UNIVERSIDADE FEDERAL DE MATO GROSSO
PROGRAMA DE RESIDÊNCIA UNIPROFISSIONAL EM MEDICINA VETERINÁRIA

The first report of Canine Morbillivirus infection in Giant anteater (*Myrmecophaga tridactyla*) described in Brazil

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CUIABÁ
2019

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Trabalho de Conclusão de Curso apresentado ao Programa de Residência Uniprofissional em Medicina Veterinária, como requisito para obtenção do título de Especialista em Patologia Clínica Veterinária.

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2019

Dados Internacionais de Catalogação na Fonte.

D286t Debesa Belizário Granjeiro, Melissa.
The first report of Canine Morbillivirus infection in Giant anteater (Myrmecophaga tridactyla) described in Brazil / Melissa Debesa Belizário Granjeiro. -- 2019
13 f. : il. color. ; 30 cm.

Orientadora: Adriane Jorge Mendonça.
TCC (especialização em Medicina Veterinária) - Universidade Federal de Mato Grosso, Faculdade de Agronomia e Medicina Veterinária, Residência Uniprofissional em Medicina Veterinária, Cuiabá, 2019.
Inclui bibliografia.

1. CDV. 2. Hematology findings. 3. Lentz corpuscles. 4. Canine distemper. 5. N gene. I. Título.

Ficha catalográfica elaborada automaticamente de acordo com os dados fornecidos pelo(a) autor(a).

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PRÓ-REITORIA DE ENSINO DE PÓS-GRADUACÃO
PROGRAMA DE RESIDÊNCIA UNIPROFISSIONAL EM MEDICINA VETERINÁRIA
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FOLHA DE APROVAÇÃO

Título: "The first report of Canine Morbillivirus infection in Giant anteater (*Myrmecophaga tridactyla*) described in Brazil **".**

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Monografia defendida e aprovada em 13/02/2019.

Composição da Banca Examinadora:

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Instituição : UNIVERSIDADE FEDERAL DE MATO GROSSO

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The first report of Canine Morbillivirus infection in Giant anteater (*Myrmecophaga tridactyla*) described in Brazil

Running title: CDV in Giant anteater

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Summary
Canine distemper (CD) is a multisystemic and contagious disease caused by Canine Morbillivirus (CM), an enveloped RNA virus that belongs from family *Paramyxoviridae*, subfamily Paramyxovirinae, genus *Morbillivirus*. Canine Morbillivirus easily replicates in epithelial, nervous and lymphoid tissues; it is released in urine, feces, saliva, oral and nasal secretions and its major infection route is from respiratory system. Although in the past the virus was described infecting domestic dogs, new studies have shown that the virus can natural or experimentally infect non-domestic and wild hosts. A recent finding during a hematological exam, that showed Lentz corpuscles inside leukocytes of a Giant anteater (*Myrmecophaga tridactyla*), combined with CM rapid test, RT-PCR and pathological findings confirmed the first report of Canine Morbillivirus in the species and the second report in family *Myrmecophagidae,* order Pilosa, in the Midwestern region of Brazil.

Keywords: CDV; Hematology findings; Lentz corpuscle; Canine distemper; N gene; RT-PCR**.**

Introduction

Canine Morbillivirus (CM) is a member of the genus *Morbillivirus* of the family *Paramyxoviridae* (Amarasinghe *et* al., 2018). CM has a single negative-stranded RNA genoma enclosed in a nucleocapside of helicoidal symmetry surrounded by a lipoprotein envelope (van Regenmortel *et* al., 2000). This virus has epithelial, nervous and lymphoid tropism and is responsible for a lethal disease of dogs known by Canine Distemper (Carré, 1905). Although the virus was first isolated in 1905 (Carré, 1905),

some data affirm that the disease was carried from Peru (South America) to Spain during the 17th century and after to England, Italy and Russia (Blancou, 2004). Their main release occurs by means the urine, feces and nasal secretions and the major infection route is from respiratory system (Barret, 2010).

Among the dog clinical manifestations are depression, malaise, mucopurulent oculonasal discharge, cough, vomiting, diarrhea, fever, snout and cushion hyperkeratosis and neurological signs such as convulsions, vestibular and cerebellar disease, paresis and myoclonus (Silva *et* al., 2007; Greene, 2015).

Beside dogs, the disease and natural host range of CM include certain species of terrestrial carnivores and therefore, it was even proposed to rename the Canine Morbillivirus as "Carnivore Distemper Virus" (Terio & Craft, 2013). Wild members of order Carnivora from numerous families other than *Canidae, Felidae* and *Mustelidae* can be infected by CM. Additionally, the orders Rodentia, Primates, Artiodactyla and Proboscidea has also been shown to be susceptible to infection (Deem *et* al. 2000; Kameo *et* al., 2012; Nikolin *et* al., 2012; Martinez-Gutierrez & Ruiz-Saenz, 2016). In particular, a most recent description reports a CM infection in *Tamandua tetradactyla* a member of the order Pilosa and family *Myrmecophagidae* (Lunardi, *et* al., 2018). In order to contributing to the natural history of the CM, the present study reports the natural infection of *Myrmecophaga tridactyla*, a threatened species in Brazil named the Giant anteater.

Materials and Methods

Animal clinical history and diagnostic procedures

A young Giant anteater rescued for mistreatment was taken to the Veterinary Hospital of Federal University of Mato Grosso by the State Environmental Police to perform a battery of exams for blood (hematologic and biochemical), feces (parasitologic) and images evaluation (echocardiography, ultrasonography and radiography). These procedures revealed anemia, parasitism of *Strongiloidea,* dilated cardiomyopathy, right atrioventricular and pulmonary semilunar valves insufficiency, hydronephrosis and intestinal constipation. The images showed also that the animal had surgical pins of a previous surgery of tibula and fibia. The animal was maintained for few days at the Veterinary Hospital for treatment and it was released back to the Environmental Police with new diet and medicine prescription. One month after released, the animal returned with rectal prolapse caused by the constipation. The Giant anteater was subjected to new exams and to a surgery to fix the rectal prolapse.

Few days from the second hospitalization, the animal was getting prostate, with nasal and ocular secretion (Fig. 1), anorexia and diarrhea. From these symptoms, new sample of blood were taken for hematologic and biochemical exams. During the differential counting of leukocytes structures similar to Lentz corpuscles (Fig. 2) were detected inside lymphocytes and monocytes. Severe anemia, leukocytosis, lymphopenia, low total proteins, with low values of albumin and globulin, and high value of alanine aminotransferase were determined. The animal was not shown any neurological signals and it was free of parasites.

CDV rapid test

Due to the evidence of inclusion corpuscle similar to CM, a swab was collected from ocular mucosa using a collector moistened with saline solution (0,9% NaCl) and applied out in a CM rapid test (Fig. 3) Senspert™ according to the manufacturer instructions (Vencofarma laboratories, Brazil).

Reverse Transcriptase followed by Polymerase Chain Reaction (RT-PCR) and sequencing

In order to detect CM RNA, a sample of sterile blood and urine were collected and submitted for RNA extraction with ReliaPrep™ RNA Cell Miniprep System kit (Promega). Posteriorly, RNA was submitted for Reverse Transcriptase followed by Polymerase Chain Reaction (RT-PCR) using Master Mix AccessQuick ™ RT-PCR System (Promega) with primers designed to amplify 287 bp of the nucleoprotein (N) gene of CM; forward CDV1 (5'-ACA GGA TTG CTG AGG ACC TAT-3') and reverse CDV 2 (5'-CAA GAT AAC CAT GTA AGG TGC-3') (Aguiar *et* al., 2012). Commercial vaccine and nuclease water free were used as positive and negative control. In order to visualize the DNA fragments, agarose gel electrophoresis was performed by 1,5 % of buffer TBE pH 8.4 (TRIS 50 mM, boric acid 67mM, EDTA 1mM) colored with Biotium red gel and subsequent visualization under ultraviolet light in the BIO-RAD Molecular Imager ® ChemiDoc™ XRS+ apparatus with Image Lab™ 4.1 Software reading.

All PCR products with expected size obtained were purified using GFX PCR DNA and Gel Band Purification kit (GE Heakthcare®) and sequenced in an automatic sequencer (ABI DNA Model 3500 Series Genetic Analyzer), according to the manufacturer's protocol. Partial sequences obtained were submitted to BLAST analysis (Altschul *et* al., 1990) to determine similarities to another CM detected previously.

Treatment

The animal was treated with supportive therapy, antibiotics and blood transfusion as the anemia was becoming more severe. Unfortunately, the prognosis was poor, and the animal did not resist.

Necropsy and histopathology development

The necropsy procedure was done soon after animal death. The procedure consisted in the macroscopy and microscopy analysis of the animal body, organ and tissues. Tissues of the brain, lung, heart, liver, spleen, stomach, duodenum, jejunum, urinary bladder, kidneys and bone narrow were collected and were immersed in 10% neutral buffered formalin to fix and after routinely processed, and 2-5 μm histologic sections were obtained for staining Hematoxylin and Eosin stain (Sonne *et* al., 2009).

Results and Discussion

The first evidence of Canine Morbillivirus active infection was seen during the hematological exams, when Lentz corpuscles were seen as viral inclusion inside leukocytes. The visualization of Lentz corpuscles inside blood cells are considered a definitive diagnostic for Canine distemper' disease, as this viral inclusion results as cytopathic effect of their replication (Barbosa *et* al., 2011).

From a total of 1000 leukocytes evaluated, 2% from 3,4% of lymphocytes and 0,8% from 4,8% monocytes had Lentz corpuscles appearing as shaped oval and round format within eosinophilic color, which is characteristic for CM inclusions. In dogs, Lentz corpuscles means that the infection is occurring during the early stage of canine distemper (Walker, 2009).Despite the lack of information about CM pathogeny in wild species, specially occurring in the Order Pilosa, while this case has a history of hospitalization, we believe that our finding corresponds to an initial phase, which is supported by other findings as prostration, anorexia, nasal and ocular secretion and diarrhea. These signs are commonly observed in early stages of canine distemper (Silva *et* al., 2005).

Two additional exams were used to confirm the diagnostic of CM infection. In order to have a fast diagnostic, a sample of ocular secretion was applied for rapid

immunochromatographic CM test which resulted positive (Fig. 3). The CM rapid test normally is not performed when the Lentz corpuscles are observed inside the cells, as this visualization confirm the diagnosis (Walker, 2009), but the need for a sensitive method was necessary because it is a non-domestic species.

Urine and blood samples were collected to confirm the presence of the virus through the RT-PCR for partial amplification of *N* gene of CM and the positive results supports the viremic phase of infection. The *N* gene is used to detect CM in dogs (Castilho *et* al., 2007) and RT-PCR was also used to confirm the diagnostic of CM infection in *T. tetradactyla*, another member of Myrmecophagidae family (Lunardi *et* al., 2018). In both cases, RT-PCR has proved useful for over definitive diagnosis. Partial sequence of *N* gene generated from positive samples from blood and urine were analyzed and proved to be identical (100%) to each other and to 98-99% similar to other sequences of CM available in GenBank. The consensus sequence generated from the amplicons was deposited in the GenBank under the accession number MK552116.

Macroscopy alterations observed during necropsy were related to CD disease (Sonne *et* al., 2009). In the present report the animal showed hyperkeratosis and evidence of lobular septa was the main pulmonar alteration. Hyperkeratosis is mentioned as being a dermatologic alteration observed during the physical examination of CD disease in domestic animals, as well as the respiratory complications in positive animals (Sonne *et* al., 2009).

Histopathologic findings were associated with the presence of eosinophilic intracytoplasmic and intranuclear inclusion corpuscles in urinary bladder (Fig. 4), kidney, lung (Fig. 5), stomach, duodenum and jejunum tissues' cells. Eosinophilic intranuclear inclusion corpuscles was previously visualized in astrocytes and neurons of *T. tetradactyla* (Lunardi *et* al., 2018) and is the principal histopathologic evidence of CM infection in dog tissues (Sonne *et* al., 2009).

The present report is the first case of infection by CM in *M. tridactyla*, a threatened species occurring in the American continent. This case of CM infection in non-carnivorous species highlights the importance of biosecurity measures adopted in veterinary centers, veterinary hospitals and zoological parks due to the possibility of interspecies' transmission, especially when endangered species are maintained for treatment. The findings also reinforce the importance of novel studies regarding CM occurring in free-ranging species in order to follow up species know to be potential wild hosts, avoid disease emergence and domestic and wild population treat.

Acknowledgments

We gratefully acknowledge all the vets involved with the animal's assistance, since the health care to necropsy staff members, the Federal Agency for the Support and Improvement of Higher Education (CAPES) for scholarships awarded to Mayara L. Kavasaki, the Ministery of Education of Brazil for scholarships awarded to Melissa D. B. Granjeiro, Marisol A. de Barros, Carolina Fontana, Mateus de A. Bianchini, Aneliza de O. Souza, Amanda R. G. L. O. Santos e Lucas A. D. Pavelegini and to the National Council for Scientific and Technological Development (CNPQ) for Scientific Productivity Grants awarded to Daniel M. de Aguiar. This case report was supported by the Veterinary Hospital of Federal University of Mato Grosso, Cuiaba, Brazil.

Conflict of interest statement

The authors declare that have no conflicting interests.

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Appendices

Figure 1. Ocular and nasal secretion and hyperkeratosis.

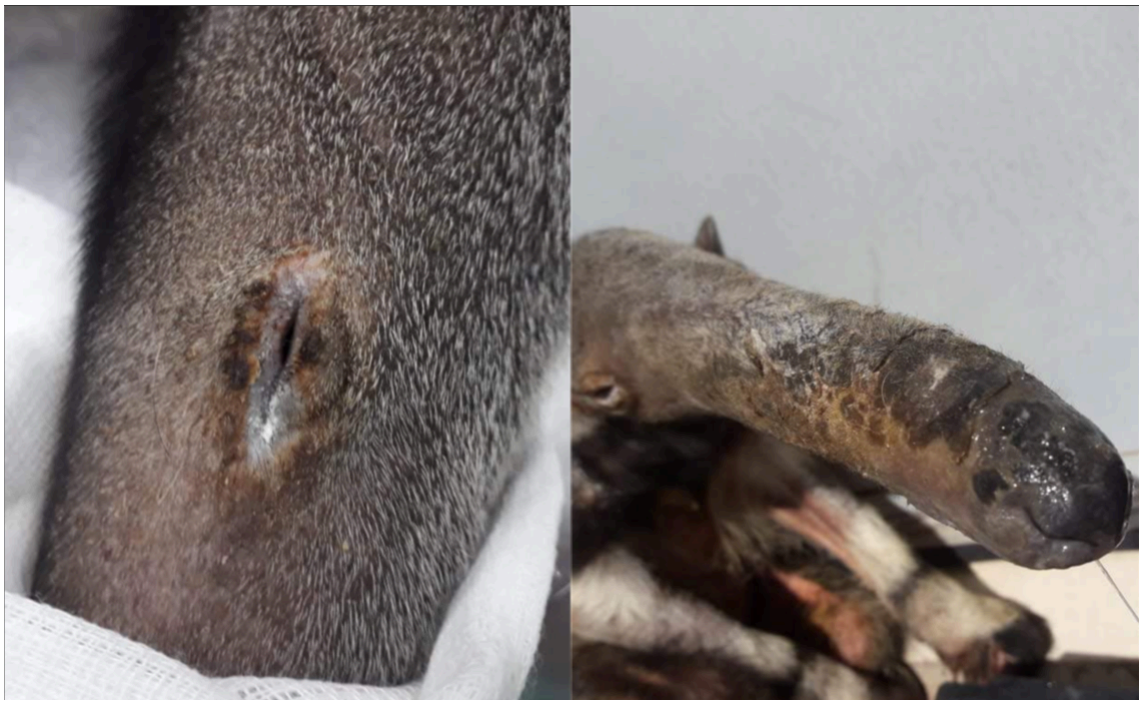

Figure 2. Lentz corpuscles inside leukocytes (Obj. 100x).

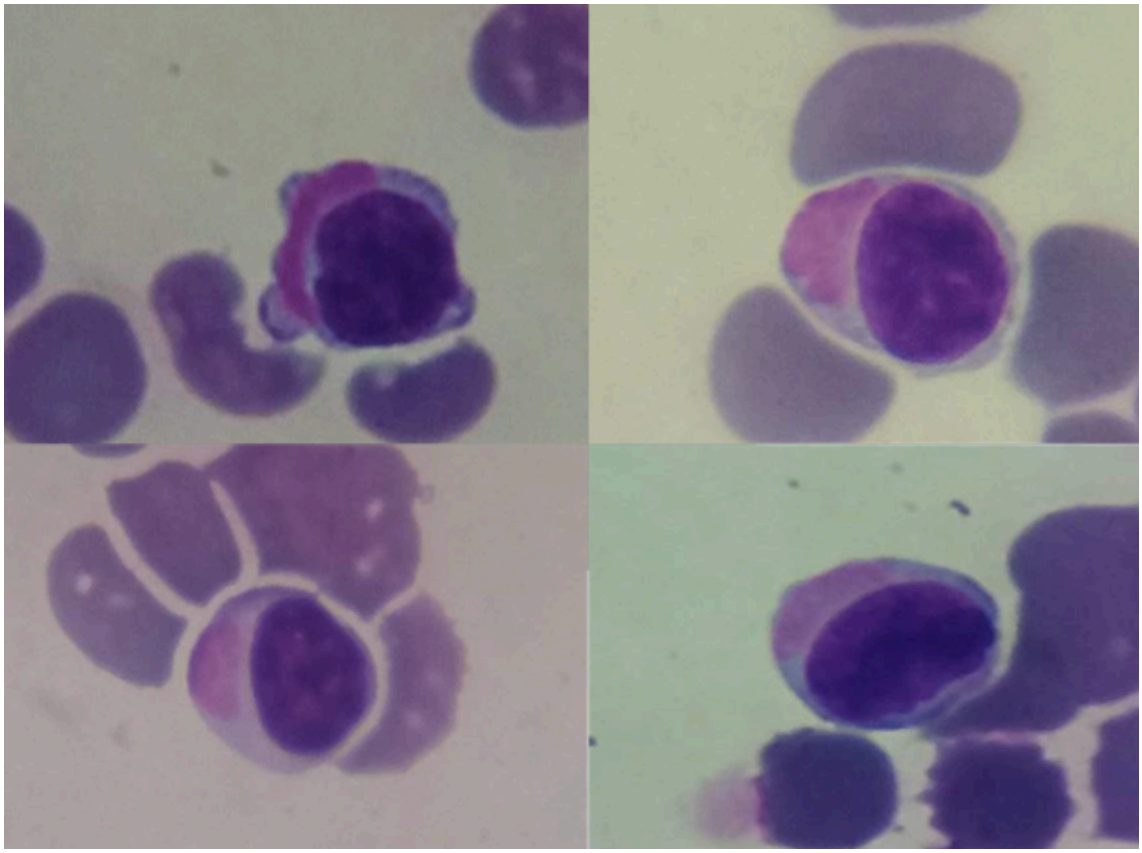

Figure 3. CM rapid test showing the control and the positive line.

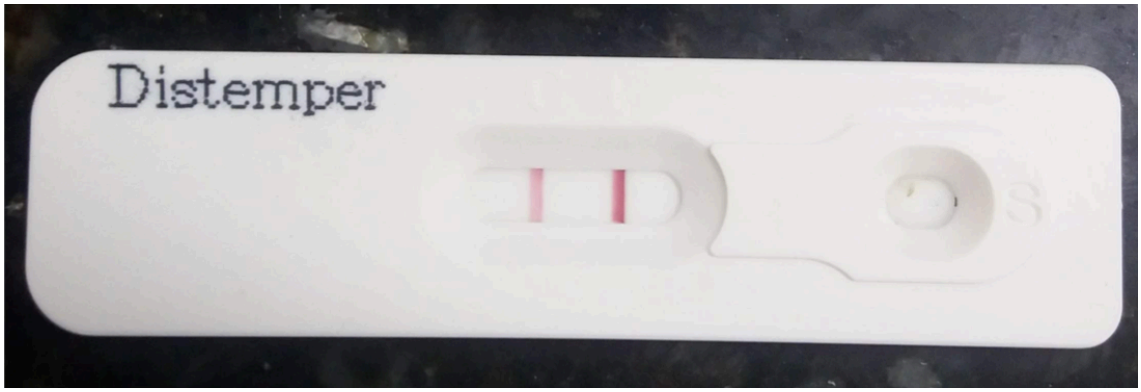

Figure 4. Balloon degeneration of the transitional epithelium with intranuclear eosinophilic inclusion corpuscles (Lentz corpuscles) in the urinary bladder tissue (Obj. 40x/Scale bar 50 micrometers).

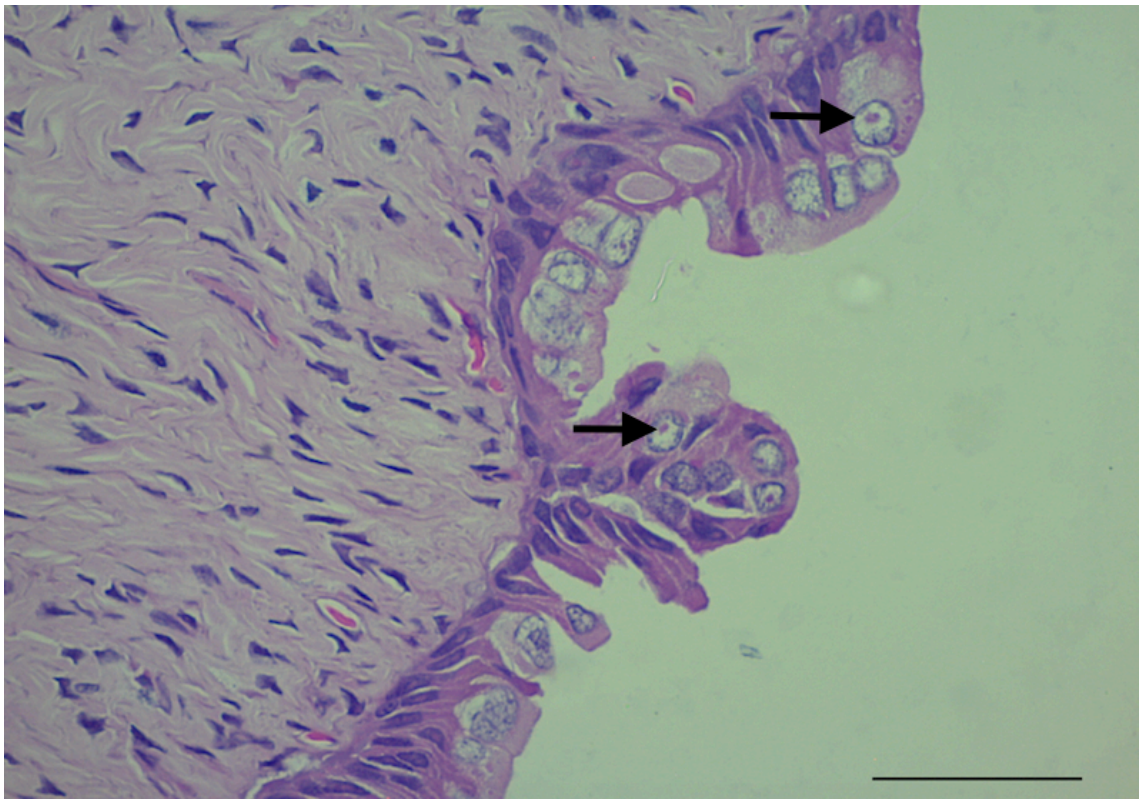

Figure 5. Intranuclear eosinophilic inclusion corpuscles (Lentz corpuscles) in the lung tissue (Obj. 40x/Scale bar 50 micrometers). Balloon degeneration of the transitional epithelium with intranuclear eosinophilic inclusion corpuscles (Lentz corpuscles) in the urinary bladder tissue (Obj. 40x/Scale bar 50 micrometers).