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# Molecular detection of feline arthropod-borne pathogens in cats in Cuiabá, state of Mato Grosso, central-western region of Brazil

Detecção molecular de patógenos transmitidos por artrópodes em gatos de Cuiabá, estado do Mato Grosso, Centro-oeste do Brasil

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## Abstract

Hemotrophic mycoplasmas (hemoplasmas), *Bartonella* sp., *Hepatozoon* sp. and *Cytauxzoon felis* are prominent pathogens that circulate between cats and invertebrate hosts. The present study aimed to detect the presence of DNA from hemoplasmas, *Bartonella* sp., *Hepatozoon* sp. and *Cytauxzoon felis*, and then confirm it by means of sequencing, in blood samples from cats in Cuiabá, MT, Brazil. From February 2009 to February 2011, blood samples with added EDTA were collected from 163 cats that were being housed in four different animal shelters in the city of Cuiabá, state of Mato Grosso, Brazil and from 15 cats that were admitted to the veterinary hospital of the Federal University of Mato Grosso (UFMT). Out of the 178 cats sampled, 15 (8.4%) were positive for hemoplasmas: four (2.2%) for *Mycoplasma haemofelis*, 12 (6.7%) for '*Candidatus* M. haemominutum' and one (0.5%) for '*Candidatus* M. turicensis'. One cat (0.5%), a patient that was attended at the veterinary hospital, was coinfected with *M. haemofelis*, '*Candidatus* M. haemominutum' and '*Candidatus* M. turicensis', based on sequencing confirmation. Four cats were positive for *Bartonella* spp.: three (1.7%) for *B. henselae* and one (0.5%) for *B. clarridgeiae*. None of the animals showed *Cytauxzoon* sp. or *Hepatozoon* sp. DNA in their blood samples. This study showed that cats housed in animal shelters in the city of Cuiabá, state of Mato Grosso, are exposed to hemoplasmas and *Bartonella* species.

**Keywords:** *Bartonella* sp., *Cytauxzoon* sp., hemoplasmas, *Hepatozoon* sp., cats, Mato Grosso.

## Resumo

Micoplasmas hemotróficos (hemoplasmas), *Bartonella* sp., *Hepatozoon* sp. e *Cytauxzoon felis* se destacam como importantes patógenos que circulam entre gatos e hospedeiros invertebrados. O presente estudo objetivou detectar e, posteriormente confirmar por seqüenciamento, a presença de DNA de hemoplasmas, *Bartonella* sp., *Hepatozoon* sp. e *Cytauxzoon felis* em amostras de sangue de gatos de Cuiabá, MT, Brasil. Entre fevereiro/2009 e fevereiro de 2011, amostras de sangue acrescidas de EDTA foram coletadas de 163 gatos mantidos em quatro diferentes abrigos na cidade de Cuiabá, estado do Mato Grosso, Brasil, e de 15 gatos atendidos no Hospital Veterinário da Universidade Federal do Mato Grosso (UFTM). Dos 178 gatos amostrados, 15 (8,4%) foram positivos para hemoplasmas: quatro (2,2%) para *Mycoplasma haemofelis*, 12 (6,7%) para '*Candidatus* M. haemominutum' e um (0,5%) para '*Candidatus* M. turicensis'. Um (0.5%) gato, atendido no Hospital Veterinário da UFMT, estava co-infectado com *M. haemofelis*, '*Candidatus* M. haemominutum' e '*Candidatus* M. turicensis', baseado na confirmação por sequenciamento. Quatro gatos mostraram-se positivos para *Bartonella* spp.: três (1,7%) para *B. henselae* e um (0.5%) para *B. clarridgeiae*. Todos os gatos amostrados mostraram-se negativos para *Cytauxzoon* sp. e *Hepatozoon* sp. Este estudo mostrou que gatos mantidos em abrigos na cidade de Cuiabá, estado do Mato Grosso, são expostos a hemoplasmas e espécies de *Bartonella* sp.

**Palavras-chave:** *Bartonella* sp., *Cytauxzoon* sp., hemoplasmas, *Hepatozoon* sp., gatos, Mato Grosso.

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## Introduction

Arthropod-borne agents have arisen as emerging pathogens over the last few decades, due to ecological and climate changes (SHAW et al., 2001). In this context, hemotrophic mycoplasmas (hemoplasmas), *Bartonella* sp., *Hepatozoon* sp. and *Cytauxzoon felis* have been seen to be important pathogens that circulate between cats and invertebrate hosts. *Bartonella* species and hemoplasmas are occasionally incriminated as pathogens in human beings. For instance, *Mycoplasma haemofelis* and *Bartonella henselae* were detected in a human immunodeficiency virus-infected patient in Brazil (DOS SANTOS et al., 2008). *Bartonella henselae* is commonly known to cause a disease transmitted to humans via cat saliva or scratches, and thus named cat-scratch disease, which is characterized by regional lymphadenopathy and fever. However, these bacteria may also cause hepatosplenic disease, bacillary angiomatosis and endocarditis (FLORIN et al., 2008).

Feline hemoplasmas comprise a group of bacteria that infect erythrocytes by attaching to the red blood cell and inducing hemolytic anemia in cats (TASKER, 2010). *Bartonella* species are bacteria that mainly infect mammalian erythrocytes and endothelial cells and cause long-lasting bacteremia in their reservoir hosts (CHOMEL et al., 2004). Cats are the major reservoirs for *B. henselae*, *B. clarridgeiae* and *B. koehlerae*, showing nonspecific or, more often, nonclinical signs of infection (BREITSCHWERDT; KORDICK, 2000; BREITSCHWERDT et al., 2010). Ticks (COTTÉ et al., 2008) and fleas have been incriminated as vectors for hemoplasmas and *Bartonella* sp. among cats (CHOMEL et al., 1996; FOIL et al., 1998; SHAW et al., 2004; WOODS et al., 2005).

While infection by hemoplasmas and *Bartonella* spp. has been documented in cats in Brazil, few reports on occurrences of tick-borne apicomplexans have been produced (DE BORTOLI et al., 2011). *Hepatozoon* sp. has been incriminated as a low-virulence agent in cats, and its main transmission route is through ingestion of a definitive hematophagous arthropod host (BANETH et al., 1998). *Cytauxzoon felis* has an intraerythrocytic phase (piroplasm) and a tissue phase consisting of large schizonts that develop in macrophages and monocytes (GREENE et al., 2007).

The present study aimed to detect the presence of DNA from hemoplasmas, *Bartonella* sp., *Hepatozoon* sp. and *Cytauxzoon felis* in blood samples of cats in Cuiabá, MT, Brazil.

## Materials and Methods

From February 2009 to February 2011, EDTA-blood samples were collected from 163 cats that were housed in four different animal shelters in the city of Cuiabá, state of Mato Grosso, Brazil, and from 15 cats that were attended at the veterinary hospital of the Federal University of Mato Grosso (UFMT). The animal shelters from which the blood samples were collected are located in different regions of the city, and vary in the size of animal population and whether or not these animals have access to the streets (Table 1). The 15 remaining samples were collected from cats that were attended within the routine service of the Small Animal Medicine sector of the veterinary hospital of the Federal University of Mato Grosso. Out of the total of 178 cats, 94 were female and 84 males; 132 were adults and 46 kittens (taking these to be animals up to 12 months of age). The cats were restrained mechanically or chemically, for intramuscular administration of ketamine (5 mg/kg) and acepromazine (0.1 mg/kg) in accordance with the protocol indicated by Natalini et al. (2007) for sedation and for performing jugular venipuncture. Samples of approximately three ml of blood was collected aseptically, and were then transported under refrigeration to the university, where they were stored at –20 °C for later analysis. The project was approved by the university's Ethics Committee under the protocol number 23108.034003/10-5.

DNA was extracted from 200 µL of whole blood sample using the QIAamp DNA blood mini-kit (QIAGEN, Valencia, California, USA), in accordance with the manufacturer's instructions.

Partial sequences of the 16S rRNA gene of *M. haemofelis,* '*Candidatus* Mycoplasma haemominutum' and '*Candidatus* Mycoplasma turicensis' were amplified by means of PCR in final-volume reaction mixtures of 25 µL containing 5 µL of template DNA, 10X (2.5 µL) PCR buffer, 1.0 mM (1 µL) $MgCl_2$, 0.2 mM (2 µL) deoxynucleotide triphosphate (dNTP) mixture, 1.5 U (0.25 µL) Taq DNA polymerase (Invitrogen, Carlsbad, California, USA) and 0.2 mM (1 µL) of primers that had previously been described (CRIADO-FORNELIO et al., 2003; SANTOS et al., 2009).

*Bartonella* genus screening was performed by means of PCR targeting the intergenic transcribed spacer (ITS), as described previously (MAGGI; BREITSCHWERDT, 2005a; DINIZ et al., 2007). The same reagent concentrations described above were also used for hemoplasma PCR assays. For further molecular characterization and species differentiation, samples that were positive in ITS amplification were tested for other genes: riboflavin synthase gene (*ribC*) (JOHNSON et al., 2003); citrate synthase gene (*gltA*) (NORMAN et al., 1995, WINOTO et al., 2005); bacteriophage-associated heme-binding protein gene (*pap31*) (MAGGI; BREITSCHWERDT, 2005b); and RNA polymerase beta subunit gene (*rpoB*) (DINIZ et al., 2007).

Previously described PCR protocols based on the 18S rRNA gene were used for *Cytauxzoon felis* (BIRKENHEUER et al., 2002) and *Hepatozoon* sp. (CRIADO-FORNELIO et al., 2006).

*Mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum' and '*Candidatus* Mycoplasma turicensis' obtained from naturally infected cats in Jaboticabal, state of São Paulo (DE BORTOLI et al., 2012) were used as positive DNA controls in PCR reactions for hemoplasmas. *Bartonella henselae* DNA, obtained from a cat in São Luís, Maranhão (BRAGA et al., 2012), was used as the positive control in *Bartonella* PCR assays. *Cytauxzoon* sp. (ANDRÉ et al., 2009) and *Hepatozoon* sp. (ANDRÉ et al., 2010) DNA obtained from naturally infected wild felids were also used as positive controls. Ultra-pure sterile water was used as the negative control. In order to prevent PCR contamination, the DNA extraction, reaction setup, PCR amplification and 1% agarose gel electrophoresis were performed in separate rooms.

The reaction products (fragments of 400 bp for *Bartonella* sp. *gltA* gene, 300 bp for *Bartonella* sp. ITS region, 600 bp for *M. haemofelis*/ '*Candidatus* Mycoplasma haemominutum' and 500 bp for '*Candidatus* Mycoplasma turicensis') were purified using Silica Bead DNA Gel Extraction Kit (Fermentas, São Paulo, SP,

Brazil). Purified amplified DNA fragments from positive samples were subjected to sequence confirmation in an automated sequencer (ABI Prism 310 genetic analyzer; Applied Biosystems, Perkin Elmer). Consensus sequences were obtained through analysis on the sense and antisense sequences using the CAP3 software (http://mobyle.pasteur.fr/cgi-bin/MobylePortal/portal.py). Comparisons with sequences deposited in GenBank were made using the basic local alignment search tool (BLAST) (ALTSCHUL et al., 1990). The CLUSTAL W (THOMPSON et al., 1994) and MEGA (KUMAR et al., 2004) software was used for alignment and phylogenetic analysis, respectively. The neighbor-joining method was used to build the phylogenetic tree (SAITOU; NEI, 1987) using a Kimura-2 parameter model. The bootstrap test with 1000 replications was applied to estimate the confidence level of branching patterns of the neighbor-joining tree (FELSENSTEIN, 1985).

## Results

Out of 178 cats sampled, 15 (8.4%) were positive for hemoplasmas: four (2.2%) for *M. haemofelis* (one cat was from the first shelter and three from the fourth one); 12 (6.7%) for '*Candidatus* M. haemominutum' (six cats were from the first shelter, two from the third and three from the fourth one) and one (0.5%) for '*Candidatus* M. turicensis'. One cat (0.5%) cat, a patient that was attended at the veterinary hospital, was coinfected with *M. haemofelis*, '*Candidatus* M. haemominutum' and '*Candidatus* M. turicensis', based on sequencing confirmation. The sequenced products showed 99% identicalness with 16S rRNA of *M. haemofelis* (CP002808), 100% identicalness with 16S rRNA of '*Candidatus* M. haemominutum' (AY150980) and 99% identicalness with 16S rRNA of '*Candidatus* M. turicensis' (DQ464425). Partial 16S rRNA hemoplasma sequences were deposited in GenBank under accession numbers KC331019 to KC331032.

Among the hemoplasma-positive cats, six were females and nine males; 13 were adults and only two were kittens (Table 2). The phylogenetic analysis based on 16S rRNA sequences confirmed that the hemoplasma DNA found was identical and showed that the isolates from cats in Cuiabá, MT, were in the same clade as other isolates from *M. haemofelis,* '*Candidatus* Mycoplasma haemominutum' and '*Candidatus* Mycoplasma turicensis' (Figure 1).

Three cats (1.7%) were positive for *B. henselae*. The analysis on sequenced products based on the ITS region (GenBank accession numbers KC331013, KC331015 and KC3310136) and *gltA* gene (GenBank accession number KC331018) showed 99% identicalness with *B. henselae* (GenBank accession numbers JQ009430 and BX897699, respectively). One cat (0.5%) was positive for *B. clarridgeiae*. The analysis on sequenced products based on the ITS region (GenBank accession number KC331014) and *gltA* gene (GenBank accession number KC331017) showed 99% identicalness with *B. clarridgeiae* (access numbers DQ683194 and FN645454, respectively). All of the *Bartonella* spp.-positive cats were adult females and were housed in the first shelter (Table 2). The phylogenetic tree based on ITS partial sequences confirmed that the *B. henselae* and *B. clarridgeiae* DNA found was identical, thus showing that the isolates from cats from Cuiabá, MT, were in the same clade as other *B. henselae* and *B. clarridgeiae* isolates (Figure 2).

None of the cats sampled were positive for *Cytauxzoon* sp. or *Hepatozoon* sp. in PCR.

## Discussion

The present study showed that hemotrophic mycoplasmas and *Bartonella* species circulate among cats housed in animal shelters in the city of Cuiabá, state of Mato Grosso, albeit at low rates. So far, hemoplasmas have been reported in cats in the states of Paraná (DE MORAIS et al., 2007), Rio de Janeiro (MACIEIRA et al., 2008), São Paulo (BATISTA, 2004; HORA, 2008; DE BORTOLI et al., 2012), Rio Grande do Sul (SANTOS, 2008) and Maranhão (BRAGA et al., 2012). The prevalences of *M. haemofelis*, '*Candidatus* M. haemominutum' and '*Candidatus* M. turicensis' were 2.2%, 6.7% and 0.5%, respectively.

**Table 1.** Characteristics of the shelters where blood samples from cats were collected.

| Place of origin | Location in Cuiabá | Cat population | Number of animals sampled | Outdoor access |
|---|---|---|---|---|
| Animal shelter 1 | Northern region | 92 | 82 | Yes |
| Animal shelter 2 | Eastern region | 10 | 7 | No |
| Animal shelter 3 | Eastern region | 14 | 8 | No |
| Animal shelter 4 | Western region | 72 | 66 | Yes |

**Table 2.** Results from the molecular survey for *Hemoplasma* and *Bartonella species* in cats in Cuiabá, MT, Brazil.

| Agent | Number of positive cats (%) | Closest GenBank entry (via BLAST®) % similarity (targeting gene) |
|---|---|---|
| *Mycoplasma haemofelis* | 4 (2.2) | CP002008 – 99% (16S rRNA) |
| '*Çandidatus* Mycoplasma haemominutum' | 12 (6.7) | AY150980 – 100% (16S rRNA) |
| '*Candidatus* Mycoplasma turicensis' | 1 (0.5) | DQ464425 – 99% (16S rRNA) |
| *Bartonella henselae* | 3 (1.7) | JQ009430 – 99% (ITS) |
| | 1 (0.5) | BX897699 – 99% (gltA) |
| *Bartonella clarridgeiae* | 1 (0.5) | DQ683194– 99% (ITS) |
| | 1 (0.5) | FN645454 – 99% (gltA) |

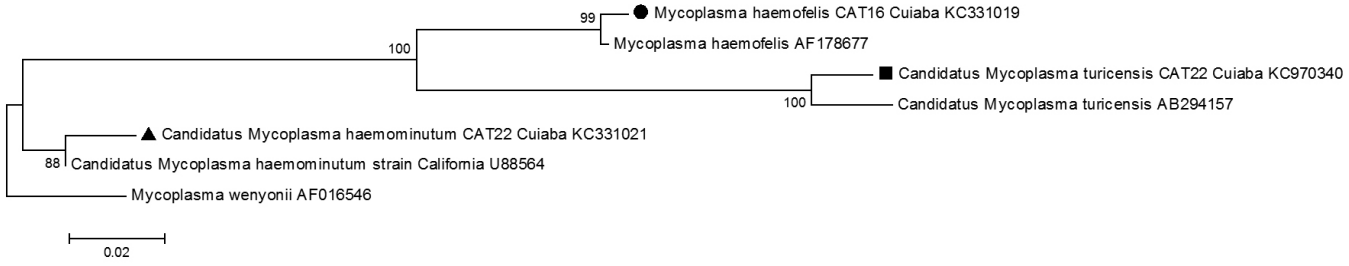


**Figure 1.** Phylogenetic position of hemoplasmas detected in domestic cats in the city of Cuiabá, based on 16S rRNA DNA sequences. The tree was constructed using the neighbor-joining kimura-2 parameter method and the numbers on the tree indicate bootstrap values for the branch points. Accession numbers are indicated.

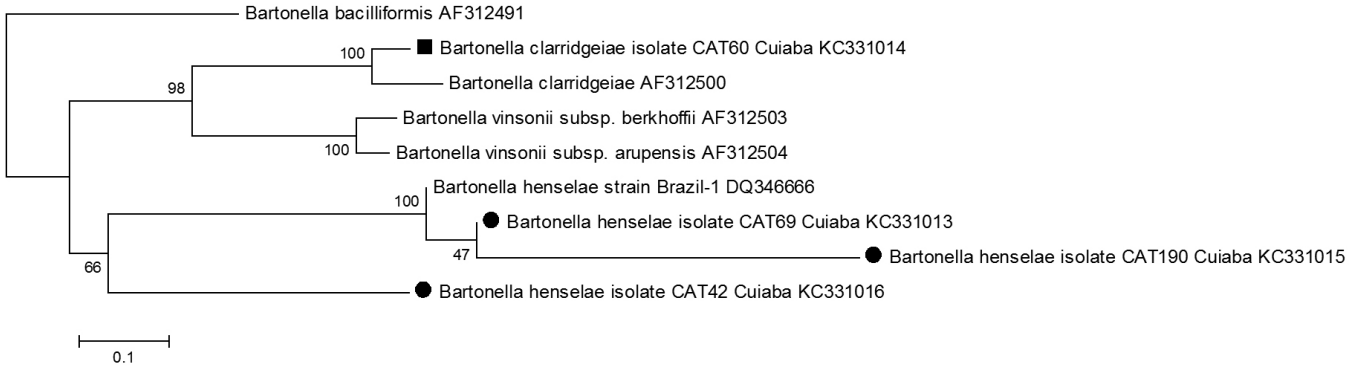


**Figure 2.** Phylogenetic position of *Bartonella* species detected in domestic cats the city of Cuiabá, based on ITS DNA sequences. The tree was constructed using the neighbor-joining kimura-2 parameter method and the numbers on the tree indicate bootstrap values for the branch points. Accession numbers are indicated.

In Brazil, the prevalences of hemoplasmas among healthy and anemic cats have been found to range from 2.1% to 38% for *M. haemofelis*; 4% to 13.5% for '*Candidatus* M. haemominutum'; and 0.37% to 2.7% for '*Candidatus* M. turicensis' (BATISTA, 2004; MACIEIRA et al., 2008; HORA, 2008; SANTOS et al., 2009; BRAGA et al., 2012; DE BORTOLI et al., 2012).

DNA from *Bartonella henselae* and *B. clarridgeiae* was detected in three and one out of the 178 cats sampled (1.7%), respectively. In Brazil, few reports on the prevalence of *Bartonella* spp. among domestic and wild felids have been produced. DNA from *Bartonella henselae* and *B. clarridgeiae* was detected in 10.6% and 6.3% of the blood samples, respectively, from 47 cats at an animal shelter in Novo Hamburgo, state of Rio Grande do Sul (STAGGEMEIER et al., 2010). The prevalence of *Bartonella* spp. among cats in the city of Vassouras, state of Rio de Janeiro, was found to be 97.3%, using molecular techniques (SOUZA et al., 2010). Furthermore, *Bartonella* spp. DNA was detected in 17 out of 40 clinically healthy cats that were treated in a spaying/neutering program in the city of Rio de Janeiro, state of Rio de Janeiro (CRISSIUMA et al., 2011). Recently, *B. henselae* DNA was detected in two out of 46 apparently healthy cats (4.3%) that were sampled in Jaboticabal, state of São Paulo (DE BORTOLI et al., 2012). In the northeastern region of the country, among 200 cats sampled in the city of São Luís, state of Maranhão, and tested for multiple genes, nine (4.5%) were positive for *Bartonella* sp: six cats for *Bartonella henselae*, and three for *Bartonella clarridgeiae* (BRAGA et al., 2012).

Fleas are considered to be potential vectors for hemoplasmas and *Bartonella* species in cats (CHOMEL et al., 1996; FOIL et al., 1998; SHAW et al., 2004; WOODS et al., 2005). The cats sampled in this study received non-regular chemical treatment against fleas and ticks, which may have favored transmission of both groups of pathogens. Several *Bartonella* species are considered to be zoonotic, including *B. henselae* and *B. clarridgeiae*. Cats are the primary reservoir and vector for transmission of *B. henselae* and probably *B. clarridgeiae* to human beings. The most frequent route for infecting humans is through contamination of scratches with flea feces (CHOMEL et al., 1996). Since the present study showed that *Bartonella* spp. occurs in the state of Mato Grosso, it is important that physicians in this region start considering cat-scratch disease as a differential diagnosis in people showing suggestive symptoms such as regional lymphadenopathy, fever and endocarditis.

None of the animals sampled was positive for *Cytauxzoon* sp. and/or *Hepatozoon* sp. Few reports on occurrences of either of these parasites in domestic cats in Brazil have been produced. Although piroplasms similar to *Cytauxzoon* sp. have been found in cat blood smears in the state of Rio de Janeiro (MENDES-DE-ALMEIDA et al., 2007), molecular confirmation of occurrences of this parasite in Brazil has only been done in wild felids

(ANDRÉ et al., 2009; FILONI et al., 2012). On the other hand, *Hepatozoon* sp. phylogenetically related to *H. canis* (RUBINI et al., 2006) and *H. felis* (DE BORTOLI et al., 2011) has been detected, although infrequently, in cats in the states of São Paulo and Maranhão, respectively.

## Conclusions

In conclusion, we showed that cats that were housed in animal shelters in Cuiabá, state of Mato Grosso, were exposed to hemoplasma and *Bartonella* species. The presence of flea-borne pathogens circulating in cats in animal shelters reinforces the importance of ectoparasite control, with the aim of preventing dissemination of these pathogens among susceptible cats and avoiding occurrences of clinical signs of hemoplasmosis and/or transmission of *Bartonella* spp. among cats and human beings. Although the occurrence rate of *Bartonella* species in sampled cats was relatively low, these animals may play a role as vectors and reservoirs for *Bartonella* spp. for transmission to human beings. Studies on the prevalence of these pathogens among human beings who come into contact with cats are much needed, with the aim of ascertaining the real role of these animals in the epidemiology of bartonellosis in Brazil.

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