Review

PATHOMORPHOLOGICAL ASPECTS OF RELEVANT GASTROINTESTINAL DISEASES IN NEWBORN AND GROWING CALVES

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ABSTRACT

The emphasis of this literature overview is placed on the etiology, epidemiology, specific gross and microscopic pathological changes of the commonest gastrointestinal diseases in newborn and growing calves in dairy and beef farms. Neonatal diarrhea in calves is a multifactorial disease accompanied with high morbidity and mortality rates. It incurs considerable economic losses for cattle industry worldwide. The gastroenteritis in calves is most commonly of infectious or parasitic etiology and the most frequently involved enteropathogens are *Cryptosporidium parvum*, bovine rotaviruses (*GRA BRV*), bovine coronaviruses (*BCoV*), mucosal disease virus - bovine viral diarrhoea (*BVDV*) and *Escherichia coli* K99 (*F5*). Affected calves are from 24th hours to 30 days of age, and the appearance of the disease is largely dependent on the immunological status of newborn calves, the amount of ingested colostrum, whether dams are vaccinated or not during the dry period, and the conditions of housing of newborns. The specific gross and microscopic lesions of the gastrointestinal tract and of some parenchymal organs are essential for the diagnostics and differential diagnostics of reviewed diseases.

Key words: calves, gastroenteritis, neonatal calf diarrhea, pathology

INTRODUCTION

The increased dairy and beef cattle populations in the world pose a higher risk from the onset and spread of gastrointestinal diseases among newborn and growing calves. At a global scale, this group of diseases is termed *neonatal calf diarrhea* (NCD) (1). Etiological agents of NCD are a variety of pathogens as bacteria, viruses, and protozoa, causing diarrhoea in calves either solely or in combination. In calves, gastrointestinal diseases are outlined with high morbidity and mortality rates and are considered to be the main reason for important economic losses for large ruminant farmers in the world. The studies indicate that about 40% of calves under one month of age show signs of gastrointestinal diseases, which are the main reason for death within this age group (2).

The rapid death in diseased calves is due to the loss of fluids of electrolytes, resulting in severe dehydration, metabolic acidosis, negative energy balance (3). From historical point of view, the etiology of gastroenteritis in newborn calves is associated with *Salmonella* spp., *E.coli* (*F5*) K99 and *Clostridium perfringens* type C (4). In about 30% of NCD cases, the diagnosis remains uncertain. Some researchers believe that about 64% of enteritis cases in calves are of infectious origin (2, 3). That is why these diseases are determined as multifactorial and dependent on the breed, feeding and rearing conditions. The immunological status of dams in the herd, the occurrence of various infectious agents and farm management are also essential (5).

Recently, bovine noroviruses (*BNoV*), bovine neboviruses (*BNeV*), bovine enteroviruses (*BEV*) and bovine toroviruses (*BToV*) were identified as potential agents of enteritis in juvenile calves (6-9). Some of them (*BNoV*, *BEV* and *BToV*) have been detected in the faeces of clinical healthy calves as well (10).

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On the basis of epidemiological studies, *BNoV* and *BToV* were reported in faeces of diarrhoeic calves, but their significance in the NCD etiology is still not acknowledged (5, 9).

Enteropathogens reported to be most commonly involved in neonatal calf diarrhea at a global scale are: *Escherichia coli* K99 (F5), bovine viral diarrhoea - mucosal disease virus (BVDV), *Cryptosporidium parvum*, bovine rotaviruses (GRA BRV) and bovine coronaviruses (BCoV) (11). GRA BRV and *C. parvum* were detected in faeces of calves with gastrointestinal diseases in 17.7% to 79.9% of cases (12). Sometimes, co-infections with more than single etiological agents are reported (13).

The prevalence of enteritis in cattle farms in the USA varies from 19.1% and 21.2% (14). Others (15) affirm that more than 50% of deaths in non-weaned calves is due to neonatal diarrhoea. Also, the NCD complex includes complicated states such as meningitis and neonatal pneumonia. Outbreaks at farms are frequent, especially when animals have been gathered from different farms with unknown immunological status (16).

The utilisation of combinations of bacteriological and virological methods of examinations along with gross anatomy and histopathological studies of fresh intestinal specimens from diarrhoeic calf carcasses is of utmost importance for detection of the severity and localization of lesions and respective enteropathogens with regard to the diagnosis and differential diagnosis of NCD (17).

COLIBACTERIOSIS

Coli-infections have a huge economic impact as they affect newborn calves, foals, pigs, lambs and chickens inflicting great losses. Colibacteriosis is an acute infectious disease affecting newborn calves in the first days of their life, manifested with signs of enterocolitis and septicaemia. The disease is caused by the Gram-negative bacterium *Escherichia coli*. Colibacteria belong to the family *Enterobacteriaceae* discovered in 1885 by Th. Escherich. The members of this large family are ubiquitous (18).

The main agents of calf diarrhea are strains from several pathovars – enterotoxigenic *E. coli* (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), diffusely adherent (DAEC) and enterohaemorrhagic (EHEC) *E. coli*. The first ETEC strain identified in calves is K99 antigen (19).

The pathogenicity of *E. coli* are also related to

the production of haemolysins and substances with necrotic effect. Endotoxins are believed to cause pathological damage of organs and systems: hyperaemia, haemorrhages, vasomotor shock, local and general Schwartzman reaction, fever, increased pulmonary blood pressure, specific dermal reaction, placental damage followed by abortion (20, 21).

Diarrhoeic syndrome epidemics in intensive farms is associated to the greater number of susceptible subjects (especially if animals are deprived of the chance to acquire colostrum immunity) and factors facilitating the alimentary transmission mechanisms via the faecal-oral route. In suckling calves, the disease rate is about 30%, but could attain up to 75%, with mortality between 10 and 50% (22).

ETEC enteritis in calves occur as early as on postpartum hour 12–18 or until the 4th day of life, less frequently in older calves and never in adults. Epidemiological surveys of gastroenteritis in suckling calves have shown that more than 80% of clinical cases were caused by strains possessing the K99 antigen (23).

The commonest clinical forms of colibacteriosis in calves are three: colisepticaemia, colienterotoxaemia and colienteritis. Mucohaemorrhagic colitis and proctitis are characteristic only for EHEC, but not for EPEC or ETEC, where only the stomach and small intestine are affected (24).

Colisepticaemia is encountered in all animal species and is caused by invasive *E. coli* strains which penetrate in tissues and bloodstream through the intestinal and nasopharyngeal mucosa, the tonsils and umbilical blood vessels. Calves, pigs and lambs with high blood serum immunoglobulin concentrations are usually protected from septicaemia. The clinical signs are due to the produced endotoxin, which causes shock. The disease is most commonly seen in newborn animals which had not been given colostrum (25).

Colienterotoxaemia is caused by enterotoxigenic *E. coli* strains capable to colonise and replicate in proximal small intestine and to produce enterotoxin. The infection is described in the literature as *isocolibacillosis* (26). It affects calves during their first week of life, usually in the first 48 hours after birth. The attachment of *E. coli* to intestinal epithelial cells is done with pili. The enterotoxic form of colibacteriosis is encountered most commonly in calves and pigs and rarely, in foals and lambs (27).

Colenteritis (or intestinal form of disease) is caused by colonisation and spread of *EPEC*, causing diarrhoea, metabolic acidosis and dehydration (28).

Gross anatomy examination showed that the gastrointestinal tract was always affected regardless of the form of colibacteriosis. The intestinal content in most cases is liquid, yellow-green, of bad odour and mixed with white bran-like fibrin flakes. Mesenteric lymph nodes are swollen, juicy and hyperaemic (29).

Macroscopic changes in intestinal colibacteriosis are characterised with dehydration, enlarged intestinal lumen filled with yellow watery content mixed with gas bubbles, and the liver is with a markedly enlarged gallbladder. In affected intestinal compartments, pseudomembranous ileitis, mucohaemorrhagic colitis and proctitis are observed (30).

Histopathological and ultrastructural changes in different segments of the alimentary system of calves with colenteritis have received little attention before due to the impossibility to reproduce the disease in experimental animals. The results from studies performed worldwide showed catarrhal-desquamative inflammation of the abomasum with hyperaemia of *propria mucosae* and oedema of submucosa and muscles. In some cases, haematomas in *propria mucosae* and bacterial emboli in some submucosal blood vessels were observed. Some authors described adhesion of intestinal villi in the region of the jejunum and ileum with neutrophilic infiltration of the mucosa and numerous attached Gram-negative bacteria (31).

BOVINE VIRAL DIARRHOEA - MUCOSAL DISEASE

Bovine viral diarrhoea - mucosal disease (*Bovine viral diarrhoea virus* - *BVDV*) is widely prevalent among calves and other ruminants, causing serious economic losses to the cattle breeding sector. The disease is caused by an enveloped virus, 45 nm in diameter and single-strand RNA in its genome. It belongs to the *Pestivirus* genus, family *Flaviviridae*. From antigenic point of view it is similar to the virus of the classical swine fever (CSFV), border disease in sheep and goats (BDV) and human rubella virus (HRV). *BVDV* is classified into two biotypes, cytopathic (*CP*) and non-cytopathic (*NCP*). Due to its high antigenic variability, it could be placed into two main antigenic groups: type 1 and type 2. Recently, in Europe was identified also type 3, comprising two subgroups of Thai and Brazilian origin. Type 1 is widely prevalent among ruminants worldwide as well as in Europe, unlike type 2 which is most

commonly encountered in the USA, Canada, Japan, South America and some European countries (32).

The disease is characterized with various clinical manifestation of the infection as respiratory diseases, acute or chronic gastrointestinal syndrome in newborn and growing calves, haemorrhagic syndrome with thrombocytopaenia, mucosal disease in cows, skin diseases and immunosuppression (33).

In pregnant animals the disease causes various reproductive disorders: early embryonic death, abortions, mummified foetuses, foetal malformations, stillbirths, birth of weak and non-vital calves (34). Embryos infected between the 60th and 90th gestational days are born as immunotolerant and persistently infected (*PI*) calves (35). Congenital malformations have been observed in newborn calves including cerebral hypoplasia, microcephaly, hydrocephaly, osteochondrosis, stunted growth, ocular neuritis, retinal degeneration, hypoplasia of the thymus, hypotrichosis, alopecia, osteosclerosis, microphthalmia and cataract (36). The virus induces degenerative and necrobiotic processes in the myocardium and lungs of intrauterinely infected calves. In sheep and goats, there are data for BVDV infection accompanied with lymphocytic myocarditis and multifocal necroses in the other parenchymal organs (37).

The erosions and shallow ulcers, but not ulcers are usually typical for BVD and the oesophageal lesions in its upper part are also very common instead the lesions in the respiratory system. The epithelium of coronary band and cleft hooves is also affected where interdigital dermatitis and coronitis develop. The BVD virus also has a strong affinity toward lymphatic tissue, especially in the area of tonsils and Peyer's patches, where dystrophic changes in immunocompetent cells and a significant decrease in the amount of lymphocytes in the germinal centers of lymph follicles are seen, which has as a consequence a strong suppression of the cellular and humoral immune response and animal death (37).

Bovine viral diarrhoea (BVD) is a problem for newborn and growing calves and is characterised with haemorrhagic-necrotic and ulcerative lesions on the mucosa of alimentary and respiratory systems (38). There is also a severe acute BVD-type 2, which is characterized by a high morbidity and mortality in all age groups, whereas the "classical bovine viral diarrhea" in calves is

characterized with a high morbidity and low mortality. Mucosal disease on the other hand is a clinicopathologic syndrome occurring in persistently infected animals commonly aged between 6 months and 2 years, when the virus causing persistent congenital infection spontaneously develops a recombination encoding NS3. The mucosal disease is characterized by low morbidity, but high mortality (38).

The main macroscopic lesions found out in calves having died from mucosal disease BVD were focused in the oral cavity, manifested with gingivitis and multiple erosions and shallow ulcers, mucosal necrosis and haemorrhages. Oesophageal lesions such as linear shallow erosions covered by shreds of necrotic epithelium are also seen most commonly in the upper part of oesophagus. The abomasus, reticulum, omasus, cecum, colon and rectum are also affected with inflammation of a catarrhal to haemorrhagic nature, small erosions, necroses or ulcers. In the chronic cases, the epithelium of coronary band and cleft hooves is affected where interdigital dermatitis and coronitis develop. The lesions in respiratory system are less obvious and only mild tracheitis, bronchitis, and bronchiolitis, which can subsequently progress to secondary bacterial pneumonia, can be seen. The BVD virus also has a strong affinity toward lymphatic tissue, especially in the area of tonsils and Peyer's patches, where dystrophic or necrotic changes in immunocompetent cells and a significant decrease in the amount of lymphocytes in the blood and in the germinal centers of lymph follicles in the spleen and lymph nodes are observed, which has as a consequence a strong suppression of the cellular and humoral immune response and animal death. Occasionally, pleuritis with pulmonary hyperaemia and oedema was present. Microscopically, the myocardium exhibited focal haemorrhages and leukocytic infiltration, and the kidneys – lymphoplasmocellular multifocal nephritis. In the brain, neuropathological lesions as neuronophagia and spongiosis of the grey matter of the brain (39).

CRYPTOSPORIDIUM ENTERITIS

Cryptosporidium spp. (type *Apicomplexa*, family *Cryptosporidiidae*) are intracellular extracytoplasmic coccidian protozoa affecting the gastrointestinal tract of a number of mammalian and amphibian species, and in birds – lungs as well (40). *Cryptosporidium parvum* and *Giardia spp.* are considered the major protozoan agents involved in calf diarrhoea. Some of the members of the family possess a marked zoonanthroponotic potential. Together

with some bacterial and viral pathogens, *Cryptosporidium parvum* is outlined as one of the commonest causative agents of neonatal gastroenteritis in calves up to 3 weeks of age. According to some researchers, the death rate is low but the morbidity rate within the population in the contact environment is high (41).

The most important clinical sign of the disease is the profuse watery diarrhoea of yellow colour, mixed with blood and mucus. The severity and duration of clinical signs depends on the involvement of viruses, bacteria and parasites. The gross appearance of small and large intestine shows that they were distended, filled with gas and watery yellowish fluid, and the mesenteric lymph nodes were enlarged. The intestinal mucosa was strongly hyperaemic and covered with yellowish intestinal content. There were no changes in parenchymal organs (42, 43).

Microscopic changes comprised severe atrophy of intestinal villi of the ileum, caecum and the colon (42). Multiple developmental forms of the protozoan bound to epithelial cells lining the ileal villi were observed. The villi were atrophied and rounded, fused one to the other, with cryptic hyperplasia and infiltration by different inflammatory cells. The *lamina propria* was infiltrated with mononuclear cells, and crypts were distended by the numerous neutrophils. Mesenteric lymph nodes were oedematous, and reticuloendothelial cells – hyperplastic. After a mild infection, no histopathological alterations of intestines were present (42, 44).

In clinical cryptosporidiosis outbreaks in calves, multiple developmental forms of the pathogen were established in the middle and distal jejunum and ileum. When the distal small intestine was colonised, the histological finding was manifested as villous atrophy, metaplasia and desquamation of the surface epithelium (42). The authors specified that similar lesions could be also observed in the duodenum, caecum and the colon. They also affirmed that the cellular infiltrate in *lamina propria* comprised mainly neutrophils (45).

ROTAVIRUS ENTERITIS

Rotaviruses provoke a number of gastrointestinal diseases in children, calves, piglets, foals, lambs, rabbits, antelopes, mice and exotic animals' species (grizzly bears, kangaroos etc.). Rotaviruses are considered to be among the first identified agents causing diarrhoea in calves under 30 days of age, with prevalence between 10% and 20%. In some countries, diarrhoea caused by rotaviruses is the main cause for high mortality among children. Group A (*gPA*) bovine rotaviruses (*GPA BRV*) belong to the family

Reoviridae with double-strand RNA in their genome, comprising 11 segments (46).

Apart *GPA BRV*, other enteropathogens as parvoviruses, pestiviruses, caliciviruses, astroviruses and adenoviruses were believed to be involved in intestinal infections of calves (47).

In newborn calves, the infection occurs without viraemic stage, after short incubation period, with profuse diarrhoea combines with severe dehydration. Rotaviruses infect selectively epithelial cells of villi, and the replication of the virus occurs in their cytoplasm with crypts remaining unaffected by the infection. There are data that rotaviruses have been isolated from the lungs and mesenteric lymph nodes of infected calves (48).

After opening of the abdominal cavity, the abomasum and the intestines were ballooned and filled with fluid (49). Also, catarrhal to and rarely catarrhal-haemorrhagic enterocolitis was observed (50).

A number of experiments have proved that small intestinal epithelial cells were the main subject to viral replication despite that viral antigens were found also in the epithelial cells of the colon and caecum of infected calves via immunofluorescence analysis (51).

Histopathological examinations of affected intestinal segments showed shortened villi, and reticular cells were distended with lymphocytic infiltration in the propria and microvilli. Morphological changes were characterised with desquamation of infected cells, followed by severe atrophy of affected villi. Atrophied villi were covered by immature cuboid cells having migrated from crypts (49).

Coronavirus enteritis

Coronaviruses induce are large enveloped viruses from the family *Coronaviridae* 100 to 120 nm in diameter, with a single-stranded RNA. Coronaviruses are divided into 3 groups depending on their source, nucleotide sequence and serological groups (52, 53).

BCoV causes diarrhoea in calves between the 3rd and 21st day of life, affecting 11-81% of the entire herd and often occurs as a mixed infection with rotaviruses. About 60% of adult cows shed the virus during the winter months. Apart being enterotropic, the BCoV is also pneumotropic as it is replicated in enterocytes of the gastrointestinal tract and upper respiratory epithelium. In suckling calves, the disease could occur between the 1st and 3rd week of life with peak prevalence 7 to 10 days after birth (54).

In some instances, pneumo-enteritis syndrome is developed, which is clinically manifested with signs of diarrhoea and pneumonia. Clinical signs of BCoV are more pronounced than those of rotavirus infection due to the more severe mucohaemorrhagic enterocolitis with hyperaemia of the nasal plane and nasal septum – signs of respiratory infection (52).

According to other literature sources, the changes in gastrointestinal tract comprise hyperaemia of the mucosa of the abomasum, caecum, colon and catarrhal-haemorrhagic enterocolitis. Very severe gross changes are established in some foci of the disease, resembling the winder dysentery and mucosal disease in cattle. Also, a pseudomembranous inflammation with multiple haemorrhages on distal colonic mucosa and enlarged mesenteric lymph nodes were observed (52, 55).

Coronaviral enteritis is characterised with atrophy of the middle part of villi of both small and large intestine, with damage of cryptic epithelium, which is not present in rotaviral enteritis affecting the upper two-thirds of villi (49). Coronavirus enteritis an atrophy or stripping of the villi of the small intestine are seen, that is often accompanied with some adhesions between the neighboring villi. In conclusion, pathological changes of the alimentary tract induced by BCoV are more severe than those caused by GPA BRV (54).

Histological changes were more serious in cases of complications with rota- and coronaviruses, enteropathogenic *E. coli* and *Cryptosporidium parvum* (56, 57).

CONCLUSIONS

In conclusion, diarrhoea in neonatal and growing calves is a disease entity of various (bacterial, viral, parasitic) etiology and multifactorial nature. The main etiological agents are *Escherichia coli* K99 (F5), bovine viral diarrhoea - mucosal disease virus (BVDV), *Cryptosporidium parvum*, bovine rotaviruses (*GPA BRV*) and bovine coronavirus (BCoV). Coronaviral enteritis is characterised with atrophy of the middle part of villi of both small and large intestine, with damage of cryptic epithelium, which is not present in rotaviral enteritis affecting the upper two-thirds of villi (49). In conclusion, pathological changes of the alimentary tract induced by BCoV are more severe than those caused by GPA BRV (54) and atrophy or stripping of the villi of the small intestine are seen, that is often

accompanied with some adhesions between the neighboring villi. Necroses in the lymphocytes in the Peyer's patches and regional lymph nodes are also characteristic for coronavirus enteritis in contrast to rotavirus enteritis. The immune status of affected calves is primarily poor and rearing management – inadequate. Losses incurred by farmers at a global scale are huge, due to the high mortality of diseased and reduced weight gain of reconvalescent calves. The establishment of specific gross and histopathological lesions in affected tissues and organs is of utmost significance for the diagnosis and differential diagnosis of these problematic disease states. In all cases, they provide a starting point for elucidation of etiological diagnosis, which takes up more time.

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This work aims to focus on etiology, spread, pathological and histopathological changes in the most common gastroenteritis in newborn and adolescent calves.

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