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First report of a canine morbillivirus infection in a giant anteater (*Myrmecophaga tridactyla*) in Brazil

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Abstract

Canine morbillivirus, also known as canine distemper virus (CDV), induces a contagious multisystemic disease caused by an enveloped RNA virus belonging to the genus *Morbillivirus* within the family *Paramyxoviridae*. CDV replicates readily in epithelial, nerve and lymphoid tissues; it is excreted in urine, feces, saliva, oral and nasal discharge; and its major route of entry for infection is through the respiratory system. Although the virus was originally believed to infect domestic dogs, new studies have shown that it can also naturally or experimentally infect non-domestic hosts. A recent blood test performed on a giant anteater (*Myrmecophaga tridactyla*) found Lentz inclusions in the animal's leucocytes. A rapid CDV test, an RT-PCR assay and pathology findings confirmed this report of canine morbillivirus in this species, which corresponds to the second report of CDV infection in the order Pilosa, family *Myrmecophagidae* in central west Brazil.

KEYWORDS
canine distemper, infection, RT-PCR, sequencing, wildlife

1 | INTRODUCTION

Canine morbillivirus, commonly known as canine distemper virus (CDV), is a virus belonging to the genus *Morbillivirus* and the family *Paramyxoviridae* (Amarasinghe et al., 2018). CDV has a single negative-stranded RNA genome enclosed in a nucleocapsid of helical symmetry surrounded by a lipoprotein envelope (van Regenmortel et al., 2000). This virus, which has epithelial, nerve and lymphoid tropism, is responsible for a deadly dog disease known as canine distemper (Carré, 1905). It is excreted mainly in the urine, feces and nasal discharge, and

its major route of entry for infection is through the respiratory system (Barret, 2010). Clinical signs of CDV in dogs include depression, malaise, mucopurulent discharge from the eyes and nose, coughing, vomiting, diarrhoea, fever, nasal and digital hyperkeratosis, and neurological signs such as convulsions, vestibular and cerebellar disorders, paresis and myoclonus (Greene & Vandevelde, 2015; Silva et al., 2007).

In addition to dogs, CDV causes severe disorders in numerous species of terrestrial carnivores, which act as hosts. Within the order Carnivora, the families *Canidae*, *Felidae* and *Mustelidae* can be infected with CDV. Additionally, the orders Rodentia, Primata, Artiodactyla and Proboscidea have also been shown to be susceptible to infection (Deem, Spelman, Yates, & Montali, 2000; Kameo et al., 2012; Martinez-Gutierrez & Ruiz-Saenz, 2016; Nikolin, Wibbelt, Michler, Wolf, & East, 2012). To exemplify, a recent report described CDV infection in *Tamandua tetradactyla*, a member of the order Pilosa and family *Myrmecophagidae* (Lunardi et al., 2018).

This paper contributes to the body of knowledge about CDV disease by reporting the case of a giant anteater, *Myrmecophaga tridactyla*, an endangered species in Brazil, naturally infected with this viral agent.

2 | MATERIALS AND METHODS

2.1 | Case history of the animal and diagnostic procedure

A young giant anteater rescued from a situation of abuse was brought to the Veterinary Hospital of the Federal University of Mato Grosso by the state environmental police. There, the animal underwent blood and biochemical tests, feces (parasitology) and imaging tests (echocardiography, ultrasonography and radiography). The giant anteater remained hospitalized for several days for treatment, and then handed over to the environmental police, along with dietary instructions and a medicine prescription. However, a month after the animal's release, it was brought back to the hospital, suffering from rectal prolapse caused by constipation. The anteater underwent new exams and surgery to repair its rectal prolapse.

A few days after its second hospitalization, the animal began presenting with prostration, nasal and eye discharge (Figure 1), anorexia and diarrhoea, so a new battery of blood and biochemical tests were performed. The mucosal surfaces of the eyes were swabbed using a SensPERT collector moistened with saline solution (0.9% of NaCl) for a rapid CDV test, following the manufacturer's instructions (Laboratórios Vencofarma do Brasil Ltda.).

2.2 | Reverse transcription followed by polymerase chain reaction (RT-PCR) and PCR sequencing

To detect the RNA of CDV, sterile blood and urine samples were collected for RNA extraction, using the ReliaPrep™ RNA Cell Miniprep System (Promega). The RNA was then subjected to an RT-PCR assay, using the Master Mix Access Quick™ RT-PCR System (Promega) and primers designed to amplify 287 bp of the nucleoprotein (N) gene (Frisk, Konig, Moritz, & Baumgartner, 1999) and 1,820 bp of the hemagglutinin (H) gene of CDV (An, Yoon, Park, No, & Park, 2008; Budaszewski et al., 2014; Harder et al., 1996; Hashimoto, Une, & Mochizuki, 2001) (Table 1). A commercial vaccine and nuclease free water were used as positive and negative controls. DNA fragments were subjected to agarose gel electrophoresis with 1.5% of TBE buffer pH 8.4, (TRIS 50 mM, boric acid 67 mM, EDTA 1 mM) and Gel Red stain (Biotium), and then examined under ultraviolet light in a molecular imager (Bio-Rad ChemiDoc XRS + system), using Image Lab™ 4.1 software.

FIGURE 1 Eye and nasal discharge, and hyperkeratosis

All the PCR products obtained of expected size were purified in a ReliaPrep™ DNA Clean-up and Concentration System (Promega®) and sequenced in an automatic sequencer (ABI DNA 3500 Series Genetic Analyzer), following the manufacturer's protocol. Partial sequences obtained were subjected to BLAST analysis (Altschul, Gish, Miller, Myers, & Lipman, 1990) to determine similarities to a previously detected CDV. The H gene sequence thus generated was aligned by means of the Clustal W programme, using MEGA v5 software, with 73 sequences of different CDV genotypes available at GenBank that represents 14 different genetic lineages circulating worldwide. Then, 1,800 characters were aligned. The phylogenetic tree for the isolate was inferred by means of the neighbour-joining method, using MEGA v5 software with the Tamura 3-parameter model of DNA substitution. The values observed here represent the percentage of 1,000 resampling bootstraps.

2.3 | Treatment

The giant anteater was treated using supportive therapy and antibiotics, and was given blood transfusions as the severity of its anaemia increased. Unfortunately, however, its prognosis was poor and the animal died.

2.4 | Necropsy and histopathology

A necropsy was carried out soon after the animal's death. The procedure consisted of a macroscopic and microscopic analysis of the

TABLE 1 Primers used for amplification of N and H genes of canine morbillivirus in *Myrmecophaga tridactyla* in Brazil

Primer	Sequence (5′ – 3′)	Gene	bp	Reference
CDV - 1F	ACAGGATTGCTGAGGACCTAT	N	287	Frisk et al. (1999)
CDV - 2R	CAAGATAACCATGTACGGTGC	N		Frisk et al. (1999)
RH3 - F	AGGGCTCAGGTACTCCAGC	H	-	Harder et al. (1996)
RH4 - R	AATGCTAGAGATGGTTTAATT	H		Harder et al. (1996)
H1F	ATGCTCTCCTACCAAGACAA	H	789	An et al. (2008)
H1R	CATGTCATTCAGCCACCGTT	H		An et al. (2008)
H2F	AATATGCTAACCGCTATCTC	H	523	An et al. (2008)
H2RB	TTTGGTTGCACATAGGGTAG	H		Budaszewski et al. (2014)
H3FB	CATATGATATATCCCGGGGC	H	253	Budaszewski et al. (2014)
H3R	TCARGGWTTTKAACGRYYAC	H		An et al. (2008)
CDVF10B	TAYCATGAYAGYARTGGTTC	H	870	Hashimoto et al. (2001)
CDVR10	ARTYYTCRACACTGRTKGTG	H		Hashimoto et al. (2001)

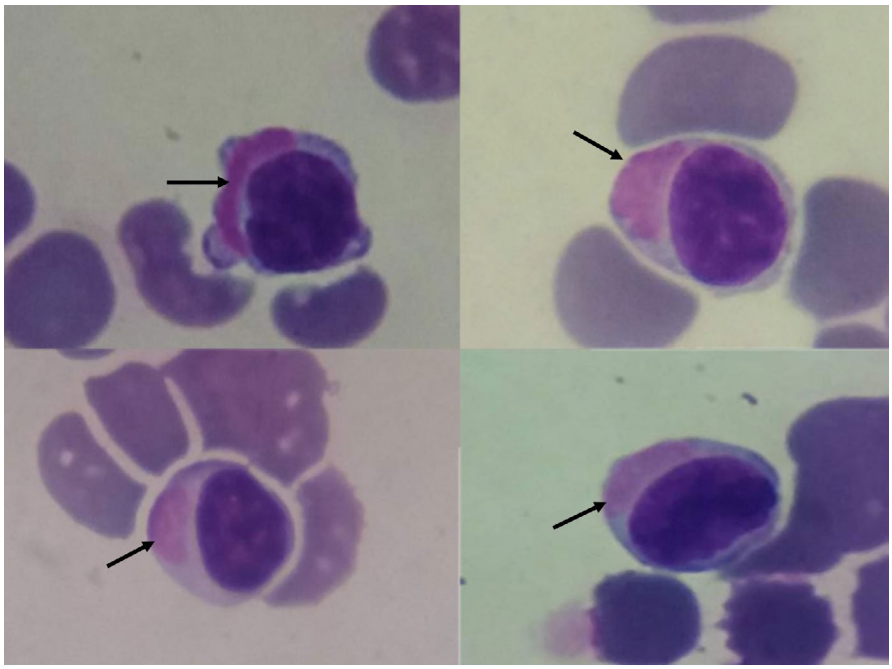

FIGURE 2 Lentz inclusions inside leucocytes (100× objective lens)

animal's body, organs and tissues. Brain, lung, heart, liver, spleen, stomach, duodenum, jejunum, urinary bladder, kidney and bone narrow tissues were collected and fixed in 10% of 'neutral-buffered formalin, routinely processed and cut into 2–5 μm histological sections, which were subjected to hematoxylin and eosin (H&E) staining (Sonne et al., 2009).

3 | RESULTS

The lab results obtained after the first examination of the anteater indicated anaemia, *Strongyloides* parasites, dilated cardiomyopathy, failure of the right atrioventricular and pulmonary semilunar valves, hydronephrosis and intestinal constipation. The images also revealed the presence of surgical pins from a previous surgery of the fibula and tibia.

After the animal was brought back to the veterinary hospital, the second blood test revealed similar structures of Lentz inclusions in lymphocytes and monocytes (Figure 2). An evaluation of leucocytes indicated that 3.4% were lymphocytes, 2.0% of which contained Lentz inclusions and 4.8% were monocytes, 0.8% of them also contained Lentz inclusions. The inclusions were oval and rounded in shape, with an eosinophilic colour characteristic of CDV inclusion bodies. Severe anaemia, leucocytosis, lymphopenia, low albumin and globulin levels and a high alanine aminotransferase level were also found. The immunochromatographic test for CDV was positive. However, despite the clinical signs (prostration, nasal and ocular discharge, anorexia and diarrhoea), no neurological disorder was detected.

The presence of CDV genetic material (RNA) in urine and blood was confirmed by the RT-PCR assay of the N gene. The partial sequence (239 bp) of N gene was found to be similar (98%–99%) to other sequences available in GenBank. (KJ933692.1,

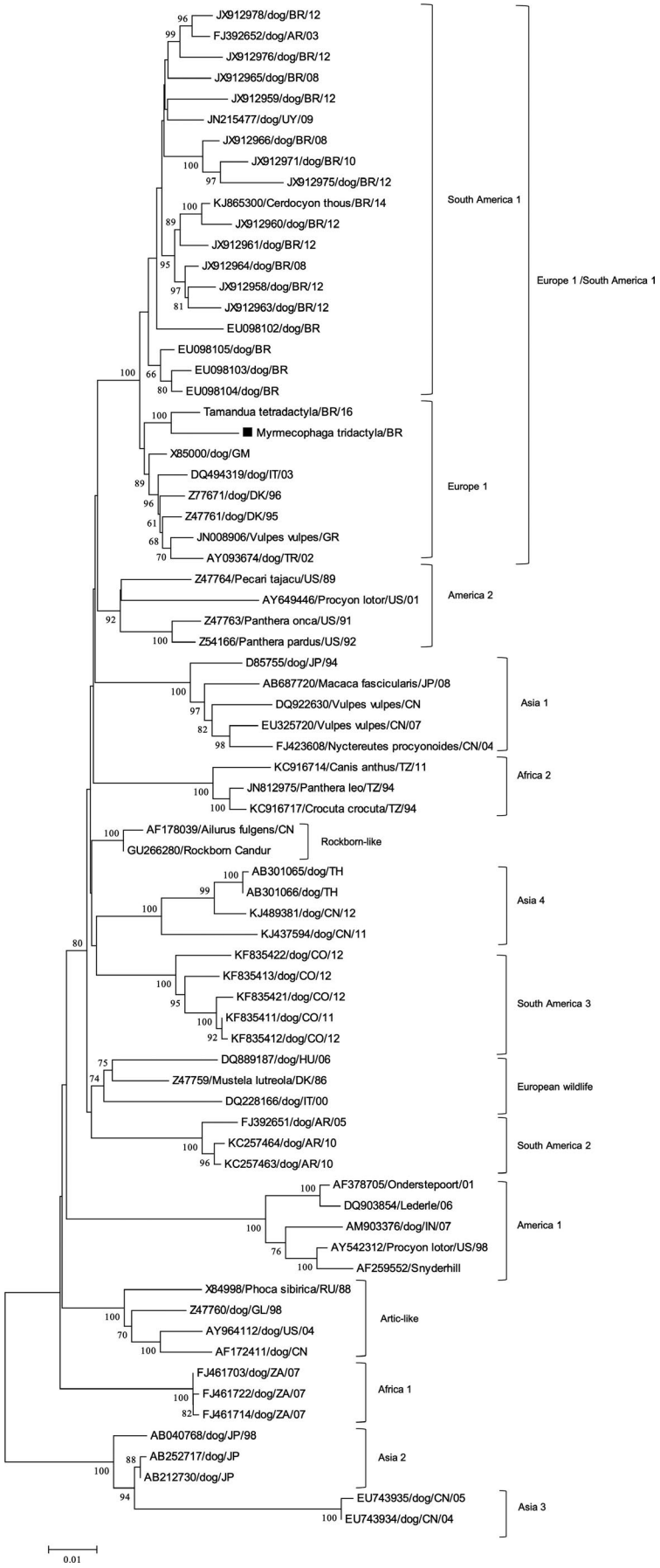

FIGURE 3 Phylogenetic neighbour-joining tree based on the complete H gene sequence of CDV infecting *Myrmecophaga tridactyla* in Brazil. The samples obtained in the present study are highlighted with a symbol ■. Bootstrap (1,000 replicates) values >60 are indicated at the nodes

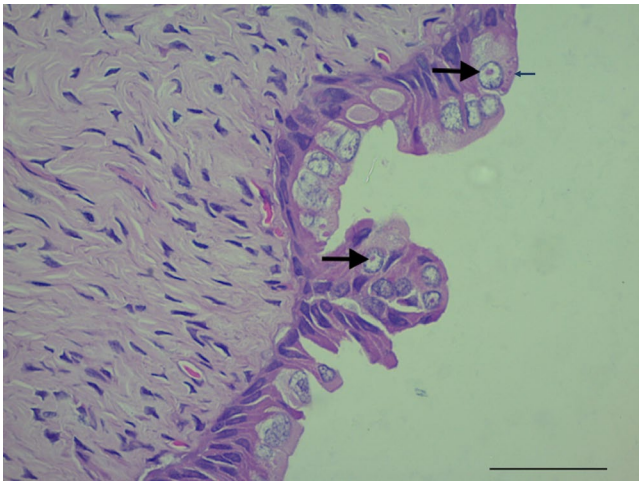

FIGURE 4 Balloon degeneration of the transitional epithelium with intranuclear (large arrow) and intracytoplasmic (low arrow) eosinophilic inclusions (Lentz inclusions) in the urinary bladder tissue (40× obj. lens/Scale bar: 50 μm)

KC812375.1, DQ005128.1). The consensus sequence generated from the N gene amplicon was deposited in GenBank under accession no. MK552116. To determine the phylogenetic position and genotype, the entire H gene was sequenced in RT-PCR assays. Based on the phylogenetic tree analysis, the H gene sequence grouped in the cluster of the Europe 1/South America 1 lineage and specifically fell within a clade composed the genotype detected previously in *T. tetradactyla* (Figure 3). The H gene sequence generated in this study was deposited in GenBank under accession no. MN208239.

The main macroscopic alteration observed in the necropsy was hyperkeratosis and the main pulmonary alteration was found in interlobular septa. The histopathological findings were associated with the presence of eosinophilic intracytoplasmic and intranuclear inclusion bodies in cells of the urinary bladder (Figure 4), kidney, lungs, stomach, duodenum and jejunum.

4 | DISCUSSION

The first evidence of active infection with canine distemper was found in the blood tests, when Lentz inclusions were visible inside leucocytes. The presence of Lentz inclusions in blood cells is considered a definitive diagnosis of canine distemper disease, because these viral inclusion bodies are the result of the cytopathic effect of their replication (Barbosa et al., 2011). In dogs, Lentz inclusions indicate that the infection started in the early stage of canine distemper (Walker, 2009). Despite the lack of information about the pathogenesis of CDV in wild species, particularly in the order Pilosa, this animal had a history of hospitalization and its symptoms started thereafter. Therefore, we believe that our findings correspond to the initial phase of the disease, because they are supported by other findings such as prostration, anorexia, nasal and ocular discharge and diarrhoea. These signs are commonly observed in the early stages of canine distemper (Silva et al., 2005).

Two additional tests were applied to confirm the diagnosis of CDV infection. To obtain a fast diagnosis, a sample of ocular discharge was subjected to a rapid immunochromatographic CDV test, which returned a positive result. Normally, a rapid CDV test is not performed when Lentz inclusions are detected inside cells, because this confirms the diagnosis (Walker, 2009). However, a sensitive testing method was needed because the animal was a non-domestic species.

In addition to the rapid test, N gene amplification was employed to confirm the diagnosis. This target has been used to detect CDV in dogs (Castilho et al., 2007), and has also been used to confirm the diagnosis of CDV infection in *T. tetradactyla*, another member of the family Myrmecophagidae found in the same region (Lunardi et al., 2018). In both cases, RT-PCR has proved useful for a definitive diagnosis. In addition to the similarity of the nucleotide sequence of N gene to other CDV samples in GenBank, the entire H gene sequence indicated that this CDV strain in *M. tridactyla* was similar to other strains diagnosed in dogs in Brazil, including the H gene sequence of CDV reported in *T. tetradactyla* (Lunardi et al., 2018). This finding reinforces our suspicion that the giant anteater of this report suffered from a CDV infection during its hospitalization.

The macroscopic alterations observed during necropsy were attributed to canine distemper disease (Sonne et al., 2009). The giant anteater of this report showed hyperkeratosis, which is described as a skin alteration detected in physical examinations of CDV in dogs, as well as respiratory complications found in CDV positive animals (Sonne et al., 2009). The histopathological findings showed eosinophilic intracytoplasmic and intranuclear inclusion bodies in several tissues. Eosinophilic intranuclear inclusion bodies have been detected previously in *T. tetradactyla* astrocytes and neurons (Lunardi et al., 2018), and constitute the main histopathological evidence of CDV infection in dogs (Sonne et al., 2009).

5 | CONCLUSIONS

This paper offers the first case report of CDV infection in *Myrmecophaga tridactyla*, an endangered species that occurs in South America. This case of CDV infection in a non-carnivorous species underscores the importance of the adoption of biosecurity measures by veterinary centres, veterinary hospitals and zoos, in view of the possibility of interspecies transmission, especially when endangered species are undergoing treatment. Our findings also reinforce the importance of new studies about CDV infections that occur among free-ranging species, aiming to monitor wild species known to be potential hosts and thereby prevent this disease from spreading and threatening the health and wellbeing of domestic and wild animal populations.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL STATEMENT

The authors confirm that they have strictly adhered to ethical policies of the journal, as set forth in its guidelines for authors. No ethical approval was required.

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