Pathogen-oriented approaches for neonatal calf diarrhea

Pathogeen-specifieke aanpak van neonatale kalverdiarree

W. van Mol, J. Clinquart, M.L. Pas, J. Bokma, B. Pardon

Department of Internal Medicine, Reproduction and Population Medicine
Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium

wouter.vanmol@ugent.be

ABSTRACT

Neonatal calf diarrhea (NCD) is the leading health concern in calves during the first weeks of their lives. In this narrative review, the potential for pathogen-oriented approaches for NCD is discussed. The literature on NCD clearly shows substantial differences in spread and characteristics between the major NCD pathogens, making pathogen-oriented approaches possible, justifying the use of etiological diagnostics. For enterotoxic *Escherichia coli*, colostrum delivery and dam vaccination, biosecurity around calving and antimicrobial therapy are key. Both for bovine coronavirus (BCV) and bovine rotavirus (BRV), biosecurity and disinfection, dam vaccination in combination with adequate and prolonged colostrum delivery are the essentials. However, a different focus concerning biosecurity is necessary given the airborne spread of BCV and higher environmental persistence of BRV. For an effective *Cryptosporidium* spp. control, the use of disinfectants that kill oocysts is crucial. Evidence supporting the prophylactic use of halofuginone lactate to reduce shedding and diarrhea, is available, but in terms of biosecurity, attention should be placed on the proper use of this product. In case of a *Salmonella enterica* outbreak, antimicrobial use remains important, and biosecurity wise, attention should be paid to shedding of periparturient cows in the calving pen and administration of infected colostrum. Both for *S. enterica* and cryptosporidiosis, farm staff should be informed on how to protect themselves against these zoonotic infections. Nutritional factors play an additional role within NCD. Improper nutrition management can induce diarrhea or can further enhance infectious NCD through osmosis or dysbiosis. In conclusion, the suggested pathogen-oriented approaches can aid to economize labor and financial investments, limit the environmental impact of NCD control and prevention and valorize tailor-made farm advisory work.

SAMENVATTING

Neonatale kalverdiarree (NKD) is het belangrijkste gezondheidsprobleem bij kalveren tijdens de eerste weken van hun leven. In dit overzicht wordt het potentieel voor een pathogeen-specifieke aanpak van NKD besproken. Uit literatuuronderzoek is duidelijk gebleken dat er aanzienlijke verschillen in de verspreiding en kenmerken bestaan tussen de belangrijkste NKD-pathogenen, waardoor pathogeen-gerichte benaderingen mogelijk zijn en het gebruik van etiologische diagnostiek gerechtvaardigd is. Voor enterotoxische *Escherichia coli* zijn de biestgift en vaccinatie van het moederdier, bioveiligheid rond het afkalven en antimicrobiële therapie van cruciaal belang. Zowel voor het boviene coronavirus (BCV) als het boviene rotavirus (BRV) zijn bioveiligheid en ontsmetting, vaccinatie van het moederdier in combinatie met adequate en verlengde biestgift van essentieel belang. Gezien de verspreiding van BCV via de lucht en de hogere persistentie van BRV in het milieu is echter een andere aanpak van de bioveiligheid noodzakelijk. Voor een doeltreffende bestrijding van *Cryptosporidium parvum* is het gebruik van ontsmettingsmiddelen die oöcysten doden cruciaal. Er zijn bewijzen voor het profylactisch gebruik van halofuginone lactaat om uitscheiding en diarree te verminderen, maar binnen de bioveiligheid moet aandacht worden besteed aan het juiste gebruik van dit product. Bij een uitbraak van *Salmonella enterica* blijft het gebruik van antimicrobiële middelen belangrijk, en op het gebied van bioveiligheid moet aandacht worden besteed aan de uitscheiding van peripariturïente koeien in de afkalfstal en de mogelijke toediening van besmette biest. Zowel voor *S. enterica* als voor cryptosporidiose moet het personeel op het bedrijf worden voorgelicht over hoe zij zich tegen deze zoönosen kunnen beschermen. Voedingsfactoren spelen een bijkomende rol bij NKD. Onjuist voedingsmanagement kan diarree veroorzaken of kan infectieuze NKD verder versterken door osmose of dysbiose. Concluderend kan worden gesteld dat de voorgestelde pathogeen-specifieke aanpak zou kunnen bijdragen tot een besparing van arbeid en financiële investeringen, een beperking van de milieueffecten van de bestrijding en preventie van NKD en een valorisatie van op maat gesneden bedrijfsadvisering.

INTRODUCTION

Despite decades of research and information campaigns, neonatal calf diarrhea (NCD) is still one of the biggest challenges for farmers and veterinarians in the first weeks of the new-born's life. Approximately, one in five calves develops NCD (Bartels et al., 2010; Windeyer et al., 2013), which is also the leading cause of mortality in the first month of life (41.9% of calf mortalities) (Florrez, 2020). The economic losses are substantial and attributable to reduced growth, animal mortality, delayed first calving, treatment costs and increased risk for other diseases like bovine respiratory disease (Waltner-Toews et al., 1986; Donovan et al., 1998; Pardon et al., 2013). NCD is referred to as a multifactorial disease, resulting from the interplay between host resilience and infectious pressure, both influenced by environmental factors in which nutrition and hygiene are key. The major underlying pathogens are enterotoxic *Escherichia coli* (ETEC), bovine rotavirus (BRV), bovine coronavirus (BCV), *Cryptosporidium parvum* and *Salmonella* spp. (Drackley, 2008; Cho and Yoon, 2014; Heller and Chigerwe, 2018; DGZ, 2020; Brunauer et al., 2021), but NCD can also be caused by multiple pathogens, nutritional factors or dysbiosis.

NCD is a well-studied problem and over the years, several key factors have been identified for a successful control and prevention. These key prevention elements are appropriate colostrum management to assure adequate transfer of passive immunity from dam to calf, appropriate biosecurity and hygiene to reduce infectious pressure, specific immunity induced by vaccination of the dam and the use of specific chemophylaxis to control *C. parvum*. This knowledge has frequently been translated or communicated to farmers and veterinarians by research institutes and pharmaceutical companies under the format of 'one size fits all', meaning that the approach offers the solution regardless of whatever pathogen is involved. In its simplest way, this was represented as a scale, balancing calf immunity and infectious pressure. Later, step-by-step and check list approaches were used, which consisted of series of risk factors to check or action points to complete (Meganck et al., 2015). Typically, the different action points can be numerous and no priority is given to one over another. Also, they remain rather vague on certain action points, such as those regarding biosecurity and hygiene. Remarkably, with the exception of halofuginone use, most of these guidelines do not strictly advice on the role of prophylactic treatment in NCD control. Although the primary involvement of bacteria in NCD is limited, antimicrobial use is still part of both treatment and control in many farms and veterinary practices (Pardon et al., 2012). This mostly refers to the individual animal, but antimicrobial metaphylaxis in NCD outbreaks is not uncommon. However, the off-label oral use of paromomycin to control cryptosporidiosis has been criticized in the framework of responsible and reduced antimicrobial use (Brainard et al., 2020).

In mastitis management, claw health and more recently also for bovine respiratory disease, the interest awoke to develop pathogen-oriented approaches (Ostergaard et al., 2005; Pardon et al., 2020). Additionally, for bovine viral diarrhea virus, bovine herpesvirus type 1 and also *Salmonella* spp. pathogens specific control and prevention programs have been designed (Nielsen et al., 2012; Raaperi et al., 2014; van Roon et al., 2020). The key principle of these approaches is that some risk factors or management measures are crucial for one pathogen, whereas they are not for another. By designing control and prevention programs tailored to the identified pathogen, attention and efforts are directed to the key factors for that pathogen. Not all of the identified risk factors are equally important for each of the possible involved pathogens. Hence, the question arises whether there is benefit for NCD in a pathogen-oriented approach, especially regarding limiting environmental pollution by use of antimicrobials, antiparasitics and/or chemical disinfectants. In this narrative review, the key elements of control and prevention of the specific pathogens are summarized and a basic framework for pathogen-oriented approaches is presented. Furthermore, control and prevention at herd level are focussed upon. The evaluation and treatment of the individual animal are not included in this review, neither is fluid therapy.

FRAMEWORK

The basic framework for pathogen-oriented approaches for the control of NCD at herd level consists of diagnosis, subsequently followed by control or prevention of pathogen specific risk factors to limit spread or disease severity. The problem assessment always begins with a proper anamnesis and detection of the affected animals, because it might be that the problem is not limited to neonatal calves only. It is recommended to at least visually inspect the maternity pen next to the individual calf hutches, as disease transmission may occur there.

The next step towards disease control is the identification of the underlying etiology, either infectious or non-infectious, with proper diagnostics. The most likely non-infectious cause lies within inappropriate nutrition that can evolve towards dysbiosis. When the cause of NCD is known, the specific risk factors can be additionally evaluated, reducing the amount of time needed for the herd visits. Present risk factors can subsequently be translated to recommendations for the control of NCD.

Regardless of the underlying infectious cause of NCD, avoidance of direct and indirect transmission of pathogens between calves is always a crucial factor. Visually, as with all infectious NCD agents, a 'follow-the-shit' approach clarifies where potential hotspots of infection are located and how transmission is possible, such as drainage of diarrhea in front of the calf pens where animal caretakers usually walk.

Sufficient administration of good quality colostrum is mandatory for every calf, but the effect on the prevention of NCD depends on the underlying pathogen. Additionally, prolonged colostrum delivery will not directly aid in the prevention for all the possible pathogens, but it has positive effects on the length and width of the villi, depth of the crypts and thickness of the mucosae of the duodenum, jejunum and ileum. This intestinal maturation increases the digestion and absorption potential of the intestines, while increasing the resilience against opportunistic pathogens, resulting in better growth (Yang et al., 2015). In Figure 1, a general overview of the pathogen specific key factors is given.

Etiology and diagnostics

NCD can be both of infectious and non-infectious nature, but is often a combination when maldigestion occurs following intestinal damage (Heller and Chigerwe, 2018). Infectious causes of NCD can be categorized as major and minor pathogens. Based on the fulfilment of the Koch postulates and a high prevalence, five major pathogens are identified: ETEC, *Salmonella enterica*, BRV, BCV and *Cryptosporidium parvum*. Mixed infections are present in 15% of the cases in the North of Belgium (Forrez, 2020). In Table 1, an overview of the prevalence of the major NCD pathogens in Belgium and several other European countries is given. These results need to be interpreted carefully since the sampled population differs between studies. In some studies, samples submitted to the laboratory or obtained from necropsies were used, whereas in other studies, on-farm sampling was performed. Hence, these studies can deviate from first-line cases, especially since the cow-side antigen-ELISA tests are frequently used by many practitioners.

Next to the major pathogens, minor NCD pathogens have been identified. In general, these are less prevalent or their etiological nature to cause diarrhea as a single agent is incompletely understood or evidenced. Minor NCD pathogens are bovine viral diarrhea virus (BVDv), norovirus, torovirus, astrovirus, nebovirus, enterohemorrhagic *E. coli. (EHEC), Enteropathogenic (EPEC)* and *Clostridium perfringens* type C (Cho and Yoon, 2014; Heller and Chigerwe, 2018; Martella et al., 2020; DGZ, 2020; Brunauer et al., 2021).

After the timely detection of the clinical problem of diarrhea, identification of the involved etiology is the next step. For that, diagnostic methods are needed. Below, an overview is given of commercially available diagnostic tests for NCD and their diagnostic accuracy.

First of all, the typical age distribution of NCD infections can be a diagnostic aid. However, this is mainly limited to the fact that ETEC infections only have a clinical effect in calves aged less than five days old (Kolenda et al., 2015). Historically, the aspect of diarrhea has sometimes been mentioned to be different between pathogens, but this is not supported by scientific evidence, with the exception of the presence of blood in the feces. ETEC cases do not have blood in the feces given the pathophysiology of the disease, while feces can contain blood in cases of *C. parvum*, *Salmonella*, BRV and BCV (Heller and Chigerwe, 2018). In contrast to infectious diarrhea, nutritional

Table 1. Prevalence estimate of the major neonatal calf diarrhea pathogens in calves admitted for necropsy at the Animal Health Care Flanders (DGZ Vlaanderen), from Wallonia (ARSIA), the Netherlands, Northern Ireland, Norway and Switzerland (Uhde et al., 2008; Gulliksen et al., 2009; Bartels et al., 2010; Forrez, 2020; Forsythe, 2020; ARSIA, 2022).

Etiology	Flanders (Belgium)	Wallonia (Belgium)	The Netherlands	Northern Ireland	Norway	Switzerland
ETEC	41 %	30%	3%	7%	3%	6%
Bovine rotavirus	13 %	26%	18%	32%	10%	59%
Bovine coronavirus	Unknown	10%	3%	4%	0	6%
C. parvum	23 %	46%	28%	35%	4%	55%
Salmonella spp.	6 %	2%	Unknown	2%	Unknown	Unknown
No pathogen identified	17%	Unknown	60 %	49%	Unknown	Unknown

diarrhea is short-lived (24 to 48 hours) after fastening of the animals. However, fastening of neonates cannot be recommended due to the risk of hypoglycemia.

Regarding the real diagnostic tests, the most widely used type of test is the cow-side antigen-ELISA. These types of tests consist of four dipsticks that test for F5-ETEC, BRV, BCV and *C. parvum.* These tests have the advantage that they can be performed cow-side and they give results within some minutes. There are multiple tests from different manufacturers available, but in Belgium, the Rainbow calf scours BIO K 306 (BIO-X, Rochefort, Belgium) is most frequently used. A point of attention is that depending on the tests, sensitivity and specificity can be highly variable for certain pathogens (Mohler et al., 2009). According to leaflet recommendations (BIO-X, Rochefort, Belgium), the Rainbow calf score has a sensitivity and specificity of over 80% for all pathogens. However, an independent evaluation of a Canadian test (Bovine Enterichek, Biovet, Quebec, Canada), showed much lower sensitivity in general and an overall low diagnostic accuracy for BCV (Cho et al., 2012). Also, to the authors' knowledge, in only a single study on *C. parvum,* antigen-ELISA for NCD in a Bayesian latent class model has been evaluated, taking the absence of a gold standard test into account. Results showed an estimated sensitivity of 59% and 76%, and a specificity of 93% and 89% for the Tetrakit (Bio-X, Rochefort, Belgium) and Techlab *Cryptosporidium* test (Techlab, Inc., Blacksburg, VA, USA), respectively. A limitation of the available antigen ELISAs is that most of them only detect ETEC F5 and no other pathogenic fimbriae, potentially leading to an underestimation of ETEC prevalence. Few exceptions test for one or two additional *E. coli* fimbriae. Overall, most tests have the lowest accuracy for BCV, which may lead to a systematic underestimation of the role of BCV in NCD. Testing multiple animals is a way to increase diagnostic sensitivity at herd level. When assuming a 100% specificity of the test, testing of five animals reliably allows to declare the presence or absence of a pathogen in the affected group (Pardon and Buczinski, 2020).

Salmonella enterica and ETEC can also be detected with bacterial culture. The detection of *S. enterica* is possible with brilliant green, xylose lysine deso-oxycholate or *Salmonella* identification agar. Enrichment in broths as tetrathionate or selenite can be necessary at 41°C – 42°C for a 24-hour period. The specificity (20% - 100%) and sensitivity (18% - 100%) depend on the used medium and enrichment broth (Waltman, 2000). *E. coli* can also be diagnosed with the use of bacterial culture, and hemolytic activity is strongly associated with the presence of virulence factors of ETEC (Weber et al., 2017). However, to confirm ETEC, identification of fimbriae with immunoassay or polymerase chain reaction (PCR) is needed after in vitro growth (Heller and Chigerwe, 2018), but this is seldom done.

All major NCD pathogens can be detected by PCR. PCR tests are assumed to have a high sensitivity and specificity, and are therefore frequently used as reference tests when evaluating antigen ELISAs. However, to the authors' knowledge, there are no peer-reviewed Bayesian latent class evaluations of PCR tests for NCD pathogens available. Previous work on PCR for bovine respiratory disease pathogens has shown that these tests do not have a perfect sensitivity and specificity (Bokma et al., 2021).

A promising new technique for the identification of pathogens is nanopore sequencing. The viral metagenome can be identified in a sample, as well as certain bacteria. The technique has already shown its added value for diagnostics in diarrheic pigs and bovine respiratory disease (Theuns et al., 2018; Bokma et al., 2021). Other techniques like immunofluorescence assay or the carbolfuchsine smear method for microscopic examination for *C. parvum* are either not feasible on farm level or no longer widely offered by veterinary laboratories, and hence outside the scope

Table 2. Overview of the different diagnostic techniques used in Belgium with the reported sensitivity, specificity and practical feasibility for every pathogen.

Test	Pathogens	Sensitivity	Specificity	Time to result	Cow-side/ laboratory	References
Antigen-ELISA	ETEC Rotavirus Coronavirus *C. parvum*	71% - 100% 42% - 96% 60% - 89% 59% - 94%	85% - 99% 98% - 100% 51% - 99% 89%- 96%	Minutes	Cow-side	Bartels et al., 2010 Cho et al., 2012 Bio-X, Rochefort, Belgium Techlab, Inc., Blacksburg, VA, USA
Bacterial culture	ETEC *S. enterica*	98% 20% - 100%	92% 18% - 100%	1-2 days	Laboratory	Weber et al., 2017 Waltman, 2000
PCR	*S. enterica*	20%	99%	Hours	Laboratory	Jensen et al., 2013

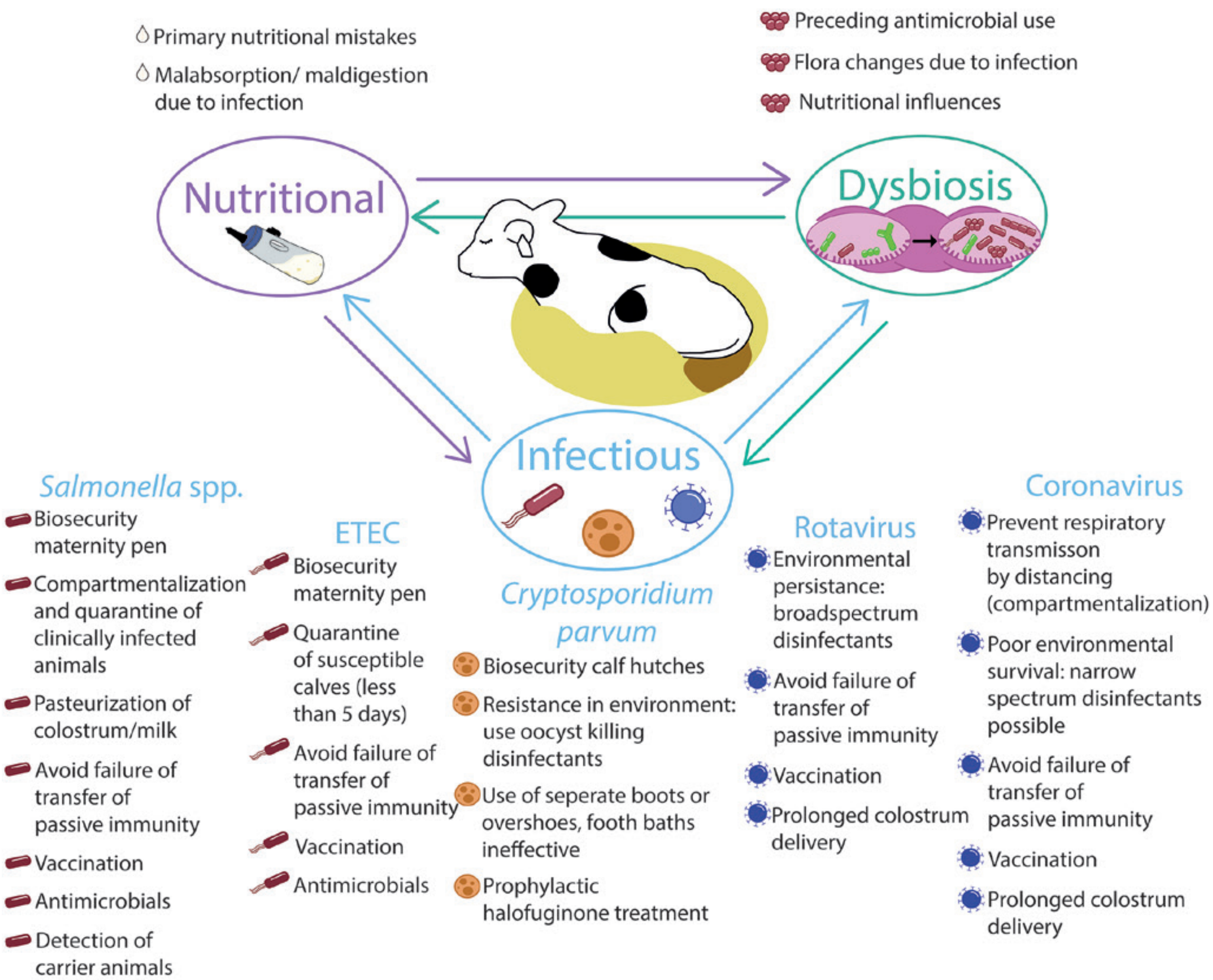

Figure 1. Schematic representation of key factors for pathogen-oriented approaches in neonatal calf diarrhea and the interaction with nutritional diarrhea and dysbiosis.

of this review. In Table 2, an overview is given of the most prevalent diagnostic techniques used in Belgium and their testing properties.

Pathogen-oriented approaches

Below, an overview of the characteristics of NCD pathogens is given, and the key elements in the control of that specific pathogen are described.

Enterotoxic *Escherichia coli*

The immature enterocytes of neonatal calves are susceptible to ETEC strains with F4- (K88), F5- (K99), F6- (K987P), F17- and F41-fimbriae, because these can attach to the immature enterocytes of the neonate (Dubreuil et al., 2016). F5- and F41-fimbriae are the most prevalent (Kolenda et al., 2015). The target sites of the F5-fimbria on the immature enterocytes decrease from 12 hours onwards (Runnels et al., 1980). The relatively short incubation time of 12 hours and possibility to attach to the immature enterocytes makes it the most prevalent underlying pathogen of NCD in calves less than five days old. The infection route is fecal-oral and the infectious dose is most likely less than the infectious dose in humans (10^8–10^{10} bacteria). Clinically affected calves can shed up to 10^{10} ETEC/ml (Acres, 1985; Kolenda et al., 2015; Dubreuil et al., 2016). ETEC produces heat stable and labile toxins that induce a secretory diarrhea through the increase of intracellular cAMP, which activates the cystic fibrosis transmembrane conductance regulator (Foster and Smith, 2009). The Heat Stable Toxin-a additionally blocks the absorption of sodium ions and induces the secretion of bicarbonate, resulting in a metabolic acidosis. The expression of fimbriae and Heat Stable Toxin-a are low in an environmental pH < 7.0. Therefore, the expression will only be increased in the distal part of the small intestine (Foster and Smith, 2009). Even though ETEC can cause severe diarrhea and massive dehydration, the damage to the intestinal wall is limited. Adult cows can have subclinical infections and serve as a reservoir (Dean-Nystrom et al., 1997; Ferens and Hovde, 2011; Kolenda et al., 2015). Periparturient dams can shed up to 10^2 – 10^4 ETEC/ml without showing clinical signs (Acres, 1985). Feces are highly contagious and *E. coli* can survive in the environment for a period between three weeks and 300 days depending on humidity and temperature. *E. coli* is generally susceptible to most classes of disinfectants, as long as the environment is appropriately cleaned and rinsed (Maule, 2000; Chauret, 2011). Key areas for improved biosecurity in an ETEC infection include all materials used around the birth process, such as for colostrum delivery (e.g. buckets, teat), resuscitation after birth, transport of the calf to a calf hutch (e.g. wheel barrow), hygiene of the calving pen (especially when calving in groups) and worker hygiene by wearing separate clothing, boots and gloves and the use of disinfection baths in contaminated situations.

To what considers prevention, neutralizing IgA antibodies directed to the fimbriae are highly efficient. Dams can transfer protective passive immunity to the calves via colostrum. However, dams can have low antibody titers against ETEC. To enhance immunity, inactivated vaccines against the K99-adhesins (F5) of ETEC have been developed for dams in gestation. High antibody concentration in colostrum can be reached after vaccination, although there is variation between different commercial vaccines (Gonzalez et al., 2019).

Given that after colostrum delivery and subsequent milk replacer feeding, ETEC antibodies remain present for a minimum of seven days in the intestine after termination of colostrum feeding, and the infectious period is limited to the first five days of life, there is no crucial role for prolonged colostrum delivery for an ETEC-problem herd (Saif and Smith, 1985).

Antibiotics may be needed in the acute phase of an ETEC outbreak and metaphylaxis may exceptionally be needed if many calves are affected or suspected to be in the incubation period. However, preventive antimicrobial use is prohibited in the European Union and there is no evidence in peer-reviewed literature for any positive effect. Antimicrobial use should by no means replace the biosecurity measures mentioned above. The absence of a functional rumen in the susceptible period for ETEC offers the opportunity to use antimicrobials orally. Parental administration of antimicrobials can be useful if sufficient intestinal distribution is reported for the product. To the authors' knowledge, comparisons of the efficacy of antimicrobials against ETEC between the different administration routes have not been published.

In summary, hygiene around birth, vaccination and adequate colostrum delivery and, when unavoidable, antimicrobial use are the key factors for ETEC control (Table 3).

Bovine rotavirus

The rotavirus is a double stranded RNA-virus without an envelope (Fritzen et al., 2019). After oral uptake, BRV replicates in the epithelial cells of the villi of the small intestines. The damaged epithelium will increase gastrointestinal motility through the release of vasoactive compounds, and increased permeability between the epithelial cells will result in elevated fluid transport to the intestinal lumen. Cytolysis of these cells will result in blunting of the villi and malabsorption diarrhea. Additionally, the expression of the NSP4-toxin will induce fluid secretion by elevating intracellular calcium. The incubation time is 12 hours to three days and clinical signs last five to nine days on average (Heller and Chigerwe, 2018).

The disease is self-limiting, but adult cattle can be an asymptomatic carrier. Increased shedding of BRV has been observed in the periparturient period (Heller and Chigerwe, 2018). Thus, periparturient cattle should not be housed close to neonatal calves. Also, if possible in the production system, calves should be immediately separated from their dam, and group calving pens should be avoided. Regardless of the calving process, the main source of infection are shedding calves and their direct environment (Dhama et al., 2009; Cho and Yoon, 2014; Heller and Chigerwe, 2018; Fritzen et al., 2019). BRV is relatively resistant in the environment and can survive for several months. Disinfection with alcohol or steam are effective ways to reduce the number of BRV particles (Barrington et al., 2002; Dhama, 2009). The use of foot baths with disinfectants, separate boots, clothing and the use of gloves to quarantine infected compartments or to protect compartments with highly susceptible animals, e.g. new-borns, are recommended. Attention needs to be paid that the disinfectant used in the foot bath is effective at the environmental temperature and in situations with excessive fecal contamination. Regular replacement is needed. The presence of BRV-specific antibodies in the intestinal lumen is important in the prevention of clinical disease. Vaccination of dams in late gestation can enhance the levels of BRV-specific antibodies (Saif and Smith, 1985; Gonzalez, 2019). Compared to ETEC, unvaccinated dams can have higher antibody levels as well (Gonzalez et al., 2019). Prolonged colostrum feeding for five days or the administration of colostrum to the milk was evidence to reduce the number of BRV infections (Saif and Smith, 1985; Dhama et al., 2009; Gonzalez et al., 2019). Prolonged colostrum delivery for the whole length of the risk period is advisable, and the required amount of colostrum depends on antibody concentration. Practically, when supported by an etiological diagnosis, it is important to persist in the strategy of prolonged colostrum delivery and realize that results can be suboptimal when the delivered antibody dose is too low (Parreno et al., 2010). Increasing the volume delivered until clinical effect is reached is the message. Pooling of colostrum from different cows does not result in a reduced antibody delivery to the calves (Barry et al., 2022). Mixed infections with BCV are common and can result in higher mortality. The odds of BCV detection after a positive test for BRV are 1.8

Table 3. An overview of the major pathogens in neonatal calf diarrhea, their environmental survival time and disinfectants with a high efficacy.

Pathogen	Environmental survival time	Effective disinfectants	References
Escherichia coli	28 days in slurry 50 – 57 days in feces 130 days in soil 21 – 300 days in water	Chlorine Chlorine Hypochlorite Cationic and anionic active compounds Lime (CaO) Pasteurization/Steam	Chaulet, 2011 Maule, 2012
Rotavirus	6 months in feces	Ethanol Phenol Formalin Lysol Pasteurization/Steam	Barrington et al., 2002 Dhama et al., 2009
Coronavirus	4 days in feces Weeks in water/slurry at 27 °C Year in water/slurry at 4 °C	Detergent Hypochlorite Quaternary ammonium Ethanol Phenol Formalin Lysol Pasteurization/Steam (≥60 °C)	Barrington et al., 2002 Casanova et al., 2009 Mullis et al., 2012
Cryptosporidium parvum	Several months in a moist environment	Quaternary ammonia compounds Hydrogen peroxide (≥6%) Steam (≥80 °C for 2 minutes)	Chalmers and Giles, 2010 Bogan, 2018
Salmonella enterica	1 day in feces compost at 64 °C 4 days in manure pile 4 months in slurry 9 months in soil	Hydrogen peroxide Quaternary ammonium Hypochlorite Phenols Peroxides	Holschbach and peek, 2018

times higher. Therefore, the presence of other pathogens should be monitored (Brunauer et al., 2021).

In summary, improving biosecurity in calving pens and in neonatal housing is essential in tackling rotavirus. Next to this, vaccination in combination with adequate and prolonged colostrum delivery tailored by clinical effect are the key factors for BRV control.

Bovine coronavirus

Bovine coronavirus is a single stranded RNA-virus with an envelope. Infections in cattle can either induce diarrhea or respiratory disease (Hodnik et al., 2020). Diarrhea in calves is mostly seen in the first two weeks of life, but also older calves and adults can be affected, i.e. winter dysentery (Cho and Yoon, 2014). Calves become infected after oral uptake of the virus or through aerosols. After ingestion, BCV primary replicates in epithelial cells of the villi, but can also invade the crypt cells. This will result in atrophy of epithelial cells of the villi and proliferation of secretory cells in the crypts. The lamina propria will become necrotic. Therefore, damage of the intestinal mucosae is more severe compared to BRV infections. This will clinically manifest as a mucohemorrhagic diarrhea and subsequently anorexia, weakness, metabolic acidosis and hypoglycemia. The incubation time is one to seven days and clinical disease will last for three to six days (Hodnick et al., 2020). Independent of the primary route of infection, virus shedding can happen both in feces and respiratory secretions (Hodnik et al., 2020). Adult cattle can become asymptomatic carriers of the disease and infect calves after development of clinical winter dysentery (Cho and Yoon, 2014; Heller and Chigerwe, 2018; Hodnik et al., 2020). Due to the possibility of spread through aerosols, prevention of both direct and indirect contact between infected and susceptible animals is of high importance (Oma et al., 2016). Airborne spread can be limited by distancing animal housings from one another, avoiding nose-nose contacts and short-distance spread. In humans, 1.5 metres distance has been recommended for SARS-CoV-2, but to the authors' knowledge, the maximum length of BCV spread through aerosols has not been determined, making it difficult to take effective measures in practice. Although environmental survival is much shorter for BCV than for BRV and despite BCV is susceptible to most disinfectants due to its envelope, high viral loads can be present on fomites, indicating a potentially high risk of indirect transmission through shared clothes and material between animals. Humans can passively carry BCV, but so far, there is no evidence that calves can be infected by this route (Barrington et al., 2002; Casanova et al., 2009; Mullis et al., 2012; Oma et al., 2018).

In the control and prevention of BCV, adequate colostrum management and prolonged colostrum delivery play a pivotal role. Adult dams can already have high antibody titres against BCV prior to vaccination, but antibody concentration will be elevated in colostrum and transition milk after vaccination in the periparturient period (Gonzalez, 2019). Although in vaccination studies, varying efficacy has been reported, potentially due to mixed infection, vaccination is regarded as an important cornerstone in the control of BCV problem herds (Gonzalez et al., 2019; Maier et al., 2022).

In summary, a BCV-oriented NCD approach should consist of biosecurity in the neonatal housings, attention to aerosol spread by compartmentalization or distancing, and the possibility to use disinfectants with more limited environmental impact, dam vaccination, adequate and prolonged colostrum delivery.

Cryptosporidium parvum

Four *Cryptosporidium* species can infect cattle (*C. bovis*, *C. ryanae* and *C. andersoni*), but only *C. parvum* is associated with clinical disease (diarrhea) in neonatal calves, predominantly at the end of the first week of life (Cho and Yoon, 2014; Thomson et al., 2017). The infection is mostly self-limiting and asymptomatic in cattle, but neonatal calves, especially when undernourished, are very susceptible to develop more severe disease due to their immunocompromised status (Tarekegn et al., 2021).

After ingestion, oocysts invade the extracytoplasmatic space of epithelial cells of the distal part of the small intestines and reproduce asexual into type I meronts and subsequently sexual into type II meronts that differentiate into micro- and macrogametocytes (Fayer and Ungar, 1986). This will cause loss of microvilli and shortening of the epithelial cells, resulting in blunting of the villi. Additionally, crypt cell proliferation is observed. The damage to the intestinal mucosae is less severe than in case of BRV or BCV infection (Heller and Chigerwe, 2018). Cryptosporidiosis will manifest as malabsorption diarrhea with the possibility of secondary nutritional diarrhea and dysbiosis (Heine et al., 1984). Due to the possibility of autoinfection, the uptake of 10^2 oocysts is enough to establish an infection (Heller and Chigerwe, 2018). Afterwards, the prepatent period is three to six days and calves can shed up to 10^7 oocysts per gram for a period of six to eight days (Fayer et al., 1998; Zambrinski et al., 2013). The initial number of ingested oocysts will influence the magnitude of oocyst shedding (Zambrinski et al., 2013). The infection route is strictly fecal-oral. Oocysts persist multiple months in the environment (Table 3). Also, for *Cryptosporidium*, contaminated areas in the farm can be visualized by 'following the shit' and clarify walking lines on farm. Contamination of feed and water with oocysts is frequent and can be the consequence of drainage of feces or indirect transmission through boots or vermin.

Within the control of cryptosporidiosis, strict biosecurity and hygiene measures are necessary, due to the low infectious dose needed and high shedding. Accumulation of infectious oocysts can result in high environmental contamination, due to high resilience against environmental conditions (Table 3). Therefore, hygiene may of all causes of NCD be the most important in the prevention of *C. parvum*. Unfortunately, *C. parvum* is resistant against the majority of disinfectants. The most well-known disinfectants with high efficacy are the quaternary ammonia releasing compounds, but also hydrogen peroxide (≥ 6% for 20 minutes) is effective on clean surfaces (Chalmers and Giles, 2010; Bogan, 2018). Steaming (2 minutes at 80 °C) is another option, but difficult to assure on farm that the required temperature is reached sufficiently long to effectively kill the pathogen (Harp and Goff, 1998; Bogan, 2018). An important difference with the other major pathogens is that the use of foot baths is generally ineffective against *Cryptosporidium*, because most of the disinfectants registered for this application do not cover *Cryptosporidium*. Furthermore, commercially available disinfectants registered against *Cryptosporidium* demand a contact time of at least two hours. Making them unsuitable for the use in foot baths (Cid Lines, Ieper, Belgium). Hence, overshoes or preferably separate boots and clothing for compartments with either infected animals or highly susceptible animals are needed, as well as attention for hand hygiene and gloves. A recent meta-analysis has shown that intensity of calf contacts, larger herd size, organic farming, warm and wet weather are risk factors for cryptosporidiosis, whereas hard flooring is a protective factor (Brainard et al., 2020). A key difference with the other four major pathogens is that colostrum management and vaccination have no direct effectiveness in the prevention of cryptosporidiosis (Kaçar et al., 2022).

Today, prophylactic treatment with halofuginone lactate is one of the cornerstones in cryptosporidiosis control and prevention. Its mode of action is unknown, but is believed to affect the merozoite and sporozoite stages (Thomson et al., 2017). The recommendation is to prophylactically start giving the drug on the second day of life and continue the treatment for seven days, or administer it therapeutically within 24 hours after the onset of diarrhea (Thomson et al., 2017). Halofuginone lactate cannot be given to calves that already have had diarrhea for more than 24 hours or that are weak and dehydrated (European Medicines Agency, 2007) (off label use). In a recent meta-analysis, it has been shown that oocyst shedding, diarrhea incidence and mortality are significantly reduced when the prophylactic therapy is initiated before calves are five days old (Brainard et al., 2021). In contrast, robust evidence of high therapeutic efficacy of halofuginone lactate against cryptosporidiosis is still lacking. Halofuginone use does not completely prevent and especially does not cure cryptosporidiosis (Thomson et al., 2017). At the time of writing, no alternative nutritional strategies have shown any advantageous effects in the prevention of clinical cryptosporidiosis. The usefulness of treatment with halofuginone lactate should therefore always be considered with special care, especially with regard to its narrow toxic margins. Its caustic effects on pharynx and esophagus may result in milk refusal. The leaflet of commercial formulations describes toxic adverse effects at twice the recommended dose. No conclusions can be drawn about the ecotoxicity of halofuginone formulations, but usage should be taken with care due to its efficacy against protozoa (EFSA, 2020). Alternatively, compounds as paromomycin or azithromycin are worldwide used as alternative prophylactic or therapeutic group treatments, but clear evidence of their efficacy in calves is still lacking (Brainard et al., 2020). Although more research on the efficacy of paromomycin on cryptosporidiosis has been recommended in a systematic review, within the framework of rational and responsible antimicrobial use, the practice of oral mass medication should absolutely be discouraged (Brainard et al., 2021).

Finally, *C. parvum* is a zoonosis and therefore, sufficient attention should be given to communication towards farm owners and staff to clarify the risk for immunocompromised persons, like children and pregnant women.

In summary, biosecurity, environmental hygiene and the use of ammonia-releasing disinfectants together with prophylactic halofuginone lactate use where deemed necessary, are the key elements of cryptosporidiosis control. Field experience is that cryptosporidiosis is most difficult to control and requires absolute compliance with farm biosecurity protocols.

Salmonella enterica

Multiple serogroups of *Salmonella enterica* subspecies I can affect calves, mostly type B, C, D and E. Serotype D is exclusive to cattle (Brenner et al., 2000; Holsbach and Peek, 2018). The most prevalent serotype in Europe is *S. enterica* serotype Dublin (*S.* Dublin), followed by *S. enterica* serotype Typhimurium (*S.* Typhimurium) (Gutema et al., 2019). Salmonellosis in calves is characterized by a hemorrhagic enterocolitis with possible clinical signs as bloody diarrhea, fever, weakness, depression, anorexia and in severe cases death. The cattle-adapted serotype *S.* Dublin is associated with pneumonia and sepsis in calves (Nielsen, 2013). Infections happen through the fecal-oral route, although *Salmonella spp.* can be shed in colostrum and milk (Holsbach and Peek, 2018; Castañeda-Salazar et al., 2021). For calves, the infectious dose is approximately 10^6 colony forming units (Holsbach and Peek, 2018). The subsequent clinical signs vary with age, but pre-weaned calves are highly susceptible. Infectious doses for adult cattle are around 10^9-10^{11} colony forming units. *Salmonella* spp. invade enterocytes in the distal jejunum and ileum. From there, they use the lymphatic system to reach the organs with the mononuclear phagocyte system, where they invade macrophages. The rate of intestinal damage, mucosal penetration and intracellular survival is strain dependent. Post clinically, animals can become persistent carriers of *S. enterica* and shed the pathogen intermittingly without developing clinical signs. The number of excreted colony forming units in asymptomatic carriers varies from 10 to 10^5 (Nielsen, 2013; Holsbach and Peek, 2018).

Antimicrobials are necessary for calves with acute signs of NCD caused by *S. enterica*, especially when the risk of sepsis is high. However, resistance can be present on a farm with varying results. Therefore, the susceptibility of the *S. enterica* strain should be evaluated and appropriate therapy should be administered (Smith, 2015). In Europe, resistance against ampicillin, tetracyclines and sulphonamides has been reported most often, but it should be evaluated case by case (EFSA, 2022). Furthermore, aminoglycosides and first- and second-generation cephalosporins can have a high in-vitro efficacy, but are not recommended against clinical salmonellosis, due to the low intracellular accumulation (CLSI, 2022). Within the framework of rational antimicrobial use, there is an ongoing discussion in Belgium on recommended antimicrobial therapy for *S. enterica*. One topic is the apparent need of fluoroquinolones to effectively treat *S. enterica* in cattle given the disappointing results that some veterinarians experience of the first-choice antimicrobial mentioned in the Belgian national formulary (sulphonamides-trimethoprim), even when the strain is susceptible. Another topic is the discussion on metaphylaxis to control a *Salmonella* outbreak situation. Both topics urgently request more research in order to support antimicrobial therapy guidelines.

A structured approach for general control of *S. enterica* on cattle farms is beyond the scope of this review and has been described by Nielsen et al. (2012). The emphasis on the control of NCD caused by *S. enterica* should be on the identification of the main routes of infection of the calves. Regardless of the involved transmission routes, strict separation of age groups in space and material is recommended. Primary infection can happen directly through contact with shedding cattle (calves or cows) or closely after birth at the maternity pen, especially if sick or carrier animals are located there. Transmission via indirect contact can happen through fomites. Dams can be asymptomatic carrier animals that start re-shedding in the periparturient period due to immunosuppression (Holsbach and Peek, 2018). Hence, limiting contact between dam and calf, and avoiding clustered births may be beneficial. Another important aspect is that colostrum can be infected with *S. enterica*, which was the case in about 15% of colostrum samples in a United States study (Houser et al., 2008). However, on the other hand, colostral antibodies can be protective and this can be enhanced by dam vaccination during late gestation using commercially available vaccines (Smith et al., 2014; Smith et al., 2015). Nevertheless, results can be variable since in an oral challenge trial with *S. enterica* var. Newport colostrum provision from a *Salmonella*-vaccinated dam did not decrease disease nor mortality (Foster et al., 2019). Colostrum pasteurization for 120 minutes at 60 °C may be an option to reduce the risk of infection by colostrum uptake (Godden et al., 2006). The effect of whole herd vaccination to control *Salmonella* is variable (Nielsen et al., 2012; Kent et al., 2021).

After the acute phase of the outbreak and after identification of the main transmission routes, carrier animals should be identified. Carrier removal can be initiated the earliest six months after infection, due to *Salmonella* antibody persistence (Nielsen, 2013). Lactating cows are at high risk of being a carrier, when they test highly positive twice on subsequent antibody ELISA with at least a 120-days' interval (Nielsen et al., 2012). Due to their risk to infect other animals and people, it is recommended to cull these animals. An important remark is that vaccination against *Salmonella* interferes with the antibody-based carrier detection and culling decision making. Also, with *Salmonella*, attention should be paid to inform farmers and staff on appropriate measures to reduce zoonotic transmission, especially to risk groups.

In summary, the use of antibiotics in the acute phase of an outbreak is inevitable. Within the control of *S. enterica*, identification of the main transmission route and carrier animals is necessary. Limiting contact between shedding animals and pasteurization of colostrum and milk will reduce the risk of transmission.

Minor pathogens

NCD pathogens of minor importance in Belgium are bovine viral diarrhea virus (BVDv), norovirus, torovirus, astrovirus, nebovirus, enterohemorrhagic *E. coli. (EHEC), Enteropathogenic (EPEC)* and *Clostridium perfringens* type C (Cho and Yoon, 2014; Heller and Chigerwe, 2018; Martella et al., 2020; DGZ, 2020; Brunauer et al., 2021). With the exception of BVDv, the absence of routine diagnostics results in the lack of a prevalence estimate for these pathogens.

Table 4. An overview of minor neonatal calf diarrhea pathogens, their known infection routes, associated clinical signs and incubation time.

Pathogen	Infection route	Associated clinical signs	Incubation time	References
Astrovirus	Fecal-oral Possibly nasal	Diarrhea Neurological signs	Unknown	Zhu et al., 2022
Clostridium perfringens type C	Present in gastrointestinal flora	Sudden death Hemorrhagic diarrhea	Multiple hours	Simpson et al., 2018
Enterohemorrhagic *Escherichia coli*	Fecal-oral	Hemorrhagic diarrhea	2-12 days	Stein and Katz, 2017
Enteropathogenic *Eschericha coli*	Fecal-oral	Debatable		Kolenda et al., 2015
Nebovirus	Fecal-oral	Mild diarrhea	1 – 3 days	Hall et al., 1984 Cho and Yoon, 2014
Norovirus	Fecal-oral	Diarrhea, no intestinal lesions	4-7 days	Van der Poel et al., 2003 Di Felice et al., 2016
Torovirus	Fecal-oral Nasal	Mild- moderate diarrhea	1 – 3 days	Hoet and Saif, 2004 Cho and Yoon, 2014

Astrovirus, torovirus and bovine caliciviruses (e.g. norovirus and nebovirus) are sometimes considered as emerging causes of NCD. However, their equal presence in healthy and diarrheic calves makes it difficult to estimate their pathogenicity (Cho and Yoon, 2014). In Table 4, an overview of known pathogenicity and incubation periods of these pathogens is given. EHEC differs from ETEC with the presence of the eae gene, responsible for attachment, and one of the two Shiga toxins. EHEC causes severe damage to the enterocytes and results in hemorrhagic diarrhea with possible pseudomembranes in calves up to three weeks of age. Adult cows can have subclinical infections and serve as a reservoir (Dean-Nystrom et al., 1997; Ferens and Hovde, 2011; Kolenda et al., 2015; Stein and Katz, 2017). When genes encoding for the Shiga toxin are lacking, then *E. coli* is classified as enteropathogenic *E. coli* (EPEC). EPEC causes disruption of the microvilli and subsequently malabsorption. The pathogenic importance of EPEC is up for debate. Both pathogens are seldom identified in NCD cases in Belgium; likely because they are not included in routine diagnostics and require molecular tests to be differentiated from other *E. coli* strains.

C. perfringens type C most commonly affects calves aged one to four weeks and is occasionally characterized by bloody diarrhea, but mainly manifests as sudden death. *C. perfringens* can naturally occur in the gastrointestinal tract, hence detection of the pathogen without detection of the toxin is meaningless. The consumption of large volumes of carbohydrates and/or proteins are believed to be the leading risk factor (Simpson et al., 2018).

Nutritional diarrhea

Nutritional diarrhea is likely the most frequent cause of diarrhea, either as the sole cause, but often as a complicating factor in infectious diarrhea. Certain infectious pathogens (*C. parvum,* BRV and BCV) can cause blunting of the villi and inflammation of the small intestine, resulting in maldigestion and malabsorptive diarrhea. Hence, a diet which resulted in adequate growth before the NCD outbreak, may no longer be optimal for NCD-affected calves, as it results in too many nutrients that are not resorbed. Consequently, fermentation of these unabsorbed nutrients can induce D-lactic acidosis and osmotic diarrhea (Foster and Smith, 2009; Lorenz and Gentile, 2014; Heller and Chigerwe, 2018). In this way, nutrition can aggravate the diarrhea problem, especially in combination with hyperosmolal oral rehydration solutions (>700 mOsm/L). However, nowadays, it is widely accepted that milk feeding needs to be continued during an NCD episode to assure feeding of enterocytes and avoid caloric-proteinic malnutrition and a negative energy balance (Constable, 2009). Therefore, it is not recommended to deprive a calf of milk or milk replacer for a period longer than 12 hours and combine it with oral rehydration solutions with low to moderate osmolality (250-600 mOsm/L) (Foster and Smith, 2009; Smith and Berchtold, 2014; Wilms et al., 2020).

The potential involvement of nutrition in a herd problem with NCD should always be evaluated, either as a primary cause (quality or formulation of feed is insufficient) or as a secondary complicating factor (associated with an intestinal infection). Too high concentrations of lactose or monosaccharides, due to too large volumes, too concentrated milk or factory formulation mistakes are among the most common causes of osmotic diarrhea. Also, some carbohydrate sources, as sucrose, cannot be digested by neonatal calves and will result in diarrhea (Drackley, 2008). An increased concentration of ashes can have similar effects (Drackley, 2008). High concentrations of fat, similar to whole milk, will not increase the incidence of diarrhea (Heller and Chigerwe, 2018; Amado et al., 2019). However, milk fat that was not emulsified properly by mixing at the appropriate temperature, is another frequent cause, which can be visualized by inspecting fat micelles (<3µm) under the microscope (Drackley, 2008). More difficult to characterize are certain combinations of protein and carbohydrate sources that result in dysbiosis, and subsequently in diarrhea with or without D-lactic acidosis (Drackley, 2008). Finally, many farmers are still afraid of increasing the nutritional plane because of a perceived risk of diarrhea. When milk is well formulated and administered under hygienic circumstances, this fear appears unjustified. In a recent study by Lorenz, et al. (2021), ad libitum milk feeding appeared to be a protective factor for the development of NCD in German dairy herds.

In the framework of this article, it is important to realize that diagnosing a nutritional diarrhea herd problem is equally important as finding the infection involved, if any.

Microbiome

A relatively new field of interest is the gastrointestinal microbiome. The microbiome itself also has an effect on the prevention of NCD, but up till now, pathogen specific knowledge is lacking. A higher abundance of *E. coli* in the intestine has been observed with diarrhea, regardless of the etiology. However, the presence of *Faecalibacterium prasunitzii* is negatively correlated with the prevalence of diarrhea. A higher gut-prevalence of *Bifidobacterium* is correlated with a lower abundance of *E. coli* at three to seven days of age. The administration of *Bifidobacterium* to neonati is also associated with a higher weight gain and feed conversion ratio and lower incidence of diarrhea (Malmuthuge et al., 2015; Slanzon et al., 2022).

Regardless of the primary infectious agent, dysbiosis, defined as disturbance of the normal gut microbiome, can occur as a complication. Dysbiosis can also occur in response to nutritional mistakes or antibiotic use, and in the last case is then referred to as 'antibiotic induced diarrhea'. Diarrhea persisting for more than ten days is most likely complicated by dysbiosis (Gomez et al., 2017). Immediate administration of colostrum after birth enhances the development of the intestinal tract and immunity directly, but also indirectly through stimulation of the microbiome. It is suspected that mucosa-attached bacteria have an effect on the gut barrier and the development of the hosts' immunity. *F. prausnitzii* and *Bifidobacterium spp.* could promote the intestinal junctions and *Blautia* and *Brevibacterium* are correlated with the development of the ileal immunity (Fischer et al., 2018; Osorio, 2020; Song et al., 2021). Unfortunately, practical implementations of the microbiome related to NCD prevention and therapy are still lacking for the moment. Further research on the microbiome could result in predictive biomarkers or new prophylactic treatments.

CONCLUSION

NCD is one of the biggest challenges for veterinarians and farmers during the first weeks of the newborn's life. In this review, substantial differences in the importance of risk factors and control measures for the different NCD pathogens were identified. This emphasizes the potential of pathogen-oriented approaches for the control of NCD on herd level, helping to economize both labor and financial resources on farm level, limiting environmental impact.

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uit het verleden

Paardenkerkhof van Childerik in Doornik

De Salische Franken, een tak van de Frankische Germanen, veroverden bij de val van het Romeinse Rijk onze streken en vestigden hun hoofdplaats in Doornik. Hun koning Childerik werd er begraven met onvoorstelbare rijkdom. Daarvan getuigen niet enkel de zogenaamde ‘schat van Childerik’, maar ook een massagraf met niet minder dan 21 paardenskeletten. C14-dateringen lieten toe dit ‘paardenkerkhof’ te situeren in de tijd van Childerik. De dieren waren begraven in drie holten in de rots gehouwen ongeveer twintig meter van het graf van de koning zelf.

Een dergelijk paardenkerkhof was uitzonderlijk bij de westelijke Germanen. Het getuigt van het aanzien, dat zowel paarden als Childerik zelf genoten. Zijn zoon Clovis (Chlodovich, Ludovik, Lodewijk, Ludwig, Louis) zou het kleine rijk uitbreiden over heel Frankrijk (het Rijk van de Franken) tot in het noorden van Spanje en over een groot gedeelte van het huidige Duitsland.

Een opstelling in het archeologisch museum van Doornik geeft daar een beeld van weer.

Met dank aan Sabine Lauwers en Luc Van Damme