First report of bacillary hemoglobinuria in buffaloes

Primeiro relato de hemoglobinúria bacilar em búfalos

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ABSTRACT

We herein describe a typical episode of bacillary hemoglobinuria in buffalloes, triggered by liver fluke invasion proved by pathognomonic macro and micro lesions of fasciolosis in the biliary ducts

and the nephrosis and hepatic focal necrosis characterizing the toxic-infectious process caused by *Clostridium haemolyticum*. This report is quite similar to another one described by AHOURAI et al. (1990) in cattle following liver fluke invasion and *C. haemolyticum* isolation and we both agree with the fact that foci of ischemic of toxic necrosis serve as a focus in which clostridial spores might vegetate and cause the disease by producing toxins. Vaccination with bacterin or toxoids is the most effective measure for preventing these histotoxic clostridial diseases since the endospores of the microorganism persist in the environment. Also, as the process is triggered by fluke liver migration, the prevention should cover the control of this trematode as well. Although the pathogenicity of bacillary hemoglobinuria is well known, there are only a few reports currently available, and to our knowledge this is the first case of the disease described in buffaloes.

Keywords: bacillary hemoglobinuria; *Clostriduim haemolyticum*; fasciolosis; buffaloes

RESUMO

Descrevemos um episódio típico de hemoglobinúria bacilar em búfalos, provocado por infestação por fasciola determinada por macro e microlesões patognomônicas de fasciolose nos ductos biliares e nefrose, e necrose focal hepática caracterizando o processo tóxico-infeccioso causado pelo *Clostridium haemolyticum*. Este relato é similar a outro descrito por AHOURAI et al. (1990) em bovinos seguindo uma invasão hepática por fasciolas e o isolamento de *C. haemolyticum* e ambos concordamos com o fato de que o foco da isquemia da necrose tóxica serviu como um local onde os esporos dos clostrídeos vegetaram e causaram a doença produzindo suas toxinas.

A vacinação com bacterinas ou toxóides é a medida mais efetiva para prevenção dessas doenças histotóxicas causadas por clostrídeos já que os endosporos do microrganismo persistem no ambiente. Como o processo é provocado pela migração hepática, a medida de prevenção deve cobrir o controle deste trematódeo também. Embora a patogenicidade da hemoglobinúria bacilar seja bem conhecida, há poucos relatos disponíveis, e este parece ser o primeiro caso da doença descrito em búfalos.

Palavras-chave: hemoglobinúria bacilar, *Clostridium haemolyticum*, fasciolose, búfalos

1 CASE REPORT

Clostridium spp. is a gender that consists in spore-former gram-positive rods spread in the soil, fresh water and marine sediments with low redox potential. Some species belong to the normal intestinal microbiota, but others can be isolated as endospores in the muscle or liver and, when activated, can germinate and induce the disease. Bacillary hemoglobinuria caused by *Clostridium haemolyticum* affects primarily cattle, and sometimes, sheep. In this endogenous infection, clostridial endospores are quiescent in the liver, and the migration of liver fluke is the triggering factor for the germination of spores and clostridial multiplication. Beta toxin, a lecithinase produced and released by vegetative cells is the responsible for intravascular hemolysis with hepatic necrosis. Due to the widespread destruction of erythrocytes, the main clinical feature observed is hemoglobinuria (QUINN et al., 2005; BALDASSI, 2005).

The present report describes the occurrence of bacillary hemoglobinuria outbreak in bufalloes from Biritiba Mirim – São Paulo State-Brazil . In the herd of 80 animals, there was no

death occurrence during four years, but three months following a flooding episode, 13 healthy animals of different ages died suddenly after presenting hemoglobinuria.

At the necropsy of two carcasses, it was observed the high infestation of *Fasciola hepatica* in the pale liver of both animals. This evidence allied to the presence of hemoglobinuria followed by sudden death, led us to suspect of bacillary hemoglobinuria.

Samples of heart, liver and kidneys were collected *in natura* and fixed into 10% buffered formalin and subjected to Instituto Biológico for histopathological, microbiological culture and polymerase chain reaction (PCR) procedures.

Fragments of organs were processed for paraffin inclusion. The histological sections (5µm thick) were stained with hematoxylin-eosin and subjected to microscopic examination. The liver samples presented thickened capsule with congested blood vessels and dilated lymphatic vessels and mononuclear cell infiltration; congested parenchyma with multifocal areas of necrosis, associated to mononuclear cells infiltrate, focal hemorrhage, vacuolar degeneration of hepatocytes; portal space with fibrosis, mononuclear cells infiltrate, replication and hyperplasia of hepatic ducts. The kidney analysis revealed eosinophilic contents (proteinuria) in the lumen of renal tubules and glomerular area of Bowman capsule; necrosis of proximal and distal convoluted tubules and congested vessels with lymphocytic marginalization. In the heart tissue a discrete mononuclear cell infiltrate and fibrin deposit among the myocardial fibers were observed.

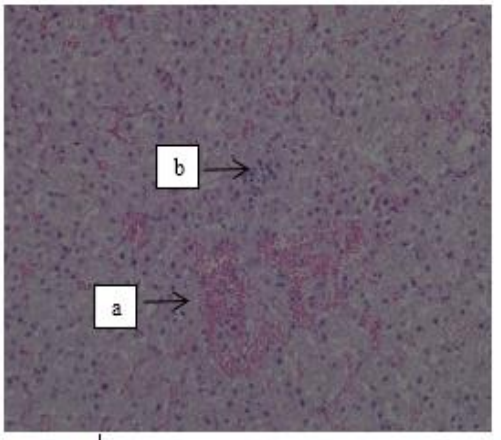


Figure 1: (a) focal hemorrhage of the parenchyma, (b) focal necrosis associated to mononuclear cells, vacuolar degeneration of hepatocytes

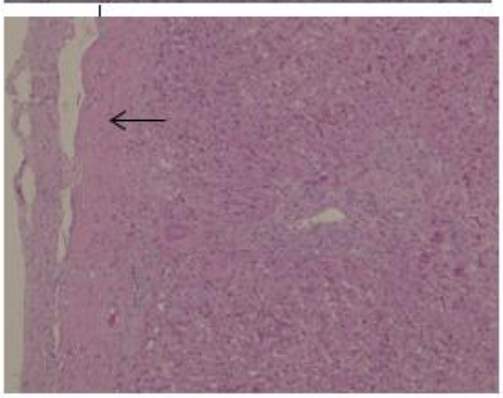


Figure 2: Hepatic capsule thickening with congested blood vessels and dilated lymphatic vessels and mononuclear cell infiltration.

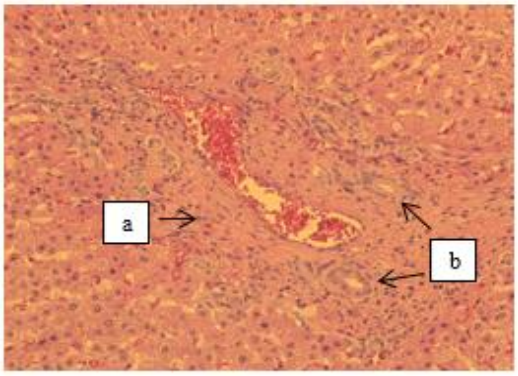


Figure 3: (a) Portal space with fibrosis, mononuclear cells infiltrate, (b) proliferation and hyperplasia of hepatic ducts

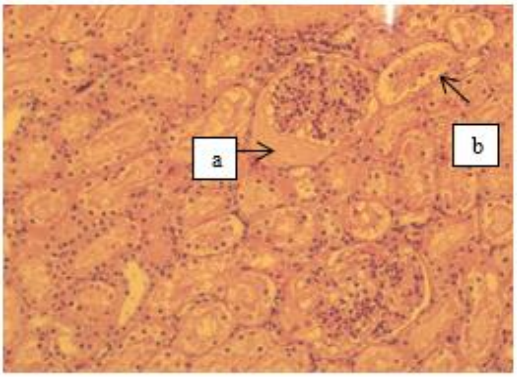


Figure 4: (a) Eosinofilic contents (proteinuria) in the lumen of renal tubules and glomerular area of Bowman capsule; (b) necrosis of proximal and distal convoluted tubules (nephrosis).

Microbiological culture was performed as prior described by Baldassi (1988). Macerated tissues were inoculated into tubes with cooked meat medium (Difco®) and maintained at 37ºC for 48h, then supernatant was plated on 5% defibrinated sheep blood agar at 37ºC for 48h under anaerobic conditions. The microbiological culture from the organs did not show strictly anaerobic isolated colonies, however, the PCR analysis of the extracted DNA from the tissues and with primers described by SASAKI et al. (2002) for detection of the *fliC* gene of *Clostridium haemolyticum, C. chauvoei, C. novyi* type A, *C. novyi* type B and *C. septicum* showed a specific band of 694 bp indicating the amplification of the *fliC* gene of *Clostridium haemolyticum*. The reaction condition was the same as described by the authors and was carried out in a PTC100 MJ Research.

The *fliC* gene codes the flagellar filament that is a part of the bacterial flagellum structure and it consists of repeating subunits of the flagellin protein (SASAKI et al., 2002). *C. haemolyticum* and *C. novyi* B are very similar in their biological characteristics, and PCR has been shown to be faster and more specific for species identification (SASAKI et al., 2001; SMITH; WILLIAMS, 1984).

2 DISCUSSION

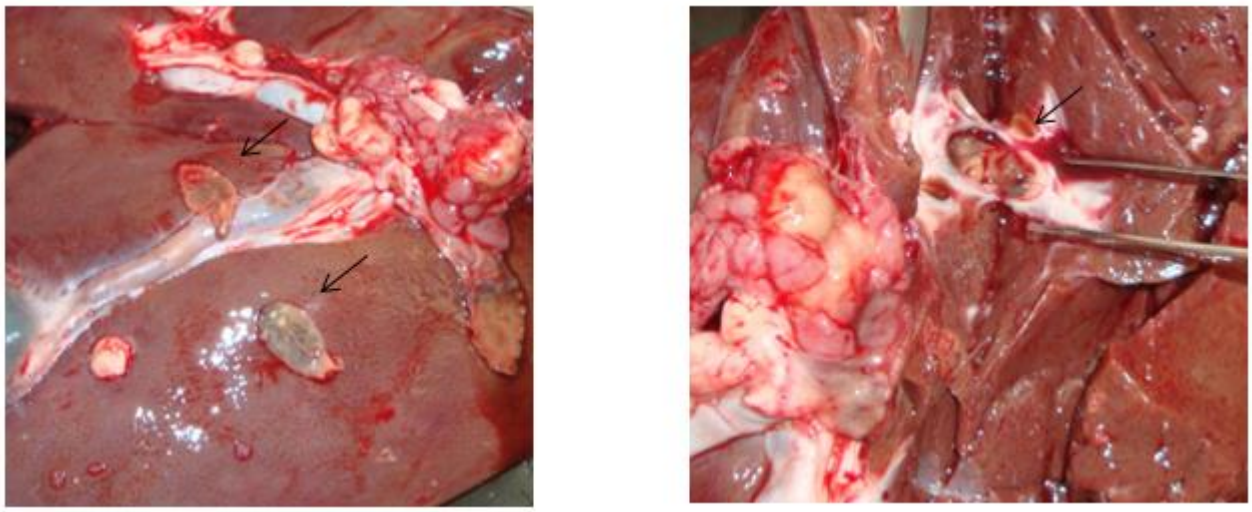
We herein described a typical episode of bacillary hemoglobinuria in buffalloes, triggered by liver fluke invasion proved by pathognomonic macro and micro lesions of fasciolosis in the biliary ducts and the nephrosis and hepatic focal necrosis associated to hepatic hemorrhage

characterize the toxic-infectious process caused by *C. haemolyticum* (CULLEN, 2007). This report is quite similar to another one described by AHOURAI et al. (1990) in cattle following liver fluke invasion and *C. haemolyticum* isolation and we both agree with the fact that foci of ischemic of toxic necrosis serve as a focus in which clostridial spores might vegetate and cause the disease by producing toxins.

Vaccination with bacterin or toxoids is the most effective measure for preventing these histotoxic clostridial diseases since the endospores of the microorganism persist in the environment. Also, as the process is triggered by fluke liver migration, the prevention should cover the control of this trematode as well.

Although the pathogenicity of bacillary hemoglobinuria is well known, there are only a few reports currently available, and to our knowledge this is the first case of the disease described in buffaloes.

Figures 5 and 6: Visualization of trematodes in the hepatic surface and in the biliary ducts



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