http://dx.doi.org/10.1590/1678-4162-12584

Arq. Bras. Med. Vet. Zootec., v.74, n.5, p.833-840, 2022

Investigation of *Chlamydia* sp., *Morbillivirus* sp., *Parvovirus* sp., *Leishmania* sp. and *Alphacoronavirus* sp. in captive giant anteaters (*Myrmecophaga tridactyla*)

[*Investigação de* Chlamydia *sp*., Morbillivirus *sp*., Parvovirus *sp*., Leishmania *sp*. *e* Alphacoronavirus *sp. em tamanduás-bandeira* (Myrmecophaga tridactyla) *em cativeiro*]

H.C.C.P. *Tessari*[1], G.R. *Paludo*[2], M.C. *Scalon*[3], J.M.M. *Silva*[4], L.Q.L. *Hirano*[2]

[1]Graduate, Universidade de Brasília, Brasília, DF, Brasil
[2]Universidade de Brasília, Brasília, DF, Brasil
[3]Veterinarian, Universidade de Brasília, Brasília, DF, Brasil
[4]Veterinarian, Instituto Estadual de Floresta de Minas Gerais, Belo Horizonte, MG, Brasil

ABSTRACT

This research aimed to investigate the occurrence of *Chlamydia* sp., *Morbillivirus* sp., *Parvovirus* sp., *Leishmania* sp. and *Alphacoronavirus* sp. in captive giant anteaters. Blood and fecal samples were taken from 16 animals in institutions from the states of Minas Gerais, Bahia and Distrito Federal, which had been in captivity for at least a year. A commercial rapid chromatographic immunoassay test was used for detecting coronavirus and parvovirus antigens, in addition to antibodies against leishmaniasis, all results being negative. In the case of the test for antibodies against distemper, four (4/16; 25%) anteaters had an average titration, two (2/16; 12.5%) a low titration and ten (10/16; 62.5%) were non-reactive. Using the DOT-ELISA (dot blotting) method for detection of immunoglobulin G, only one specimen obtained a 1:40 titration. For the polymerase chain reaction tests for *Leishmania* and *Chlamydia*, all samples were negative.

Keywords**:** Xenarthra, infectious diseases, distemper

RESUMO

Esta pesquisa teve como objetivo investigar a ocorrência de Chlamydia *sp.*, Morbillivirus *sp.*, Parvovirus *sp.*, Leishmania *sp. e* Alphacoronavirus *sp. em tamanduás-bandeira cativos. Foram colhidas amostras de sangue e fezes de 16 animais em instituições dos estados de Minas Gerais, Bahia e Distrito Federal, que estavam em cativeiro há pelo menos um ano. Um teste comercial rápido de imunoensaio cromatográfico foi usado para detectar antígenos de coronavírus e parvovírus, além de anticorpos contra a leishmaniose, sendo todos os resultados negativos. No caso do teste para anticorpos contra a doença, quatro (4/16; 25%) tamanduás apresentaram titulação média, dois (2/16; 12,5%) uma titulação baixa e dez (10/16; 62,5%) não foram reativos. A partir do método DOT-ELISA (dot blotting) para detecção de imunoglobulina G, apenas um espécime obteve uma titulação de 1: 40. Para os testes de reação em cadeia da polimerase para* Leishmania *e* Chlamydia*, todas as amostras foram negativas.*

Palavras-chave: Xenarthra, doenças infecciosas, cinomose

INTRODUCTION

In Brazil, the giant anteater (*Myrmecophaga tridactyla* Linnaeus, 1758) is part of the group of 110 taxa of endangered mammals (Young *et al*., 2003), with a conservation status classified as "vulnerable" (Miranda *et al*., 2014). The main threats to the species include being run over, habitat destruction, predatory hunting, fires, and attacks by dogs (Braga and Santos, 2009).

The extinction and fragmentation of ecosystems (Jorge *et al*., 2010) result in the proximity of native fauna to anthropic environments. This situation may impact the lives of wild animals, such as contagion by diseases whose reservoirs are domestic species (Dobson and Carper, 1996).

Corresponding author: hedermy.cerqueira@gmail.com
Submitted: January 5, 2022. Accepted: April 20, 2022.

Furthermore, many of the emerging zoonoses arose from changes in the interaction of fauna promoted by humans, which resulted in the contact of pathogens with new hosts (Chang, 2020).

There is little research on the effects of proximity between domestic animals and wild specimens, especially with the species *M. tridactyla* (Miranda, 2008). In the case of free-living mammals, Vilela and Guedes (2014) highlighted the impact of the presence of domestic dogs in protected areas, as in addition to preying on and competing with native species for territory and food, they can transmit infectious and contagious diseases.

When it comes to xenarthras, distemper was reported in seven two-toed sloths (*Choloepus didactylus*) by Sheldon *et al.* (2017), with diagnosis made by polymerase chain reaction (PCR) test and gene sequencing. A positive result was also found in an anteater (*Tamandua tetradactyla*) by Lunardi *et al*. (2018), from post-mortem PCR tests, immunohistochemical assay and gene sequencing. In *M. tridactyla*, Granjeiro *et al*. (2020) reported the presence of Lentz inclusions in an animal from Mato Grosso state, that died with positive results also in the rapid test for canine distemper antigen, PCR of gene N and phylogenetic analysis of gene H.

Additionally, for giant anteaters, there are reports of some infectious agents occurring in domestic animals, such as *Toxoplasma gondii* (Ferrari, 2016) and bacteria of the genera *Brucella* and *Leptospira* in free-living animals (Miranda, 2008), in addition to influenza virus A in captive specimens (Nofs *et al.*, 2009). Cases of interspecific transmission of infectious agents highlight the importance of biosecurity measures and monitoring of wild animals under human care (Granjeiro *et al*., 2020). Determining the health status of captive individuals is essential in the case of threatened species, since it is possible to include them in reintroduction, translocation, or population invigoration programs.

Due to the scarcity of information about the occurrence of pathogens in anteaters, this study aimed to investigate the occurrence of *Chlamydia* sp., *Morbillivirus* sp., *Parvovirus* sp., *Leishmania* sp. and *Alphacoronavirus* sp. in captive *M. tridactyla*.

MATERIAL AND METHODS

The methodology of this study was approved by the Animal Use Ethics Committee of the University of Brasília (CEUA-UnB), protocol no. 55/2019, and the Biodiversity Information and Authorization System (SISBIO), no. 68635-4. Blood and feces samples from 16 giant anteaters (*M. tridactyla*) were used, seven being males and nine females. One of the specimens was born in captivity, while the others were received as puppies by the institutions and kept under human care for at least a year.

The samples were collected from seven animals from the TamanduAsas rehabilitation project and two from the Parque do Sabiá Municipal Zoo, both in the municipality of Uberlândia, MG. Four animals were under the care of the Fundação Jardim Zoológico de Brasília, DF, and three were from Parque Vida Cerrado, in Luís Eduardo Magalhães, BA (Table 1).

The anteaters were captured and immobilized with the aid of a catching net for the application of drugs for chemical restraint, with variation of the protocols according to what was established by each institution. The physical examination included inspection, palpation, and auscultation of the anteaters, which had a body score between 2.5 and 3 (scale 1 to 5), mean rectal temperature of 33 °C, normohydrated, well-formed stools, with no change in coat or lesions to the skin. Blood collection for blood count and serum biochemistry was also performed.

Blood was punctured from the cephalic (Figure 1A), lateral saphenous or femoral vein, using a 5 mL syringe and 25 × 0.70 mm disposable needle. The samples were placed in tubes containing ethylenediamine tetraacetic acid (EDTA) and a tube without anticoagulant. In the case of feces, samples were collected fresh directly in the enclosure when defecation was witnessed, and by rectal swab (Figure 1B), with storage in a universal collector flask and Eppendorf tubes containing phosphate-buffered saline (PBS), respectively.

Table 1. Information of sex, age (approximate) and institution responsible for the specimens of *Myrmecophaga tridactyla* used in the study

Individual	Sex	Weigth	Age	Origin
1 – TA1	Female	15kg	2 years old	TamanduAsas, Uberlândia, MG, Brazil
2 – TA2	Female	19kg	2 years old	
3 – TA3	Female	7kg	1 year old	
4 – TA4	Male	26kg	1 year old	
5 – TA5	Male	37kg	2 years old	
6 – TA6	Female	28kg	2 years old	
7 – TA7	Male	40kg	1 year old	
8 – PS1	Male	38kg	1 year old	Parque do Sabiá, Uberlândia, MG, Brazil
9 – PS2	Male	37kg	13 years old	
10 – ZB1	Female	30kg	2 years old	Jardim Zoológico de Brasília, DF, Brazil
11 – ZB2	Female	30kg	1 year old	
12 – ZB3	Female	32kg	19 years old	
13 – ZB4	Male	35kg	2 years old	
14 – VC1	Male	37kg	10 years old	Parque Vida Cerrado, Luís Eduardo Magalhães, BA, Brazil
15 – VC2	Female	35kg	10 years old	
16 – VC3*	Female	26kg	1 year old	

*Animal born in captivity. The other specimens were born in the wild and were rescued.

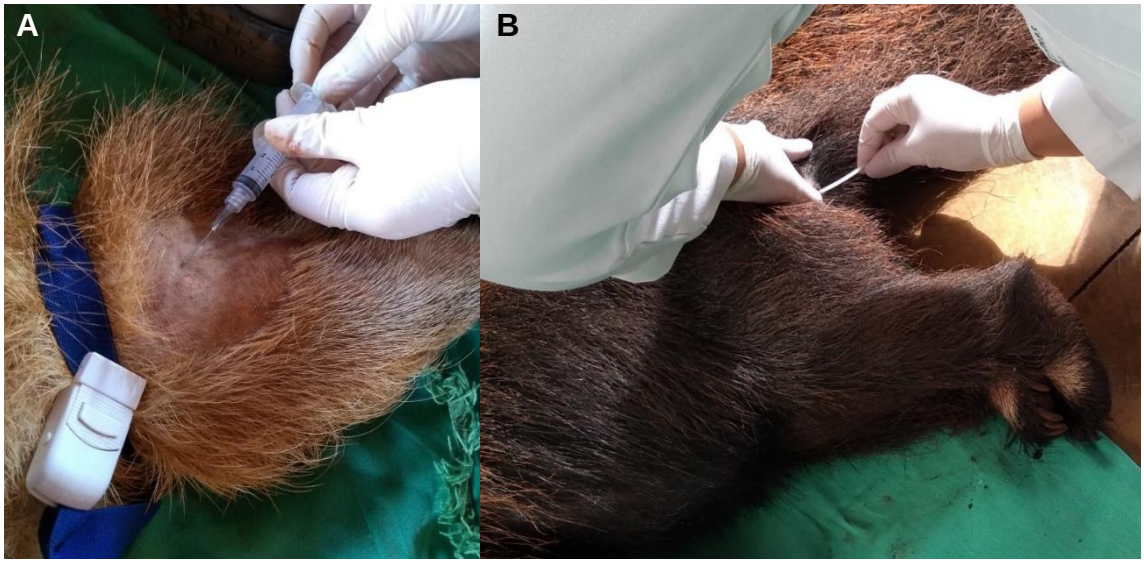

Figure 1. Collection of biological samples in *Myrmecophaga tridactyla*. A: blood collection in the right cephalic vein. B: rectal swab sample collection

Immediately after the collection of biological samples, rapid immunochromatographic tests (Alere S.A. – Bionote, Belo Horizonte, MG, Brazil) were performed to detect antibodies against distemper and *Leishmania infantum* (rK9, rK39 and rK26 antigens), using whole blood sampling. Additionally, the same types of tests were also used to investigate the presence of parvovirus and canine coronavirus antigens (Test Corona/Parvo Ag®, Alere S.A. – Bionote, Belo Horizonte, MG, Brazil) in stool samples and rectal swabs. All procedures performed in this step followed the manufacturer's laboratory recommendations.

The molecular testing began with the extraction of deoxyribonucleic acid (DNA) from blood samples stored in EDTA tubes and feces in PBS

tubes. For that, a commercial kit was used (Illustra Blood genomicPrep Mini Spin Kit®, GE Healthcare, Piscataway, NJ, USA) and the procedures followed the manufacturer's recommendations. The material obtained was stored in Eppendorf tubes in a freezer at −20 °C until the time of performing the PCR tests.

All samples were submitted to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) test to verify the extraction quality, the integrity of the obtained DNA and the absence of PCR inhibitors. The mixture for this first step consisted of 16.55 µL of water; 2.5 µL of PCR buffer; 1.5µL of magnesium chloride (50mM $MgCl_2$/µL); 1µL of each oligonucleotide (Table 2) (10pmol/µL GAPDH F and GAPDH R); 0.2µL of phosphated deoxyribonucleotides (25mM/µL DNTP); 0.25µL of Taq DNA polymerase (5U/µL; Invitrogen®, Vila Guarani, SP, Brazil); and 2µL of the sample (DNA), with a final volume of 25µL. After preparation, the compound was amplified by denaturation steps. For this, initially a temperature of 95°C for 5 min was used, followed by 40 cycles of amplification at 94°C for 30s, 52°C for 1 min, 72°C for 1 min and final extraction at 72°C for 5 min, as described by Birkenheyer *et al*. (2003).

Table 2. Sequence of oligonucleotides, gene of origin, size of amplification products and references of polymerase chain reactions with deoxyribonucleic acid samples used in the research

Primer	Sequence 5'-3'	Gene	Size	Reference
GAPDH	**GAPDH F:** CCTTCATTGACCTCAACTACAT **GAPDH R:** CCAAAGTTGTCATGGATGACC	GAPDH GAPDH	400 pb	Birkenheyer *et al*. (2003)
Leishmania 18S / R221 R332	GGTTCCTTTCCTGATTTACG GGCCGGTAAAGGCCGAATAG	18S	600 pb	Van Eys *et al*. (1992)
Leishmania cinetoplasto/ LBW LFW	**LBW:** CCGCCCCTATTTTACACCAACCCC **LFW:** GGGTAGGGGCGTTCTGCGAA	LBW LFW	120 pb	Disch *et al*. (2003)
MOMP / ChLS A ChLS B	**ChLS A:** CAGGATATCTTGTCTTGTCTGGC TTTAA **ChLS B:** GCAAGGATCGCAAGGATC	MOMP MOMP	260 pb	Raso *et al*. (2006); Silva (2013)

After performing the PCR test to confirm the integrity of the DNA and the absence of inhibitors, each sample was submitted to the protocol for detection of *Leishmania* and *Chlamydia*. The Leishmania 18S PCR mixture was composed of 16µL of water; 2.5µL of PCR buffer; 1.5µL of $MgCl_2$ (50mM/µL); 1µL of primers R221 and R332 (10pmol/µL); 0.2µL of 25µM DNTP (25mM/µL); 0.2µL of Taq DNA polymerase; and 2.5µL of the sample (DNA) with a final volume of 25µL. The Leishmania kinetoplast PCR mixture was composed of 17.35µL of water; 2.5µL of PCR buffer; 0.75µL of $MgCl_2$ (50mM/µL); 1µL of LFW and LBW primers; 0.2µL of 25µM DNTP (25mM/µL); 0.2µL of Taq polymerase; and 2µL of the sample (DNA) with a final volume of 25µL. Amplification was performed in three steps, starting with denaturation at 94°C for 5 min, followed by 40 cycles of amplification, with denaturation at 94°C for 30 s, annealing at 63°C for 30 s and extension at 72°C for 5 min, as indicated by Disch *et al*. (2003).

The mix for *Chlamydia* was composed of 17.05µL of water; 2.5µL of PCR buffer; 1.0µL of $MgCl_2$ (50mM/µL); 1.0µL of Chls A and Chls B primers; 0.2µL of 25µM DNTP (25mM/µL); 0.2 µL of Taq polymerase (Invitrogen®, Vila Guarani, SP, Brazil); and 2µL of the sample (DNA) in a final volume of 25µL. Then, the mixture was submitted to the amplification protocol, starting from initial denaturation at 94°C for 10 min, followed by 34 cycles of

denaturation at 94°C for 1 min, annealing at 54°C for 1 min and extension at 72°C for 1 min. Finally, the mixture underwent final extension at 72°C for 4 min and cooling at 4°C, according to the methodology of Silva (2013).

All PCR tests were performed in a thermocycler device (Bio-Rad® C1000TM Thermal Cycler, Hercules, CA, USA) and their products were visualized on a 2% agarose gel by electrophoresis. To assess the size of the bands of the amplified compound, the gel was stained with ethidium bromide (Vetec, Sigma-Aldrich®, St. Louis, MO, USA) and photographed in a transilluminator (UV Transilluminator UVP®, Upland, CA, USA) under ultraviolet (UV) light. In all tests, a molecular marker (Invitrogen®, Vila Guarani, São Paulo, Brazil) was used, with a molecular weight range of 100bp. The negative control was carried out with sterile water devoid of DNA (autoclaved Milli-Q), and the positive control with samples from dogs diagnosed and confirmed by the Laboratory of Microbiology and Molecular Pathology of the Veterinary Hospital of the University of Brasília and vaccine for detection of *Chlamydia*.

The samples were analyzed for IGG detection against distemper and parvovirus using the DOT-ELISA (dot blotting) technique. Results were interpreted as follows: score zero = negative; score 1 and 2 = low positive, score 3 = medium positive, and score 4 or greater = high positive. Subsequently, using the indirect immunofluorescence reaction (IFAT) method, the results were obtained in the table of conversion of titers to 1:40; 1:80; 1:160 or 1:320. The laboratory analysis of ELISA was performed by the laboratories TECSA® (Animal Health Technology Laboratory, Belo Horizonte, MG, Brazil) and CIVET (Uberlândia, MG, Brazil) with blood serum.

RESULTS

For the investigated infectious agents, all 16 animals showed non-reactive results for coronavirus and parvovirus antigens, as well as for antibodies against *Leishmania*. All animals tested were positives for GAPDH and negative for Leishmania 18S, Leishmania kinetoplast and *Chlamydia* sp. in PCR. None of the samples evaluated were reactive for parvovirus virus in the DOT-ELISA test.

In the rapid test for detection of antibodies against distemper, four (4/16; 25%) animals had a medium titer marking, two (2/16; 12.5%) had a low titer and ten (10/16; 62.5%) were non-reactive (Table 3). When comparing the rapid test with the laboratory test, the agreement was 68.75% (11/16), with 31.25% (5/16) of the samples showing false-positive results by the rapid test.

Table 3. Results of the rapid immunochromatographic test and the enzyme-linked immunosorbent assay (DOT-ELISA) for detection of antibodies against distemper in captivity *Myrmecophaga tridactyla*

Sample	Quick test	DOT-ELISA IGG
1 – TA1	Low titration	Negative
2 – TA2	Average titration	Negative
3 – TA3	Negative	Negative
4 – TA4	Negative	Negative
5 – TA5	Negative	Negative
6 – TA6	Negative	Negative
7 – TA7	Low titration	Negative
8 – ZB1	Negative	Negative
9 – ZB2	Negative	Negative
10 – ZB3	Negative	Negative
11 – ZB4	Negative	Negative
12 – PS1	Negative	Negative
13 – PS2	Negative	Negative
14 – VC1	Average titration	Negative
15 – VC2	Average titration	Low positive
16 – VC3	Average titration	Negative

Only sample VC2 was positive by DOT-ELISA (1/16, 6.25%), with a score of 1 and a 1:40 titer for canine distemper virus. The VC2 animal, which showed medium titration results in the rapid immunochromatographic test, was received by the Brasília Zoo as a puppy, without clinical signs of disease, and at 1 year of age was transferred to the Parque Vida Cerrado, in Bahia. The three animals of the institution are housed in neighboring rooms separated by a wire mesh and showed positive results in the rapid test. Animals VC1 and VC2 were mated in 2018, resulting in the birth of VC3.

DISCUSSION

The scarcity of data about the sanitary conditions of wildlife species represents one of the challenges for conservation strategies, as this information can be used to estimate the risk of extinction (Radchuk *et al*., 2016). Surveys about the occurrence of agents causing diseases such as distemper, parvovirus, leishmaniasis and chlamydiasis in specimens of *M. tridactyla* kept under human care are rare or non-existent, this being the first study in which rapid and comparative laboratory tests were carried out to investigate antibodies against the morbillivirus in the species.

Of the six anteaters positive in the rapid antibody detection test for distemper, only one confirmed a reaction in the DOT-ELISA IGG laboratory test. This specific anteater did not show clinical signs of disease and there are reports that stray dogs sporadically invade the park. Studies in this regard are important since Lunardi *et al*. (2018) followed the occurrence of death of giant anteaters by distemper with signs similar to those presented by canids, but there is still a lack of further information about the morbidity and mortality of the disease for the species and this is the first investigation of this disease in xenarthras from the Distrito Federal, and Bahia and Minas Gerais states.

The *M. tridactyla* positive for antibodies against distemper in the laboratory test was rescued as a puppy and has been in captivity for 10 years, so it is not possible to determine when and where its contact with the morbillivirus occurred. According to Monti (2004), dogs that were experimentally exposed to canine distemper virus presented antibody titers on the ninth day after exposure and a study showed that the duration of immunity against the disease can extend for a period greater than 9 years (Angelico and Pereira, 2012).

The use of rapid tests in veterinary medicine is interesting because of the routine possibility of immediate results, especially for free-living animals whose management depends on chemical restraint, such as those of the species *M. tridactyla*. Additionally, although the rapid immunochromatographic test used had a sensitivity and specificity of 100% for dogs (ALERE® Cinomose Ac Test Kit, Bula do teste, Bionote Inc, Gyeonggi-do, 2013). In this study false-positive results were observed in 31.25% of the samples from anteaters. Therefore, confirmation by laboratory tests for *M. tridactyla* is interesting, especially for positive results in the rapid test.

The genus *Leishmania* has been described in several wild mammal species and the xenarthras are known as protozoan reservoirs. There are reports of detection of this pathogen in sloths, armadillos, and anteaters, but without observation of clinical manifestations (Araújo *et al*., 2012; Roque and Jansen, 2014; Espinosa *et al*., 2016). Although the samples tested had negative results in this study, as it is an important zoonosis for public health, the investigation of *Leishmania* sp. was relevant since there are records of the occurrence of canine leishmaniasis in cities close to the institutions where the anteaters are housed (Leite, 2014; Sousa *et al*., 2015; Reis *et al*., 2020).

In the case of *Alphacoronavirus* 1, this is the first investigation of the occurrence of a Coronaviridae virus in xenarthras. Because of recent emerging zoonotic diseases, mainly because of the Covid-19 pandemic, the Coronaviridae family is one of the three viral families closely monitored on the planet. There are four Coronaviridae genera, *Alphacoronavirus* (αCoV), *Betacoronavirus* (βCoV), *Gammacoronavirus* (γCoV) and *Deltacoronavírus* (δCoV). Species of the first genus mainly infect domestic dogs and cats, but there are species that infect pigs and humans, and a study has already detected the circulation of *Alphacoronavirus* in wild bats, mice, and rabbits in France (Monchatre-Leroy *et al*., 2017; Stout *et*

al., 2020). Due to the high mutation potential of coronaviruses, including as zoonotic agents, and the previous reports of infectious diseases from dogs in anteaters (Sheldon *et al.*, 2017; Lunardi *et al*., 2018; Granjeiro *et al*., 2020), investigations such as the one in the present study are important for their health.

Chlamydia is a bacterium that causes reproductive disturbance in several species of domestic mammals and stands out for its zoonotic potential and for causing economic damage (Lima *et al.*, 2019). The Institute for Research on the Conservation of Anteaters in Brazil (IPCTB) described an individual of the species *M. tridactyla* from captivity in a zoo in the state of São Paulo, with a positive diagnosis for the disease after miscarriage (Miranda, 2008). All animals sampled in this study were negative for *Chlamydia*, similarly to the research carried out by Miranda (2008) with free-living specimens.

Understanding how diseases are transmitted, how they progress and how to prevent their spread is important to ensure the sanitary health of wild animals under human care. Some anteaters in this research are part of rehabilitation projects; therefore, the performance of sanitary tests is essential to assess the health of animals and reduce the chances of transmission of infectious agents to free-living populations. The data obtained in this study can support decisions to be implemented in the National Action Plan (PAN) for conservation of the species *M. tridactyla*.

CONCLUSION

The specimens of *M. tridactyla* from captivity showed negative results for the investigation of *Chlamydia* sp., *Morbillivirus* sp., *Parvovirus* sp., *Leishmania* sp. and *Alphacoronavirus* sp. in the tests performed. Six anteaters obtained a positive reaction in the rapid immunochromatographic test for detection of antibodies against *Morbillivirus*, but only one was confirmed by the DOT-ELISA technique, with an occurrence of 31.25% of false-positive results.

REFERENCES

BIRKENHEUER, A.J.; LEVY, M.G.; BREITSCHWERDT, E,B. Development and evaluation of a seminested PCR for detection and differentiation of *Babesia gibsoni* (Asian genotype) and *B. canis* DNA in canine blood samples. *J. Clinic. Microbiol.*, v.41, p.4172-4177, 2003.

ANGÉLICO, S.M.R.; PEREIRA, C.A.D. Novas diretrizes vacinais para cães – uma abordagem técnica e ética. *Rev. Clín. Vet.*, v.17, p.62-81, 2012.

ARAÚJO, V.A.L.; BOITÉ, M.C.; CUPOLILLO, E. *et al*. Mixed infection in the anteater *Tamandua tetradactyla* (Mammalia: Pilosa) from Pará State, Brazil: *Trypanosoma cruzi*, *T. rangeli* and *Leishmania infantum. Parasitology*, v.140, p.455-460, 2012.

BRAGA, F.G.; SANTOS, R.E.F. Relações entre a fauna e o fogo. In: SOARES, R.V.; BATISTA, A.C.; NUNES, J.R.S. *Incêndios florestais no Brasil:* o estado da arte. Curitiba: Produção Independentes, 2009. cap.7, 246p.

CHANG, J. China needs to establish a directory system of wildlife prohibited from hunting, breeding, transferring or eating urgently. *Derecho Anim.*, v.11, p.59-64, 2020.

DISCH, J.; MACIEL, F.C.; OLIVEIRA, M.C.D.E.; ORSINI, M.; RABELLO, A. Detection of circulating *Leishmania chagasi* DNA for the non-invasive diagnosis of human infection. *Trans. R. Soc. Trop. Med. Hyg.*, v.97, p.391-395, 2003.

DOBSON, A.P.; CARPER, E.R. Infectious diseases and human population history. *BioScience*, v.46, p.115-126, 1996.

ESPINOSA, O.A.; SERRANO, M.G.; CAMARGO, E.P. *et al*. Na appraisal of the taxonomy and nomenclature of trypanosomatids presently classified as *Leishmania* and *Endotrypanum*. *Parasitology*, v.145, p.430-442, 2016.

FERRARI, M.V. *Isolamento e caracterização genética de Toxoplasma gondii em Myrmecophaga tridactyla (Linnaeus, 1758).* 2016. 47f. Dissertação (Mestrado em Genética Animal e Evolução) - Universidade Estadual Paulista, São José do Rio Preto, SP.

GRANJEIRO, M.D.B.; KAVASAKI, M.L.; MORGADO, L.A.D.P. *et al*. First report of a canine morbillivirus infection in a giant anteater (*Myrmecophaga tridactyla*) in Brazil. *Vet. Med. Sci.*, v.6, p.606-611, 2020.

JORGE, R.S.P.; ROCHA, F.L.; MAY JÚNIOR, J.A.; MORATO, R.G. Ocorrência de patógenos em carnívoros selvagens brasileiros e suas implicações para a conservação e saúde pública. *OEcologia Australis*, v.14, p.686-710, 2010.

LEITE, M.D.X. *Leishmaniose visceral canina* – distribuição e delimitação de área de risco em Barreiras, Bahia. 2014. 123f. Dissertação (Mestrado em Ciência Animal nos Trópicos) - Universidade Federal da Bahia, Salvador, BA.

LIMA, A.M.C.; SANTOS, M.D.; DAMASCENO, M.S. *et al*. Inquérito sorológico da infecção por *Chlamydophila abortus* em ovinos pertencentes à mesorregião dos Sertões Cearenses. *Rev. Bras. Reprod. Anim.*, v.43, p.525, 2019.

LUNARDI, M.; DAROLD, G.M.; AMUDE, A.M. *et al*. Canine distemper virus active infection in order Pilosa, Family Myrmecophagidae, species *Tamandua tetradactyla*. *Vet.Microbiol.*, v.220, p.7-11, 2018.

MIRANDA, F.; BERTASSONI, A.; ABBA, A. M. *Myrmecophaga tridactyla*: the IUCN red list of threatened species, 2014. Available in: http://dx.doi.org/10.2305/IUCN.UK.2014-1.RLTS.T14224A47441961.en. Accessed in: 15 Mar. 2022.

MIRANDA, F.R. *Pesquisa de anticorpos contra bactérias do gênero Brucella spp., Leptospira spp., Chlamydophila spp. em tamanduás-bandeira (Myrmecophaga tridactyla, Linnaeus, 1758), da RPPN SESC Pantanal, Parque Nacional da Serra da Canastra (PNSC) e Parque Nacional das Emas (PNE)*. 2008. 118f. Dissertação (Mestrado em Ecologia Aplicada) - Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, SP.

MONCHATRE-LEROY, E.; BOUÉ, F.B.; BOUCHER, J. *et al*. Identification of Alpha and Beta coronavirus in wildlife species in France: bats, rodents, rabbits, and hedgehogs. *Viruses*, v.29, p.364, 2017.

MONTI, F. S. *Anticorpos contra o virus da cinomose em cães vacinados em diferentes estabelecimentos da área urbana do município de Viçosa/MG*. 2004. 67f. Dissertação (Mestrado em Medicina Veterinária) - Universidade de Viçosa, Viçosa, MG.

NOFS, S.; ELDAIM, M.A.; THOMAS, K.V. *et al*. Influenza virus A (H1N1) in giant Anteaters (*Myrmecophaga tridactyla*). *Emerg. Infect. Dis.*, v.15, p.1081-1083, 2009.

RADCHUK, V.; OPPEL, S.; GROENEVELD, J. *et al*. Simples ou complexo: impacto relativo da disponibilidade de dados e propósito do modelo na escolha dos tipos de modelo para análises de viabilidade populacional. *Ecol. Modelo*, v.323, p.87-95, 2016.

RASO, T.F.; SEIXAS, G.H.F.; GUEDES, N.M.R.; PINTO, A.A. *Chlamydophila psittaci* in free-living Bluefronted Amazon parrots (*Amazona aestiva*) and Hyacinth macaws (*Anodorhynchus hyacinthinus*) in the Pantanal of Mato Grosso do Sul, Brazil. *Vet. Microbiol*., v.117, p.235-241, 2006.

REIS, F.G.R.C.; ANDRADE, E.F.F.A.; TEIXEIRA, P.A.; JUNQUEIRA JUNIOR, D.G. Prevalência de Leishmaniose canina em cães de abrigo no município de Uberlândia – Minas Gerais – estudo de caso. *Encicl. Biosfera*, v.17, p.186, 2020.

ROQUE, A.L.R.; JANSEN, A.M. Wild and synanthropic reservoirs of *Leishmania* species in Americas. *Int. J. Parasitol. Parasit. Wildl.*, v.3, p.251-262, 2014.

SHELDON, J.D.; CUSHING, A.C.; WILKES, R.P. *et al*. Serologic response to canine distemper vaccination in captive Linnaeus's two-toed sloths (*Choloepus didactylus*) after a fatal canine distemper virus outbreak. *J. Zoo Wildl. Med.*, v.48, p.1250-1253, 2017.

SILVA, S.S. *Avaliação e diagnóstico da ocorrência de Chlamydophila psittaci em calopsitas (Nymphicus hollandicus) de criadores comerciais de Brasília DF*. 2013. 87f. Dissertação (Mestrado em Saúde Animal) - Faculdade de Agronomia e Medicina Veterinária, Universidade de Brasília, Brasília, DF.

SOUSA, T.C.; FRANCISCO, A.K.P.R.; SANTOS, I.B. Leishmaniose canina em Brasília, DF: revisão de literatura. *Tempus Actas Saúde Coletiva*, v.9, p.187-202, 2015.

STOUT, A.L.E.; ANDRÉ, A.N.M.; JAIMES, A.J.A.; MILLET, A.J.K.; WHITTAKERA, G.R. Coronaviruses in cats and other companion animals: where does SARS-CoV-2/COVID-19 fit? *Vet. Microbial.*, v.247, p.1-6, 2020.

VAN EYS, G.J.J.M.; SCHOONE, G.J.; KROON, N.C.M.; EBELING, S.B. Sequence analysis of small subunit ribosomal RNA genes and its use for detection and identification of Leishmania parasites. *Mol. Biochem. Parasitol.*, v.51, p.133-142,1992.

VILELA, A.L.O.; GUEDES, V.L. Cães domésticos em unidades de conservação: Impacto e controle. *HOLOS Environ.*, v.14, p.199, 2014.

YOUNG, R.J.; COELHO, C.M.; WIELOCH, D.R. A note on the climbing abilities of giant anteaters, *Myrmecophaga tridactyla* (Xenarthra, Myrmecophagidae). *Bol. Museu Biol. Mello Leitão*, v.15, p.41-46, 2003.