Review Article Compte rendu

Viral enteritis in calves

Diego E. Gomez, J. Scott Weese

Abstract — A complex community of bacteria, viruses, fungi, protists, and other microorganisms inhabit the gastrointestinal tract of calves and play important roles in gut health and disease. The viral component of the microbiome (the virome) is receiving increasing attention for its role in neonatal calf diarrhea (NCD). Rotavirus and coronavirus have for a long time been associated with NCD and commercial vaccines have been produced against these agents. Recently, several other viruses which may play a role in diarrhea have been discovered in calf fecal samples, mostly by sequence-based methods. These viruses include torovirus, norovirus, nebovirus, astrovirus, kobuvirus, and enterovirus. Most studies have involved epidemiologic investigations seeking to show association with diarrhea for each virus alone or in combination with potential pathogens. However, determining the contribution of these viruses to calf diarrhea has been challenging and much uncertainty remains concerning their roles as primary pathogens, co-infection agents, or commensals.

Résumé — Entérite virale chez les veaux. Une communauté complexe de bactéries, de virus, de champignons, de protistes et d'autres micro-organismes habitent dans le tube gastro-intestinal des veaux et joue des rôles importants dans la santé et les pathologies du tractus digestif. La composante virale du microbiome (le virome) reçoit de plus en plus d'attention pour son rôle dans la diarrhée néonatale du veau (DNV). Le rotavirus et le coronavirus sont depuis longtemps associés à la DNV et des vaccins ont été produits contre ces agents. Récemment, plusieurs autres virus, qui peuvent jouer un rôle dans la diarrhée, ont été découverts dans des échantillons de fèces des veaux, surtout par des méthodes de séquençage. Ces virus incluent le torovirus, le norovirus, le nébovirus, l'astrovirus, le kobuvirus et l'entérovirus. La plupart des études ont comporté des enquêtes épidémiologiques pour découvrir l'association de chaque virus avec la diarrhée, seul ou en combinaison avec des agents pathogènes potentiels. Cependant, la détermination de la contribution de ces virus à la diarrhée du veau a été difficile et il reste encore beaucoup d'incertitude concernant leurs rôles en tant qu'agents pathogènes primaires, agents de co-infection ou commensaux.

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Introduction

Diarrhea is the most important cause of disease in calves < 30 d of age and is a major cause of economic loss to cattle producers (1). The financial losses arise not only from mortality, but also from the cost of medication (especially antimicrobials), labor needed to treat sick calves, delayed growth of calves, and higher age at first calving (2,3). The 2007 National Animal Health Monitoring System for US dairy cattle stated that 57% of calf deaths before weaning resulted from neonatal calf diarrhea (NCD), with most cases occurring in calves < 1 mo of age (1). Similar mortality rates due to diarrhea in dairy calves were recently reported in Korea (53%) (4) and Iran (58%) (5).

The cattle industry has made great improvements with herd management, animal facilities and care, feeding and nutrition, and timely use of bio-pharmaceuticals; however, calf diarrhea is still problematic, likely because of the multi-factorial nature of the disease (6). Investigation of diarrhea has been focused on individual pathogens, namely *Escherichia coli, Salmonella* spp., rotavirus, coronavirus, and *Cryptosporidium* spp.; however,

Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1.

Address all correspondence to Dr. Diego E. Gomez; e-mail: diegogomeznieto@ufl.edu

Dr. Gomez's current address is Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, Florida 32610, USA.

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Table 1. Reported detection rates of 8 viruses in healthy and diarrheic calves, and rates of detection of each virus as a sole potential pathogen or along with other infectious agents in diarrheic calves.

Virus	Healthy calves	Diarrheic calves	Diarrheic calves: Unique Agent	Diarrheic calves: Co-infection	References
Rotavirus	2% to 45%	7% to 80%	16% to 27%	29% to 31%	23, 24, 26, 91, 92
Coronavirus	1% to 8.2%	3% to 79%	1.4% to 4.2%	8% to 13%	23, 44, 91, 92
Torovirus	6% to 12%	14% to 28%	28%	7.6%	44, 57, 58
Norovirus	10%	1.6% to 76%	4%	20%	64, 65, 66, 68
Nebovirus	0% to 1.6%	7% to 21%	ND	ND	26, 65, 67, 70
Astrovirus	ND	46%	8% to 13%	24% to 87%	68, 73
Kobuvirus	4.8% to 24%	5.3% to 37%	ND	ND	75, 80
Enterovirus	32%	5%	ND	ND	26

ND — not determined.

recent studies in humans have suggested that co-infection (simultaneous infection of a host by multiple pathogens) might be important in the pathophysiology of gastrointestinal diseases (7).

A complex community of bacteria, viruses, fungi, protists, and other microorganisms inhabit the gastrointestinal tract of calves. Recent studies have demonstrated that this complex community (the microbiota) and its total genetic complement (the microbiome) play important roles in gut health and disease. While the bacterial component of the microbiota is the most abundant, the viral component of the microbiome (the virome) is receiving increasing attention. Various pathogenic viruses have been well-characterized in cattle, causing a range of diseases *via* acute, persistent, or latent infections (8). Viruses that infect animal cells represent a small proportion of the gut virome when compared to bacteriophages (viruses that infect bacteria), but animal viruses are among the most important etiologic agents of acute NCD. This review focuses on animal viruses and their role in NCD.

Viral enteritis: A brief history

Escherichia coli was considered to be the main cause of NCD during the first decades of the 20th century (9). In 1943, a filterable virus was suspected to cause diarrhea and pneumonia in calves younger than 1 mo (10). Soon after this, viruses such as bovine viral diarrhea virus (11), adenoviruses (12), parvoviruses (13), and enteroviruses (13) were also suggested as possible causes of this syndrome. In 1970, 2 viruses were isolated from cases of NCD on Nebraska ranches. The first one was a reo-like virus that induced disease generally within the first 96 h of life, causing diarrhea characterized by yellow liquid feces (14). The second virus isolated was a coronavirus-like agent that was reported to infect calves between 5 d and 6 wk of age (15). Shortly thereafter, the reo-like (now known as rotavirus) and coronavirus-like viruses, as well as an adenovirus, were identified in dairy calves from Quebec and Ontario (16). Subsequently, other viruses were identified in feces of calves with gastroenteritis, such as calicivirus (17,18), torovirus (BToV) (19), astrovirus (BAsV) (17), nebovirus (BNoV) (17), and enterovirus (BEnV) (20). Some of these viruses can be identified in feces from clinically healthy calves (Table 1), which makes assessing the clinical relevance of these viruses very difficult and the role of some of these viruses in NCD still remains undetermined.

Viruses known to cause diarrhea in calves

Bovine rotavirus

Rotaviruses are non-enveloped RNA viruses that have 3 important antigenic specificities: group, subgroup, and serotype. Group A rotaviruses are major pathogens in calves, with Group B playing a minor role. Group A rotaviruses consist of 11 segments of double-stranded RNA, encoding 6 structural viral proteins (VP1 to VP4, VP6 and VP7) and 6 non-structural proteins (NSP 1 to NSP 6) (21).

Bovine rotavirus (BRoV) typically causes diarrhea in calves $<$ 3 wk of age (22). Clinical signs are non-specific as is characteristic of NCD. Typically, pale yellow, non-bloody, profuse diarrhea is observed, often containing large amounts of mucus. Diarrhea usually lasts between 4 to 8 d. Fever can be present and the calves are usually dull and reluctant to drink. Limited studies have reported prevalence rates of 7% to 80% for shedding of BRoV by diarrheic calves (23,24). Two case-control studies from Brazil and the USA detected BRoV in feces of 11% and 30% of diarrheic calves, respectively, compared to 0% in healthy calves (25,26). However, other studies have demonstrated that BRoV can be detected in both healthy and diarrheic calves, including reports of BRoV in 2% to 12% of non-diarrheic and 7% to 30% of diarrheic fecal samples from dairy calves in Europe (27–29) and Central America (23). One study from France also reported BRoV in 49% of diarrheic and 45% of healthy beef calves (28). A recent study from Brazil also determined that BRoV was detected at significantly higher ($P < 0.0001$) frequency in the feces of dairy calves with diarrhea compared with the feces of non-diarrheic calves (30). Differences among studies include the age of the calves sampled, geographic location, management practices, experimental design, and assays to detect BRoV [e.g., polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), and chromatographic lateral flow immunoassay]. Additionally, most of these studies were cross-sectional in design and the health status of the control (healthy) group was not followed up to determine if calves that were shedding BRoV developed diarrhea after the time of sampling. Overall, these results make it difficult to determine the clinical relevance of BRoV as a primary pathogen or a potential co-infection agent. Similarly, determination of the impact of BRoV is challenging since its role in disease is unclear. Mortality rates from 5% to

80% have been reported (31) but whether mortality was attributable to BRoV is difficult to discern. As with most causes of NCD, the prognosis is good if supportive care is administered promptly. Regardless of the role of BRoV in diarrhea, this virus is predominantly found in young calves. After 3 months of age, calves are not usually susceptible to infection.

Bovine coronavirus

Coronaviruses are single stranded RNA viruses that can infect a wide range of hosts. Animal coronaviruses are divided into 3 antigenic groups: Group 1 has no hemagglutinin-esterase (HE), Group 2 has HE and includes BCoV (32), and Group 3 contains avian viruses including infectious bronchitis virus. Bovine coronavirus (BCoV) has been associated with gastrointestinal and respiratory diseases in cattle including diarrhea in neonatal calves (33), winter dysentery (34), and respiratory tract illness (35). In dairy and beef calves, BCoV can cause enteritis with naturally infected calves showing clinical signs of disease between 5 and 30 d of life. As with some other potential causes of NCD, BCoV can commonly be found in both healthy and diarrheic calves, complicating the assessment of its role as a primary pathogen (29). Some studies involving a limited number of calves ($n < 100$) have identified numerical, but not statistical, associations between the detection of BCoV in fecal or nasal samples and clinical signs (36–38). In contrast, 1 case-control study involving 380 calves found a statistical association between BCoV and diarrhea in dairy calves in Costa Rica (23). Discrepancies among studies can be explained, at least in part, by differences in the sample sizes in those investigations. The prevalence of BCoV was investigated in dairy farms from Ontario at 3 periods: 1982, 1990 to 1991, and 1995 to 1997 (39–41). The overall prevalence ranged from of 5% to 17%. In one study, the prevalence rates of BCoV in healthy and diarrheic calves were 13% (15/118) and 2.3% (1/43), respectively (40), but the remaining 2 studies failed to differentiate the prevalence of BCoV in both groups. Higher prevalence rates of BCoV in diarrheic calves have been reported recently compared to those identified 2 to 3 decades ago (42). Further, new strains of BCoV have been described worldwide during the last decade (43,44). At present, the prevalence of BCoV in healthy and diarrheic calves from dairy farms in Canada, the association of BCoV with diarrhea and whether new strains are circulating among dairy farms are unknown.

Clinical signs begin approximately 2 d after exposure and continue for 3 to 6 d. Typically, coronavirus infection causes profuse watery diarrhea, and feces can contain blood clots. Calves become moderately depressed, the suckling reflex is weak, and dehydration can develop rapidly. Decreased food intake, fluids, and electrolyte loss can result in dehydration, metabolic acidosis, and hypoglycemia. The diagnosis of BCoV enteritis can be achieved using viral culture, antigen-capture ELISA, hemagglutination assay using mouse erythrocytes, and PCR (45). Recently, a pancoronavirus reverse transcription (RT) PCR assay (PanCoV-RT) was described to identify human CoV from samples of humans with respiratory diseases (46). The utility of PanCoV to detect BCoV in samples of animals with clinical diseases has not been described.

As with other viral causes, treatment is supportive in nature. Clinically recovered calves may continue to shed low levels of virus for weeks (15,47).

Prevention of diarrhea caused by rotavirus and coronavirus

The basic tenets of preventing viral gastroenteritis is enhancing host immunity and reducing the load of viral agents in the environment. Infection control practices dealing with reducing exposure are beyond the scope of this review. The importance of good colostrum management, leading to an adequate passive transfer in the prevention of calf diarrhea is without debate (2,48). Most cows are seropositive to BRoV due to field exposure; however, antibody titers of milk decline to non-protective levels after parturition in unvaccinated cattle (49). It is unclear if vaccinating cows late in gestation improves calf antibody titers or whether the practice improves resistance of calves to disease. Colostral and milk antibodies against BRoV and BCoV can be enhanced *via* parenteral vaccination of the cows during the dry period (passive immunization). The success of the passive immunity against enteric viral infection depends on the continuous presence of a protective level of specific antibody in the gut lumen (50,51). BRoV and BCoV normally cause diarrhea between 5 and 14 d of age, a time that has been associated with a major decline in specific antibody concentration, as ingestion of high colostral antibody is replaced by ingestion of milk, which has much lower antibody concentration (52,53). Therefore, optimal protection would be from vaccines that can be given to cattle during pregnancy and that increase both colostrum and milk antibodies for a period of at least 3 wk. The efficacy of parenteral vaccines for prevention of diarrhea caused by BRoV and BCoV is unclear. In 1985, a field trial evaluating the efficacy of a vaccine consisting of modified live BRoV and BCoV with a F5-positive *E. coli* bacterin failed to detect differences in rates of diarrhea and associated mortality in calves from vaccinated and unvaccinated cows on dairy farms in Ontario (54). Importantly, colostral antibodies to BRoV and BCoV were similar in both groups, suggesting that the vaccine resulted in limited impact on passive transfer of immunity. Minimal increases of antibodies in milk or serum of calves from cows vaccinated with inactivated BCoV antigen were identified in some studies (55,56). Yet, several other studies have reported that pregnant cows vaccinated against BRoV and BCoV had increased titers of antibodies in colostrum and milk (39,51,53). A later study evaluated the concentration and persistence of antibodies in colostrum and milk against BRoV, BCoV and *E. coli* F5 antigens after cows were vaccinated 1 mo before expected calving date with a single dose of a vaccine containing an inactivated BRoV (serotype G6-P5), inactivated BCoV (originally isolated from a calf with diarrhea) and purified cell-free *E. coli* F5. This study demonstrated a 4-fold increase, for at least 28 days, in antibodies against BRoV and BCoV in colostrum and milk of vaccinated cows compared to the control group (53). However, this study failed to demonstrate vaccine efficacy through an animal challenge model. These results suggest that newer vaccines could be effective in the prevention and control of viral diarrhea in calves; however, randomized field trials are required to prove their efficacy.

Several studies have reported discrepancies between the genotypes of rotavirus (25) and coronavirus (43) in the commercial vaccine and those of the strains circulating in both beef and dairy herds. Differences between the vaccine and field strains suggest that the vaccines may not be protective against circulating strains (43). To provide optimal immunity, vaccine antigens should be as similar as possible to the circulating strains. Therefore, future studies should focus on epidemiological surveillance in order to avoid potential causes of vaccination failure.

Other viruses that might cause enteritis

Numerous animal viruses can be found in diarrheic calves. As sequence-based methods become more affordable and easy to use, it is almost certain that many more new viruses will be identified. However, identification of a virus in a diarrheic calf is typically much easier than determining what role, if any, it has in disease. The presence of viruses in both healthy and diarrheic calves does not rule out the potential for them to cause disease, but it complicates determination of their pathogenicity. Some viruses that may play a role in NCD, either as primary pathogens or co-infection agents, are discussed below.

Bovine torovirus

Torovirus is a genus of enveloped RNA viruses of the Coronaviridae family. Toroviruses are similar in appearance to the crown-like coronaviruses but often have a donut-shaped structure within the particle. Toroviruses have been identified in humans, horses, cattle, and swine with gastroenteritis worldwide, but their role in disease etiology is still unclear. Similarly, bovine torovirus (BToV) has been identified in feces of diarrheic and healthy calves (57). One study identified BToV in 43/118 (36%) diarrheic and 5/43 (12%) healthy calves on Ontario farms, both as the sole detected pathogen (28% of diarrheic calves) and along with other pathogens (7.6%) (41). Another study failed to detect any association of BToV with calf diarrhea (58). Despite the passage of time since the first identification of BToV, its role in disease remains poorly characterized. Mixed results have been reported with attempted experimental infection, as some experimental infections have failed to produce clinical signs or histopathological lesions (59).

Natural infection usually occurs in calves between 2 and 5 d of age, but calves up to 4 mo appear to be susceptible (19,60,61). Diarrheic calves $<$ 1 mo of age that are shedding BToV appear to be the major source of the virus (60,61). After ingestion or nasal exposure, the virus infects the epithelium of the distal half of the jejunum, the ileum, and colon. Viral replication is cytoplasmic and entrance into the enterocytes is achieved by attachment of the viral S protein to host cell receptors, which mediates endocytosis. Microscopic lesions consist of necrosis of the crypt and villous enterocytes and atrophy of the villi (62,63). Similar to coronavirus, lesions in the intestine caused by torovirus infection are expected to result in malabsorptive and hypersecretory diarrhea (48). Clinical signs observed in naturally occurring outbreaks include a yellow to white semisolid or profuse watery diarrhea (19). If the calf survives, it can be fully protected from infection but can intermittently shed BoTV (58,63). Specific preventive measures are not available.

Bovine norovirus

Noroviruses are non-enveloped RNA viruses that are members of the family Caliciviridae. On the basis of phylogenetic relationships inferred from the VP1 sequences, noroviruses have been divided into 6 genogroups (GI to GVI), with bovine noroviruses (BNoV) classified as GIII (64). The pathogenesis of BNoV is poorly understood; however, extrapolation from other species, especially humans, suggests that BNoV can be transmitted *via* the fecal/oral route, through contaminated food or water (65). The prevalence of BNoV in cattle has not been well established. Results from limited studies have reported ranges of 1.6% and 72% in Canadian dairy calves and USA veal calves, respectively (64,65), and up to 10% in healthy calves in Europe (66,67). One study identified BNoV as the sole detected pathogen in 4% of samples from diarrheic calves and along with other pathogens in 20% (68); however, this study only evaluated the presence of viral agents (BRoV, BAstV, BNoV, BCoV, BToV, and BVDV) and failed to investigate the presence of bacterial and parasitic agents. Therefore, conclusions regarding the true role of BNoV as a primary pathogen or co-infection were limited.

Gnotobiotic calves infected with the GIII BNoV strain exhibited anorexia and diarrhea associated with necrosis of the intestinal epithelium and villous atrophy (69). However, evidence that BNoV is a significant (or even rare) cause of diarrhea in calves in the field is limited. The potentially high prevalence of BNoV in healthy calves and lack of a significant difference in shedding between healthy and diarrheic calves (26,70) suggest this virus may be of limited clinical relevance, at least as a primary pathogen. Whether BNoV can be pathogenic in some situations, either as the sole infectious agent or a co-infecting agent, is as yet unknown. Therefore, a definitive causal relationship between BNoV and calf diarrhea remains to be determined.

Bovine nebovirus

Similar to norovirus, nebovirus is a non-enveloped member of the family Caliciviridae. Outbreaks of bovine nebovirus (BNebV) associated gastroenteritis were initially reported in diarrheic calves in England (17) and Germany (18), but BNebV has been detected in cattle worldwide (66,67). In Italy, BNebV was detected in feces of diarrheic calves but not in healthy animals (67). In France, the United Kingdom and Korea, the prevalence of BNebV in diarrheic calves ranged from 7% to 9% (67,70,71). In North America, BNebV has been identified in 21% (43/199) of fecal samples of diarrheic calves and 1.6% (4/245) of samples from healthy calves. BNebV was commonly detected in feces also positive for BCoV, *Cryptosporidium parvum* or BToV (26). Experimental infection of gnotobiotic calves with BNebV causes lesions in the jejunum similar to those described for BNoV, and these lesions have been associated with malabsorption (72). The mechanism of diarrhea due to BNebV remains poorly understood but malabsorptive and hypersecretory diarrhea can be expected (72). Described clinical signs in gnotobiotic calves infected with BNebV included depression, anorexia, and diarrhea (72). However, despite the potential over-representation of BNebV in diarrheic calves and experimental reproduction of disease in gnotobiotic calves, the role of BNebV in diarrhea in the field remains unclear.

Bovine astrovirus

The family Astroviridae includes 2 genera, Mamastroviruses and Avastroviruses that infect mammals and birds worldwide, respectively. Bovine astrovirus (BAstV) was initially isolated from a diarrheic calf in England in 1978 (17). However, exposure of gnobiotic calves to this virus failed to produce clinical signs and, therefore, that strain of BAstV was considered to be non-pathogenic (17). In 1984, a similar BAstV was isolated from a diarrheic calf from USA that was also positive for BRoV (62). Experimental infection of gnotobiotic calves with the BAstV-USA strain caused infection and cytopathology of M-cells of the dome epithelium covering the Payer's patches of the calf ileum but did not cause clinical signs. Interestingly, when BAstV-USA strain was mixed with BRoV or BToV, gnotobiotic calves developed severe diarrhea and more extensive BAstV infection (62). Study of BAstV in field situations has been limited. A recent study demonstrated a high prevalence (46%) of BAstV in diarrheic calves, with co-infection with other viruses, including BEnV, BCoV, BRoV and BVDV identified in 88% of those animals (73). Another study identified BAstV as the sole detected pathogen in 8% of the fecal samples and along with other viral agents in 24% (68).

One study from Scotland found that BAstV was common in calves [present in 74% (85/115) of samples] but uncommon in adult cattle [present in 15% (3/20) of samples]. However, no association was found between the presence of BAstV and calf diarrhea or the presence of a specific AstV lineage and calf diarrhea (74). The lack of comparative data with a healthy control group limits what can be concluded from that study. As with various other viruses, it is unclear whether BAstV is a relevant primary pathogen, a potential cause of disease with co-infections, or a clinically irrelevant virus.

Bovine kobuvirus (aichivirus B)

Kobuvirus, a genus of non-enveloped RNA viruses from the family Picornaviridae, contains 2 officially recognized species, Aichivirus and bovine kobuvirus (BKoV, now referred to as Aichivirus B) and 1 candidate species, porcine kobuvirus (75). Aichivirus was first isolated from a person with acute enteritis in Japan (76), although its role in disease in humans remains unclear. Initially, BKoV was only identified in bovine serum and feces from clinically healthy cattle (77), then, in 2008, it was isolated from feces of cattle with diarrhea (78). It has been suggested that BKoV can play a role in the pathogenesis of enteritis in calves; however, the role of BKoV infection in NCD still needs to be clarified because of limited data and the presence of this virus in clinically normal animals (75). While this virus has been isolated from diarrheic calves (78,79), studies comparing diarrheic and healthy calves are limited. Two recent studies compared the prevalence of BKV in healthy and diarrheic calves from Italy (80) and Korea (75). In the Italian study, the prevalence of BKV was similar in diarrheic (5.3%; $n = 38$) and non-diarrheic (4.8%; $n = 104$) calves, whereas in the Korean study, BKV was found in 37% (32/86) of diarrheic and 24% (5/21) of non-diarrheic samples. Both studies failed to investigate the presence of other etiologic agents causing diarrhea in calves and therefore it is not possible to attribute a causal association between BKV and NCD.

Bovine enterovirus

Bovine enterovirus (BEnV) belongs to the genus Enterovirus in the family Picornaviridae, a group of non-enveloped RNA viruses that includes numerous human and animal pathogens. The enterovirus genus consists of 12 species; 9 enteroviruses (A to J) and 3 rhinoviruses (81). The BEnVs are now classified into 2 subgroups E (1 to 4) and F (1 to 6) (81). Since 1959, BEnV has been isolated from cattle suffering from respiratory, gastrointestinal and reproductive diseases (82,83). However, respiratory and gastrointestinal disease could not be reproduced experimentally using viral isolates from affected calves. The pathogenesis and virulence of BEnV in cattle are largely unknown. One study described the pathogenesis associated with acute infection of BEnV in calves experimentally inoculated with the BEnV but found no clinical signs following acute infection (84). More importantly, the control group (unexposed group) used in this study was inadvertently infected with the inoculated BEnV, which largely limited the conclusions of the study.

Recently, BEnV was isolated from feces of diarrheic cattle from dairy herds in China (85). BEnV was detected in 25% of healthy and diarrheic calves, but the authors failed to report the prevalence in healthy and diarrheic calves separately. Therefore, conclusions regarding a potential association with disease cannot be made. Another recent study identified BEnV in calves with severe diarrhea from dairy herds in Egypt (86). Although the investigated diarrheic calves were negative for BCoV, BRoV, and BVDV on cell culture, other bacterial and parasitic causes of diarrhea were not investigated. There was also no corresponding study of healthy calves, so association between the presence of BEnV and diarrhea could not be investigated.

A case-control study conducted to assess the prevalence of 11 infectious agents in fecal samples from calves from Midwest USA revealed that prevalence of BEnV in healthy calves was significantly higher (32%) than in diarrheic calves (5%) (26). Difficulties in reproducing clinical signs following experimental infection, and the fact that BEnV appears to be more prevalent in healthy than diarrheic calves suggest that BEnV plays little to no role in neonatal calf diarrhea.

Co-infection and calf diarrhea

As testing becomes more comprehensive, identification of co-infection with known or potential pathogens becomes more common. Co-infection with multiple pathogens has been identified among children with diarrhea and has been associated with more severe diarrhea than infection with a single pathogen. For instance, one case-control study in children from China reported multiple pathogens in 185 (40%) diarrheic feces and in 69 (15%) controls (7). High rates of co-infection have also been reported in diarrheic foals (87), with co-infections being more frequent in the diarrheic foals (15 mono-infections *versus* 22 co-infections) than in the healthy group (12 *versus* 4, respectively, $P = 0.0002$). Metagenomic studies have demonstrated that healthy piglets excrete enteric pathogenic viruses, but at lower concentrations when compared with diarrheic piglets. Interestingly, piglets that shed 6 or more distinct viruses were more likely to suffer from diarrhea (88). One study that evaluated the etiological agent in fecal samples of diarrheic piglets submitted to the Animal Health Laboratory of the University of Guelph identified rotavirus in 28 out of 237 samples. RoV was identified as the single etiological agent in 18 cases and associated with other pathogens (co-infection) in 10 cases (89). Furthermore, dogs with diarrhea can also simultaneously excrete several enteric viruses including rotavirus, coronavirus, parvovirus, norovirus, astrovirus, distemper virus, and paramyxovirus (90).

Studies investigating the interaction between viral microorganisms and other microorganisms of the gastrointestinal tract of the calf are scarce; however, several studies investigating calf diarrhea have demonstrated high rates of co-infection (24,26,34,91,92). One study evaluated the prevalence of 5 known pathogens causing diarrhea and found rates of co-infection of 15% in diarrheic calves (33), whereas another study determined a rate of co-infection of 71% when fecal samples were tested for 4 known pathogens (24). A recent study testing for 11 pathogens associated with NCD documented a rate of co-infection of 55% in fecal samples from diarrheic calves. Notably, in this study the rate of co-infection in healthy calves was only 3% (26). One study in Ontario (Canada) farms, evaluating the presence of viruses (BToV, BCoV, BRoV, BVDV and small round-structured viruses) in feces of dairy calves reported a rate of co-infection of 14% (17/118) in diarrheic calves, whereas co-infection was not detected in clinically normal calves (41). This study failed to evaluate co-infection with bacterial or parasitic agents.

The pathogens associated with co-infection in diarrheic calves vary among studies. One study reported the most common combination of pathogens in diarrheic calves was *C. parvum* and BRoV (19%) followed by BRoV and *E. coli* K-99 (91). Similar results were reported in calves suffering from diarrhea in Australia in which the combination of *C. parvum* and BRoV accounted for 25% of the co-infections (24). Remarkably, a recent investigation determined that the most common co-infection in diarrheic calves from Unites States were viral pathogens with *C. parvum* (28%), viral and bacterial co-infection (7.5%), and viral, bacterial pathogens and *C. parvum* co-infection (1.5%). Furthermore, the presence of more than one pathogen increased the odds of diarrhea occurring in 2 studies (26,29). These studies suggest that co-infections with a large number of pathogens rather a single entity may be responsible for the diarrhea in a subset of neonates, likely by overwhelming the gut mechanisms of defense against pathogens.

In conclusion, viral gastroenteritis remains as an important cause of morbidity and mortality in neonatal calves. A large number of viruses in the gastrointestinal tract of calves are yet to be identified. Description of novel viruses will occur in the near future as next-generation sequencing technologies have facilitated virus discovery. Therefore, future clinical research should focus on determining the clinical relevance of the novel viruses, the role of co-infection in calf gastroenteritis, and the efficacy of vaccines in prevention and control of neonatal calf diarrhea.

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