

## CASE REPORT

### MOLECULAR DETECTION OF “*Candidatus Mycoplasma haemominutum*” IN A LION (*Panthera leo*) FROM A BRAZILIAN ZOOLOGICAL GARDEN

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#### SUMMARY

Although *Mycoplasma haemofelis* and “*Candidatus Mycoplasma haemominutum*” infections have been reported in wild cats from United States, their presence among native and captive wild cats in Brazil is still unknown. A 12 year old healthy male lion (*Panthera leo*) from the Zoological Garden of Curitiba, Brazil was anesthetized for transportation and dental evaluation. A blood sample was obtained for a complete blood cell count (CBC) and PCR analysis. DNA was extracted and fragments of *Mycoplasma haemofelis* and “*Candidatus Mycoplasma haemominutum*” 16S ribosomal RNA gene were amplified in PCR assays. CBC results were within reference intervals. A weak band of 192 pb for “*Candidatus Mycoplasma haemominutum*” was observed, and no band was amplified from *Mycoplasma haemofelis* reaction. A weak PCR band associated with normal CBC results and without visible parasitemia or clinical signs may suggest a chronic subclinical infection with “*Candidatus Mycoplasma haemominutum*”. The lack of clinical signs may also represent the low pathogenicity of this organism; however, it is noteworthy that immune suppression caused by management and/or corticoids treatment may induce parasitemia and anemia in this animal. This detection suggests further studies in captive wild cats in Brazilian Zoological Gardens.

**KEYWORDS:** *Mycoplasma haemofelis*; “*Candidatus Mycoplasma haemominutum*”; Lion; *Panthera leo*; Feline infectious anemia; *Haemobartonella*.

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#### INTRODUCTION

*Mycoplasma haemofelis* and “*Candidatus Mycoplasma haemominutum*” (formerly *Haemobartonella felis* Ohio and California strains, respectively) are small and pleomorphic bacteria that parasitize feline red blood cells. Haemoplasmosis due to *M. haemofelis* causes a life-threatening hemolytic anemia in domestic cats known as feline infectious anemia<sup>2</sup>. “*Candidatus M. haemominutum*” is smaller than *M. haemofelis* and appears to be less pathogenic, resulting in minor or no clinical signs<sup>5</sup>. However, concurrent disease or immune suppression may predispose a “*Candidatus M. haemominutum*” infected cat to develop a life-threatening anemia<sup>3</sup>.

Both microorganisms have been reported in domestic cats worldwide<sup>8</sup> including in Brazil<sup>6</sup>; however, their distribution among nondomestic cats are still unknown. In a previous study<sup>7</sup>, two captive tigers (*Panthera tigris*) from United States tested positive for *M. haemofelis* by PCR amplification. In the same study, eight lions (*P. leo*) tested negative for both *M. haemofelis* and “*Candidatus M. haemominutum*” infection. Thus, the present study describes the first

case of “*Candidatus M. haemominutum*” infection in a captive lion (*P. leo*) from a Zoological Garden in Curitiba, Brazil.

#### CASE REPORT

A 12 year old healthy vasectomized male lion (*P. leo*) from the Zoological Garden of Curitiba, Brazil, maintained in a solitary cage, was anesthetized with tiletamine HCl and zolazepam HCl (Zoletil 50, Virbac Animal Health, Carros Cedex, France) for transportation and dental evaluation. A blood sample was obtained by jugular venipuncture and placed into an EDTA tube for a complete blood cell count (CBC) and PCR analysis. Animal procedures were performed under regulations of the Brazilian Institute for the Environment and the Renewable Resources (IBAMA).

DNA was extracted from blood using a commercially available kit (Generation Capture Column, Genra Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions. Fragments of *M. haemofelis* and “*Candidatus M. haemominutum*” 16S ribosomal RNA gene were amplified using sets of primers already described<sup>1,4</sup>. Fragments of the expected size from *M. haemofelis* and “*Candidatus*

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*M. haemominutum*” cloned into pGEM T-Easy Vector System II (Promega, Madison, WI, USA) were used as positive controls. “*Candidatus M. haemominutum*” and *M. haemofelis* PCR reactions were performed in separate using 5 µL of DNA template per 25 µL reaction containing a final concentration of 50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100, 2.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 0.2 mM of each primer, 1.0 U of *Taq* DNA polymerase (Promega, Madison, WI, USA), and 11.3 µL of water. The cycling conditions for both haemoplasmas consisted of 94 °C for four minutes; followed by 35 cycles of 94 °C for 30 seconds, 53 °C for one minute, and 70 °C for 45 seconds; with a final extension of 70 °C for five minutes and a cooling at 4 °C. The predicted products were separated by electrophoresis in a 1% agarose gel containing ethidium bromide, 5 µg/mL. The gels were photographed under UV light with an Alpha Imager 2200 imaging system (Alpha Innotech Corporation, San Leandro, CA, USA).

CBC results were within the reference intervals. The hematocrit was 38% (reference range: 35% to 40%) and the total plasma protein was 8.0 g/dL (reference range: 5.0 g/dL to 8.5 g/dL). No parasites were observed on erythrocytes via manual evaluation of the stained blood smear. A weak band of 192 pb for “*Candidatus M. haemominutum*” was observed, and no band was amplified from *M. haemofelis* reaction.

Additionally, the lion has also shown a history of arthrosis with periodical anti-inflammatory corticoid treatments; however, at the blood sampling time he was not under such medications or disease peak period. Moreover, blood-sucking arthropods were not found during the sampling.

## DISCUSSION

Haemoplasma infection in domestic cats has been observed in Curitiba, Brazil<sup>6</sup>, and feral cats that have been commonly seen at this Zoological Garden may justify the origin of this infection in the lion. In fact, this possibility was not confirmed. However, transmission via blood-sucking arthropods has been reported in domestic cats<sup>9</sup>, and fleas may be responsible for the transmission even if they were not found on the lion during the sampling. The detection of a weak PCR band associated with normal CBC results and without visible parasitemia or clinical signs may suggest a chronic subclinical infection with “*Candidatus M. haemominutum*”. The lack of clinical signs may also represent the low pathogenicity of this organism; however, it is noteworthy that immune suppression caused by management stress, concurrent disease, and/or corticoids treatment may induce parasitemia and consequent anemia in this animal. Afterwards, it will be important to monitor CBC values of this animal during possible anti-inflammatory corticoid treatments to control probable parasitemia and anemia. Further studies are in progress that will better evaluate the prevalence of haemoplasma infections among captive nondomestic cats in Brazil.

## RESUMO

### Deteção molecular do “*Candidatus Mycoplasma haemominutum*” em um leão (*Panthera leo*) de um zoológico brasileiro

Embora a infecção por *Mycoplasma haemofelis* e “*Candidatus*

*Mycoplasma haemominutum*” tenha sido reportada em felinos selvagens dos Estados Unidos, sua presença entre felinos selvagens de vida livre e de cativeiro no Brasil ainda é desconhecida. Um leão macho, saudável, com 12 anos de idade, residente no Zoológico de Curitiba, Brasil, foi anestesiado para transporte e avaliação dentária. Uma amostra de sangue foi coletada para a realização do hemograma completo e análise pela Reação em Cadeia da Polimerase (PCR). O DNA foi extraído e fragmentos do gene 16SrRNA do *Mycoplasma haemofelis* e “*Candidatus Mycoplasma haemominutum*” foram submetidos à metodologia da PCR. O hemograma apresentou valores normais. Uma banda de baixa intensidade de aproximadamente 192 pb do “*Candidatus Mycoplasma haemominutum*” foi detectada, e nenhuma banda foi observada pela PCR na detecção de *Mycoplasma haemofelis*. A banda de baixa intensidade, o hemograma normal e a ausência de parasitemia e sinais clínicos podem sugerir uma infecção crônica subclínica por “*Candidatus Mycoplasma haemominutum*”. A ausência de sinais clínicos pode também indicar a baixa patogenicidade desse microrganismo; entretanto, a imunossupressão por manejo e/ou tratamento com corticóides podem levar a parasitemia e conseqüente anemia neste animal. Este achado sugere novos estudos em felinos selvagens de cativeiro em zoológicos brasileiros.

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