PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 45:e07679, 2025
DOI: 10.1590/1678-5150-PVB-7679

Original Article
Livestock Diseases

Bovine abortion caused by *Ureaplasma diversum* infection in Midwest Brazil[1]

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ABSTRACT.- Ribeiro M, Cavasani JPS, Santos ÍG, Costa MTS, Nakazato L, Olaya CEB, Marques RR, Silva MERJ, Siqueira FM, Souza MA, Watanabe TTN, Pescador CA. **Bovine abortion caused by *Ureaplasma diversum* infection in Midwest Brazil.** *Pesquisa Veterinária Brasileira 45:e07679, 2025.* Faculdade de Medicina Veterinária, Universidade Federal de Mato Grosso, Avenida Fernando Corrêa da Costa 2367, Boa Esperança, Cuiabá, MT 78060900, Brazil E-mail: carolinepescador@gmail.com

Ureaplasma diversum has been reported to infect the respiratory and genital systems in cattle, leading to numerous reproductive alterations, including abortions. However, detailed and comprehensive studies of fetal pathology in naturally infected cases are lacking in the scientific literature to this date. Therefore, this study describes the pathological lesions in three fetuses spontaneously aborted by three heifers naturally infected with *U. diversum* localized in three municipalities from Mato Grosso State, Brazil. The fetal necropsy revealed diffusely reddened and unexpanded lungs. Histologically, diffuse moderate subacute to chronic neutrophilic and histiocytic bronchointerstitial pneumonia with bronchial-associated lymphoid tissue hyperplasia. *U. diversum* was identified in fetal tissues by molecular approaches and sequencing. To our knowledge, this is the first description of bovine abortion caused by *U. diversum* infection in the Midwest of Brazil.

INDEX TERMS: Fetal loss, pneumonia, PCR, Mollicutes, Brazil.

RESUMO.- [**Aborto bovino causado por *Ureaplasma diversum* na região Centro-Oeste do Brasil.**] *Ureaplasma diversum* infecta os sistemas respiratório e genital de bovinos, levando a várias alterações reprodutivas, inclusive abortos. No entanto, até o momento, não há na literatura científica, estudos detalhados e abrangentes no Brasil sobre as lesões fetais em casos naturalmente infectados. Portanto, este artigo descreve as lesões em três fetos espontaneamente abortados por três novilhas naturalmente infectadas por *U. diversum* localizadas em três municípios do estado de Mato Grosso, Brasil. A necropsia fetal revelou pulmões difusamente avermelhados e não expandidos. Histologicamente, havia pneumonia neutrofílica e histiocítica broncointersticial subaguda a crônica difusa e moderada com hiperplasia do tecido linfoide associado aos brônquios. *U. diversum* foi identificado em tecidos fetais após a realização de técnicas moleculares e sequenciamento. Esta parece ser a primeira descrição de aborto bovino causado por infecção por *U. diversum* na região Centro-Oeste do Brasil.

TERMOS DE INDEXAÇÃO: Perda fetal, pneumonia, PCR, Mollicutes, Brasil.

INTRODUCTION

Ureaplasma diversum is a bovine bacterium that was first isolated in the reproductive tract in Canada in 1967. Although the mechanisms of virulence and pathogenicity in cattle are primarily unknown, *Ureaplasma* spp. consumes urea through the urease enzyme, releasing ammonia that causes damage

[1] Received on April 15, 2025.
Accepted for publication on June 2, 2025.
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in the mucous membranes of the reproductive tract, leading to prostaglandin E2 and F2 decrease by endometrial cells and consequently causing difficulties in embryo implantation and gestation (Santos-Junior et al. 2021).

Ureaplasma diversum is a widespread commensal and pathogenic bacterium associated with vulvitis, endometritis, abortion, and infertility in cattle (Doig et al. 1980). In bulls, it is a venereal microorganism that may cause low sperm motility, seminal vesiculitis, and epididymitis (Marques et al. 2013). Heifers have a higher risk of infection, which results in a low birth rate (León et al. 1995). Although *U. diversum* is a bacteria transmitted through coitus, reproductive biotechnologies widely used in cattle herds also represent potential sources of transmission of these bacteria (Crane & Hughes 2018). Economic harm and costs for veterinarians and treatment are also major concerns in commercial livestock (Gambarini et al. 2009), mainly in major advanced beef-producing countries such as the USA, China, and India. Brazil is the holder of the second-largest herd of cattle in the world, and the ureaplasmal frequency in animals with vulvovaginitis pustular or reproductive disorders usually varies from 38.8% to 83.8% (Gaeti et al. 2014, Voltarelli et al. 2018).

Granular vulvovaginitis is Brazil's main clinical reproductive problem reported in cows and heifers (Gaeti et al. 2014, Azevedo et al. 2017). This seems to be the first comprehensive description of ureaplasmal fetal loss in beef cattle associated with pneumonia in Brazil. Therefore, the objective of this study is (i) to describe the epidemiological and microscopic lesions as well as (ii) to emphasize the diagnostic tools for cases of infection by *U. diversum* in aborted bovine fetuses, and (iii) to highlight the importance of considering *U. diversum* infection as a differential diagnosis for fetal loss in beef cattle.

MATERIALS AND METHODS

Ethical approval. This study was not submitted to the Ethics Committee on the Use of Animals (CEUA) of the "Universidade Federal de Mato Grosso" (UFMT), as the fetuses analyzed are part of the diagnostic routine of the Veterinary Pathology Laboratory of UFMT (LPV-UFMT) and the legislation provides for the need for submission only in cases of use of live animals for experimentation.

Study population. All fetuses were forwarded by autonomous veterinarians along with epidemiological data and clinical history. Cattle of three beef herds derived from three municipalities of Mato Grosso State (MT), Brazil, had a clinical history of reproductive problems characterized by pregnancy losses. All the farms practiced routine immunizations against bovine herpesvirus (BoHV-1), bovine viral diarrhea virus (BVDV), *Leptospira* sp., and *Brucella abortus*. Abortion at these farms was restricted to the final third of pregnancy. Cattle at all farms were maintained predominantly on green pastures and supplemented with commercial mineral salt.

Post mortem examinations of the fetuses were performed. Estimation of the gestational age according to the crown-rump length (Barr et al. 1990), which also included the presence or lack of hair on lips, eyebrows, and muzzle, the eruption of incisor teeth, and the presence or absence of horn pits were recorded. Representative brain, lung, thymus, heart, liver, spleen, kidney, and skeletal muscle specimens were collected, fixed in 10% neutered-buffered formalin, and processed routinely for histological examination. Brain, liver, spleen, thymus, kidney, heart, lung and abomasal fluid samples were maintained at -20 °C until processed for molecular diagnostics.

DNA extraction and PCR assay. Genomic DNA extraction from lung and abomasal fluid was performed by PureLink™ Genomic DNA Mini Kit (Invitrogen, Massachusetts, USA), according to the manufacturer's instructions.

The DNAs were subjected to multiplex PCR assays to detect *Ureaplasma diversum*, *Mycoplasma bovis*, and *Mycoplasma bovigenitalium*, using a combination of a set of primers described in the literature. In detail, the sequencing primer 16SF-5'-GTTTGATCCTGGCTCAGGAT-3' with the reverse primers: 16SR-5'-CTCATAAGCGAGCATCATT-3' (*U. diversum*), 16SR-5'-CAAACGCTTCCTTTTATATTAC-3' (*M. bovis*), and 16SR-5'-AAGGTACATTCAATATAGTGG-3' (*M. bovigenitalium*), provided PCR amplicons with 838 bp, 198 bp, and 476 bp of length to *U. diversum, M. bovis*, and *M. bovigenitalium*, respectively. Multiplex PCR reactions were performed in a final volume of 24 µl, including 1 U of Taq DNA Polymerase (Quatro G, Porto Alegre, Brazil), 1x reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM of dNTPs, 0.4 µM of each primer and 10 ng of DNA template. The optimum PCR conditions were initial denaturation at 95 °C for 15 min, followed by 40 cycles at 95 °C for 1 min, annealing for 1 min at 52 °C, and extension for 1 min at 72 °C, concluding with a final extension at 72 °C for 10 min. All reactions were performed in a thermocycler MiniAmpThermal Cycler (Applied Biosystems, Massachusetts, USA). The amplified products were analyzed in 1.5% agarose gel electrophoresis and observed under ultraviolet light.

Positive controls used for multiplex PCR reactions were purified and sequenced by the Sanger method, followed by assembly using Geneious v.10.2.3 (Biomatters), and analyzed for similarity in the NCBI platform. The sequences were compared with the sequence found in GenBank by the BLAST program.

The phylogenetic analysis was performed using multiple alignment software "Muscle," and a tree was constructed using the neighbor-joining distance method with 1,000 bootstraps in the program Phylogenetic.fr.

Furthermore, fresh kidney and liver samples were tested for *Leptospira* spp. using PCR as previously described (Ahmed et al. 2012), thymus and spleen samples were tested for BVDV-1 (Vilček et al. 1994), lung, liver, and spleen samples for BoHV-1 (Silva et al. 2007) and lung and abomasum contend for *Brucella* spp. (Silva et al. 2009).

RESULTS

The aborted fetuses were from three farms in three municipalities of the Mato Grosso State in Midwest Brazil. Abortion was defined as the interruption of pregnancy occurring from 42 to 260 days of gestation (Mee 2020), and the age of the fetuses varied from seven to eight months of gestational age. The first fetus was a female, Nelore, from a property in the municipality of Vila Bela da Santíssima Trindade/MT. The second fetus was a mixed-breed male sent from a property in Novo Mundo/MT. The third animal was a female, Angus, from Alta Floresta/MT. No clinical signs or gross injuries were reported by the autonomous veterinarians' doctors who performed clinical evaluated these cows.

At necropsy, all fetuses had atelectatic lungs (Fig. 1). The third fetus had skin covered with blackened material, consistent with meconium. The placenta was not submitted for gross examination in any of the cases. Microscopically, the lungs from all fetuses had similar findings with differences in severity (Table 1). The alveolar septa were expanded by macrophages and fewer lymphocytes (Fig. 2). Multifocal alveolar spaces and bronchiolar lumina contained low numbers of viable and degenerate neutrophils mixed with edema and fibrin and multinucleated giant cells

containing brownish intracytoplasmic material compatible with meconium are observed (Fig. 3). Multifocal peribronchiolar lymphoid aggregates were mildly compressing the adjacent airways (Fig. 4). Interlobular septa were diffusely expanded by edema. All the vasculatures were congested. No other significant lesions were found in the different tissues.

The three fetuses were positive for *Ureaplasma diversum* by PCR. The DNA obtained from two samples was submitted to molecular characterization and genome sequence, confirming that it is the bacterium *U. diversum* and not another bacterium connected to the Mollicutes class. Sequence analysis (UFMT A and C) shows 100% identification to *U. diversum* (CP096123). Additional PCR tests for BVDV-1, BoHV-1, *Leptospira* sp., *Brucella* spp., *Mycoplasma bovis* and *Mycoplasma bovigenitalium* were negative. Table 1 summarizes the macroscopic, histopathological, and PCR results. Based on DNA identity and phylogenetic analysis tree, the samples were closest to *U. diversum* available in GenBank (Fig. 5).

DISCUSSION

Determining the etiology of the abortion and perinatal cattle death investigation is inherently challenging (Mee 2020). In this study, ureaplasmal abortion confirmation was made through the association of microscopic changes and molecular detection. Additionally, abortifacient agents routinely considered in this study were ruled out through molecular technique.

The microscopic fetal pulmonary lesions were similar to those developed in the aborted fetuses following experimental intra-amniotic inoculation with *Ureaplasma diversum* (Miller et al. 1994). The potential of ureaplasmas to incite a defense mechanism in neonates' bloodstream and airways has been investigated to characterize how these organisms can produce pathological changes when they reach the lungs. Suppurative bronchopneumonia may vary from mild to moderate, as observed in the cases reported herein. Similarly, this evidence is seen in human fetuses infected by *Ureaplasma urealyticum,* which are commonly isolated from amniotic fluid

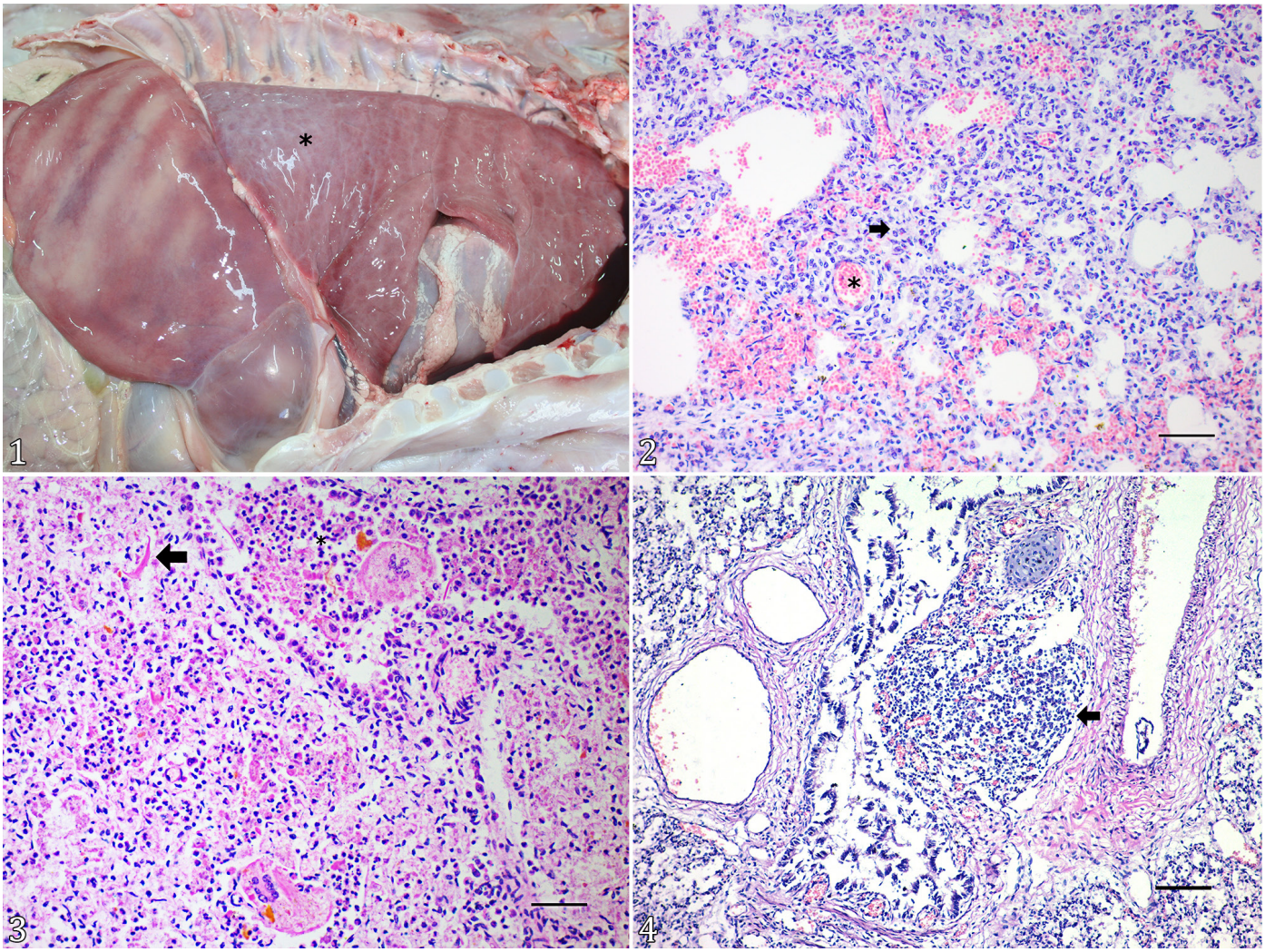

Fig. 1-4. Ureaplasma diversum infection in bovine fetuses. Lung. (**1**) The lungs are diffusely atelectasis (asterisk), indicating a lack of aeration. (**2**) Alveolar septa (arrow) are expanded by lymphocytes and fewer macrophages. The vasculature is diffusely congested (asterisk). HE, obj. 20x, Bar = 50 mm. (**3**) Alveolar and bronchiolar lumina contain abundant numbers of neutrophils and fewer multinucleated giant cells containing intracytoplasmic brown pigment (consistent with meconium) mixed with small keratin fragments (arrow). HE, obj. 20x, Bar = 50 mm. (**4**) Peribronchiolar lymphoid aggregates mildly compress the adjacent airways (bronchus). HE, obj. 20x, Bar = 50 mm.

from women (Viscardi et al. 2002) as well as it has also been reported in the lung of mice inoculated by *U. diversum* (Da Silva et al. 2021). *Ureaplasma diversum* causing pulmonary infection induces an early influx of neutrophils into the large airways and alveolar spaces and subsequent production of pro-inflammatory mediators (Da Silva et al. 2021).

In this study, bovine fetuses had a pattern of neutrophilic and histiocytic bronchointerstitial pneumonia; prominent peribronchial and peribronchiolar lymphoid aggregates often forming lymphoid follicles; and thickening of the alveolar septa. These findings closely resemble those previously described in the chronic stages of *Mycoplasma hyopneumoniae* infection in swine (Sarradell et al. 2003). Therefore, it is highly suggestive that cellular infiltrates composed of neutrophils and macrophages may be recruited early in the *U. diversum* infection, and lymphocytic hyperplasia may develop in a chronic phase of the disease. Ureaplasmal abortion usually occurs in late gestation, which may be linked to the follicular hyperplasia pattern of the lungs usually observed in the chronic stages of infection. In the case reported herein, the fetuses' ages ranged from seven to eight months, which aligns with the findings reported in the literature (Santos-Junior et al. 2021). Therefore, *U. diversum* invades fetal lungs through the placenta and amniotic fluid for an extended period, leading to adverse pregnancy outcomes (Santos-Junior et al. 2021).

Abortion due to *U. diversum* infection has been reported worldwide (Ruhnke et al. 1984, Barkallah et al. 2014), although, in Brazil, the role of *U. diversum* as an abortigenic agent is not well determined. To the best of our knowledge, this is the first serial case report on the detection of *U. diversum* as a cause of bovine abortions in Brazil. *Ureaplasma diversum* has been identified in bovine and linked with several diseases in the genital and respiratory systems. In Brazil, the prevalence of vulvovaginitis by *U. diversum* in cows has been focused only on clinical manifestations of this disease (Voltarelli et al. 2018). In Mato Grosso State, cases of ureaplasmal vulvar inflammation in beef (Gaeti et al. 2014) and dairy cattle (Azevedo et al. 2017) have been reported, emphasizing that chronic vulvovaginitis can develop endometritis by ascending route and results in reproductive failure (Barkallah et al. 2014).

Additionally, all abortion problems occurred in heifers, suggesting that animals with none or one delivery had a higher risk of infection than those with several parturitions (León et al. 1995). This can be explained by the individual's immunological behavior against the primary infection. The cow, when coming into contact with the bacteria for the first time, through intercourse

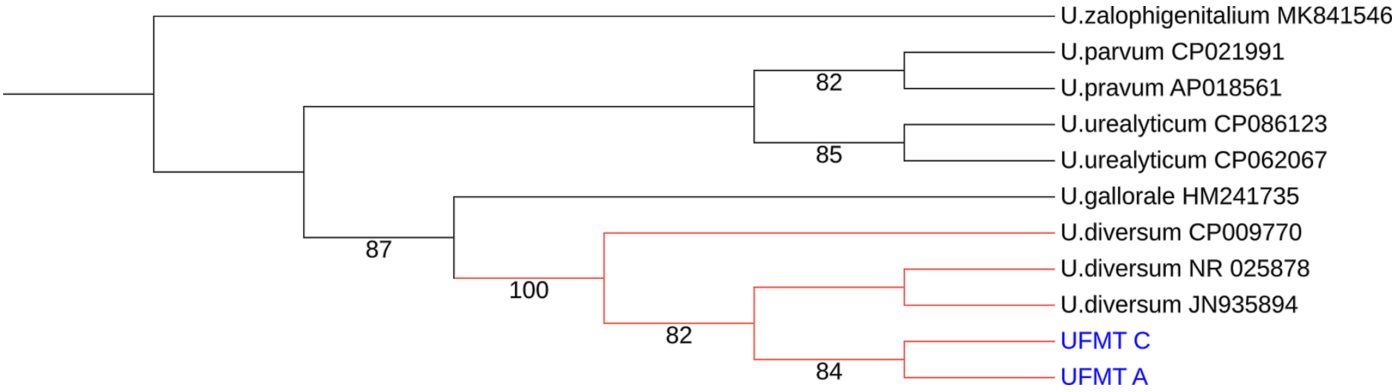

Fig. 5. Phylogeny of Ureaplasma diversum detected in bovine fetus using PhyML with the GTR substitution model based on the nucleotide sequence partial ribosomal gene. The scale bars represent the number of substitutions per site. *Mycoplasma bovis* was outgroup.

Table 1. Microscopic findings of bovine fetuses in abortion caused by *Ureaplasma diversum*

Microscopic lung lesions	Fetus 1	Fetus 2	Fetus 3
Alveolar septa			
Expanded	+	+	++
Macrophages	+	+	+
Lymphocytes	++	+	+
Fibrin	-	+	+
Congested vessels	+	++	+
Edema	-	+	
Lumina alveolar/bronchiolar			
Viable neutrophils	+	-	+++
Degenerate neutrophils	++	-	++
Lymphocytes	-	-	++
Fibrin	-	-	++
Meconium	+	++	++
Giant cells	-	-	++
Peribronquiolar lymphoid aggregates	+++	++	++

NE = not evaluated. Tissue not submitted for laboratory analysis.

or insemination, generates an immune response that lasts until the fourth parturition approximately (Doig et al. 1980). These findings strongly highlight that *Ureaplasma* spp. is present within cattle herds of the Mato Grosso state and is a potential cause of abortion. Importantly, this bacterial microorganism is most likely neglected as an important infectious disease agent to livestock reproductive pathogens in Brazil. Most Brazilian veterinary diagnostic laboratories that work with abortion have a standard diagnostic protocol for bovine specimens for *post mortem* investigation, but PCR testing for *Ureaplasma* spp. is not performed if pustular lesions in the genital tract of the affected cows. Asymptomatic cows and chronically infected with *Ureaplasma* spp. are usually the primary source of the dissemination of bacteria in the herd.

Typical macroscopic lesions include a relatively fresh fetus and frequently retained and inflamed placenta (Britton et al. 1987). In the cases reported here, the bovine fetuses were sent frozen and without placenta to the diagnostic laboratory. Frozen fetuses and no placenta submissions are the common abortion submissions from cattle farms to diagnostic laboratories in Brazil and around the world, including all major cattle-producing countries. In Brazil, it is highly speculated that this is due to two main factors: the first is the significant distance between the farms and the state diagnostic laboratory, as well as poor and lack of transportation options, which makes it necessary to freeze the fetus to prevent advanced *post mortem* changes during shipment. The second factor is the difficulty in locating fetal products and placenta due to the large size of the properties and the consumption of these samples by predators.

CONCLUSION

We describe three beef bovine abortion cases with ureaplasmal pneumonia in Midwest Brazil. In a diagnostic setting, *Ureaplasma diversum* should be considered a pathogen that causes interstitial pneumonia associated with BALT hyperplasia in aborted bovine fetuses in Brazil. Thus, it is essential to unravel, more broadly, the participation of Mollicutes in the genesis of bovine abortion in cattle belonging to farms in the state of Mato Grosso, Brazil.

Acknowledgments.- The study was supported by "Fundação de Amparo a Pesquisa do Estado de Mato Grosso" (FAPEMAT), project # FAPEMAT-PRO. 000566/2023. M. Ribeiro is supported by a fellowship from "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq).

Conflict of interest statement.- Author Tatiane Terumi Negrão Watanabe is employed by Antech Diagnostics, Mars Petcare Science & Diagnostics. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Credit author statement.- Marlon Ribeiro, Caroline A. Pescador, João Paulo S. Cavasani, Ícaro Guilherme dos Santos, Camilo Eduardo B. Olaya - Performing post mortem evaluation and sample processing. Marlon Ribeiro, Caroline A. Pescador- Performing histological evaluation. Marco Túlio S. Costa, Luciano Nakazato - Performing molecular evaluation. Rafaela R. Marques, Maria Eduarda R.J. Silva, Franciele M. Siqueira - Performing genetic Sequencing. Marlon Ribeiro, Caroline A. Pescador, Marcos A. Souza - Data analysis and article writing. Caroline A. Pescador, João Paulo S. Cavasani, Ícaro Guilherme dos Santos, Tatiane T.N. Watanabe - Article review.

Data availability statement.- The authors confirm that the data supporting the findings of this study are deposited in the archives of the LPV, Veterinary Hospital, UFMT, Cuiabá/MT, Brazil, and will be made available upon request to the corresponding author (Pescador CA).

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