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Case report

CLINICAL AND MORPHOLOGICAL INVESTIGATIONS IN A SPONTANEOUS *CRYPTOSPORIDIUM* ENTERITIS OUTBREAK IN CALVES

I. KALKANOV[1], I. DINEV[1], K. DIMITROV[1] & P. ILIEV[2]

[1]Department of General and Clinical Pathology, [2]Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

Summary

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The purpose of this report was to present the results of histopathological examination during the course of a natural *Cryptosporidium parvum* outbreak, as well as the morphology of cells infiltrating affected gastrointestinal tract areas in newborn calves. The study included 18 calves exhibiting a marked diarrhoeic syndrome, between 1 and 8 days of age. Sporulated *C. parvum* oocysts were demonstrated in faecal smears stained by the method of Henriksen. Coproantigens of *C. parvum* were detected by the rapid Rainbow calf scour 5 BIO K 306 test. Gross lesions were mainly present in the gastrointestinal tract, together with inflammation in regional mesenteric lymph nodes. Microscopic histopathological lesions consisted mainly in desquamative catarrh of intestinal mucosa and intestinal villous atrophy. The affected intestinal epithelium contained multiple *Cryptosporidium* spp. forms at different stage of the life cycle. The results from histopathological studies of the outbreak allowed confirming some main morphogenetic features of the disease caused by *C. parvum* in newborn and growing calves.

Key words: calves*, Cryptosporidium parvum,* cryptosporidiosis, pathology

Gastrointestinal diseases in newborn and juvenile calves are the commonest problems in this livestock category. The losses are due to high mortality and morbidity rates, reduced weight gain after remission from the disease and disease treatment costs. Neonatal diarrhoea in calves is a multifactorial disease influenced by the breed, feeding regimen and farming conditions. The immunological status of the dams from the herd, farm management and the occurrence of different infectious agents are also important (Bendali *et al*., 1999; Scott *et al*., 2004). The risk for occurrence of the disease is the highest during the first month, then decreases as age

advances (Frank & Kaneene, 1993; Bendali *et al*., 1999). At a global scale, neonatal diarrhoea is associated with five primary enteropathogens: *Cryptosporidium parvum*, rotaviruses, bovine coronavirus (BCV), enterotoxigaenic *Escherichia coli* (*E. coli* K99) (Garcia *et al*., 2000), and *Salmonella,* all causing diarrhoea. Outbreaks at farms are often observed, especially when the animals originate from different farms with unknown immunological status (Naylor, 2002).

Cryptosporidium spp. (phylum Apicomplexa*,* family Cryptosporidiidae) are coccidian protozoa affecting the gastrointestinal tract in several mammalian and amphibian species and only the lungs in birds (Helmy *et al*., 2013). Some of the members of this family possess also well expressed zoo-anthropogenic potential (Castro-Hermida *et al*., 2002). The possibility for subclinical course and parasite shedding explain the broad geographic spread of cryptosporidiosis. According to Joachim *et al*. (2003), the prevalence of *Cryptosporidium* spp. in calves in European countries varies between 20% and 40%. Together with some bacterial and viral pathogens, *Cryptosporidium parvum* is considered as one of the most frequent agents of neonatal gastroenterites in calves up to 3 months of age (Chartier *et al*., 2013). The relative death rate is low, but morbidity within the general population in the contact environment is high (Castro-Hermida *et al*., 2002). The most important clinical sign reported by Joachim *et al*. (2003) is profuse diarrhoea. Its duration and intensity correspond to the localisation of the infection and the extent of morphological aberrations of the intestinal mucosa. The histological findings of colonised distal small intestine compartments consist in villous atrophy, metaplasia and desquamation of the surface epithelium (Fayer & Xiao, 2007). The authors explained that similar lesions could be also observed in the duodenum, caecum and the colon. In their view, the cell infiltration in *lamina propria* consisted mainly of neutrophil leukocytes.

The purpose of this report was to establish the histopathological alterations during the course of a natural *C. parvum* outbreak, as well as the morphology of cells infiltrating affected gastrointestinal tract areas in newborn calves.

The study was conducted on 18 calves with clearly manifested diarrhoeic syndrome aged between 1 and 8 days.

Case history. A sudden increase in mortality rates in calves from the 24th h of life to 6–8 days of age was reported at a cattle farm housing 200 animals from the Limousin and Hereford breeds, grazing freely on pasture. Clinically, bloody diarrhoea, rapid dehydration and death, without fever, were observed. The palliative general fluid therapy and parenteral treatment with macrolide antibiotics were of no use. At that time, no preventive vaccination in cows have been performed during the dry period.

Coprological samples were obtained manually from *ampula recti* of each animal and stored at 4 ^{o}C until the analysis. Sporulated oocysts were detected on faecal smears stained by the method of Henriksen (Emmonya Biotech, Chelopech) – modified Ziehl-Neelsen staining for acid-fast microorganisms. The presence of *C. parvum* coproantigens was confirmed through Rainbow calf scour 5 BIO K 306 (BIOX Diagnostics) rapid test, designed for detection of rotavirus*,* coronavirus*, E. coli – F5, Cryptosporidium parvum* and *Clostridium perfringens* type A.

Gross and histological examinations. Eight carcasses were submitted to routine necropsy using the standard protocol. Tis-

sue samples (size 2.5 cm) were collected from the affected gastrointestinal tract areas (abomasum, duodenum, jejunum with mesenteric lymph nodes, ileum, caecum, colon, rectum) for histopathological examination. Samples (2.5×2.5×1 cm) were also obtained from parenchymal organs – liver, lungs, kidneys, spleen and heart. Specimens for histological examination were fixed in 10% neutral buffered formalin and embedded in paraffin. From paraffin blocks, 4 μm cross sections were cut on a Leica RM 2235 microtome and conventionally stained with haematoxylin-eosin.

The external examination of calf carcasses revealed a strong dehydration and staining of the perianal areas with diarrhoeic faeces. Gross lesions were observed predominantly in the gastrointestinal tract, together with reactive regional mesenteric lymph nodes. They were enlarged 1–2 times, hyperaemic, and haemorrhages were present in some of them. Along the entire intestinal wall, multiple subserous petechial haemorrhages could be perceived and at some areas, single haematomas were seen on the mesentery (Fig. 1). The content of all small intestinal compartments and the caecum was watery, with yellow-greenish colour, mixed with gas bubbles and milk coagula (Fig. 2). The intestinal mucosa was oedematous, hyperaemic, spattered with haemorrhages, and in some loci, especially in the caecum, erosive lesions were present. There were no macroscopic alterations in the other studied visceral organs.

Faecal smears from tested calves stained by Henriksen exhibited sporulated *Cryptosporidium spp*. oocysts (Fig. 3). The presence of the etiological agent was confirmed by the rapid Rainbow calf scour 5 BIO K 306 test.

Microscopic histopathological lesions consisted mainly in desquamative catarrh of the intestinal mucosa and intestinal villous atrophy. The villi were with rounded edges, and at some places, were fused. An usual finding was the local or diffuse infiltration of the mucosa and submucosa of some areas of small intestine and the caecum with neutrophil leukocytes. The affected intestinal epithelium contained a lot of developmental forms of *Cryptosporidium spp*. (Fig. 4).

The performed histopathological investigations on *Cryptosporidium* enteritis in calves demonstrated the presence of

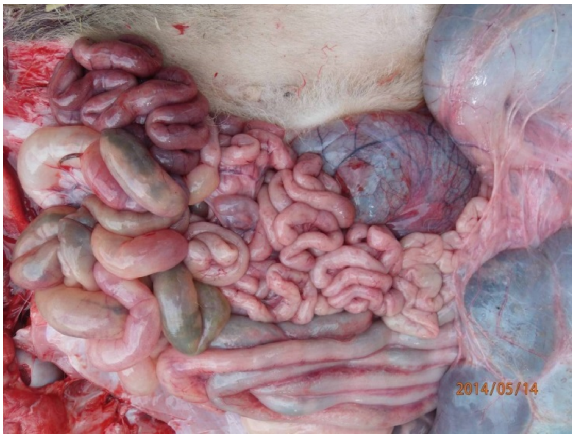

Fig. 1. Catarrhal haemorrhagic enterocolitis – a typical gross finding.

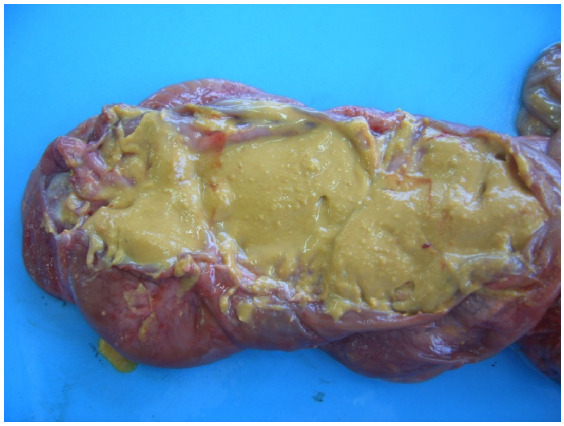

Fig. 2. Specific yellow-greenish content mixed with milk coagula, oedema and hyperaemia of caecal mucosa.

Cryptosporidium spp. in 100% of animals along the entire intestinal tract. The observed intensive loss of intestinal villi, especially in the distal small intestinal compartment, is associated exactly with established extensive *Cryptosporidium* in fection (Rosales *et al*., 1998). At the same time, we believe that the observed signs of regeneration and repair, mainly under the form of crypt hyperplasia in about one quarter of cases (5 calves) are an attempt for restoration of mucosal cell barrier, although at an insufficient extent. This fact, as well as the reported high-degree atrophy of intestinal villi predispose to secondary complications, facilitating the colonisation of other pathogens (Argenzio *et al*., 1990). The hypothesis is confirmed by the results from a number of animal experimentations (Tzipori *et al*., 1982; Heine *et al.,* 1984; Argenzio *et al*., 1990; Moore *et al*., 1995; Gookin *et al*., 2002).

The intensive loss of enterocytes is attributed to the pathogenetic mechanism of *Cryptosporidium* infection, namely formation of parasitic vacuole after penetration of the agent into the cytoplasm (de Graaf *et al*., 1990; O'Handley & Olson, 2006).

Cryptosporidium infection and neutrophilic infiltration in described case report was established not only in the distal small intestinal compartment, but also in *lamina propria* of the proximal colon. We agree with the thesis of some researchers that these lesions are at the background of bloody diarrhoea (Jewis *et al*., 1966) and that in cases when only small intestine is affected and the colon is intact, faeces were free of blood (Jewis *et al*., 1966; Kovatch & White, 1972).

The observed outbreak of neonatal calf diarrhoea associated with *Cryptosporidium* spp. confirms that the pathogen is responsible for a considerable share of gastrointestinal tract diseases in newborn and juvenile calves. We also affirm that the susceptibility to this infection is the highest until the 3rd week of life. Our studies gave us reason to acknowledge, similarly to other research teams, that at this age, the etiology of enterites in calves could be complicated with other agents, mainly of bacterial or viral origin (Chartier *et al*., 2013). The histological results allowed confirming some main morphogenetic features of *Cryptosporidium par-*

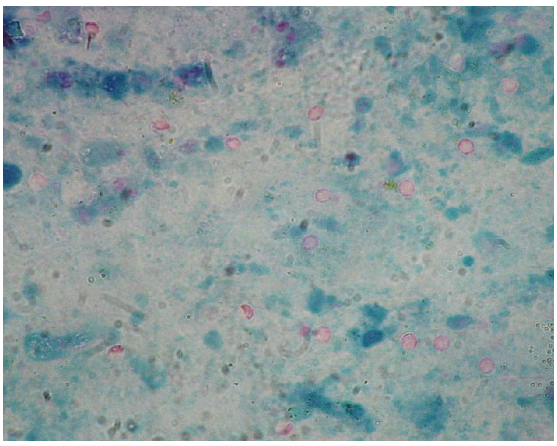

Fig. 3. *Cryptosporidium* spp. oocysts, faecal smear, Ziehl-Neelsen, ×1000.

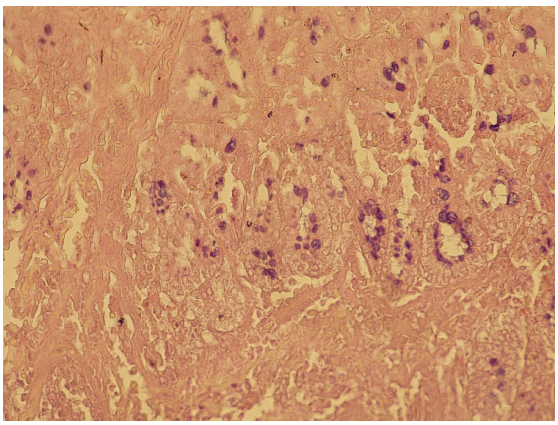

Fig. 4. Multiple developmental forms of *Cryptosporidium* spp. in mucous ileal crypts, H&E, ×300.

vum attachment to the surface of enterocytes in the distal small intestine and proximal colon (Gay *et al*., 2012). We also believe that poor hygienic conditions and production system deficiencies, as well as the insufficient amount of colostrum could be factors for appearance of the disease (Castro-Hermida *et al*., 2002).

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Correspondence:

Ismet Kalkanov, DVM
Department of General and Clinical Pathology
Faculty of Veterinary Medicine,
6000 Stara Zagora, Bulgaria,
e-mail: ismet_88@abv.bg