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PATHOLOGY

Polioencephalomalacia in buffaloes (*Bubalus bubalis*) in a feedlot

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ABSTRACT: Polioencephalomalacia (PEM) is a nervous disease that affects ruminants, has a worldwide distribution and causes significant economic losses. PEM is abundantly described and studied in cattle and small ruminants, but there is little information about the disease in buffaloes, especially in Brazil. The objective of this work is to describe the clinical and pathological aspects of an outbreak of PEM in buffaloes in a feedlot in the state of Pará, Brazil. The outbreak occurred in a flock of 76 Murrah buffaloes, with an average age of five months, in a feedlot. Twenty-eight of these buffaloes became ill, and thirteen showed neurological signs and died. Two buffaloes underwent necropsy. The brain and fragments of various abdominal and thoracic cavity organs were fixed in 10% formalin and sent for histopathological examination. In the frontal, parietal, and occipital telencephalic lobes, the convolutions were flattened and yellowish, and when cut, there was a clear line of demarcation between the superficial and deep layers. Histologically, the intermediate layers of the cerebral cortex showed mild to moderate vacuolization of the neuropil, moderate enlargement of the perivascular, perineuronal, and periastrocyte spaces, moderate swelling and edema of astrocytes, and a large number of necrotic neurons. The work demonstrates that, despite being poorly documented in Brazil, PEM should be considered in the differential diagnosis of neurological syndromes in buffaloes.
Key words: buffaloes, pathology, cerebrocortical necrosis, laminar necrosis, thiamine.

Polioencefalomalacia em búfalos (*Bubalus bubalis*) em confinamento

RESUMO: Polioencefalomalacia (PEM) é uma doença nervosa complexa que afeta ruminantes, tem distribuição mundial e causa perdas econômicas significativas. A PEM é abundantemente descrita e estudada em bovinos e pequenos ruminantes, porém há poucas informações sobre a doença em bubalinos, especialmente no Brasil. O objetivo deste trabalho é descrever os aspectos clínicos e patológicos de um surto de PEM em búfalos em um confinamento no estado do Pará, Brasil. O surto ocorreu em um lote de 76 búfalos da raça Murrah, com idade média de cinco meses, em um sistema de confinamento. Vinte e oito desses bubalinos adoeceram e treze apresentaram sinais neurológicos e morreram. Dois bubalinos foram submetidos à necropsia. O encéfalo e fragmentos de diversos órgãos da cavidade abdominal e torácica foram fixados em formol a 10% e enviados para exame histopatológico. Nos lobos frontal, parietal e occipital do telencéfalo as circunvoluções estavam achatadas e amareladas e ao corte havia uma clara linha de demarcação entre as camadas superficial e profunda. Na histologia as camadas intermediárias do córtex cerebral apresentavam vacuolização leve a moderada do neurópilo, aumento moderado dos espaços perivascular, perineuronal e periastrocitário, tumefação e edema moderados de astrócitos e grande número de neurônios necróticos. O trabalho demonstra que, apesar de pouco documentada no Brasil, a PEM deve ser considerada no diagnóstico diferencial das síndromes neurológicas em bubalinos.
Palavras-chave: bubalinos, patologia, necrose cérebro-cortical, necrose laminar, tiamina.

INTRODUCTION

Polioencephalomalacia (PEM) is a significant disease within the category of neurological disorders affecting ruminants, as It has a global distribution and leads to considerable economic losses (SANT'ANA et al., 2010). The causes of PEM can be classified as deficient, toxic, or infectious (SANT'ANA et al., 2010; MACHADO et al., 2017; ASSIS, 2021). Morphologically, the disease is characterized by necrosis, followed by the softening (malacia) of the brain's grey matter (polio). Other terms to describe this specific type of brain injury include cerebrocortical necrosis and cerebral cortical laminar necrosis. Initially, the term PEM was exclusively used when thiamine deficiency caused the injury. However, it is now used to describe this distinct morphological pattern of brain injury, regardless of its cause (SANT'ANA et al., 2010; DAL MAS et al., 2017; MILLER & ZACHARY, 2017; BUERGELT et al., 2018).

PEM is extensively researched and documented in cattle and small ruminants (SANT'ANA et al., 2010), but there is limited

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information on the disease in buffaloes (TANWAR et al., 1993; DWIVEDI et al., 2001; MOULI & BABU, 2004; GUIMARÃES et al., 2008; JANA & GOSH, 2009; MAHAJAN et al., 2011). In Brazil, there is a report of a PEM outbreak in buffaloes kept in open fields (GUIMARÃES et al., 2008).

The aim of this study is to describe the clinical, epidemiological, and pathological aspects of a PEM outbreak in buffaloes in the state of Pará, Brazil.

MATERIALS AND METHODS

The outbreak was observed in Murrah buffaloes at a feedlot in the municipality of Tomé-Açu, located in the northeastern mesoregion of the state of Pará. Clinical and epidemiological data were provided by the veterinarian overseeing the farm, who conducted necropsies on two animals (Buffalos 1 and 2). The brains of these animals were fixed in 10% formaldehyde; fragments were received from Buffalo 1, while the brain from Buffalo 2 was intact. Fragments of lung, liver, kidney, spleen, heart, and skeletal muscle were also collected and fixed in 10% formaldehyde. All fixed tissues underwent routine histopathological processing, were sliced at 5µm thickness, and were stained with hematoxylin and eosin (HE).

RESULTS AND DISCUSSION

The buffaloes that fell ill were part of a group of 76 Murrah calves, approximately 5 months old, consisting of 42 males and 34 females. On the farm, calves were separated from adult buffaloes on the second day after birth. Their diet consisted of milk, milk replacer, feed, and *Cynodon* sp. grass, provided in a trough. The quantity of each food item varied according to the calf's age, up until they reached 70 days old. At that point, the calves began receiving 1 kg of feed per day. Between the ages of 70 and 90 days, and weighing between 80 and 100 kg, the animals were transferred to another farm. They were kept in a feedlot and exclusively fed corn silage there.

Twenty days after they were transferred to a feedlot, 28 animals fell ill. Of these, 15 showed apathy, and 13 showed mild paresis of the pelvic limbs, blindness, difficulty in standing upright, and opisthotonus. These symptoms progressed to lateral recumbency, nystagmus, limb spasticity, and pedalling movements (Figure 1A), evolving over two to three days until death. After the first deaths, all symptomatic buffaloes were treated with a single 10ml dose of 0.2% dexamethasone, but showed no clinical response. Subsequently, the group was moved from the feedlot to pastures of *Cynodon* spp. and *Brachiaria humidicula*. Following the death of the 13 animals, a single 2ml dose of 10% thiamine (vit. B1) was administered as treatment, resulting in the recovery of the fifteen animals that had initially showed mild apathy. After 30 days, all the calves received a second dose of thiamine and were returned to the feedlot; no new disease cases were observed. The morbidity, mortality, and case fatality rates were 36.8%, 17.1%, and 46.4%, respectively.

In the brains of both buffaloes, it was observed that the convolutions were flattened and yellowish, with some areas appearing softened. In the brain of Buffalo 2, which was more intact, these lesions were found to specifically affect the dorsolateral portions of the cerebral hemispheres (Figure 1B). Upon sectioning, it was observed that the yellowing predominantly affected the superficial layers of the cerebral cortex, with a distinct line of demarcation separating the superficial and deep layers. These lesions were bilateral and relatively symmetrical and were observed in the frontal, parietal, temporal, and occipital lobes.

The primary histopathological changes were observed in the cerebral cortex (Buffalo 1 and 2). Moderate neuropil vacuolization was observed in the middle layer and mild vacuolization in the more superficial layers (Figure 1C). This was accompanied by a moderate enlargement of the perivascular, perineuronal, and periastrocytic spaces. Astrocytes in these areas showed moderate nuclear swelling, and it was occasionally possible to observe a small amount of eosinophilic cytoplasm around the nucleus. Neurons in these vacuolated regions were retracted, showing increased cytoplasmic eosinophilia, and pyknosis, karyorrhexis, and karyolysis. Swelling of the endothelial cells in the blood capillaries was also observed (Figure 1D). Other rare findings in the histopathological examination included neuronophagia and haemorrhages around leptomeningeal vessels and nervous parenchyma.

Other areas of the brain (rostral colliculi, thalamus, cerebellum, and pons), skeletal muscles, and kidneys showed no changes (Buffalo 1 and 2).

The diagnosis of PEM in this study was based on epidemiological, clinical, and pathological data. These data are similar to those described for the disease in buffaloes (TANWAR et al., 1993; MOULI & BABU, 2004; GUIMARÃES et al, 2008; JANA & GOSH, 2009; MAHAJAN et al., 2011) and in other ruminants (SANT'ANA et al., 2010; DAL MAS et al., 2017; MILLER & ZACHARY, 2017; BUERGELT et al., 2018; PAULA et al., 2018).

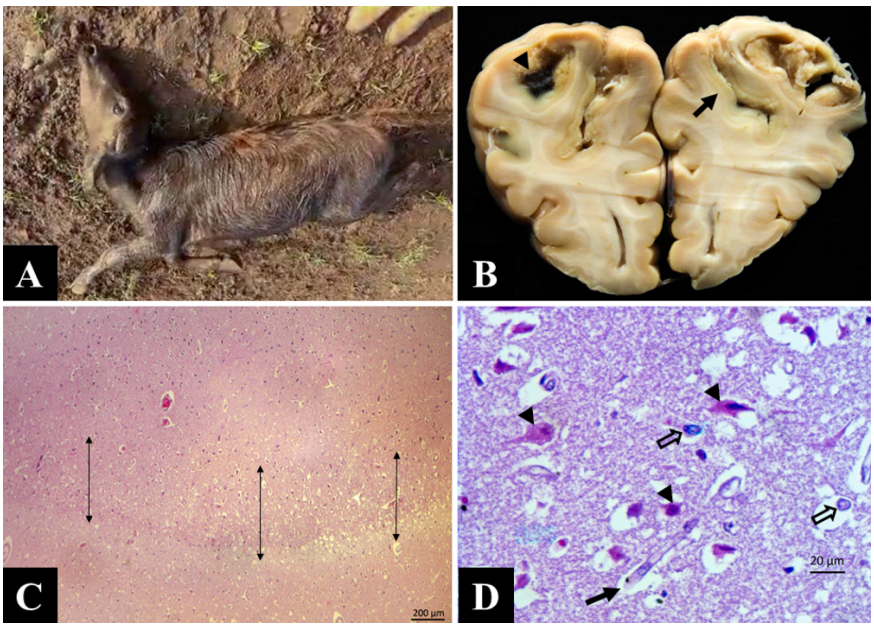

Figure 1 - Polioencephalomalacia. Buffalo 2. (A) Animal in lateral recumbency showing opisthotonus; (B) Cross-section of the fixed brain. In the frontal cortex region, there was a slight flattening of the convolutions and yellowish coloration of the grey matter, and hemorrhagic areas (arrowheads). Clear demarcation with increased yellowing intensity in the superficial areas of the cortex, with a demarcation line separating the outer layer from the inner layer of the grey matter (arrow); (C) Evident cerebral cortex edema indicated by moderate vacuolization of the neuropil in the intermediate layer and mild in the most superficial layer (at the top of the figure), corresponding with the demarcation line observed in the macroscopic view. HE, obj.10x; (D) Vacuolation of the neuropil and an enlargement of the perivascular, perineuronal, and periastrocytic spaces. Several necrotic neurons with increased cytoplasmic eosinophilia, pyknosis, and karyolysis (arrowheads). Swelling in the endothelial cells of blood capillaries (arrow). Astrocytes with nuclear swelling (open arrow). HE, obj.40x.

The rates of morbidity (36.8%) and mortality (17.1%), except for case fatality rate (46.4%), in the current PEM outbreak were higher than those reported in a previously described outbreak in buffaloes on pasture in Brazil, which had rates of 6.5%, 4.3%, and 66.7%, respectively (GUIMARÃES et al., 2008). The higher rates in the present study may be attributable to differences in food management across the two systems. The rates in the present study also exceed those typically observed in PEM outbreaks in cattle, which usually show morbidity and mortality rates ranging from 0.04% to 14%. However, the case fatality rate in the present outbreak is similar to that reported in cattle (SANT'ANA et al., 2010).

In cattle, the disease can occur in young animals kept in a feedlot as well as in adult animals grazing in pastures (SANT'ANA et al., 2010; CÂMARA et al., 2018). Cases of PEM have been reported in buffaloes between the ages of 6 months and 2.5 years (MOULI & BABU, 2004; GUIMARÃES et al., 2008). This is similar to the findings in the current outbreak, where the affected animals were around 5 months old. In the outbreak described by GUIMARÃES et al. (2008), the buffaloes were raised in extensive conditions and received concentrate supplementation. This differs from the current report, where the animals were kept in a feedlot, a condition also identified as significant for cases of PEM in cattle (SANT'ANA et al., 2010).

The clinical signs of PEM observed in the buffaloes in the present study were indicative of a cerebral syndrome, similar to that described in the literature on the disease (LORENZ et al., 2010; SANT'ANA et al., 2010). For example, the blindness reported in the buffaloes was associated with brain lesions located in the occipital cortex region (FEITOSA, 2020).

The macroscopic and microscopic lesions observed in the two buffaloes subjected to necropsy

in this study are consistent with those previously described in PEM in buffaloes (TANWAR et al., 1993; GUIMARÃES et al., 2008) and other ruminants (SANT'ANA et al., 2010). The lesions in the current case were considered acute and aligned with the short clinical progression time reported for buffaloes. Acute macroscopic lesions, similar to those observed in the buffaloes in this study, may be absent or challenging to detect (SANT'ANA et al., 2010). However, signs of cerebral edema were evident in the fixed brain tissue, such as flattening and yellowing of the convolutions. Additionally, a clear demarcation was observed between the affected superficial layers and the unaltered deep layers of the cerebral cortex, corresponding to areas of more intense spongiosis upon microscopic examination.

On histopathology, the lesions showed an evident laminar pattern. Spongiosis and neuronal necrosis were most intensely observed in the middle layers of the cerebral cortex, affecting the upper layers to a lesser degree and sparing the deeper areas, as reported in cattle and sheep (SANT'ANA et al., 2009; PAULA et al., 2018). In humans, this laminar pattern of cerebral cortical necrosis is attributed to the greater vulnerability of specific layers to ischemia. It is well-established that, in humans, of the six layers constituting the cerebral cortex, the third layer (external pyramidal) is the most vulnerable to hypoxia. The fifth (internal pyramidal) and sixth (fusiform) layers are slightly less susceptible, while the second (external granular) and fourth (internal granular) layers are the most resistant (HAN et al., 2011). Variations in the distribution of PEM lesions in the layers of the cerebral cortex across different reports may be related to the extent of metabolic impairment in the nervous tissue and, consequently, the clinical progression of the cases. A similar pattern was observed in an experiment involving lead poisoning in 3-month-old cattle. In the acute condition, damage was present in the second to fourth layers, while in the chronic condition, injury was found in the third to sixth layers of the cerebral cortex (WELLS et al., 1976).

Considering the affected group and the dietary changes that occurred, it is likely that the PEM that affected the buffaloes in this study was related to ruminal acidosis and subsequent thiamine deficiency. PEM is most commonly observed in young ruminants fed diets rich in carbohydrates, which leads to ruminal acidosis and alters the rumen microbiota. In adult animals with a well-balanced diet, thiamine is synthesized by rumen bacteria. This synthesis also occurs in monogastric animals and calves but not in sufficient quantities to eliminate the need for dietary supplementation. Therefore, thiamine deficiency states can be easily induced in these animals (JONES et al., 2000; RADOSTITIS et al., 2006). Healthy calves and sheep have significantly lower blood levels of thiamine compared to other animals, suggesting that these groups are more prone to thiamine deficiency (HORINO, 2001). In the present study, the likely cause of PEM associated with thiamine deficiency could have been confirmed by measuring thiamine levels in the blood or tissues of affected animals (GOULD, 1998). However, blood was not collected during the outbreak, and tissues obtained during necropsy were fixed in formaldehyde.

The presumptive diagnosis of PEM due to thiamine deficiency can be established by observing the recovery of affected animals following the administration of this vitamin (RIET-CORREA et al., 2007; RADOSTITIS et al., 2006). In the outbreak discussed here, buffaloes with mild clinical signs who received thiamine treatment recovered, and the other buffaloes in the same group treated with thiamine did not show clinical signs. A positive response to thiamine treatment has been reported in spontaneous cases of PEM in buffaloes (TANWAR et al., 1993; MOULI & BABU, 2004; GUIMARÃES et al., 2008; JANA & GOSH, 2009; MAHAJAN et al., 2011).

The primary differential diagnoses considered in this case were rabies, lead poisoning, and listeriosis. Rabies is the most critical disease to consider in the differential diagnosis and is believed to be underreported in buffaloes (BIANCHI et al., 2017). Histopathological studies on rabies in buffaloes have shown lesions largely similar to those in cattle, featuring non-suppurative encephalomyelitis that mainly affects the brain stem, cerebellum, and spinal cord, along with the presence of Negri bodies (JAMADAGNI et al., 2007; BIANCHI et al., 2017). These lesions are entirely different from those observed in the buffaloes in the present study. Lead poisoning has been described in buffaloes (DWIVEDI et al., 2001) as well as other ruminants (WELLS et al., 1976). However, no potential lead sources were found in the areas where the animals were kept. Additionally, in this outbreak, lesions in the basal ganglia and brain stem described in previous studies were not observed, nor were lesions in other organs such as the kidneys (WELLS et al., 1976). Listeriosis was also considered in the differential diagnosis due to the buffaloes' diet of corn silage, as this disease is often linked to this type of food, especially when of low quality (BUERGELT et al., 2018). PRADO et al. (2019) reported the encephalic form of listeriosis in

buffaloes, presenting with neurological symptoms that included ataxia, quadriplegia, reduced skin sensitivity, lateral recumbency, and death, progressing over the course of 24 to 48 hours. Other clinical signs such as circling, pressing the head against objects, facial nerve paralysis, nystagmus, blindness, tongue protrusion, and drooping ears can also be observed (JONES et al., 2000; BUERGELT et al., 2018). The typical lesion in the encephalic form of listeriosis consists of microabscesses in the brain stem, surrounded by multifocal perivascular cuffs containing neutrophils, histiocytes, and lymphocytes (PRADO et al., 2019). In this outbreak, neither the sender reported clinical signs related to the brain stem nor macro or microscopic lesions in the brain were observed, as mentioned above. In cattle, it is important to distinguish between PEM and bovine herpesvirus infection (BoHV). Both conditions show extensive areas of cortical necrosis, but BoHV is also associated with non-suppurative inflammation and may occasionally feature intranuclear inclusion bodies in neurons and glial cells (SANT'ANA et al., 2010). This disease was not included in the differential diagnosis for this case report, as there is no documented evidence of this disease in buffaloes.

CONCLUSION

The present study shows that, although poorly documented in Brazil, polioencephalomalacia (PEM) should be considered in the differential diagnosis of neurological syndromes in buffaloes. The PEM outbreak in the buffaloes in this study was attributed to introducing a carbohydrate-rich diet without proper adaptation.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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