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Bovine enzootic hematuria in the state of tocantins and use of ultrasonography as a diagnostic method

Hematúria enzoótica bovina no estado do Tocantins e o uso da ultrassonografia como método de diagnóstico

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ABSTRACT: Bovine enzootic hematuria (BEH) is a chronic disease caused by ingestion of *Pteridium esculentum* subsp. *arachnoideum* (Kaulf.) Thomson. This disease has great importance because it affects the animal wellbeing, causes economic losses, and hinders public health due to the toxic principle that is present in the milk of cows that ingest this plant. Early diagnosis assists in the decision making for disposal and replacement of animals in endemic regions. Despite ultrasonography is a useful tool for diagnosis of this disease, there is a lack of studies evaluating BEH in the state of Tocantins, Brazil, using ultrasound and epidemiological data and clinical signs; however, the histopathological examination is the method that provides the definitive diagnosis. The objective of this study was to describe the occurrence of BEH in the state of Tocantins and evaluate the efficiency of bladder ultrasound examination for diagnosis and prognosis of BEH. Ten animals that presented intermittent hematuria and hypo-colored mucous membranes were subjected to clinical and complementary exams. The urine physical analysis presented light red color, high turbidity, and positive sedimentation. The ultrasonography showed a thick, irregular mass in the bladder wall, containing an echogenic liquid of high cellularity and pedunculated papillary lesions. Macro and microscopic analyses of the animals' bladders showed areas with vascular proliferation in the sub-mucous membranes associated with mononuclear inflammatory cell infiltrate and proliferation of the epithelium. Ultrasonography is an excellent option and, combined with clinical and laboratory exams of blood and urine, enables the diagnosis and prognosis of BEH.

KEYWORDS: Bracken fern; *Pteridium esculentum* subsp. *arachnoideum* (Kaulf.) Thomson.; bladder; cattle.

RESUMO: Hematúria enzoótica bovina (HEB) doença crônica causada pela ingestão de Pteridium esculentum subsp. arachnoideum (Kaulf.) Thomson. Essa intoxicação tem grande importância no bem-estar animal, causando prejuizos econômicos e na saúde pública, uma vez que o princípio tóxico está presente no leite de vacas que ingerem a planta. Em regiões endêmicas o diagnóstico precoce auxilia na tomada de decisões, como o descarte e a substituição dos animais. Apesar da ultrossonografia ser uma ferramenta diagnóstica muito útil, há uma carência de estudos de HEB no estado do Tocantins no que diz respeito aos dados ultrassonográficos, epidemiológicos e sinais clínicos; entretanto, o exame histopatológico é o diagnóstico definitivo. O objetivo deste estudo foi descrever a ocorrência da doença neste estado e avaliar a eficácia do exame ultrassonográfico da bexiga urinária para o diagnóstico e prognóstico de HEB. Foram 10 animais submetidos a exames clínicos e complementares, ao exame apresentavam hematúria intermitente e mucosas hipocoradas. A análise física da urina apresentou-se vermelho vivo, turva e com sedimentação positiva. A ultrassonografia revelou massa na parede da bexiga urinária, espessa e irregular, contendo líquido ecogênico de alta celularidade e com lesões papilares pedunculadas. A análise macro e microscópica da bexiga do animal mostrou áreas com proliferação vascular na submucosa, associadas a infiltrado inflamatório mononuclear e proliferação do epitélio. Pode-se concluir que a ultrassonografia é uma excelente opção e, associada aos exames clínicos e laboratoriais de sangue e urina, possibilitou o diagnóstico e o prognóstico da doença.

PALAVRAS-CHAVE: samambaias; Pteridium esculentum subsp. arachnoideum (Kaulf.) Thomson.; bexiga urinária; gado.

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INTRODUCTION

Bovine enzootic hematuria is a chronic and debilitating cattle disease caused by prolonged ingestion of bracken fern plants [*Pteridium esculentum* subsp. *arachnoideum* (Kaulf.) Thomson]. This is a cosmopolitan plant that is present in all continents. In Brazil, this plant occurs mainly in mountainous regions, from southern state of Bahia to Rio Grande do Sul. In addition, bracken fern plants are found in hot-climate lowland areas in the Amazon region. Buds are the most toxic parts of the plant and the rhizome has higher carcinogenic activity due to the presence of ptaquiloside (TOKARNIA et al., 2012).

The clinical forms of the disease are related to the time and amount of plant ingested and can be hemorrhagic diathesis, upper gastrointestinal tract carcinomas, and bovine enzootic hematuria (BEH), which consist in progressive weight loss, hypo-colored mucous membranes, and hematuria (TOKARNIA et al., 2012). Laboratory evaluations have shown progressive anemia, with decreases in the hematocrit and hemoglobin, lymphocytosis, neutropenia, hypoproteinemia, hypoalbuminemia, decreases in plasma calcium levels, normal levels of phosphorus and magnesium, and inflammatory, hyperplastic, and metaplastic lesions in the bladder (SÁNCHEZ-VILLALOBOS et al., 2004).

The diagnosis is based on history, clinical status, and necropsy findings (TOKARNIA et al., 2012). Ultrasonography is used as a tool to assist in the diagnosis of lesions present in the urinary tract (HOQUE et al., 2002). Breeders in northern Tocantins have reported outbreaks of bracken fern toxicity; however, no studies on diagnosis or incidence of this disease have been conducted for this region (COSTA et al., 2011). Thus, the objective of this work was to describe the incidence of BEH in the state of Tocantins, Brazil, and evaluate the efficiency of ultrasound examination of the urinary vesicle for diagnosis and prognosis of BEH.

CASE REPORT

Researchers of the Ruminant Clinical Medicine Sector (SCMR) of the Veterinary Medicine Hospital of the Federal University of Tocantins (UFT) were asked to conduct exams in properties of two Settlement Projects (SP-1 and SP-2), located at 07°09'35"S, 48°31'39"W, in the municipality of Aragominas, state of Tocantins, Brazil.

Visits to the properties: Significant amounts of bracken fern plants [*Pteridium esculentum* subsp. *arachnoideum* (Kaulf.) Thomson] presenting signs of ingestion by animals were found during the visits to the properties, as well as animals with clinical signs of hematuria. Samples of these plants were collected for botanical identification, and animals with BEH symptoms were examined according to the method described by Marçal (2003). Ten bovines with BEH symptoms in the SP-1 were selected for complementary exams.

Botanical identification: Samples of the plants were maintained pressed in absorbent papers, which were exchanged daily until the total drying of the samples (14 days), and, then, sent to the Department of the Botany Course of the UFT for taxonomical identification.

Blood and urine examinations: Blood and urine samples were collected from the from the animals. A 5mL blood sample was withdrawn from the jugular vein of each animal by using a 25×0.8 vacutainer coupled to a collection pipe with EDTA. The blood samples were stored in an isothermal box at 2 °C and sent to the Laboratory of Clinical Pathology (LPC) of the UFT to carry out an hemogram, according to the technique described by Jain (1993). A 20ml urine sample was collected from each animal, by making a soft massage in the perineal and vulvar regions, and stored in sterile bottles, which were placed in an isothermal box at 2 °C and sent to the LPC-UFT for urinalysis (KANEKO; HARVEY; BRUSS, 2008).

Ultrasound examination: The ultrasonography was carried out via transrectal by using a HS-1500 device (Honda Electronics) with a transducer HLV-375M 50 mm (multifrequency of 5, 7.5, and 10 MHz) in B mode and real time at frequency of 7.5 MHz to capture images. The transducer was lubricated with ultrasound gel and introduced into the animal's rectum in close contact with the rectal floor. Screening movements were carried out longitudinally and transversally above the urinary vesicle. The urinary vesicle examination consisted of evaluation of its topography, distension, wall surface regularity, wall thickness, liquid content, and echogenicity. The presence of mass, tumor, or pedunculated papillary lesions was verified. All images were saved in the device memory and, then, transferred to a computer.

Macroscopic and histological analyses: During the visits to the properties evaluated, an animal presenting BEH symptoms was slaughtered by the owner for consumption, but the animal's bladder was collected for histopathological analysis. The material was fixed in 10% buffered formalin and sent to the Laboratory of Veterinary Pathology of the UFT for macroscopic and microscopic analyses. Fragments were dehydrated, diaphanized, fixed in paraffin, cut in 5µm sections, stained in hematoxylin and eosin, and evaluated in an optical microscope.

Epidemiological data: A total of 150 rural properties were visited in 19 municipalities of the state of Tocantins. However, the only place that the SCMR researchers identified presence of bracken fern plants and cases of BEH was in the municipality of Aragominas, in two Settlement Projects: SP-1 and SP-2. SP-1 contained 200 properties: 40% of them presented cases of BEH and, on average, 28% of the animals of each property presented the disease. SP-2 contained 187 properties: 30% of them presented cases of BEH and, on average, 25% of the animals of each property presented the disease.

Clinical status: All bovine examined presented intermittent hematuria and progressive weight loss; 40% of them presented slightly, and 60% presented moderately hypo-colored

mucous membranes; the other parameters were within the physiological limits (DIRKSEN; GRÜNDER; STÖBER, 1993).

Blood and urine examinations: The laboratory exams showed that the animals presented results below the reference values for the species (KANEKO; HARVEY; BRUSS, 2008). Decreases in numbers of erythrocytes and hematocrits were the most common changes in the animals studied, as well as decreases in hemoglobin content and mean corpuscular hemoglobin concentration (MCHC). Nine (90%) of the animals presented normochromic microcytic anemia and one animal (10%) presented hypochromic normocytic anemia. The mean hemoglobin rate was 5.26 g dL^{-1}, and the mean MCHC was 26.33%. The physical and chemical analyses showed that all animals presented urine with light red color, high turbidity, positive sedimentation, presence of blood, and increases in proteins. The sediment analysis showed numerous red cells, urothelial epithelial cells, coagulum, and mucus. The other parameters were within the normal ranges.

Ultrasound examination: Eight of the ten animals evaluated were subjected to ultrasonography, and six of them had urinary vesicle with lesions noticeable on ultrasound. Considering the urinary vesicles of the six animals that presented lesions, all of them had the usual topography; four presented moderate distension and the other two presented little and highly distension; all presented thickened and irregular walls, with thickness ranging from 5.3 to 10.8 mm; three showed liquid contents with limpid and anechoic aspects and three presented an echogenic content with high cellularity. In addition, four of the six animals that presented noticeable alterations presented pedunculated papillary lesions from the urinary vesicle wall (Figure 1).

Two animals presented mass in the urinary vesicle wall, with dimensions of 17.3 × 14.2 mm and 33.3 × 34.2 mm (Figure 2).

The two other animals with lesions noticeable on ultrasound presented anechoic urine with absence of crystals or cell debris; the images were compatible with polypus, masses, or calculi inside the bladder.

Macro and microscopic analyses: Macroscopically, the bladder wall was thick and the mucous membranes had multiple reddish-brown areas. The microscopic exam showed that the bladder presented areas with vascular proliferation in the sub-mucous membranes, often associated with mononuclear inflammatory cell infiltrate and proliferation of the epithelium, and lesions that are compatible with many hemangiomas associated with hyperplasia of mucous membranes.

RESULTS AND DISCUSSION

The diagnosis of BEH was established using epidemiological data, presence of bracken fern plants [*Pteridium esculentum* subsp. *arachnoideum* (Kaulf.) Thomson] in the grazing field, and bovine animals presenting clinical signs of hematuria, combined with clinical laboratory results, ultrasound findings, and histological evaluation of the bladder collected.

Among the 19 municipalities visited in the state of Tocantins, only Aragominas was identified with BEH cases and presence of bracken fern plants. According to Marçal (2003), the occurrence of BEH is directly related to the presence of plants infesting the grazing field, since the animals could ingest the plants.

SP-1 presented 40% (80/200) of the properties with cases of BEH and, on average, 28% of the animals of each property had the disease. SP-2 presented 30% (56/187) of the properties with cases of BEH and, on average, 25% of the animals of each property presented the disease. It denotes the

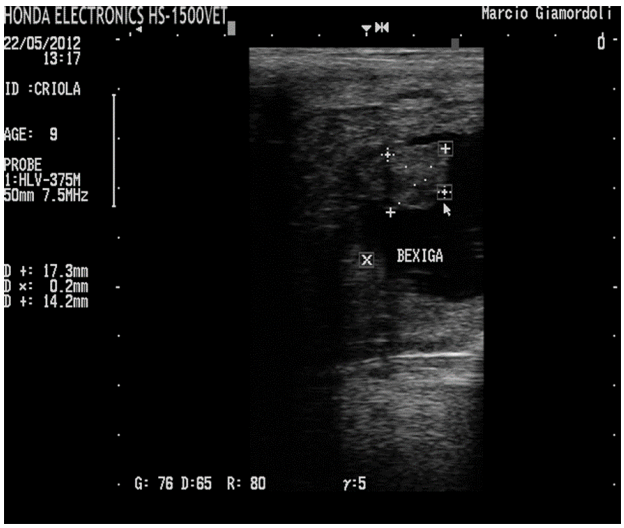

Figure 1. Ultrasound image of urinary vesicle of a bovine animal presenting: usual topography; urinary vesicle moderately distended by urine; thickened and irregular walls; urine with moderate cellularity and echogenic content; presence of pedunculated papillary lesions from the urinary vesicle wall; presence of a mass with dimensions of 17.3 × 14.2 mm.

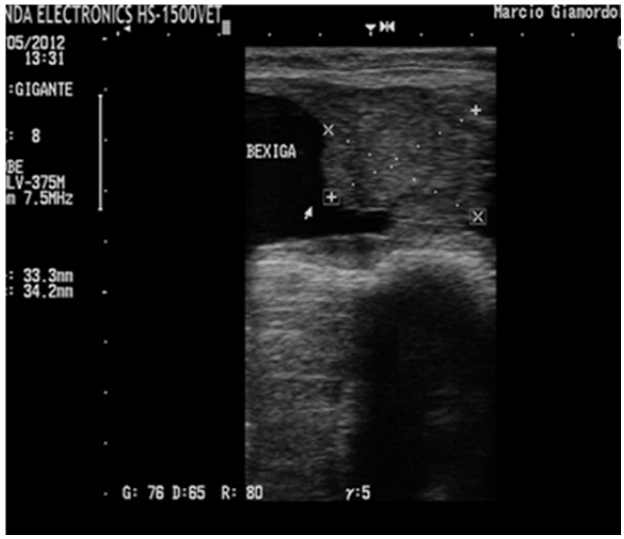

Figure 2. Ultrasound image of urinary vesicle of a bovine animal presenting: usual topography; urinary vesicle moderately distended by urine; thickened and irregular walls, measuring 5.3 mm; urine with apparently limpid aspect and anechoic content; presence of pedunculated papillary lesions from the urinary vesicle wall; presence of a mass with dimensions of 33.3 × 34.2 mm.

economic importance and impact of this disease in regions with bracken fern plants. Studies on incidence of BEH have been conducted in other regions of Brazil. In the Southwest region, Galvão et al. (2012) found that 71 (97%) of the 73 properties that had bracken fern plants presented cases of BEH. In the North region of Tocantins, the disease was found in 37.5% (15/40) of the visited properties. The incidence of the disease was low in the region studied (1.8%), however, it varied between properties (0.8-16.6%) (FURLAN et al., 2014), which can be related to different factors, such as the degree of infestation in each property or region and the availability of food for the animals.

The incidence of BEH in other countries was reported in 2013, in Oxapampa (Peru), where the disease is endemic (HERENCIA; FALCÓN; GARCÍA, 2013). In Bhutan, it was recently reported as the second most important disease in 16 districts of the country (HIDANO et al., 2016).

The different carcinogenicity of bracken fern plants [*Pteridium esculentum* subsp. *arachnoideum* (Kaulf.) Thomson] in different areas indicated that the toxicity of this plant varies according to the geographical distribution (HIRONO et al., 1972); the different incidence indexes found in different regions of Brazil and other countries may be related to this characteristic.

According to Ribeiro & Soto-Blanco (2020), clinical signs of BEH vary according to the site of installation and size of the neoplasia; the main symptoms are progressive weight loss, dysphagia, bloat, and regurgitation. These neoplasms are usually found at the tongue base, pharynx, esophagus, and rumen (TOKARNIA et al., 2012). Hematuria presents a slow and progressive course with frequent intervals of variable duration ranging from some weeks to months. It may cease subtly and slowly and reappear after a period, which may repeat until the death of the animal (PAMUKCU; PRICE; BRYAN, 1976).

In the present study, all animals examined presented intermittent hematuria and progressive weight loss; 40% of them had slightly hypo-colored mucous membranes and 60% had moderately hypo-colored mucous membranes; the other parameters were within the physiological limits. Hematuria, signs of anemia, and other changes such as seldom urinary incontinence and death may also be found (TOKARNIA et al., 2012).

The laboratory exams showed that the animals presented results below the reference values for the species (KANEKO; HARVEY; BRUSS, 2008). The decrease in number of erythrocytes and hematocrits was the more evident change in the animals, as well as the low hemoglobin content and mean corpuscular hemoglobin concentration (MCHC). In addition, 90% of the animals presented normochromic microcytic anemia, which is characteristic of iron deficiency in progress; and 10% presented hypochromic normocytic anemia, which is commonly found at the initial iron deficiency stage (SÁNCHEZ-VILLALOBOS et al., 2004). The bovines with BEH presented a significant decrease in hematocrit and hemoglobin, with progressive anemia due to blood loss through urine, which may also explain the weight loss of the animals. According to Marçal (2003), the anemia progresses as the loss of undamaged red blood cells through urine increases, and it regresses as the hematuria ceases. These authors found normal hemoglobin concentration and number of red blood cells. This normal erythrogram result can be explained by the fact that most animals examined were at the microhematuria stage remained presenting erythrogram results within the normal range until the development of macrohematuria (SÁNCHEZ-VILLALOBOS et al., 2004).

Chronic cases present decreases in hemoglobin and red blood cells, indicating secondary anemia (PAMUKCU; PRICE; BRYAN, 1976). In an experimental study of toxicity by bracken fern plants in 18 animals, 11 showed significant hematological changes; the main characteristic was progressive decrease in number of white blood cells and platelets in the peripheral blood; the leucopenia was associated with decreases in number of polymorphs; and the inversion of number of polymorphs and lymphocytes indicated that the ingestion of the plant seriously damages the bone marrow (PAMUKCU; PRICE; BRYAN, 1976). These findings are consistent with those found in the present work, in which 60% of the animals presented levels of total leukocytes and neutrophils below or at the lower limit, 100% presented eosinophils below the physiological parameter, and 70% presented lymphocyte counting above or at the higher limit of the reference used (KANEKO; HARVEY; BRUSS, 1997).

The results of the urinalysis showed that 100% of the animals presented macrohematuria, i.e., were at the clinical stage of the disease, differing from other studies that evaluated animals with macro and microhematuria (PAMUKCU; PRICE; BRYAN, 1976). The samples evaluated in the present work also showed proteinuria. The sediment analysis showed urothelial epithelial cells, probably due to the lesions in the urinary vesicle wall (SANDOVAL et al., 2002).

The changes found in the ultrasound examination were similar to those found by Sandoval et al. (2002); however, two animals presented no lesions noticeable on ultrasound, despite clinical signs of BEH had been observed. Hematuria in some animals is associated with non-neoplastic changes, such as ectasia, congestion, and capillary proliferation (TOKARNIA et al. 2012), which would explain the fact that these bovines present clinical signs of the disease with no changes noticeable on ultrasound. Few studies have used ultrasonography as a technique for the evaluation and diagnosis of BEH (HOQUE et al., 2002; SANDOVAL et al., 2002; TOKARNIA et al., 2012).

Ultrasound presents many advantages over other methods; it is a safe, non-invasive procedure that provides readily available information of lesions (HOQUE et al., 2002). However, it requires the participation of a trained operator to assure an adequate scan of the organ under evaluation and

a correct interpretation of the images. The use of ultrasound combined with other evaluations, such as clinical and laboratory exams, allowed for a better evaluation of the disease and even the establishment of a prognosis for the affected animal, since the ultrasound examination revealed the urinary vesicle problems, such as presence or absence of structures suggestive of tumor masses and other lesions. In addition, ultrasonography can be used to detect subclinical cases of BEH and provide an early diagnosis of bladder tumors in which hematuria is still not developed (HOQUE et al., 2002).

Regarding the anatomical-pathological exam of the bladder collected, the macroscopic aspect was consistent with that described by Tokarnia et al. (2012), denoting lesions characteristic of BEH, such as dilation and thickening of the bladder wall, congestion of mucous membranes, hemorrhage spots, coalescent lesions, firm tumor masses with yellowish, irregular surface projecting to the mucous membranes as multiple reddish polypoid formations. The microscopic findings of this bladder were compatible with studies that histologically evaluated urinary vesicles of animals with BEH (TOKARNIA et al., 2012), which confirms the diagnosis of disease in the study site.

CONCLUSIONS

This is the first report of bovine enzootic hematuria (BEH) diagnosed in the state of Tocantins, Brazil. This kind of work is important for the mapping of diseases and to provide information about diseases that occur in a specific region and have not known treatment, since their prognosis assist producers in decision making for disposal and replacement of affected animals. The ultrasonography showed to be an excellent tool that, combined with clinical and laboratory exams of blood and urine, enables the diagnosis and prognosis of BEH.

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