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Cytauxzoon felis and ‘*Candidatus* *Mycoplasma haemominutum*’ coinfection in a Brazilian domestic cat (*Felis catus*)

Co-infecção por *Cytauxzoon felis* e ‘*Candidatus* *Mycoplasma haemominutum*’ em um gato doméstico (*Felis catus*) no Brasil

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Abstract

This article describes the first detection of *Cytauxzoon felis*, using molecular techniques, in a naturally infected domestic cat from Brazil, South America. Coinfection with ‘*Candidatus* *Mycoplasma haemominutum*’ was also found. The molecular identification of the piroplasmid species was performed by Polymerase Chain Reaction (PCR) and sequencing analysis. A 284 pb fragment of the gene encoding the 18S ribosomal RNA region was amplified and showed 99% identity with other *C. felis* strains from North America. In addition, PCR-RFLP (restriction fragment length polymorphism) analysis, which amplifies a 595 bp fragment of the gene encoding 16S ribosomal RNA of some bacterial species, identified the co-infecting species as ‘*Candidatus* *M. haemominutum*’.

Keywords: Cytauxzoonosis, hemoplasmosis, PCR, sequencing, feline.

Resumo

Este artigo descreve a primeira detecção de *Cytauxzoon felis* em um gato doméstico naturalmente infectado no Brasil, América do Sul, através de técnicas moleculares. Também foi encontrada co-infecção com ‘*Candidatus* *Mycoplasma haemominutum*’. A detecção molecular da espécie do piroplasmídeo foi realizada através da reação em cadeia pela polimerase (PCR) e sequenciamento. Um fragmento de 284 pb do gene codificador da região 18S do RNA ribossomal do parasito foi sequenciada e mostrou 99% de identidade com outros isolados de *C. felis* da América do Norte. Ademais, através da análise por meio de PCR-RFLP (Polimorfismo no comprimento de fragmentos de restrição), que amplifica um fragmento de 595 pb do gene codificador da porção 16 do RNA ribossomal de algumas espécies de bactérias, concluiu-se que a espécie com-infectante era ‘*Candidatus* *M. haemominutum*’.

Palavras-chave: Cytauxzoonose, hemoplasmoze, PCR, sequenciamento, felino.

Cytauxzoon felis, a protozoan parasite that infects domestic and wild felids, was first reported in domestic cats from the United States of America (the USA) in the mid-seventies (WAGNER, 1976). In the USA, bobcats (*Lynx rufus*) are the natural reservoir hosts for this parasite with high prevalence, but other felids such as Florida panthers (*Puma concolor coryi*) and domestic cats may also maintain long-term parasitemias and serve as reservoir hosts

(SHOCK et al., 2011). Two tick species, *Dermacentor variabilis* and *Amblyomma americanum*, are the only known vectors for *C. felis* in the USA (REICHARD et al., 2009).

In Brazil, natural *Cytauxzoon* sp. infections have been confirmed by molecular techniques in wild/exotic felids (PEIXOTO et al., 2007; ANDRÉ et al., 2009; FILONI et al., 2012), but few studies regarding natural cytauxzoonosis in domestic cats were solely based on the identification of parasites on blood smears (MENDES-DE-ALMEIDA et al., 2007).

This article describes the first report of *C. felis* natural infection in a domestic cat in Brazil, South America, using molecular techniques (PCR and sequencing analysis). Furthermore, the

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occurrence of '*Candidatus Mycoplasma haemominutum*' co-infection was also confirmed by PCR-RFLP (restriction fragment length polymorphism) analysis.

A 5-year-old crossbred domestic cat was taken to a veterinary practice in the municipality of Areal, State of Rio de Janeiro, Brazil, presenting dehydration, vomiting, prostration and anorexia. At the time of admission, blood samples were collected for hematological and serum biochemical analyses: alanine aminotransferase (ALT), aspartate aminotransferase (AST), and Blood Urea Nitrogen (BUN). The animal was also tested for the presence of Feline Immunodeficiency Virus (FIV) antibodies and Feline Leukemia Virus (FeLV) infection using a commercial ELISA kit (Snap FIV Antibody/FeLV Antigen Combo; IDEXX Laboratories, Westbrook, ME, USA). Despite supportive therapy and antibiotics treatment (doxycycline 5 mg/kg, given by oral route every 12 hours), the animal died four days after the onset of symptoms.

Laboratorial findings consisted of neutrophilia with a mild left shift, severe lymphopenia, thrombocytopenia, and increased ALT, AST activities and BUN concentration. Piroplasms within erythrocytes, reactive monocytes and platelet clumps were observed during Giemsa-stained blood smear evaluation. The cat tested negative for both FIV antibodies and FeLV antigen (Snap FIV antibody/FeLV antigen Combo, Idexx Laboratories, Westbrook, ME, USA).

Genomic DNA was extracted from the cat's blood sample using a commercial kit (Illustra Blood Genomic Prep Mini Spin Kit, GE Healthcare, Piscataway, NJ, USA) according to the instructions provided by the manufacturer. Polymerase Chain Reaction (PCR) was performed with primer pair specific for *C. felis* 18S rRNA gene, as previously described by Birkenheuer et al. (2006). Both positive (known positive sample gently provided by Dr. Aivaldo Henrique da Fonseca, UFRRJ, RJ, Brazil) and negative (Ultra-Pure DNase/RNase-Free Distilled Water, Invitrogen, Carlsbad, CA, USA) controls were used in each set of reactions. The 284 bp DNA fragment was purified using the PureLink PCR Purification Kit (Invitrogen, Carlsbad, CA, USA) and subjected to direct sequencing in both directions. Alignment and sequence analysis were performed using the BioEdit Sequence Alignment Editor 7.0.1 (<http://mbio.ncsu.edu/BioEdit/bioedit.html>), and the sequence (GenBank Access number JX393072) was compared with different *Cytauxzoon* spp. sequences available in the GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>). The alignment of this sequence with other isolates of *C. felis* from North America (AF399930, AY679105, and AY531524) and a captive felid from Brazil (GU903911) showed 99% nt identity.

A second PCR was performed targeting *Mycoplasma* spp. 16S rRNA, according to Criado-Fornelio et al. (2003), which amplifies a 618 bp product for '*Candidatus M. haemominutum*'. RFLP assay with *EcoRI* endonuclease was performed and the two fragments obtained (269 and 349 bp) were consistent with '*Candidatus M. haemominutum*' infection (Figure 1). Known positive samples containing DNA obtained from *M. haemofelis* and '*Candidatus M. haemominutum*' (kindly provided by Dr. Ana Márcia de Sá Guimarães, Purdue University, West Lafayette, IN, USA), and negative – water (Ultra-Pure DNase/RNase-Free Distilled Water, Invitrogen, Carlsbad, CA, USA) controls were also used.

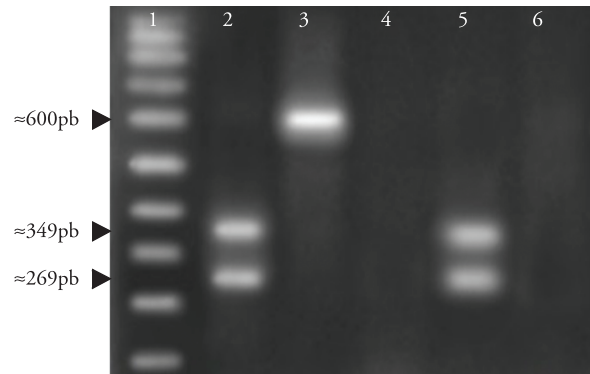


Figure 1. Agarose gel analysis of PCR for hemoplasmas 16S rRNA gene combined with *EcoRI* digestion results. Line 1 – Molecular weight marker (100 bp ladder; BioTools, Madrid, Spain); line 2 – '*Candidatus Mycoplasma haemominutum*' positive control; line 3 – *M. haemofelis* positive control; line 4 – Hemoplasma negative control; line 5 – '*Candidatus M. haemominutum*' positive sample; line 6 – Negative sample.

This is the first report of *C. felis* and '*Candidatus M. haemominutum*' natural coinfection in a domestic cat in Brazil. *Cytauxzoon felis* may cause hematological and clinical chemistry changes in infected cats, according to Meinkoth and Kocan (2005), and some of these changes (neutrophilia, lymphopenia, thrombocytopenia, increased ALT and AST activities and BUN concentration) were observed herein. '*Candidatus Mycoplasma haemominutum*' is a hemotrophic bacterial species that can induce anemia in cats – both alone or in combination with other pathogens (BIONDO et al., 2009; SANTOS et al., 2009). In this case, although anemia was not described, the association of both vector-borne organisms may have contributed to the aggravation of the clinical and hematological alterations that resulted in the fatal outcome.

The diagnosis of a domestic cat positive for *C. felis* DNA represents the first report of such infection under natural conditions confirmed by molecular techniques in South America. In Brazil, future research efforts using molecular techniques are needed to study these protozoa natural host(s), vector(s) and pathogenicity.

In addition, infections caused by both *C. felis* and hemoplasmas should be screened when there is suspicion of blood parasites in Brazilian domestic cats.

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