisms can form additional Type I meronts or Type II meronts, which form micro- and macrogamonts. Micro- and macrogamonts reproduce sexually to create thin- and thick-walled oocysts. The thin-walled oocysts lead to autoinfection, whereas the thick-walled oocysts pass out with feces to contaminate the environment. These oocysts are infective immediately, and remain viable in the environment for extended periods of time.<sup>55-57</sup>

*C. parvum* oocyst shedding occurs as early as 3 days of age, peaks at 2 weeks of age, and can continue to occur in adult cattle. However, diarrhea caused by *C. parvum* rarely occurs after 3 months of age.<sup>57-63</sup> After infection, clinical signs peak at 3 to 5 days and last 4 to 17 days.<sup>60,64</sup> Some studies have shown that up to 100% of dairy calves become infected with *C. parvum*,<sup>58,65</sup> and become the major source of environmental contamination because calves shed up to  $10^7$  oocysts per gram of feces.<sup>60</sup> Shedding in beef calves is much less frequent and occurs in less than 5% of calves.<sup>59,66</sup> Calves appear to be resistant to subsequent infection after the initial episode of *C. parvum* diarrhea.<sup>63</sup> Severity of diarrhea and incidence of clinical signs in calves shedding oocysts can be variable within and between farms, leading some to question the true importance of *C. parvum* as a primary pathogen,<sup>67</sup> however, it has been repeatedly isolated independent of other known pathogens in clinical cases.<sup>57</sup>

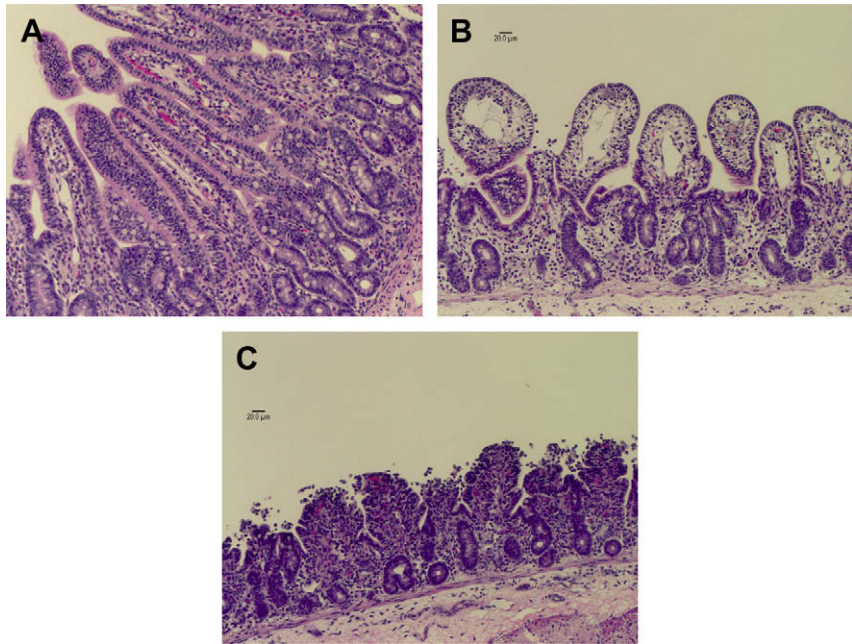
### **Malabsorptive Diarrhea Caused by *Cryptosporidium parvum***

Infection with *C. parvum* has been shown to induce severe villous atrophy (**Fig. 2**) in calves and other food animal species.<sup>64,68,69</sup> This atrophy is caused by the loss of villous enterocytes and the subsequent retraction of the villous to maintain a continuous epithelial barrier. Crypt hyperplasia also occurs in an effort to replace the lost epithelial cells, however in severe infections, disruption of the epithelial barrier can occur despite these efforts.<sup>64,70,71</sup> Furthermore, both cell culture and animal models have shown an increase in epithelial permeability after *C. parvum* infection when the loss of epithelial surface area is taken into account.<sup>70,72</sup> In spite of this well recognized consequence of *C. parvum* infection, the precise mechanism of cell loss remains elusive. It is still not understood whether the cell loss is an effect of the pathogen or is part of the host response in an effort to resolve the infection.

There are two potential mechanisms for the increased loss of epithelial cells in *C. parvum* infections. The first is a direct cytotoxic effect of the organism on the intestinal epithelium, but this is not well supported by the current literature. In a few cell culture models of *C. parvum* infection, the cytosolic enzyme, lactate dehydrogenase, has been shown to leak into the cell media.<sup>73-75</sup> However, this may simply be caused by the deformation of the apical membrane by the organism as it attaches to and is enveloped by the membrane.<sup>70</sup>

The second and more likely mechanism for cell loss is apoptosis because apoptotic cells are consistently found in both in vitro and in vivo models of infection.<sup>76-82</sup> Yet there is evidence in cell culture models that the loss of epithelial cells is minimized by the inhibition of apoptosis during the infection,<sup>78,83</sup> and many infected cells are not apoptotic.<sup>79</sup> Specifically, research has shown that the activation and inhibition of apoptosis is





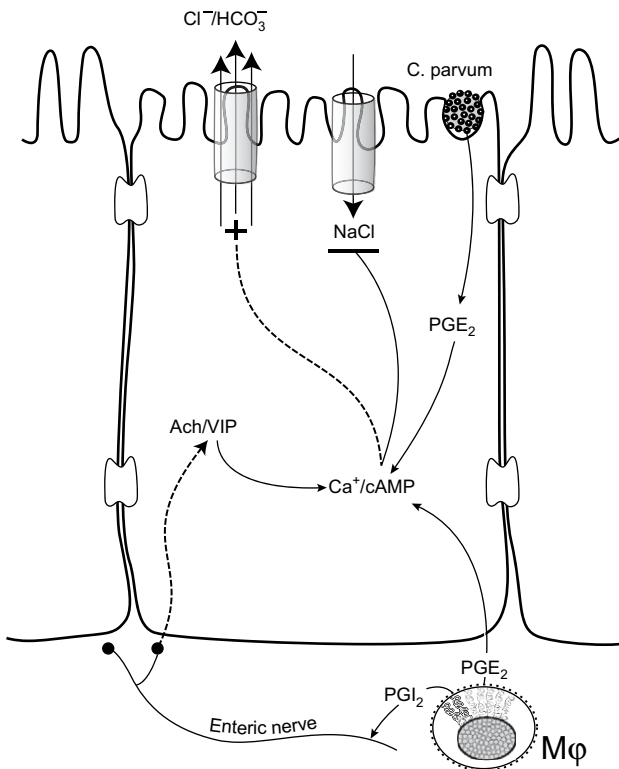
**Fig. 2.** Normal and *C parvum*-infected intestinal mucosa from a calf ileum at 100 $\times$  magnification. (A) Normal calf ileal mucosa. (B) and (C). Calf ileal mucosa experimentally infected with *C parvum*. Note the blunting of the villi and the hyperplasia of the crypts. There are more severe histologic changes in (C), because the villi are more atrophied and the mucosa no longer completely covers the lamina propria (hematoxylin and eosin).

related to the life stage of *C parvum*, and that apoptosis is inhibited during the trophozoite stage when the organism is most dependent on the host, but then increases later during the infection. Furthermore, the incidence of apoptosis will vary over time between infected cells and uninfected neighboring cells. This may be beneficial to the host to limit spread of the organism, limit the severity of cell loss, and/or speed clearance of the organism.<sup>83</sup> Pharmacologically induced apoptosis in infected cell cultures is also prevented, indicating that apoptosis mechanisms are actively inhibited,<sup>78,83</sup> which has been shown to be mediated by NF- $\kappa$ B.<sup>78</sup> Additional research is needed to elucidate the ultimate beneficiary of this apoptotic regulation: the organism, to maintain its intracellular habitat, or the host, to limit cell loss and spread of infection.

Irrespective of how or why epithelial cell loss and villous atrophy occurs, this leads to a malabsorptive diarrhea. The net absorption of fluid is caused by the movement of sodium coupled with either chloride or other nutrients in the villous tip versus the secretion of anions in the crypts. Therefore, absorption is impaired because of the loss of the mature villous epithelial cells and their associated transporters as well as a decrease in total surface area.<sup>64,70,84,85</sup> Absorption of sodium and water can still occur to some degree in the crypts when coupled with glucose or neutral amino acids (eg, glutamine), which can be used to improve absorption of oral rehydration solutions,<sup>86,87</sup> but overall absorption of carbohydrates, lipids, and amino acids is reduced.<sup>88–91</sup> This malabsorption leads to diarrhea that can range from very mild to life threatening, depending on the dose of organism and coinfection with other pathogens. However *C parvum* has not been shown to decrease overall growth in calves once the infection has resolved.<sup>55</sup>

### Prostaglandin-Mediated Diarrhea Due to *Cryptosporidium parvum*

Epithelial cell loss, villous atrophy, and malabsorption cannot account for all the fluid loss seen in *C parvum* infections, and studies have documented a prostaglandin-mediated anion secretion ( $\text{Cl}^-$  or  $\text{HCO}_3^-$ ) and inhibition of neutral NaCl absorption (Fig. 3). The prostaglandins  $\text{PGE}_2$  and prostaglandin  $\text{I}_2$  ( $\text{PGI}_2$ ) are found at higher concentrations in infected tissue, and blocking the secretion of these prostaglandins reverses the anion secretion and inhibition of NaCl absorption.<sup>70,84</sup> However, in vivo, inhibition of prostaglandins exacerbated the villous atrophy, indicating that this approach is unlikely to be useful therapeutically.<sup>92</sup> The source of prostaglandins in the infected tissue is unknown, but is believed to be leukocytes that infiltrate the lamina propria in the infection. Macrophages appear to be the most likely source because they invade the lamina propria after infection and can induce prostaglandin secretion from mesenchymal cells,<sup>64,70,92,93</sup> whereas inhibition of neutrophil migration into infected tissue had no effect on prostaglandin synthesis.<sup>94</sup> Furthermore, NO, which has been shown to be important in defense against *C parvum* infections,<sup>95</sup> stimulates prostaglandin-mediated secretion when NO production is augmented by arginine supplementation.<sup>96</sup>



**Fig. 3.** Infection of intestinal epithelial cells with *C parvum* induces the epithelial cell to secrete  $\text{PGE}_2$  and leads to activation of macrophages (M4) in the lamina propria. This leads to secretion of  $\text{PGE}_2$  and  $\text{PGI}_2$  from the mesenchymal cells.  $\text{PGI}_2$  activates the enteric nervous system to secrete acetylcholine (Ach) and VIP. The secretion of Ach, VIP, and  $\text{PGE}_2$  leads to an increase in intracellular calcium and cAMP, which activates anion secretion ( $\text{Cl}$  and  $\text{HCO}_3$ ) and inhibits neutral sodium and chloride absorption (NaCl).



The mechanism of action of the two prostaglandins differs because PGE<sub>2</sub> acts on the enterocyte directly, whereas PGI<sub>2</sub> exerts its effect through the ENS. PGI<sub>2</sub> causes 75% of the secretion in *C parvum* infection by stimulating the nicotinic ganglia and the VIP-ergic and cholinergic motor neurons that innervate the intestinal mucosa. Prostaglandin secretion ultimately leads to increases in calcium and cAMP that increase anion secretion and decrease sodium absorption.<sup>42,70,92</sup> Inhibiting the effects of prostaglandins on the ENS is a potential method of decreasing the diarrhea associated with *C parvum* without exacerbating the villous atrophy. Specifically, peptide YY, which is naturally found in the intestinal epithelium, is a potent inhibitor of VIP and can abolish the secretory response to PGI<sub>2</sub>.<sup>97</sup> Furthermore, if the inhibition caused by prostaglandins can be blocked, the intestine is capable of absorbing NaCl and water despite the villous atrophy, indicating that the transporters are fully functional, even in the immature enterocytes.<sup>85–87</sup>

### ***Treatment and Prevention of Cryptosporidium parvum Diarrhea***

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Because of the privileged location of *C parvum*, drug delivery can be difficult. Drugs in the lumen of the intestine may pass through without actually reaching the organism, whereas drugs that penetrate the intestinal epithelial cell would concentrate in the cytoplasm instead of the extracytoplasmic parasitophorous vacuole. Despite these challenges, multiple drugs have been studied as potential treatments with varying degrees of success, and none are currently licensed for calves in North America.<sup>55,57</sup> Halofuginone is licensed for prevention of *C parvum* infection in Europe when administered during the first 7 days of life. Unfortunately, clinical trials have not shown it to be consistently effective. When used as directed in a study of 31 bull calves, there was no difference in the incidence of or treatment rates for diarrhea between the treated and control calves. There was a significant delay in shedding of oocysts, but upon withdrawal of the drug, the treated calves began to shed a similar number of organisms. There was no difference in milk intake, weight gain, or age at weaning between the two groups.<sup>98</sup> Another study in which halofuginone treatment was initiated at 7 to 10 days of age saw no difference in the number of calves shedding oocysts or in the incidence of diarrhea. However, the total number of oocysts shed was reduced for the 7 days during treatment as well as the following 7 days. Oocyst shedding then rebounded and was greater than in control animals 21 days after the start of treatment.<sup>99</sup> Other studies have shown more favorable results in which the incidence of diarrhea and excretion of oocysts was reduced. Re-excretion of oocysts after stopping treatment continued to be a problem, but was less if a lower dose was used.<sup>100–102</sup> In an experimental infection, the only difference noted with treatment was a decrease in the number of calves dying. However, calves began excreting oocysts after treatment was discontinued, even though they had been housed individually and reinfection was unlikely. This indicates that halofuginone is cryptosporidiosis-static but is unlikely to effectively kill oocysts.<sup>103</sup> Halofuginone appears to be effective at decreasing oocyst shedding only when it is being administered. It may or may not reduce clinical signs in the calf, and has not been consistently shown to be effective as a treatment for *C parvum* diarrhea.

Paromomycin was shown to be effective in one trial of experimental infection as a prophylaxis for *C parvum* infection. The drug was administered 1 day prior to infection and continued for a total of 11 days. Oocyst shedding and diarrhea were decreased, but calves began to shed organisms at the end of the treatment period, and the shedding continued after treatment was stopped.<sup>104</sup> Decoquinatate has also been used to control *C parvum* in calves, but trials have not consistently shown it to reduce diarrhea or oocyst shedding in calves.<sup>57,99,105</sup> In a study from Turkey,

azithromycin was shown to be effective as a treatment of calves that were known to be shedding *C parvum* when it was administered at a dose of 1,500 mg/calf/day for 7 days. Treatment decreased oocyst shedding and diarrhea, and improved weight gain.<sup>106</sup> However, the cost of azithromycin in the United States would likely prevent its use to treat cryptosporidiosis at this time. In a small study, activated charcoal with wood vinegar liquid was effective in stopping diarrhea and oocyst shedding when administered after the start of experimentally induced *C parvum* diarrhea. This effect was noted 24 hours after addition of this product to the milk replacer.<sup>107</sup> It remains to be seen if this effect can be duplicated in a large-scale field trial.

Similar to chemotherapeutic agents, both active and passive vaccination have not been consistently successful in preventing *C parvum* infection, diarrhea, and oocyst shedding.<sup>108</sup> Vaccination of dry cows with whole organisms<sup>109</sup> or a recombinant protein<sup>110</sup> both reduced oocyst shedding and clinical signs, but neither have been validated under field conditions. An oral vaccine to be given to calves at birth prior to colostrum administration showed promise initially,<sup>111</sup> but was ineffective in a field trial.<sup>112,113</sup>

Because of the questionable benefit of mass medication or vaccination for control, prevention should be focused on decreasing exposure to the organism by appropriate hygiene and husbandry. Because *C parvum* is a zoonotic agent, appropriate personal hygiene is also important for public health and farm employee safety.<sup>112</sup> Specific treatment for *C parvum*-infected calves also cannot be recommended in the United States at this time, although the extralabel use of azithromycin or activated charcoal with wood vinegar appears promising. In general, treatment should be focused on appropriate fluid therapy and supportive care because most calves will recover from cryptosporidiosis if there is not an overwhelming infection or coinfection with another pathogen.

## ROTAVIRUS

Rotavirus was one of the first identified viral causes of diarrhea, and was initially known as neonatal calf diarrhea virus. Subsequently, it has been found throughout the world and has been identified as a significant pathogen of children and most other mammals.<sup>114,115</sup> Antibodies to rotavirus can be found in more than 90% of unvaccinated cattle,<sup>116</sup> and the virus was isolated from 94% of dairy calves at a large dairy and calf ranch during the first 2 weeks of life.<sup>117</sup> It has also been isolated from approximately 20% of calf diarrhea samples,<sup>118,119</sup> and from at least one calf on 63% of farms.<sup>120</sup> Calves become infected after ingesting the virus from fecal contamination of the environment, because the virus remains quite stable if the temperature does not get near freezing. The virus typically affects calves less than 3 weeks old, with a peak incidence at 6 days of age. After ingestion of the virus, the incubation period is approximately 24 hours, with resolution of diarrhea in uncomplicated cases in 2 days.<sup>115</sup> Classically, rotavirus diarrhea is thought to be primarily a malabsorptive diarrhea, but recent evidence indicates that there is also a toxin-mediated secretory component as well.

### ***Malabsorptive Diarrhea Caused by Rotavirus***

Similar to *C parvum*, rotavirus preferentially targets the mature villous enterocytes and spares the crypts, generally causing moderate villous damage. The virus attaches to these cells via specific receptors and invades through an unknown mechanism. The virus replicates within the cells, leading to enterocyte death. Malabsorption will then occur because of the loss of surface area, and unabsorbed glucose and other

carbohydrates create an osmotic load pulling fluid into the lumen. Furthermore, fluid secretion from the crypts increases the amount of fluid in the intestinal lumen relative to villous absorption, which leads to diarrhea.<sup>21,114,115</sup> However, the severity of clinical signs does not always correlate with histologic damage to the villi. This has led to speculation that there may be another mechanism contributing to the diarrhea seen with rotaviral infections, and that enterocyte damage is less critical than previously believed.

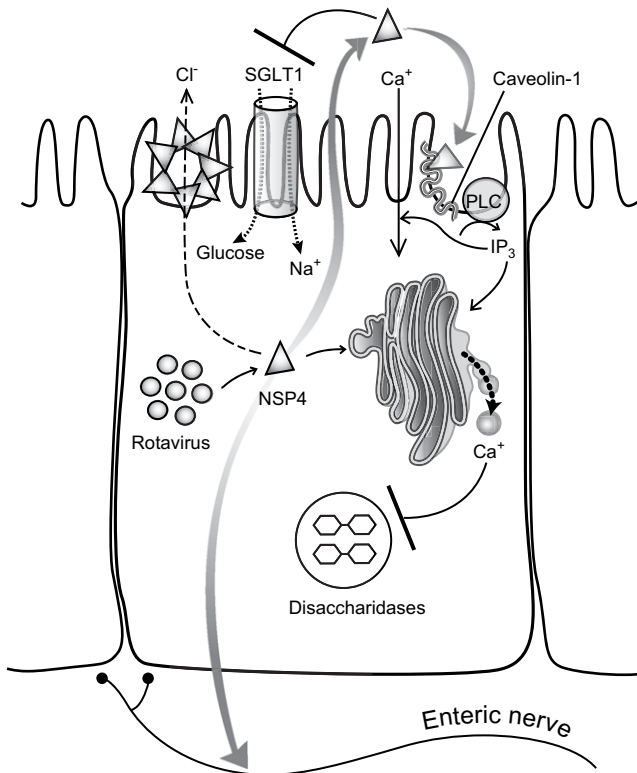
In the mid-1990s, a viral enterotoxin was demonstrated to be crucial to the pathogenesis of rotaviral diarrhea.<sup>121</sup> This was the first time an enterotoxin could be identified in a viral diarrheal pathogen, and this changed our fundamental understanding of rotavirus diarrhea.<sup>122</sup> The rotavirus protein, nonstructural glycoprotein 4 (NSP4), was found to induce a dose- and age-dependent diarrhea that is clinically similar to rotavirus diarrhea. Unlike the bacterial enterotoxins, diarrhea due to NSP4 is unrelated to cAMP, cGMP, or CFTR.<sup>123,124</sup> The protein is initially produced during intracellular viral replication and acts on the infected cell. It is secreted or released upon cell death, and acts in a paracrine manner.<sup>125</sup> Exogenous exposure of intestinal epithelial cells to NSP4 allows binding to caveolae, special lipid rafts within the endoplasmic reticulum (ER) and cell membrane. It specifically binds to caveolin-1, a transmembrane, hairpin protein unique to these rafts.<sup>126,127</sup> Binding to caveolin-1 leads to an increase in intracellular calcium concentrations by causing the release of calcium from ER stores and increasing movement across the plasma membrane. This is mediated by phospholipase C (PLC), which increases the level of intracellular inositol 1,4,5-triphosphate (IP<sub>3</sub>),<sup>124,128–130</sup> however intracellular NSP4 causes the release of calcium independent of the PLC pathway.<sup>131</sup>

Extracellular and intracellular exposure to NSP4 causes several changes in the movement of nutrients and water across the epithelium (**Fig. 4**). Increases in intracellular calcium inhibit the translocation of disaccharidases from the intracellular vesicles to the luminal surface, decreasing the ability to digest carbohydrates and leading to maldigestion and exacerbation of the diarrhea.<sup>132–134</sup> NSP4 also directly inhibits sodium glucose cotransporter SGLT1, the primary sodium and glucose cotransporter that is critical for effective water absorption, significantly contributing to the pathogenesis of rotaviral diarrhea.<sup>135</sup> The actions of NSP4 better account for the maldigestion and malabsorption that are seen in rotavirus diarrhea, and is likely more important to the pathogenesis than is histologic damage to the epithelium.

### ***Secretory Diarrhea Caused by Rotavirus***

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NSP4 has also been implicated in causing secretion of chloride through the increase in intracellular calcium,<sup>124</sup> but the importance of this finding is being increasingly questioned because the increase is relatively mild and only occurs early in the course of diarrhea.<sup>136,137</sup> As previously mentioned, the actions of NSP4 are independent of CFTR, so the ion channel that is important for this chloride movement is unknown, and has been hypothesized to be created by NSP4.<sup>114,124</sup> An alternate mechanism that may explain the chloride secretion occurring in rotaviral diarrhea is activation of the ENS. Pharmacologically inhibiting the ENS dramatically reduces the diarrhea seen with rotavirus infections, although the mechanism by which the virus activates ENS-dependent secretion is unknown.<sup>42,114,138</sup> Prostaglandins and other inflammatory mediators may also play a role in causing secretion by affecting the ENS,<sup>137</sup> similar to other intestinal pathogens such as *C parvum*.<sup>42,70,92</sup> The ENS appears to play a critical role in rotavirus-induced secretion, but the mechanism responsible for this effect is unclear.



**Fig. 4.** Once rotavirus replicates in an intestinal epithelial cell, the enterotoxin NSP4 is produced. It has autocrine effects by causing calcium (Ca) release from the endoplasmic reticulum. NSP4 has paracrine effects by being secreted and binding to caveolin-1. This activates PLC, which increases cytoplasmic IP<sub>3</sub>. IP<sub>3</sub> increases intracellular calcium by increasing release from the endoplasmic reticulum and increasing calcium movement across the luminal membrane. The increased intracellular calcium inhibits movement of disaccharidases to the luminal surface. NSP4 directly inhibits the SGLT1 which decreases the absorption of sodium (Na) and glucose, and increases chloride (Cl) secretion by an unknown mechanism, but may involve a channel created by NSP4. NSP4 also activates the enteric nervous system by an unknown mechanism.

### **Treatment and Control of Rotavirus Diarrhea**

Treatment of rotavirus diarrhea is focused solely on rehydration because there are no currently available pharmacologic methods of controlling the infection in calves. Whereas the inhibition of SGLT1 and subsequent decrease in sodium and glucose absorption mediated by NSP4 would theoretically make sodium- and glucose-containing oral rehydration solutions less effective, this has not been shown clinically or experimentally. Currently, the Centers for Disease Control and Prevention recommend a low-osmolality rehydration solution that contains sodium and glucose for children with acute gastroenteritis. This recommendation is irrespective of the cause, yet rotavirus is likely involved in a majority of the cases.<sup>139</sup> Similar fluids would be expected to be effective in rehydrating calves with rotaviral diarrhea as well.

Enhancing colostral antibody transfer to the calf from the dam appears to be the most effective method of control for rotaviral diarrhea in calves. First, proper

colostrum management is critical to ensure that an adequate IgG mass is delivered to each calf. This can be enhanced by administering vaccines to cows in the dry period to increase the amount of rotavirus-specific antibodies in the colostrum.<sup>140</sup> Experimental evidence has shown these vaccines to be effective at decreasing clinical signs,<sup>141–144</sup> however, some authors feel that the commercial vaccines are not as effective clinically.<sup>145</sup> There appears to be protection initially that is caused by the presence of specific antibodies in the intestinal lumen, but immunity in subsequent weeks is dependent on the resecretion of IgG into the intestine.<sup>142</sup> Oral vaccination of the calves at birth has been found to be less successful, and is not recommended.<sup>145</sup> Similar to other gastrointestinal pathogens, reducing exposure is also critical for control of rotaviral diarrhea in calves. Therefore, appropriate housing, stocking density, and hygiene cannot be ignored.

## **CORONAVIRUS**

The epidemiology and pathophysiology of coronavirus diarrhea in calves overlaps significantly with that caused by rotavirus. Antibodies to coronavirus are ubiquitous in cattle,<sup>146</sup> and the virus is frequently found in both normal and diarrheic feces of calves.<sup>147</sup> Coronavirus typically affects calves with the first 3 weeks of life, and peak incidence occurs between days 7 and 10. The virus is ingested from the environment, which is contaminated by other calves or adult cattle.<sup>115,148</sup> Clinical signs begin approximately 2 days later and continue for 3 to 6 days.<sup>148,149</sup> Diarrhea secondary to coronavirus is mainly caused by intestinal epithelial cell loss and malabsorption. This virus has also been implicated in respiratory disease outbreaks in older calves as well as a diarrheal disease of adult cattle (winter dysentery), but discussion of these syndromes is beyond the scope of this report.

### ***Malabsorptive Diarrhea Caused by Coronavirus***

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Coronavirus infection begins in the proximal small intestine, but then usually spreads throughout the jejunum, ileum, and colon. Initially, the virus attaches to the enterocyte via the spike and hemagglutinin glycoproteins, which also allow fusion of the viral envelope with the cell membrane or endocytotic vesicles.<sup>148,150–152</sup> Once in the cell, the virus replicates and is released using normal secretory mechanisms and upon cell death.<sup>148,153</sup> Diarrhea begins at the time of virus entry into the cell (before cell death occurs), but it is unknown if this is the result of secretion, malabsorption, or both.<sup>115</sup>

Infected cell loss is significant by 2 days after onset of diarrhea, and villous blunting occurs. The mature villous epithelial cells are the primary target for the virus, but crypt enterocytes are also affected.<sup>154,155</sup> As in rotavirus and *C parvum* infections, maldigestion and malabsorption lead to diarrhea. Because the crypt enterocytes and the colonocytes can be affected by coronavirus, the clinical signs often have a longer duration.<sup>149,154</sup>

### ***Treatment and Control of Coronavirus Diarrhea***

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Like rotavirus diarrhea, there are no specific treatment methods for coronavirus infections, and little research has been done to confirm specific control measures for coronavirus in calves. Oral electrolyte solutions should be provided to prevent dehydration and treat acidosis. Methods aimed at controlling rotavirus infections (proper housing and hygiene, good colostrum management, and dry cow vaccination) are believed to be the best measures for control of coronavirus as well. Furthermore,

most dry cow vaccinations targeted for neonatal calf diarrhea contain both rotavirus and coronavirus.

### OTHER CAUSES OF INFECTIOUS DIARRHEA IN NEONATAL CALVES

Salmonella is the other major infectious cause of diarrhea in calves, but it is discussed in a separate article in this issue. A few other minor causes of diarrhea in calves are worth brief discussion.

#### *Attaching and Effacing E coli*

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In addition to ETEC, there are several other types of *E coli* that are potential pathogens in calves, and these fall into the broad category of attaching and effacing *E coli*. These bacteria are characterized by the presence of the *eae* gene,<sup>156</sup> which encodes the protein intimin, a key component of the outer membrane that mediates attachment to the intestinal epithelium.<sup>157</sup> If these bacteria do not secrete any enterotoxins, they are classified as enteropathogenic *E coli* (EPEC).<sup>157</sup> EPEC organisms attach to the epithelium, disrupt the microvilli, and cause malabsorption.<sup>158</sup> They also use a Type III secretory protein to inject effector proteins into the host cell, inducing a secretory response by an undefined mechanism. Furthermore, they disrupt tight junctions between epithelial cells and lead to inflammation; all of which contribute to diarrhea.<sup>159</sup> The importance of EPEC as a pathogen of calves is debatable. It can be found in abnormal fecal samples, but is also frequently found in healthy calves<sup>160</sup> or not found at all.<sup>161</sup>

Enterohemorrhagic *E coli* (EHEC) are typically defined as expressing the *eae* gene and Shiga toxin. Strains that lack *eae* but secrete Shiga toxin are designated STEC.<sup>162</sup> Shiga toxin mediates many of the systemic effects that are seen in humans with *E coli* O157:H7 infections, and may cause some intestinal damage in some species.<sup>158</sup> Many epidemiological studies have shown that EHEC and STEC are commonly found in calves with normal and abnormal feces, and there is significant interest in these bacteria from the public health standpoint.<sup>160,161,163–166</sup> However, Shiga toxin receptors cannot be found in the intestine of calves or adult cattle, and no diarrhea was seen following experimental infection,<sup>167</sup> calling into question claims that these bacteria are pathogens of calves.

#### *Clostridium difficile*

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Diarrhea caused by *Clostridium difficile* appears to be an emerging problem in both humans and veterinary patients. Diarrhea caused by *C difficile* is mediated by bacterial toxins that lead to epithelial cell death, damage to epithelial cell tight junctions, inflammation of the mucosa and submucosa, and activation of the ENS.<sup>168</sup> *C difficile* and its toxins can be found in the feces of both normal and diarrheic calves,<sup>169–172</sup> but its role as a pathogen has not been clearly established. Purified toxins will cause epithelial damage and an increase in luminal fluid in a calf intestinal loop model,<sup>169</sup> however experimental infection has not been successful.<sup>172</sup>

#### *Giardia*

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*Giardia* organisms can be found in the feces of calves with diarrhea throughout the world, but is also commonly found in the feces of normal calves.<sup>58,59,66,173–182</sup> Some of these studies also found other pathogens along with *Giardia*, and none were controlled experiments. Only a single study has documented attempted experimental infection of calves with *Giardia*. In that study, histologic changes were found in only 2 of 12 calves, clinical signs were simply described as not severe, and

the incidence of diarrhea was not reported.<sup>183</sup> *Giardia* has been documented to cause villous atrophy in naturally infected calves,<sup>184</sup> and is known to cause a malabsorptive diarrhea in other species.<sup>185,186</sup> Therefore, some have proposed that it may not be a significant cause of disease, but could still negatively impact calf growth.<sup>55</sup> This has also not been experimentally proven. Although *Giardia* is commonly found in the feces of both dairy and beef calves, it is unknown if it is truly a pathogen.

### **Torovirus**

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In the early 1980s, an infectious agent similar to coronavirus was identified in a herd of beef cattle in Iowa.<sup>187</sup> It was initially named Breda virus, but has subsequently been renamed torovirus. Since that time, it has been identified in both beef and dairy calves throughout the world, and 94% of adult cattle are seropositive.<sup>188</sup> Torovirus is found in calves with normal and abnormal feces, but is isolated more frequently in diarrheic feces. The incidence in calves with diarrhea ranged from 5% to 35%, while it was never isolated from more than 12% of normal calves. Other pathogens were commonly, but not always, found in conjunction with torovirus, but none appeared to be consistently associated with torovirus infection.<sup>189–193</sup> After ingestion, the virus infects the epithelium of the distal half of the jejunum, the ileum, and colon. Histopathologic lesions in experimental infections include necrosis of the crypt and villous enterocytes and villous atrophy, but infection does not consistently lead to clinical signs or histologic damage.<sup>187,194,195</sup> Although it has not been conclusively shown, these lesions would be expected to lead to a malabsorptive diarrhea. There is no specific information on control of torovirus, but as with other viruses, proper housing, decreasing exposure to adult cattle, and good hygiene are likely important to prevent its spread.

### **SUMMARY**

Pathophysiologic mechanisms of infectious diarrhea in calves can be generally divided into malabsorptive/maldigestive, secretory, or both, and research into these mechanisms at the cellular level may ultimately lead to more specific control and treatment methods. Currently, most information must be extrapolated from other research models because calves are not commonly used. Further elucidation of the mechanisms by which these pathogens affect calves is critical because diarrhea is a significant cause of morbidity and mortality in both dairy and beef cattle.

### **REFERENCES**

1. NAHMS. Reference of 1997 beef cow-calf production management and disease control. USDA survey 1998.
2. NAHMS. Dairy heifer morbidity, mortality, and health management focusing on preweaned heifers. USDA survey 1994.
3. Acres SD. Enterotoxigenic *Escherichia coli* infections in newborn calves: a review. *J Dairy Sci* 1985;68(1):229–56.
4. Acres SD, Saunders JR, Radostits OM. Acute undifferentiated neonatal diarrhea of beef calves: the prevalence of enterotoxigenic *E. coli*, reo-like (rota) virus and other enteropathogens in cow-calf herds. *Can Vet J* 1977;18(5):113–21.
5. Sherwood D, Snodgrass DR, Lawson GH. Prevalence of enterotoxigenic *Escherichia coli* in calves in Scotland and northern England. *Vet Rec* 1983; 113(10):208–12.



6. Myers LL, Guinea PA. Occurrence and characteristics of enterotoxigenic *Escherichia coli* isolated from calves with diarrhea. *Infect Immun* 1976;13(4):1117–9.
7. Smith HW. The development of the flora of the alimentary tract in young animals. *J Pathol Bacteriol* 1965;90(2):495–513.
8. Smith HW. Observations on the flora of the alimentary tract of animals and factors affecting its composition. *J Pathol Bacteriol* 1965;89:95–122.
9. Hadad JJ, Gyles CL. The role of K antigens of enteropathogenic *Escherichia coli* in colonization of the small intestine of calves. *Can J Comp Med* 1982;46(1):21–6.
10. Hadad JJ, Gyles CL. Scanning and transmission electron microscopic study of the small intestine of colostrum-fed calves infected with selected strains of *Escherichia coli*. *Am J Vet Res* 1982;43(1):41–9.
11. Holland RE. Some infectious causes of diarrhea in young farm animals. *Clin Microbiol Rev* 1990;3(4):345–75.
12. Mainil JG, Bex F, Jacquemin E, et al. Prevalence of four enterotoxin (STaP, STaH, STb, and LT) and four adhesin subunit (K99, K88, 987P, and F41) genes among *Escherichia coli* isolates from cattle. *Am J Vet Res* 1990;51(2):187–90.
13. Shin SJ, Chang YF, Timour M, et al. Hybridization of clinical *Escherichia coli* isolates from calves and piglets in New York State with gene probes for enterotoxins (STaP, STb, LT), Shiga-like toxins (SLT-1, SLT-II) and adhesion factors (K88, K99, F41, 987P). *Vet Microbiol* 1994;38(3):217–25.
14. Francis DH, Allen SD, White RD. Influence of bovine intestinal fluid on the expression of K99 pili by *Escherichia coli*. *Am J Vet Res* 1989;50(6):822–6.
15. Mylrea PJ. Functioning of the digestive tract of young fasted calves. *Res Vet Sci* 1968;9(1):1–4.
16. Mylrea PJ. Gastro-intestinal disorders and the functioning of the digestive tract of young calves. *Res Vet Sci* 1968;9(1):14–28.
17. Smith HW. Observations on the aetiology of neonatal diarrhoea (scours) in calves. *J Pathol Bacteriol* 1962;84:147–68.
18. Constable PD. Pathophysiology of calf diarrhea: new concepts. Presented at the ACVIM Forum Proceedings. Charlotte, NC, June 3–7, 2003.
19. 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(1):298–300.
20. Pearson GR, Logan EF. The pathogenesis of enteric colibacillosis in neonatal unsuckled calves. *Vet Rec* 1979;105(8):159–64.
21. Argenzio RA. Pathophysiology of neonatal calf diarrhea. *Vet Clin North Am Food Anim Pract* 1985;1(3):461–9.
22. Field M, Semrad CE. Toxigenic diarrheas, congenital diarrheas, and cystic fibrosis: disorders of intestinal ion transport. *Annu Rev Physiol* 1993;55:631–55.
23. Mainil JG, Moseley SL, Schneider RA, et al. Hybridization of bovine *Escherichia coli* isolates with gene probes for four enterotoxins (STaP, STaH, STb, LT) and one adhesion factor (K99). *Am J Vet Res* 1986;47(5):1145–8.
24. Acosta-Martinez F, Gyles CL, Butler DG. *Escherichia coli* heat-stable enterotoxin in feces and intestines of calves with diarrhea. *Am J Vet Res* 1980;41(7):1143–9.
25. Saeed AM, Magnuson NS, Gay CC, et al. Characterization of heat-stable enterotoxin from a hypertoxigenic *Escherichia coli* strain that is pathogenic for cattle. *Infect Immun* 1986;53(2):445–7.
26. Giannella RA, Mann EA. *E. coli* heat-stable enterotoxin and guanylyl cyclase C: new functions and unsuspected actions. *Trans Am Clin Climatol Assoc* 2003;114:67–85 [discussion: 85–6].

27. Dreyfus LA, Robertson DC. Solubilization and partial characterization of the intestinal receptor for *Escherichia coli* heat-stable enterotoxin. *Infect Immun* 1984;46(2):537–43.
28. Krause WJ, Cullingford GL, Freeman RH, et al. Distribution of heat-stable enterotoxin/guanylin receptors in the intestinal tract of man and other mammals. *J Anat* 1994;184(Pt 2):407–17.
29. Al-Majali AM, Asem EK, Lamar CH, et al. Studies on the mechanism of diarrhoea induced by *Escherichia coli* heat-stable enterotoxin (STa) in newborn calves. *Vet Res Commun* 2000;24(5):327–38.
30. Al-Majali AM, Asem EK, Lamar CH, et al. Characterization of the interaction of *Escherichia coli* heat-stable enterotoxin (STa) with its putative receptor on the intestinal tract of newborn calves. *FEMS Immunol Med Microbiol* 2000;28(2):97–104.
31. al-Majali AM, Robinson JP, Asem EK, et al. Age-dependent variation in the density and affinity of *Escherichia coli* heat-stable enterotoxin receptors in mice. *Adv Exp Med Biol* 1999;473:137–45.
32. Jaso-Friedmann L, Dreyfus LA, Whipp SC, et al. Effect of age on activation of porcine intestinal guanylate cyclase and binding of *Escherichia coli* heat-stable enterotoxin (STa) to porcine intestinal cells and brush border membranes. *Am J Vet Res* 1992;53(12):2251–8.
33. Golin-Bisello F, Bradbury N, Ameen N, et al. STa and cGMP stimulate translocation to the surface of villus enterocytes in rat jejunum and is regulated by protein kinase G. *Am J Physiol, Cell Physiol* 2005;289(3):C708–16.
34. Thiagarajah JR, Broadbent T, Hsieh E, et al. Prevention of toxin-induced intestinal ion and fluid secretion by a small-molecule CFTR inhibitor. *Gastroenterology* 2004;126(2):511–9.
35. Sellers ZM, Childs D, Chow JY, et al. Heat-stable enterotoxin of *Escherichia coli* stimulates a non-CFTR-mediated duodenal bicarbonate secretory pathway. *Am J Physiol Gastrointest Liver Physiol* 2005;288(4):G654–63.
36. Tantisira MH. Effects of heat-stable *Escherichia coli* enterotoxin on intestinal alkaline secretion and transepithelial potential difference in the rat intestines in vivo. *Scand J Gastroenterol* 1990;25(1):19–28.
37. Lucas ML, Thom MM, Bradley JM, et al. *Escherichia coli* heat stable (STa) enterotoxin and the upper small intestine: lack of evidence in vivo for net fluid secretion. *J Membr Biol* 2005;206(1):29–42.
38. Lucas ML. A reconsideration of the evidence for *Escherichia coli* STa (heat stable) enterotoxin-driven fluid secretion: a new view of STa action and a new paradigm for fluid absorption. *J Appl Microbiol* 2001;90(1):7–26.
39. Mitchell ID, Tame MJ, Kenworthy R. Conditions for the production of *Escherichia coli* enterotoxin in a defined medium. *J Med Microbiol* 1974;7:395–400.
40. Mourad FH, Nassar CF. Effect of vasoactive intestinal polypeptide (VIP) antagonism on rat jejunal fluid and electrolyte secretion induced by cholera and *Escherichia coli* enterotoxins. *Gut* 2000;47(3):382–6.
41. Peterson JW, Whipp SC. Comparison of the mechanisms of action of cholera toxin and the heat-stable enterotoxins of *Escherichia coli*. *Infect Immun* 1995;63(4):1452–61.
42. Jones SL, Blikslager AT. Role of the enteric nervous system in the pathophysiology of secretory diarrhea. *J Vet Intern Med* 2002;16(3):222–8.
43. Lucas ML, Duncan NW, O'Reilly NF, et al. Lack of evidence in vivo for a remote effect of *Escherichia coli* heat stable enterotoxin on jejunal fluid absorption. *Neurogastroenterol Motil* 2008;20(5):532–8.

44. Eklund S. The net fluid secretion caused by cyclic 3'5'-guanosine monophosphate in the rat jejunum in vivo is mediated by a local nervous reflex. *Acta Physiol Scand* 1986;128(1):57–63.
45. Eklund S. The enteric nervous system participates in the secretory response to the heat stable enterotoxins of *Escherichia coli* in rats and cats. *Neuroscience* 1985; 14(2):673–81.
46. Rolfe V, Levin RJ. Enterotoxin *Escherichia coli* STa activates a nitric oxide-dependent myenteric plexus secretory reflex in the rat ileum. *J Physiol* 1994;475(3):531–7.
47. Nzegwu HC, Levin RJ. Role of the enteric nervous system in the maintained hypersecretion induced by enterotoxin STa in the nutritionally deprived intestine. *Gut* 1994;35(9):1237–43.
48. Nzegwu HC, Levin RJ. Fluid hypersecretion induced by enterotoxin STa in nutritionally deprived rats: jejunal and ileal dynamics in vivo. *Exp Physiol* 1994; 79(4):547–60.
49. Nzegwu HC, Levin RJ. Neurally maintained hypersecretion in undernourished rat intestine activated by *E. coli* STa enterotoxin and cyclic nucleotides in vitro. *J Physiol* 1994;479(Pt 1):159–69.
50. Ahmed AF, Constable PD, Misk NA. Effect of feeding frequency and route of administration on abomasal luminal pH in dairy calves fed milk replacer. *J Dairy Sci* 2002;85(6):1502–8.
51. Smith HW, Halls S. Observations by the ligated intestinal segment and oral inoculation methods on *Escherichia coli* infections in pigs, calves, lambs and rabbits. *J Pathol Bacteriol* 1967;93(2):499–529.
52. Constable PD. Antimicrobial use in the treatment of calf diarrhea. *J Vet Intern Med* 2004;18(1):8–17.
53. Mosier DA, Oberst RD. Cryptosporidiosis. A global challenge. *Ann NY Acad Sci* 2000;916:102–11.
54. MacKenzie WR, Schell WL, Blair KA, et al. Massive outbreak of waterborne cryptosporidium infection in Milwaukee, Wisconsin: recurrence of illness and risk of secondary transmission. *Clin Infect Dis* 1995;21(1):57–62.
55. O'Handley RM, Olson ME. Giardiasis and cryptosporidiosis in ruminants. *Vet Clin Food Anim* 2006;22(3):623–43.
56. Tzipori S, Ward H. Cryptosporidiosis: biology, pathogenesis and disease. *Microbes Infect* 2002;4(10):1047–58.
57. de Graaf DC, Vanopdenbosch E, Ortega-Mora LM, et al. A review of the importance of cryptosporidiosis in farm animals. *Int J Parasitol* 1999;29(8):1269–87.
58. 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(3):391–6.
59. 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(2):113–22.
60. Fayer R, Gasbarre L, Pasquali P, et al. Cryptosporidium parvum infection in bovine neonates: dynamic clinical, parasitic and immunologic patterns. *Int J Parasitol* 1998;28(1):49–56.
61. 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(2):103–17.
62. Langkjaer RB, Vigre H, Enemark HL, et al. Molecular and phylogenetic characterization of *Cryptosporidium* and *Giardia* from pigs and cattle in Denmark. *Parasitology* 2007;134(Pt 3):339–50.

63. Harp JA, Woodmansee DB, Moon HW. Resistance of calves to *Cryptosporidium parvum*: effects of age and previous exposure. *Infect Immun* 1990;58(7):2237–40.
64. Argenzio RA, Liacos JA, Levy ML, et al. Villous atrophy, crypt hyperplasia, cellular infiltration, and impaired glucose-Na absorption in enteric cryptosporidiosis of pigs. *Gastroenterology* 1990;98(5 Pt 1):1129–40.
65. Xiao L, Herd RP. Infection pattern of *Cryptosporidium* and *Giardia* in calves. *Vet Parasitol* 1994;55(3):257–62.
66. Gow S, Waldner C. An examination of the prevalence of and risk factors for shedding of *Cryptosporidium* spp. and *Giardia* spp. in cows and calves from western Canadian cow-calf herds. *Vet Parasitol* 2006;137(1–2):50–61.
67. Anderson BC. Cryptosporidiosis in bovine and human health. *J Dairy Sci* 1998; 81(11):3036–41.
68. Tzipori S, Angus KW, Campbell I, et al. Experimental infection of lambs with *Cryptosporidium* isolated from a human patient with diarrhoea. *Gut* 1982;23(1):71–4.
69. Heine J, Pohlenz JF, Moon HW, et al. Enteric lesions and diarrhea in gnotobiotic calves monoinfected with *Cryptosporidium* species. *J Infect Dis* 1984;150(5): 768–75.
70. Gookin JL, Nordone SK, Argenzio RA. Host responses to *Cryptosporidium* infection. *J Vet Intern Med* 2002;16(1):12–21.
71. Moore R, Tzipori S, Griffiths JK, et al. Temporal changes in permeability and structure of piglet ileum after site-specific infection by *Cryptosporidium parvum*. *Gastroenterology* 1995;108(4):1030–9.
72. Chen XM, Levine SA, Tietz P, et al. *Cryptosporidium parvum* is cytopathic for cultured human biliary epithelia via an apoptotic mechanism. *Hepatology* 1998;28(4):906–13.
73. Adams RB, Guerrant RL, Zu S, et al. *Cryptosporidium parvum* infection of intestinal epithelium: morphologic and functional studies in an in vitro model. *J Infect Dis* 1994;169(1):170–7.
74. Laurent F, Eckmann L, Savidge TC, et al. *Cryptosporidium parvum* infection of human intestinal epithelial cells induces the polarized secretion of C-X-C chemokines. *Infect Immun* 1997;65(12):5067–73.
75. Griffiths JK, Moore R, Dooley S, et al. *Cryptosporidium parvum* infection of Caco-2 cell monolayers induces an apical monolayer defect, selectively increases transmonolayer permeability, and causes epithelial cell death. *Infect Immun* 1994;62(10):4506–14.
76. Buret AG, Chin AC, Scott KG. Infection of human and bovine epithelial cells with *Cryptosporidium andersoni* induces apoptosis and disrupts tight junctional ZO-1: effects of epidermal growth factor. *Int J Parasitol* 2003;33(12):1363–71.
77. Chen XM, Gores GJ, Paya CV, et al. *Cryptosporidium parvum* induces apoptosis in biliary epithelia by a Fas/Fas ligand-dependent mechanism. *Am J Phys* 1999; 277(3 Pt 1):G599–608.
78. Chen XM, Levine SA, Splinter PL, et al. *Cryptosporidium parvum* activates nuclear factor kappaB in biliary epithelia preventing epithelial cell apoptosis. *Gastroenterology* 2001;120(7):1774–83.
79. McCole DF, Eckmann L, Laurent F, et al. Intestinal epithelial cell apoptosis following *Cryptosporidium parvum* infection. *Infect Immun* 2000;68(3):1710–3.
80. Ojcius DM, Perfettini JL, Bonnin A, et al. Caspase-dependent apoptosis during infection with *Cryptosporidium parvum*. *Microbes Infect* 1999;1(14):1163–8.
81. Motta I, Gissot M, Kanellopoulos JM, et al. Absence of weight loss during *Cryptosporidium* infection in susceptible mice deficient in Fas-mediated apoptosis. *Microbes Infect* 2002;4(8):821–7.

82. Widmer G, Corey EA, Stein B, et al. Host cell apoptosis impairs *Cryptosporidium parvum* development in vitro. *J Parasitol* 2000;86(5):922–8.
83. Mele R, Gomez Morales MA, Tosini F, et al. *Cryptosporidium parvum* at different developmental stages modulates host cell apoptosis in vitro. *Infect Immun* 2004; 72(10):6061–7.
84. Argenzio RA, Lecce J, Powell DW. Prostanoids inhibit intestinal NaCl absorption in experimental porcine cryptosporidiosis. *Gastroenterology* 1993;104(2):440–7.
85. Argenzio RA, Rhoads JM, Armstrong M, et al. Glutamine stimulates prostaglandin-sensitive Na(+)-H+ exchange in experimental porcine cryptosporidiosis. *Gastroenterology* 1994;106(6):1418–28.
86. Blikslager A, Hunt E, Guerrant R, et al. Glutamine transporter in crypts compensates for loss of villus absorption in bovine cryptosporidiosis. *Am J Physiol Gastrointest Liver Physiol* 2001;281(3):G645–53.
87. Cole J, Blikslager A, Hunt E, et al. Cyclooxygenase blockade and exogenous glutamine enhance sodium absorption in infected bovine ileum. *Am J Physiol Gastrointest Liver Physiol* 2003;284(3):G516–24.
88. Topouchian A, Huneau JF, Barbot L, et al. Evidence for the absence of an intestinal adaptive mechanism to compensate for *C. parvum*-induced amino acid malabsorption in suckling rats. *Parasitol Res* 2003;91(3):197–203.
89. Topouchian A, Kapel N, Huneau JF, et al. Impairment of amino-acid absorption in suckling rats infected with *Cryptosporidium parvum*. *Parasitol Res* 2001; 87(11):891–6.
90. Klein P, Kleinova T, Volek Z, et al. Effect of *Cryptosporidium parvum* infection on the absorptive capacity and paracellular permeability of the small intestine in neonatal calves. *Vet Parasitol* 2008;152(1–2):53–9.
91. Holland RE, Herdt TH, Refsal KR. Pulmonary excretion of H<sub>2</sub> in calves with *Cryptosporidium*-induced malabsorption. *Dig Dis Sci* 1989;34(9):1399–404.
92. Argenzio RA, Armstrong M, Rhoads JM. Role of the enteric nervous system in piglet cryptosporidiosis. *J Pharmacol Exp Ther* 1996;279(3):1109–15.
93. Kandil HM, Berschneider HM, Argenzio RA. Tumour necrosis factor alpha changes porcine intestinal ion transport through a paracrine mechanism involving prostaglandins. *Gut* 1994;35(7):934–40.
94. Zadrozny LM, Stauffer SH, Armstrong MU, et al. Neutrophils do not mediate the pathophysiological sequelae of *Cryptosporidium parvum* infection in neonatal piglets. *Infect Immun* 2006;74(10):5497–505.
95. Gookin JL, Chiang S, Alle J, et al. NF-kappaB-mediated expression of iNOS promotes epithelial defense against infection by *Cryptosporidium parvum* in neonatal piglets. *Am J Physiol Gastrointest Liver Physiol* 2006;290(1): G164–74.
96. Gookin JL, Foster DM, Coccaro MR, et al. Oral delivery of L-arginine stimulates prostaglandin-dependent secretory diarrhea in *Cryptosporidium parvum*-infected neonatal piglets. *J Pediatr Gastroenterol Nutr* 2008;46(2):139–46.
97. Argenzio RA, Armstrong M, Blikslager A, et al. Peptide YY inhibits intestinal Cl- secretion in experimental porcine cryptosporidiosis through a prostaglandin-activated neural pathway. *J Pharmacol Exp Ther* 1997;283(2):692–7.
98. Jarvie BD, Trotz-Williams LA, McKnight DR, et al. Effect of halofuginone lactate on the occurrence of *Cryptosporidium parvum* and growth of neonatal dairy calves. *J Dairy Sci* 2005;88(5):1801–6.
99. Lallemand M, Villeneuve A, Belda J, et al. Field study of the efficacy of halofuginone and decoquinone in the treatment of cryptosporidiosis in veal calves. *Vet Rec* 2006;159(20):672–6.

100. Villacorta I, Peeters JE, Vanopdenbosch E, et al. Efficacy of halofuginone lactate against *Cryptosporidium parvum* in calves. *Antimicrobial Agents Chemother* 1991;35(2):283–7.
101. Peeters JE, Villacorta I, Naciri M, et al. Specific serum and local antibody responses against *Cryptosporidium parvum* during medication of calves with halofuginone lactate. *Infect Immun* 1993;61(10):4440–5.
102. Lefay D, Naciri M, Poirier P, et al. Efficacy of halofuginone lactate in the prevention of cryptosporidiosis in suckling calves. *Vet Rec* 2001;148(4):108–12.
103. Naciri M, Mancassola R, Yvore P, et al. The effect of halofuginone lactate on experimental *Cryptosporidium parvum* infections in calves. *Vet Parasitol* 1993; 45(3–4):199–207.
104. Fayer R, Ellis W. Paromomycin is effective as prophylaxis for cryptosporidiosis in dairy calves. *J Parasitol* 1993;79(5):771–4.
105. Moore DA, Atwill ER, Kirk JH, et al. Prophylactic use of decoquinate for infections with *Cryptosporidium parvum* in experimentally challenged neonatal calves. *J Am Vet Med Assoc* 2003;223(6):839–45.
106. Elitok B, Elitok OM, Pulat H. Efficacy of azithromycin dihydrate in treatment of cryptosporidiosis in naturally infected dairy calves. *J Vet Intern Med* 2005; 19(4):590–3.
107. Watarai S, Tana, Koiwa M. Feeding activated charcoal from bark containing wood vinegar liquid (nekka-rich) is effective as treatment for cryptosporidiosis in calves. *J Dairy Sci* 2008;91(4):1458–63.
108. de Graaf DC, Spano F, Petry F, et al. Speculation on whether a vaccine against cryptosporidiosis is a reality or fantasy. *Int J Parasitol* 1999;29(8):1289–306.
109. Fayer R, Andrews C, Ungar BL, et al. Efficacy of hyperimmune bovine colostrum for prophylaxis of cryptosporidiosis in neonatal calves. *J Parasitol* 1989;75(3):393–7.
110. Perryman LE, Kapil SJ, Jones ML, et al. Protection of calves against cryptosporidiosis with immune bovine colostrum induced by a *Cryptosporidium parvum* recombinant protein. *Vaccine* 1999;17(17):2142–9.
111. Harp JA, Goff JP. Protection of calves with a vaccine against *Cryptosporidium parvum*. *J Parasitol* 1995;81(1):54–7.
112. Harp JA, Goff JP. Strategies for the control of *Cryptosporidium parvum* infection in calves. *J Dairy Sci* 1998;81(1):289–94.
113. Harp JA, Jardon P, Atwill ER, et al. Field testing of prophylactic measures against *Cryptosporidium parvum* infection in calves in a California dairy herd. *Am J Vet Res* 1996;57(11):1586–8.
114. Ramig RF. Pathogenesis of intestinal and systemic rotavirus infection. *J Virol* 2004;78(19):10213–20.
115. Torres-Medina A, Schlafer DH, Mebus CA. Rotaviral and coronaviral diarrhea. *Vet Clin North Am Food Anim Pract* 1985;1(3):471–93.
116. Schlafer DH, Scott FW. Prevalence of neutralizing antibody to the calf rotavirus in New York cattle. *Cornell Vet* 1979;69(3):262–71.
117. Chinsangaram J, Schore CE, Guterbock W, et al. Prevalence of group A and group B rotaviruses in the feces of neonatal dairy calves from California. *Comp Immunol Microbiol Infect Dis* 1995;18(2):93–103.
118. Theil KW, McCloskey CM. Molecular epidemiology and subgroup determination of bovine group A rotaviruses associated with diarrhea in dairy and beef calves. *J Clin Microbiol* 1989;27(1):126–31.
119. Alfieri AA, Parazzi ME, Takiuchi E, et al. Frequency of group A rotavirus in diarrhoeic calves in Brazilian cattle herds, 1998–2002. *Trop Anim Health Prod* 2006;38(7–8):521–6.

120. Lucchelli A, Lance SE, Bartlett PB, et al. Prevalence of bovine group A rotavirus shedding among dairy calves in Ohio. *Am J Vet Res* 1992;53(2):169–74.
121. Ball JM, Tian P, Zeng CQ, et al. Age-dependent diarrhea induced by a rotaviral nonstructural glycoprotein. *Science* 1996;272(5258):101–4.
122. Ciarlet M, Estes MK. Interactions between rotavirus and gastrointestinal cells. *Curr Opin Microbiol* 2001;4(4):435–41.
123. Morris AP, Estes MK. Microbes and microbial toxins: paradigms for microbial–mucosal interactions. VIII. Pathological consequences of rotavirus infection and its enterotoxin. *Am J Physiol Gastrointest Liver Physiol* 2001;281(2):G303–10.
124. Morris AP, Scott JK, Ball JM, et al. NSP4 elicits age-dependent diarrhea and Ca(2+)mediated I(-) influx into intestinal crypts of CF mice. *Am J Physiol* 1999;277(2 Pt 1):G431–44.
125. Zhang M, Zeng CQ, Morris AP, et al. A functional NSP4 enterotoxin peptide secreted from rotavirus-infected cells. *J Virol* 2000;74(24):11663–70.
126. Parr RD, Storey SM, Mitchell DM, et al. The rotavirus enterotoxin NSP4 directly interacts with the caveolar structural protein caveolin-1. *J Virol* 2006;80(6):2842–54.
127. Storey SM, Gibbons TF, Williams CV, et al. Full-length, glycosylated NSP4 is localized to plasma membrane caveolae by a novel raft isolation technique. *J Virol* 2007;81(11):5472–83.
128. Dong Y, Zeng CQ, Ball JM, et al. The rotavirus enterotoxin NSP4 mobilizes intracellular calcium in human intestinal cells by stimulating phospholipase C-mediated inositol 1,4,5-trisphosphate production. *Proc Natl Acad Sci U S A* 1997;94(8):3960–5.
129. Brunet JP, Cotte-Laffitte J, Linxe C, et al. Rotavirus infection induces an increase in intracellular calcium concentration in human intestinal epithelial cells: role in microvillar actin alteration. *J Virol* 2000;74(5):2323–32.
130. Brunet JP, Jourdan N, Cotte-Laffitte J, et al. Rotavirus infection induces cytoskeleton disorganization in human intestinal epithelial cells: implication of an increase in intracellular calcium concentration. *J Virol* 2000;74(22):10801–6.
131. Berkova Z, Morris AP, Estes MK. Cytoplasmic calcium measurement in rotavirus enterotoxin-enhanced green fluorescent protein (NSP4-EGFP) expressing cells loaded with Fura-2. *Cell Calcium* 2003;34(1):55–68.
132. Collins J, Candy DC, Starkey WG, et al. Disaccharidase activities in small intestine of rotavirus-infected suckling mice: a histochemical study. *J Pediatr Gastroenterol Nutr* 1990;11(3):395–403.
133. Jourdan N, Brunet JP, Sapin C, et al. Rotavirus infection reduces sucrase–isomaltase expression in human intestinal epithelial cells by perturbing protein targeting and organization of microvillar cytoskeleton. *J Virol* 1998;72(9):7228–36.
134. Martin-Latil S, Cotte-Laffitte J, Beau I, et al. A cyclic AMP protein kinase A-dependent mechanism by which rotavirus impairs the expression and enzyme activity of brush border–associated sucrase–isomaltase in differentiated intestinal Caco-2 cells. *Cell Microbiol* 2004;6(8):719–31.
135. Halaihel N, Lievin V, Ball JM, et al. Direct inhibitory effect of rotavirus NSP4(114–135) peptide on the Na(+)-D-glucose symporter of rabbit intestinal brush border membrane. *J Virol* 2000;74(20):9464–70.
136. Lorrot M, Benhamadouche-Casari H, Vasseur M. Mechanisms of net chloride secretion during rotavirus diarrhea in young rabbits: do intestinal villi secrete chloride? *Cell Physiol Biochem* 2006;18(1-3):103–12.



137. Lorrot M, Vasseur M. How do the rotavirus NSP4 and bacterial enterotoxins lead differently to diarrhea? *Virology* 2007;4:31–7.
138. Lundgren O, Peregrin AT, Persson K, et al. Role of the enteric nervous system in the fluid and electrolyte secretion of rotavirus diarrhea. *Science* 2000;287(5452):491–5.
139. King CK. Managing acute gastroenteritis among children: oral rehydration, maintenance, and nutritional therapy. *MMWR Recomm Rep* 2003;52(RR-16):1–16.
140. Kohara J. Enhancement of passive immunity with maternal vaccine against newborn calf diarrhea. *J Vet Med Sci* 1997;59(11):1023–5.
141. Fernandez FM, Conner ME, Hodgins DC, et al. Passive immunity to bovine rotavirus in newborn calves fed colostrum supplements from cows immunized with recombinant SA11 rotavirus core-like particle (CLP) or virus-like particle (VLP) vaccines. *Vaccine* 1998;16(5):507–16.
142. Parreno V, Bejar C, Vagnozzi A, et al. Modulation by colostrum-acquired maternal antibodies of systemic and mucosal antibody responses to rotavirus in calves experimentally challenged with bovine rotavirus. *Vet Immunol Immunopathol* 2004;100(1-2):7–24.
143. Saif LJ, Smith KL. Enteric viral infections of calves and passive immunity. *J Dairy Sci* 1985;68(1):206–28.
144. Cornaglia EM. Reduction in morbidity due to diarrhea in nursing beef calves by use of an inactivated oil-adjuvanted rotavirus–*Escherichia coli* vaccine in the dam. *Vet Microbiol* 1992;30(2-3):191–202.
145. Radostits OM, Gay CC, Blood DC, et al. Viral diarrhea in calves, lambs, kids, piglets, and foals. In: *Veterinary medicine: a textbook of the diseases of cattle, sheep, pigs, goats, and horses*. 9th edition. New York: WB Saunders and Co, LTD; 1999. p. 1115.
146. Rodak L, Babiuk LA, Acres SD. Detection by radioimmunoassay and enzyme-linked immunosorbent assay of coronavirus antibodies in bovine serum and lacteal secretions. *J Clin Microbiol* 1982;16(1):34–40.
147. Snodgrass DR, Terzolo HR, Sherwood D, et al. Aetiology of diarrhoea in young calves. *Vet Rec* 1986;119(2):31–4.
148. Clark MA. Bovine coronavirus. *Br Vet J* 1993;149(1):51–70.
149. Lewis LD. Pathophysiologic changes due to coronavirus-induced diarrhea in the calf. *J Am Vet Med Assoc* 1978;173(5 Pt 2):636–42.
150. Schultze B, Gross HJ, Brossmer R, et al. The S protein of bovine coronavirus is a hemagglutinin recognizing 9-O-acetylated sialic acid as a receptor determinant. *J Virol* 1991;65(11):6232–7.
151. Schultze B, Wahn K, Klenk HD, et al. Isolated HE-protein from hemagglutinating encephalomyelitis virus and bovine coronavirus has receptor-destroying and receptor-binding activity. *Virology* 1991;180(1):221–8.
152. Payne HR, Storz J, Henk WG. Initial events in bovine coronavirus infection: analysis through immunogold probes and lysosomotropic inhibitors. *Arch Virol* 1990;114(3-4):175–89.
153. Payne HR, Storz J. Scanning electron microscopic characterization of bovine coronavirus plaques in HRT cells. *Zentralbl Veterinarmed B* 1990;37(7):501–8.
154. Storz J, Doughri AM, Hajer I. Coronavirus morphogenesis and ultrastructural changes in intestinal infections of calves. *J Am Vet Med Assoc* 1978;173(5 Pt 2):633–5.
155. Park SJ, Kim GY, Choy HE, et al. Dual enteric and respiratory tropisms of winter dysentery bovine coronavirus in calves. *Arch Virol* 2007;152(10):1885–900.
156. Moon HW, Whipp SC, Argenzio RA, et al. Attaching and effacing activities of rabbit and human enteropathogenic *Escherichia coli* in pig and rabbit intestines. *Infect Immun* 1983;41(3):1340–51.

157. Donnenberg MS, Kaper JB. Enteropathogenic *Escherichia coli*. Infect Immun 1992;60(10):3953–61.
158. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. Clin Microbiol Rev 1998; 11(1):142–201.
159. Hecht G. Microbes and microbial toxins: paradigms for microbial–mucosal interactions. VII. Enteropathogenic *Escherichia coli*: physiological alterations from an extracellular position. Am J Physiol Gastrointest Liver Physiol 2001;281(1):G1–7.
160. Holland RE, Wilson RA, Holland MS, et al. Characterization of eae+ *Escherichia coli* isolated from healthy and diarrheic calves. Vet Microbiol 1999;66(4):251–63.
161. Wieler LH, Sobjinski G, Schlapp T, et al. Longitudinal prevalence study of diarrheagenic *Escherichia coli* in dairy calves. Berl Munch Tierarztl Wochenschr 2007;120(7–8):296–306.
162. Levine MM. *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. J Infect Dis 1987;155(3):377–89.
163. Leomil L, Aidar-Ugrinovich L, Guth BE, et al. Frequency of shiga toxin–producing *Escherichia coli* (STEC) isolates among diarrheic and non-diarrheic calves in Brazil. Vet Microbiol 2003;97(1–2):103–9.
164. Lee JH, Hur J, Stein BD. Occurrence and characteristics of enterohemorrhagic *Escherichia coli* O26 and O111 in calves associated with diarrhea. Vet J 2008; 176(2):205–9.
165. Kang SJ, Ryu SJ, Chae JS, et al. Occurrence and characteristics of enterohemorrhagic *Escherichia coli* O157 in calves associated with diarrhoea. Vet Microbiol 2004;98(3–4):323–8.
166. Osek J, Gallien P, Protz D. Characterization of shiga toxin–producing *Escherichia coli* strains isolated from calves in Poland. Comp Immunol Microbiol Infect Dis 2000;23(4):267–76.
167. Pruijboom-Brees IM, Morgan TW, Ackermann MR, et al. Cattle lack vascular receptors for *Escherichia coli* O157:H7 Shiga toxins. Proc Natl Acad Sci U S A 2000;97(19):10325–9.
168. Keel MK, Songer JG. The comparative pathology of *Clostridium difficile*–associated disease. Vet Pathol 2006;43(3):225–40.
169. Hammitt MC, Bueschel DM, Keel MK, et al. A possible role for *Clostridium difficile* in the etiology of calf enteritis. Vet Microbiol 2008;127(3–4):343–52.
170. Keel K, Brazier JS, Post KW, et al. Prevalence of PCR ribotypes among *Clostridium difficile* isolates from pigs, calves, and other species. J Clin Microbiol 2007;45(6):1963–4.
171. Rodriguez-Palacios A, Stampfli HR, Duffield T, et al. *Clostridium difficile* PCR ribotypes in calves, Canada. Emerging Infect Dis 2006;12(11):1730–6.
172. Rodriguez-Palacios A, Stampfli HR, Stalker M, et al. Natural and experimental infection of neonatal calves with *Clostridium difficile*. Vet Microbiol 2007; 124(1–2):166–72.
173. Bjorkman C, Svensson C, Christensson B, et al. *Cryptosporidium parvum* and *Giardia intestinalis* in calf diarrhoea in Sweden. Acta Vet Scand 2003;44(3–4):145–52.
174. Huetink RE, van der Giessen JW, Noordhuizen JP, et al. Epidemiology of *Cryptosporidium* spp. and *Giardia duodenalis* on a dairy farm. Vet Parasitol 2001;102(1–2):53–67.
175. Xiao L, Herd RP, Rings DM. Concurrent infections of *Giardia* and *Cryptosporidium* on two Ohio farms with calf diarrhea. Vet Parasitol 1993;51(1–2):41–8.
176. St Jean G, Couture Y, Dubreuil P, et al. Diagnosis of *Giardia* infection in 14 calves. J Am Vet Med Assoc 1987;191(7):831–2.

177. Geurden T, Geldhof P, Levecke B, et al. Mixed *Giardia duodenalis* assemblage A and E infections in calves. *Int J Parasitol* 2008;38(2):259–64.
178. Mendonca C, Almeida A, Castro A, et al. Molecular characterization of *Cryptosporidium* and *Giardia* isolates from cattle from Portugal. *Vet Parasitol* 2007;147(1–2):47–50.
179. Becher KA, Robertson ID, Fraser DM, et al. Molecular epidemiology of *Giardia* and *Cryptosporidium* infections in dairy calves originating from three sources in Western Australia. *Vet Parasitol* 2004;123(1–2):1–9.
180. Maddox-Hyttel C, Langkjaer RB, Enemark HL, et al. *Cryptosporidium* and *Giardia* in different age groups of Danish cattle and pigs—occurrence and management associated risk factors. *Vet Parasitol* 2006;141(1–2):48–59.
181. Matsubayashi M, Kimata I, Abe N. Identification of genotypes of *Giardia intestinalis* isolates from a human and calf in Japan. *J Vet Med Sci* 2005;67(3):337–40.
182. Smith KE, Stenzel SA, Bender JB, et al. Outbreaks of enteric infections caused by multiple pathogens associated with calves at a farm day camp. *Pediatr Infect Dis J* 2004;23(12):1098–104.
183. Uehlinger FD, O’Handley RM, Greenwood SJ, et al. Efficacy of vaccination in preventing giardiasis in calves. *Vet Parasitol* 2007;146(1–2):182–8.
184. Ruest N, Couture Y, Faubert GM, et al. Morphological changes in the jejunum of calves naturally infected with *Giardia* spp. and *Cryptosporidium* spp. *Vet Parasitol* 1997;69(3–4):177–86.
185. Buret A, Hardin JA, Olson ME, et al. Pathophysiology of small intestinal malabsorption in gerbils infected with *Giardia lamblia*. *Gastroenterology* 1992;103(2):506–13.
186. Buret A, Gall DG, Olson ME. Effects of murine giardiasis on growth, intestinal morphology, and disaccharidase activity. *J Parasitol* 1990;76(3):403–9.
187. Woode GN, Reed DE, Runnels PL, et al. Studies with an unclassified virus isolated from diarrheic calves. *Vet Microbiol* 1982;7(3):221–40.
188. Koopmans M, van den Boom U, Woode G, et al. Seroepidemiology of Breda virus in cattle using ELISA. *Vet Microbiol* 1989;19(3):233–43.
189. Duckmanton L, Carman S, Nagy E, et al. Detection of bovine torovirus in fecal specimens of calves with diarrhea from Ontario farms. *J Clin Microbiol* 1998;36(5):1266–70.
190. Hoet AE, Nielsen PR, Hasoksuz M, et al. Detection of bovine torovirus and other enteric pathogens in feces from diarrhea cases in cattle. *J Vet Diagn Invest* 2003;15(3):205–12.
191. Hoet AE, Smiley J, Thomas C, et al. Association of enteric shedding of bovine torovirus (Breda virus) and other enteropathogens with diarrhea in veal calves. *Am J Vet Res* 2003;64(4):485–90.
192. Kirisawa R, Takeyama A, Koiwa M, et al. Detection of bovine torovirus in fecal specimens of calves with diarrhea in Japan. *J Vet Med Sci* 2007;69(5):471–6.
193. Haschek B, Klein D, Benetka V, et al. Detection of bovine torovirus in neonatal calf diarrhoea in Lower Austria and Styria (Austria). *J Vet Med B Infect Dis Vet Public Health* 2006;53(4):160–5.
194. Fagerland JA, Pohlenz JF, Woode GN. A morphological study of the replication of Breda virus (proposed family Toroviridae) in bovine intestinal cells. *J Gen Virol* 1986;67(Pt 7):1293–304.
195. Pohlenz JF, Cheville NF, Woode GN, et al. Cellular lesions in intestinal mucosa of gnotobiotic calves experimentally infected with a new unclassified bovine virus (Breda virus). *Vet Pathol* 1984;21(4):407–17.