



## SHORT COMMUNICATION

# Co-infection with *Mycoplasma haemofelis* and ‘*Candidatus Mycoplasma haemominutum*’ in three cats from Brazil<sup>☆</sup>

Helio A de Moraes DVM, PhD, Dip ACVIM<sup>1</sup>, Ana Marcia S Guimarães DVM<sup>2</sup>,  
Odilon Vidotto DVM, PhD<sup>4</sup>, Aline Baumann DVM<sup>5</sup>,  
Alexander W Biondo DVM, PhD<sup>2,3</sup>, Joanne B Messick VMD, PhD, Dip ACVCP<sup>6\*</sup>

<sup>1</sup>Department of Medical Sciences,  
University of Wisconsin, Madison,  
WI 43210, USA

<sup>2</sup>Departamento de Medicina  
Veterinária, Universidade Federal  
do Paraná, Curitiba, PR 80035,  
Brazil

<sup>3</sup>University of Illinois, Urbana, IL  
61802, USA

<sup>4</sup>Departamento de Medicina  
Veterinária Preventiva,  
Universidade Estadual de Londrina,  
Londrina, PR 86051, Brazil

<sup>5</sup>Clinilab, Curitiba, PR 82540,  
Brazil

<sup>6</sup>Department of Comparative  
Pathobiology, School of Veterinary  
Medicine, Purdue University,  
725 Harrison Street,  
West Lafayette, IN 47907, USA

The two most common haemotropic *Mycoplasma* of cats, *Mycoplasma haemofelis* and ‘*Candidatus Mycoplasma haemominutum*’ have been identified using molecular techniques in all continents, except Antarctica. We report the first molecular characterization in South America of a dual infection with *M haemofelis* and ‘*Candidatus Mycoplasma haemominutum*’ in three domestic cats. The 16S ribosomal RNA gene was amplified in three anaemic cats in which haemoplasma organisms were seen attached to the erythrocytes in the peripheral blood smear. Bands of the expected size for *M haemofelis* and ‘*Candidatus Mycoplasma haemominutum*’ were observed in all three cats. The 393 bp segment of one of the amplicons had a similarity value of 100% to *M haemofelis*, whereas the other amplicon, a 192 bp segment, was 100% similar to ‘*Candidatus Mycoplasma haemominutum*’. After diagnosis, two cats received blood transfusion and they were all treated with doxycycline. All three cats recovered uneventfully.

Date accepted: 9 May 2007

© 2007 ESFM and AAFP. Published by Elsevier Ltd. All rights reserved.

**M***ycoplasma haemofelis* is the causative agent of feline infectious anaemia (FIA). This red cell parasite of cats was originally named, *Eperythrozoon felis*, and subsequently renamed, *Haemobartonella felis* (Neimark et al 2001). Its description in domestic cats in Brazil was first reported in 1976 (Massard et al 1976). More recently, sequence analysis has shown that *Haemobartonella felis* and related haemotropic bacteria belong to the genus *Mycoplasmas*

(Neimark et al 2001). The trivial name, haemoplasmas was given to these red cell parasites, which form two subclusters within the pneumoniae group of *Mycoplasma*. *Mycoplasma haemofelis*, *M haemocanis*, and *M haemomuris* have a characteristic truncation of about 10 bp in a segment corresponding to positions 453–481 of the 16S rRNA gene sequence of *Escherichia coli* (Johansson et al 1999). This deletion is not present in the other subcluster of haemoplasmas that includes ‘*Candidatus M haemominutum*’, another haemoplasma that infects the cat (Messick et al 2002). ‘*Candidatus M haemominutum*’ is smaller than *M haemofelis* and was previously called *H felis* California (Foley and Pedersen 2001). A third

\*This work was presented in abstract form at the XXVII Congresso Brasileiro de Clínicos Veterinários de Pequenos Animais in Vitória, Espírito Santo, Brazil, 2006.

\*Corresponding author. Tel/Fax: +1-765-496-1748. E-mail: jmessic@purdue.edu

feline haemoplasma, 'Candidatus *Mycoplasma turicensis*' was also recently described in a cat with haemolytic anaemia in Switzerland (Willi et al 2005). Haemoplasmosis caused by *M haemofelis* causes life-threatening haemolytic anaemia in cats, whereas clinical signs in haemoplasmosis caused by 'Candidatus *Mycoplasma haemominutum*' are minor or absent (Foley and Pedersen 2001). We report the first molecular characterization in South America of a dual infection with *M haemofelis* and 'Candidatus *Mycoplasma haemominutum*' in three domestic cats.

Three adult domestic longhair cats, two females and one male were presented with lethargy, anorexia, and increased body temperature. One cat had pale mucous membranes, whereas the remaining two were icteric. All cats were anaemic (packed cell volume: 21%, 9%, and 6%) and haemoplasma organisms were seen attached to the erythrocytes in the peripheral blood smear. Blood from the cats was lysed and DNA was extracted using a commercially available kit (Gentra Systems, Minneapolis, USA). Reaction mixtures for amplification were prepared under a hood, which was subsequently irradiated by ultraviolet light. To avoid contamination, a separate set of pipettes and aerosol-guarded tips were used exclusively for the preparation of reaction mixtures. Using a universal primer set, nearly complete 16S ribosomal RNA genes were amplified (Messick et al 1998) and produced a band of the expected size (approximately 1400 bp), which identified a bacterial infection in the blood of all three cats. A fragment of 16S ribosomal RNA gene sequence of *M haemofelis* (Berent et al 1998) and 'Candidatus *Mycoplasma haemominutum*' (Foley et al 1998, Foley and Pedersen 2001) was amplified by polymerase chain reaction (PCR) using species-specific primers as previously described. Bands of the expected size for *M haemofelis* (393 bp) and 'Candidatus *Mycoplasma haemominutum*' (192 bp) were observed in all three cats. All products were separated by electrophoresis in a 1% agarose gel containing 5 µg/ml of ethidium bromide, and photographed under ultraviolet light with an Alpha Imager 2200 imaging system (Alpha Innotech, San Leandro, CA, USA). Following gel-purification of the 393 bp and 192 bp fragments of the 16S ribosomal RNA gene of *M haemofelis* and *M haemominutum*, respectively, were cloned and sequenced in the sense and antisense directions by use of a dideoxy terminator method as previously described (Messick et al 1998).

Gene sequence analysis (GenBank) for the larger amplicon (393 bp) was identified as *M haemofelis*. A sequence similarity score of 100% was observed with the Oklahoma isolate of *M haemofelis* (AF178677). Similarly, the smaller amplicon (192 bp) was identified by sequence analysis as 'Candidatus *Mycoplasma haemominutum*'. This fragment showed 100% identity with 'Candidatus *Mycoplasma haemominutum*' strains from California (U88564), Australia (AY150978), South Africa (AY150979), United Kingdom (AY150980), with two mutations when compared to the Israeli isolate (AY150974) and to a small haemoplasma observed in a dog in Brazil (AY297712). This small variation was not unexpected. Despite amplifying a conserved region of the 16S ribosomal RNA gene, haemoplasma isolates from different continents have shown minor variations in these regions (Tasker et al 2003).

After diagnosis, two cats received blood transfusion and they were all treated with doxycycline (2.5–5 mg/kg of body weight, PO, q 12 h, for 21 days). All three cats recovered uneventfully. Our results confirm that the haemotropic mycoplasmal species previously identified morphologically in cats from South America are *M haemofelis* and 'Candidatus *Mycoplasma haemominutum*'. Clinical signs and laboratory abnormalities were similar to the ones observed in other parts of the world. It has been previously shown that the cat flea *Ctenocephalides felis* is a potential vector for both haemoplasma of cats (Woods et al 2005). This flea is common in cats in Brazil (Pereira and Santos 1998) and may serve as the vector in South America.

## References

- Berent LM, Messick JB, Cooper SK (1998) Detection of *Haemobartonella felis* in cats with experimentally induced acute and chronic infections, using a polymerase chain reaction assay. *American Journal of Veterinary Research* **59**, 1215–1220.
- Foley JE, Harrus S, Poland A, Chomel B, Pedersen NC (1998) Molecular, clinical, and pathologic comparison of two distinct strains of *Haemobartonella felis* in domestic cats. *American Journal of Veterinary Research* **59**, 1581–1588.
- Foley JE, Pedersen NC (2001) 'Candidatus *Mycoplasma haemominutum*', a low-virulence epierythrocytic parasite of cats. *International Journal of Systematic and Evolutionary Microbiology* **51**, 815–817.
- Johansson KE, Tully JG, Bolske G, Pettersson B (1999) *Mycoplasma caviopharyngis* and *Mycoplasma fastidiosum*, the closest relatives to *Eperythrozoon* spp and *Haemobartonella* spp. *FEMS Microbiology Letters* **174**, 321–326.
- Massard CL, Serra Freire NMS, Flausino W (1976) *Haemobartonella felis* Flint and McKelvie, 1955 (Rickettsiales:

- Anaplasmatecae) em hemáceas de *Felis catus domesticus* L. no continente Sul Americano. XV Congresso Brasileiro de Medicina Veterinária, Rio de Janeiro, p. 1.
- Messick JB, Berent LM, Cooper SK (1998) Development and evaluation of a PCR-based assay for detection of *Haemobartonella felis* in cats and differentiation of *H felis* from related bacteria by restriction fragment length polymorphism analysis. *Journal of Clinical Microbiology* **36**, 462–466.
- Messick JB, Walker PG, Raphael W, Berent L, Shi X (2002) 'Candidatus Mycoplasma haemodidelphidis' sp. nov., 'Candidatus Mycoplasma haemolamae' sp. nov. and *Mycoplasma haemocanis* comb. nov., haemotrophic parasites from a naturally infected opossum (*Didelphis virginiana*), alpaca (*Lama pacos*) and dog (*Canis familiaris*): phylogenetic and secondary structural relatedness of their 16S rRNA genes to other mycoplasmas. *International Journal of Systematic and Evolutionary Microbiology* **52**, 693–698.
- Neimark H, Johansson KE, Rikihisa Y, Tully JG (2001) Proposal to transfer some members of the genera *Haemobartonella* and *Eperythrozoon* to the genus *Mycoplasma* with descriptions of 'Candidatus Mycoplasma haemofelis', 'Candidatus Mycoplasma haemomuris', 'Candidatus Mycoplasma haemosuis' and 'Candidatus Mycoplasma wenyonii'. *International Journal of Systematic and Evolutionary Microbiology* **51**, 891–899.
- Pereira MC, Santos AP (1998) *Ctenocephalides felis felis*: biologia, ecologia e controle integrado (1ª parte – biologia e ecologia). *Clínica Veterinária* **16**, 34–38.
- Tasker S, Helps CR, Day MJ, Harbour DA, Shaw SE, Harrus S, Baneth G, Lobetti RG, Malik R, Beaufils JP, Belford CR, Gruffydd-Jones TJ (2003) Phylogenetic analysis of hemoplasma species: an international study. *Journal of Clinical Microbiology* **41**, 3877–3880.
- Willi B, Boretti FS, Cattori V, Tasker S, Meli ML, Reusch C, Lutz H, Hofmann-Lehmann R (2005) Identification, molecular characterization, and experimental transmission of a new hemoplasma isolate from a cat with hemolytic anemia in Switzerland. *Journal of Clinical Microbiology* **3**, 2581–2585.
- Woods JE, Brewer MM, Hawley JR, Wisnewski N, Lappin MR (2005) Evaluation of experimental transmission of 'Candidatus Mycoplasma haemominutum' and *Mycoplasma haemofelis* by *Ctenocephalides felis* to cats. *American Journal of Veterinary Research* **66**, 1008–1012.

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

