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PATHOMORPHOLOGICAL AND ETIOLOGICAL STUDIES IN A CLINICAL CASE OF NECROHEMORRHAGIC ENTERITIS IN BUFFALOES

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ABSTRACT

Clostridia are obligate anaerobic, spore–forming Gram–positive bacteria. *Clostridium perfringens* type A has been implicated as the most common etiologic agent causing enteritis, abomasitis, and enterotoxemia in large ruminants. Necrohemorrhagic enteritis in large ruminants caused by *C. perfringens* type A is characterized by sudden death, sometimes without clinical manifestation. Clinical signs occur with initial depression, dyspnea, pyrexia, bloody diarrhea, anemia or jaundice of mucous membranes, abdominal pain, and hemoglobinuria. The present report describes a clinical case of necrohemorrhagic enteritis in young buffaloes, which occurred with 30% morbidity and 100% mortality in a private buffalo farm in Northern Bulgaria. Pathological, antigenic, histopathological and microbiological studies were performed to diagnose the disease. The described macro– and microlesional changes in the affected organs and systems could be used in the diagnosis and differential diagnosis of gastrointestinal diseases in large ruminants.

Key words: histopathology, patoanatomy, necrohemorrhagic enteritis, buffalo.

Introduction

Clostridia are obligate anaerobic, spore–forming Gram–positive bacteria. Genus Clostridium consists of dozens of strains characterized by different pathogenicity, many of which are able to cause disease in humans and animals (Compiani *et al.,* 2021). Pathogenicity is expressed through their replication under favorable environmental conditions, and their sporulation is carried out through the release of toxins. Clostridial exotoxins are biologically active proteins that are antigenic in nature. The exotoxins and extracellular enzymes produced by for each clostridial strain have characteristic pathological changes of tissues and organs. (Hatheway, 1990). Based on toxin production, *Clostridium perfringens* isolates are divided into five toxitypes (A, B, C, D, and E), and based on the presence of toxin–encoding genes: alpha, beta, epsilon, and iota toxin. In addition to the specified toxins, *C. perfringens* strains can also produce additional toxins, such as B–like toxin (NetB) (Keyburn *et al.,* 2008).

Based on their toxic activity, clostridial species are classified into three groups: enterotoxic, histotoxic, and neurotoxic, acting on target organs and systems (Rood *et al.,* 1997). *C. perfringens* type A has been implicated as the most common etiologic agent causing enteritis, abomasitis, and enterotoxemia in large ruminants (Roeder *et al.,* 1988).

Although Clostridia are more common in young ruminants, *C. perfringens* type A has been associated with hemorrhagic enteritis in older cattle and sheep and hemolytic enterotoxemia in goats (Constable *et al.,* 2017).

Necrohemorrhagic enteritis in large ruminants caused by *C. perfringens* type A is characterized by sudden death, sometimes without a clinic. Clinical signs occur with initial depression, dyspnea, pyrexia, bloody diarrhea, anemia or jaundice of mucous membranes, abdominal pain, and hemoglobinuria. The disease is often fatal and animals are likely to die within 12 hours of the onset of clinical signs. C. perfringens type A also causes jejunal haemorrhagic syndrome in dairy cows (Elhanafy *et al.*, 2013).

Pathologicanatomical changes are shown with hemorrhagic to necrotic lesions, often occurring together as necrohemorrhagic lesions affecting the thin sections of the gastrointestinal tract. The disease usually affects calves and young buffaloes in good condition receiving large amounts of milk or milk replacers, the infection is characterized by sudden death without previous signs of gastrointestinal disease. Morbidity is usually around 30%, in contrast to mortality that reaches up to 100%, which is associated with large economic losses for farmers (Lebrun *et al.*, 2010).

Case history

The present report describes a clinical case of necrohemorrhagic enteritis in foals, which occurred with 30% morbidity and 100% mortality in a private buffalo farm in Northern Bulgaria, diagnosed on the basis of pathoanatomical, antigenic, histopathological and microbiological studies.

It concerns a herd of buffaloes of the Bulgarian Murra breed, numbering 230 animals, newborns and growing buffaloes (60) and (170) adult buffaloes. As the growing buffaloes were aged from the 24th hour after birth to the 25th day. Within 96 hours, 22 baby buffaloes fell ill and died. Signs of lethargy were observed, stools were brown and blood–tinged, and internal body temperature was subnormal. After several hours of illness, the animals died. In some animals, death occurred suddenly without clinical signs. Attempts were made to treat with parenteral antibiotic therapy accompanied by intravenous infusions, but without success. The growing animals in the buffalo farm were not vaccinated preventively against infectious diseases. They were fed mainly with milk substitutes. Two carcasses of young buffaloes were provided for necropsy and subsequent diagnostic studies by the farmer.

Contents from the small and large intestines were used for rapid antigen diagnostics of enteropathogens (5 valent antigen tests, *Rainbow calf scour 5 BIO K 306 Detection of Rotavirus, Coronavirus, E. coli F5, Cryptosporidium parvum and Clostridium perfringens* type A (*BIOX Diagnostics, Belgium*). After that, tissue samples for histopathological examination measuring 1 cm x 1 cm were obtained from the heart, kidneys, liver, spleen, as well as from the affected parts of the proximal and distal parts of the gastrointestinal tract: abomasum, duodenum, jejunum with mesenteric lymph nodes, ileum, cecum, colon and rectum – 2.5 cm long. Materials for histopathological examination were fixed in 10% neutral buffered formalin for 48–72 h and embedded in paraffin. Sections with a thickness of 4 μm were prepared from the obtained paraffin blocks using a *"Leica" RM 2235* microtome and stained conventionally with hematoxylin–eosin (H/E).

Samples from the parenchymal organs (lung, liver, spleen and kidney), blood from the heart and a ligated section of the small intestine were used for microbiological studies. Cultures were prepared on blood agar with 5% sheep erythrocytes, McConkey agar, trypticase–soy broth, selenite broth. All these cultures were incubated at 37°C for 24 hours under aerobic conditions. For the isolation of anaerobic microorganisms, Zeisler's blood sugar agar and thioglycolate medium (fluid thioglycolate medium; Difco Laboratories) were used, and cultures were cultivated for 24–48 hours

at 37°C and anaerobic conditions. Anaerobic conditions for the semi–solid medium were achieved by pouring sterile liquid paraffin into the tubes, and for solid mediums by AnaeroPack with the addition of a palladium catalyst. From the thioglycolate medium, after 24 incubation, smears and cultures were prepared on Zeisler's blood sugar agar for 24–48 hours at 37°C and anaerobic conditions. Identification of isolates was performed by routine microbiological methods.

Results and discussion

From the pathoanatomical examination, the following macroscopic changes were found – the carcass was highly swollen, the mucous membranes of the mouth and nasal cavity were pale. The conjunctivae were anemic. The perianal area and base of the tail with hemorrhagic faecal contents. On skinning the carcass, the subcutaneous connective tissue was dotted with petechial hemorrhages. There were also such on the superficially lying cadaveric lymph nodes. When the abdominal cavity was opened, ballooning of the gastrointestinal tract was found. The intestines had a blue–violet color and were dotted with punctate hemorrhages on the serosa (Fig. 1.). On section, the intestinal contents were dark red in color and mixed with gas bubbles, and the mucosa was edematous, hemorrhagic imbibed and dotted with hemorrhages, especially in the distal small intestine and colon (Fig. 2). The macroscopic lesions in the gastrointestinal tract analyzed in report largely coincide with the macrolesions described in calves with Clostridium perfringens type A infection, by authors such as (Valgaeren *et al.,* 2013). Similar to our intestinal macro–lesions were also described by Manteca *et al.,* (2000) in dairy cows with hemorrhagic–jejunal syndrome. In addition, petechial hemorrhages were observed under the capsule of the spleen and liver, which was brownish–yellow in color and crumbly in appearance, and the kidneys were mushy and flaccid in consistency and pale in color (Fig. 3). The abomasum was catarrhally hemorrhagically inflamed. A section of the pericardium showed the presence of about 300 ml. pink fluid, and the epicardium was dotted with numerous hemorrhages along the coronary margin. Haemorrhages were also observed on the surface of the endocardium. The pathological changes in the affected organs and systems described correspond to those described by Petrov *et al.,* (2014) in *Clostridium perfringens* type A infection in bison.

Figure 1: Flatulence, blue–violet color and hemorrhages on the serosa of the small intestine. A buffalo with necrohemorrhagic enteritis.

Figure 2: Bloody intestinal contents mixed with gas bubbles, and the mucosa is edematized and hemorrhagic imbibed. A buffalo with necrohemorrhagic enteritis.

Figure 3: Kidneys with a pale color and a soft flaccid consistency (degeneration). A buffalo with necrohemorrhagic enteritis.

From the antigen tests performed, a positive result was obtained for the presence of C. perfringens type A in intestinal contents from the small intestine (Fig. 4).

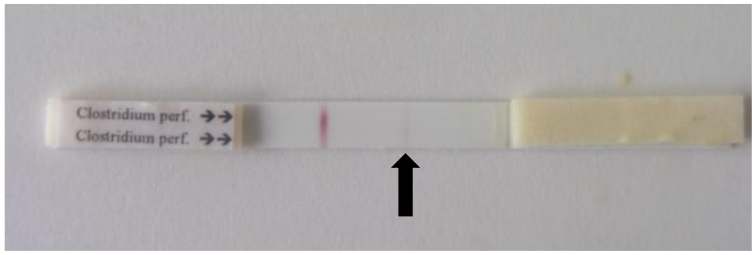

Figure 4: Positive result (arrow) for the presence of *Clostridium Perfringens* type A in intestinal contents from the small intestine.

The microlesional changes registered by us in the individual segments of the gastrointestinal tract were expressed in desquamation with dystrophic–necrobiotic changes of the epithelial cells covering the intestinal villi, without affecting the intestinal crypts. Submucosal edema, hyperemia of the vessels was observed (Fig. 5), and extensive hemorrhages and mononuclear proliferations, as well as more prominent lesional changes, were observed on individual segments of the small intestine. In the liver, a strong granular and fatty degeneration of the hepatocytes was found, reaching in places to necrosis of the liver parenchyma. Multiple hemorrhages were also visualized. Epithelial cells covering the renal tubules had desquamation, disintegration and highly pronounced processes of necrobiosis. Hyaline cylinders were found in the lumen of single renal tubules (Fig. 6). Strong hyperemia and hemorrhages were found in the spleen. Strong hyperemia of blood vessels was observed in the myocardium and numerous extensive hemorrhages, and some of the muscle fibers showed hyalinization with fragmentation, cloudy swelling, and granular dystrophy. According to us and Petrov *et al.*, (2014) the described macro– and microscopic changes in the affected organs and systems are due to the three types of toxins produced by C. perfringens type A. Alpha toxin having hemolytic and necrotizing properties, beta toxin causing intestinal inflammation and mucosal damage and enterotoxin acting on the intestinal mucosa, mainly in the jejunum and ileum and causing severe diarrhea.

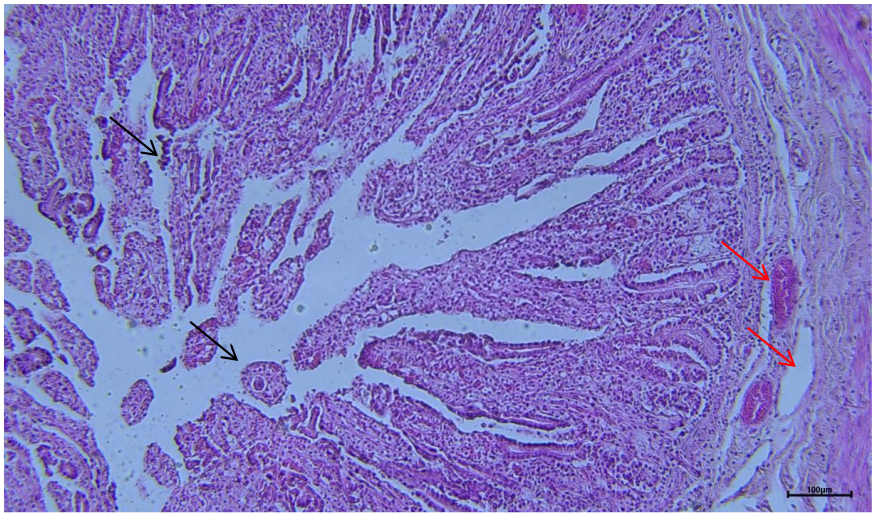

Figure 5: Desquamation and degenerative–necrobiotic processes of the epithelial cells covering the intestinal villi (black arrow), submucosal edema with hyperemia of the vessels, mononuclear proliferates in the submucosa (red arrow), H/E, bar=10 µm.

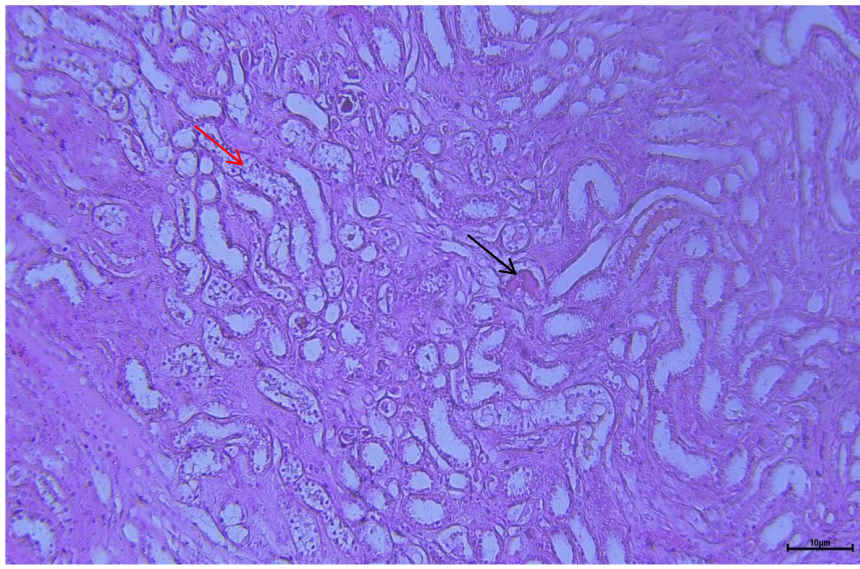

Figure 6: Desquamation, disintegration and necrobiosis of the epithelial cells covering the renal tubules (red arrow), hyaline cylinders in the lumen of the renal tubules (black arrow), kidney, H/E, bar=10 µm.

Based on the obtained results, we share the opinion of authors such as Manteca *et al.*, (2001) and Pardon *et al.*, (2012) that clostridia and in particular C. perfringens type A cause lethal gastrointestinal infections in large ruminants of any age, with the most pronounced micro– and macroscopic changes in the thin part of the gastrointestinal tract. According to Pardon *et al.*, (2012) they are most common in calves up to 4 months of age, as well as in highly productive dairy cows.

The cultural study under aerobic conditions did not show the presence of pathogenic microorganisms in any of the examined parenchymal organs, the intestinal tract and in the blood from the heart.

At 24 hours of incubation in the semi–solid culture medium (thioglycolate medium), numerous gas bubbles were observed under the layer of liquid paraffin. Microscopic examination of the smears revealed microbial cells with the characteristics of Clostridium species.

Under anaerobic conditions, a pure culture of gray–white, hemolytic colonies 1–2 mm in diameter was established on Zeisler's blood sugar agar (Fig. 7 subculture of thioglycolate medium). Microscopy of the prepared smears showed clostridial microorganisms (Fig. 8).

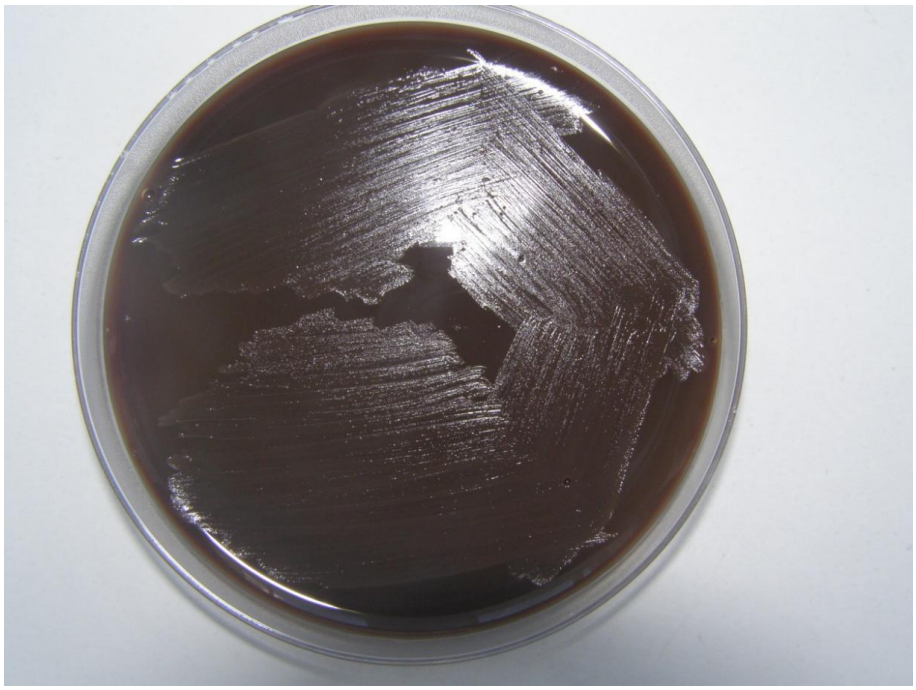

Figure 7: Zeisler's blood sugar agar, showing growth of gray–white, hemolytic colonies.

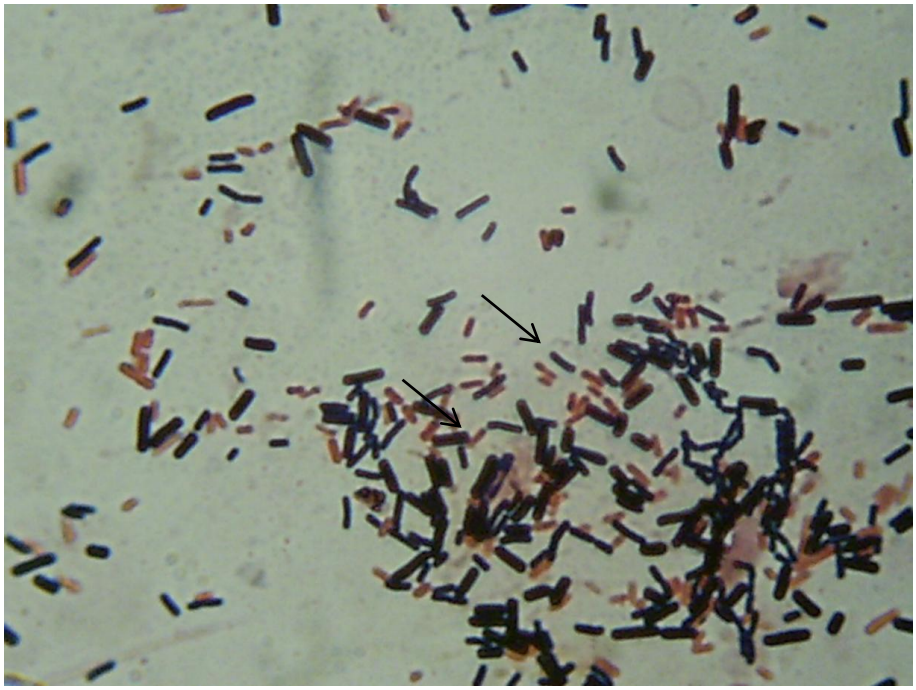

Figure 8: Identification of bacterial growth, multiple Gram–stained clostridial organisms (arrows).

The results obtained by us from the performed antigenic and microbiological studies support the thesis of (Akiba *et al.*, 2000) and (Filho *et al.*, 2009) that in 90% of cases of necrohemorrhagic enteritis in buffaloes and cattle there is a correspondence between the clostridial isolates . This statement is also proved by the results of studies by (Petrov *et al.*, 2014) in bison. The analysis of the data from the anamnesis, the pathoanatomical, pathomorphological, antigenic and microbiological studies of the carcasses of the buffaloes submitted for autopsy proved *Clostridium Perfringens* type A as the etiological agent.

Conclusion

The pathoanatomical and histopathological changes in the organs and various intestinal parts found in the described clinical case could be beneficial for the pathomorphological diagnosis of this disease. On the other hand, they could be important in the differential diagnosis, both between this and other diseases, such as Eimeria (coccidiosis), salmonellosis colibacteriosis, cryptosporidiosis, coronavirus and rotavirus infection in newborn and adolescent large ruminants.

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