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Molecular Diagnosis of *Mycoplasma haemofelis* and '*Candidatus Mycoplasma haemominutum*' in Domestic Feline: A Case Report

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Case Report

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ABSTRACT

The aim of this work was to report the molecular diagnosis of *Mycoplasma haemofelis* and '*Candidatus Mycoplasma haemominutum*' in a domestic feline. Mycoplasma spp. are pleomorphic bacteria that parasitize the surface of red blood cells in several domestic species, mainly cats, being responsible for feline *mycoplasmosis*, which can cause hemolytic anemia. Cats can become infected through blood transfusions, social interactions via fights and flea bites. In this report, the patient exhibited in the clinical exam, pale and icteric mucous membranes, and enlargement of the popliteal and submandibular lymph nodes. Based on the symptoms presented, a blood sample was collected to search for hemoparasites using blood smears and also molecular examination by polymerase chain reaction (PCR). The blood smear revealed inclusions characteristic of Mycoplasma spp. Observed in red blood cells, and the diagnosis of *mycoplasmosis* was confirmed by PCR. This report highlights the importance of screening for hemoparasites in the feline clinical routine, with molecular tests being the most recommended due to their high sensitivity and specificity.

Keywords: Cats; hemotropic mycoplasma; PCR.

1. INTRODUCTION

Feline *mycoplasmosis* also called Feline hemotrophic *mycoplasmosis* (FHM) or Feline infectious anemia is caused by the bacteria of the genus Mycoplasma. *Mycoplasma haemofelis* (Mhf), '*Candidatus Mycoplasma haemominutum*' (CMhm) and 'Candidatus Mycoplasma turicensis' (CMt) are gram-negative bacteria, showing a coccoid form, that parasitize the surface of red blood in felines [1]. Infection occurs through blood transfusions, vertical transmission, social interactions via fights and flea bites (*Ctenocephalides felis*) [2].

The majority of related cases of *mycoplasmosis* are subclinical adhesion of this bacteria to the surface of erythrocytes can lead to its destruction by the mononuclear phagocytic system, causing hemolytic anemia, from mild to severe [3]. The majority of infected cats are asymptomatic, but when the symptoms appear, the clinical signs include anorexia, fever, loss of weight and pale and/or jaundiced mucous membranes [4]. Splenomegaly, jaundice, and lymphadenopathy may also be present [5].

The infection is considered opportunistic, occurring mainly in immunosuppressed animals, such as those that are stressed animals or showing co-infections, mainly by retroviruses, like feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) [6].

The clinic manifestation depends on the pathogenicity of the bacteria, M. haemofelisbeing the most pathogenic species, able to cause disease in immunocompetent animals, while the infection by '*Candidatus Mycoplasma haemominutum*' is considered less pathogenic, being detected many times through PCR in healthy animals [7].

The diagnosis is based on the bacteria observation in blood smears, although it presents low sensitivity. Therefore the molecular methods, as Polymerase chain reaction (PCR), are the recommendation for the diagnosis, because they have high sensitivity and specificity [1].

The aim of this study was to relate the molecular diagnosis of *Mycoplasma haemofelis* and '*Candidatus Mycoplasma haemominutum*' in domestic feline.

2. PRESENTATION OF CASE

A feline, male, neutered, adult, and mixed breed was attended at the veterinary hospital of the Federal University of Pelotas. Upon inspection, skin lesions were found on the back, with pruritus and an ulcerated nodule on the ear. In the anamnesis, the owner reported that they had adopted the animal about two months ago, already showing the aforementioned lesions. On clinical examination, pale and icteric mucous membranes were observed, as well as enlarged popliteal and submandibular lymph nodes. The other evaluated parameters were within the physiological limits for the feline species.

As complementary exams, a blood sample was collected by venipuncture for blood count and biochemical examination. Due to the symptoms observed, an aliquot was sent for hemoparasite

research through blood smear and molecular examination by polymerase chain reaction (PCR). An ultrasound imaging examination was also performed and due to the skin lesions, collection of material for cytology was performed with the aid of a cervical brush.

3. RESULTS AND DISCUSSION

The Complete Blood Count Test (Table 1) showed macrocytic/hypochromic anemia, with regeneration due to the presence of reticulocytes. Anisocytosis, polychromasia and the presence of Howell-Jolly bodies were also observed. There are two possible mechanisms for the occurrence of anemia: direct damage to the red blood cell membrane by the bacteria and immunomodulation, resulting in a decline in the number of red blood cells due to hemolysis (2). Some studies indicate that Mycoplasma spp. is a predisposing condition for anemia and demonstrate that positive cats are more likely to be anemic than negative cats [8,9,10].

Thrombocytopenia was also observed and the presence of jaundiced plasma. Decrease in platelets was described by other authors, as Martinez et al. [11], that realized hematologic analysis in domestic felines diagnosed with *mycoplasmosis* in the municipality of Osasco, found thrombocytopenia in 46.7% of the cats and by Raimundo et al. [9], in Rio de Janeiro, who observed that of the nine positive cats for M. haemofelis, eight had thrombocytopenia. However, in these same studies, negative animals also had low platelet concentrations, indicating that thrombocytopenia is probably related to other causes, since changes in platelet counts are not consistent with hemoplasma infections [12].

Another alteration observed in the blood count was an increase in total plasma proteins (TPP) (9.0 g/dL). The concentration of total proteins is normally within reference values, but in some cases it may be increased due to hyperglobulinemia, associated with the host's immune response, or in cases of dehydration [13].

Ultrasonography detected heterogeneous splenomegaly with splenic lymph node enlargement and hepatomegaly, which may be related to extramedullary hematopoiesis, erythrocyte sequestration and/or increased activity of splenic macrophages [2].

In the investigation of hemoparasites through blood smears, characteristic inclusion bodies of Mycoplasma spp. in red blood cells (Fig. 1). To confirm the diagnosis found in the direct examination, PCR was performed. Total genomic DNA (gDNA) extraction using the PetNAD™ Nucleic Acid Co-prep Kit column was performed. To detect *Mycoplasma haemofelis* DNA (Mhf) and/or '*Candidatus Mycoplasma haemominutum*' (CMhm) a conventional PCR was performed, in which primers directed to the 16S rRNA gene for Mhf and CMhm were used [7,14] where the forward primer (Hf-F, 5'-ACGAAAGTCTGATGGAGCAATA-3') and reverse primer (Hf-R, 5'-ACGCCCAATAAATCCGRATAAT-3') produced amplicons of 170 bp of Mhf and 193 bp of CMhm, confirming the diagnosis of *mycoplasmosis* (Fig. 2).

Although the bacteria was observed in the smear and the PCR was positive, some authors comparing the two diagnostic techniques indicated greater sensitivity and specificity of PCR, as Raimundo et al. [9], in Rio de Janeiro and Pekel and Duru [15] in Turkey, who, analyzing blood samples from domestic cats finding 11.2% and 22.8% of positive samples for Mycoplasma spp. using PCR and 6% and 13% with smear. Despite being commonly used for Mycoplasma spp. research, the blood smear technique has low specificity and sensitivity, with the possibility of false positive and negative results [16].

Table 1. Result of the feline blood count diagnosed with *Mycoplasmosis*

Eritrogram	Result	Reference value	Leucogram	Result	Reference value
Red Blood cells	3.1 milhões/ul	5-10 milhões/ul	Total leukocytes	9800 /uL	5500-19500 /uL
Hemoglobine	6.0 g/dL	8-15 g/dL	Neutrophils	3430 /uL	2500-12500 /uL
Hematocrit	22.8 %	24-45 %	Lymphocytes	5586/uL	1500-7000/uL
MCV	74.3 fL	39-55 fL	Monocytes	686 /uL	0-850 /uL
MCHC	26.3 %	31-35 %	Eosinophils	98 /uL	0-1500 /uL
Platelets	63 (mil/uL)	300-800 (mil/uL)	TPP	9.0 g/dL	6-8 g/dL

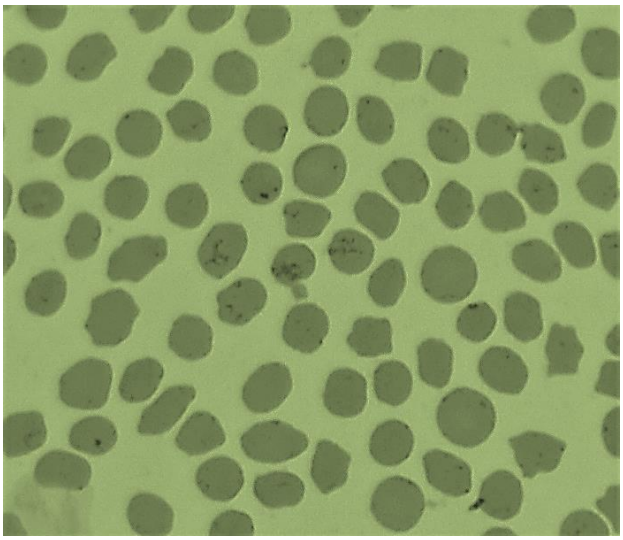

Fig. 1. Photomicrograph showing inclusion bodies in red blood cells, characteristic of *Mycoplasma* spp., observed through optical microscopy, 1000x magnification.

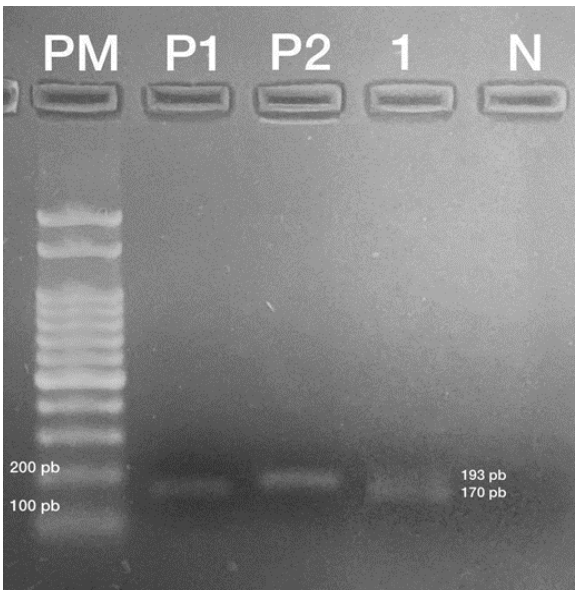

Fig. 2. 1.5% agarose gel after electrophoresis. *Mycoplasma* detection by amplification of the 16S rRNA gene for Mhf (170 bp) and CMhm (193 bp) by conventional PCR. PM: Molecular weight marker (100 bp), P1: Mhf positive control; P2: CMhm positive control; 1: Mhf and CMhm positive sample; N: Negative control

Candidatus Mycoplasma haemominutum' (CMhm) is the most cited hemotropic Mycoplasmaof felines, when compared to *Mycoplasma haemofelis* (Mhf). Some studies confirm this, such as Aragão-de-Souza [17] and Raimundo et al. [9] that reported a prevalence of 7.96% e 11.7% for CMhm and 1.96% and 4.6% for Mhf. These authors along with Jenkins et al. [18] and Kamyingkird et al. [19], noted the same association of both species, in the feline of this report.

Cats with coinfections, mainly by retrovirusis are more susceptible to infection, due to immunosuppression. Some studies were performed with the aim to determine the prevalence and risk factors for hemoplasmas infections in domestic cats naturally infected by feline immunodeficiency virus (FIV) and/or feline leukemia (FeLV), as Duarte et al. [20] who observed that 45% of FIV+ cats and 23.5% of FeLV+ cats were positive for Mycoplasma spp. Maciel et al. [21] in southern Brazil, also found

that of the 18 cats positive for *Mycoplasma haemofelis*, 33.3% had co-infection with FeLV, 5.6% with FIV, and 5.6% with FIV and FeLV. In a study carried out by Vergara et al. [22] in Chile, FIV positive cats had 3.77 times more risk of being infected by hemoplasma infection than negative ones.

Outdoor male felines are more predisposed to infection, due to greater exposure to vectors, as well as being more subject to fights and co-infections [23]. Maciel et al. [21], while analysing factors associated with *Mycoplasma haemofelis* infection in cats in the state of Santa Catarina, Brazil, observed that male cats were seven times more likely to be infected than females (OR 7.07), and those that had access to the street were twice as likely as those who lived only indoors (OR 2.26). Petry et al. [24], determining the prevalence of hemotropic mycoplasmas in cats in the central region of Rio Grande do Sul, observed that of the infected animals, 75% (21/28) were males and 25% (7/28), females. In New Zealand, Jenkins et al. [18], identified a fivefold higher risk of infection in males.

In the analysis of the skin cytology of the patient in the present report, the presence of Sporothrix spp. was observed, indicating a case of sporotrichosis, in addition, he was rescued from the street, corroborating the hypothesis that cats that present co-infections and outdoor acess are more predisposed to developing *mycoplasmosis*.

The recommended therapy consisted of adminstration of Doxycycline, 10 mg/kg, orally, SID, for 21 days. Doxycycline is a broad-spectrum antibiotic, which does not exhibit nephrotoxicity and with reduced side effects and hepatotoxicity in cats [2]. Furthermore, this drug is bacteriostatic, liposoluble, rapidly absorbed, reaching satisfactory serum and intracellular concentrations [25].

It is important to emphasize that oral administration of doxycycline can lead to a severe inflammatory process, resulting in esophagitis and esophageal stenosis in cats. Thus, to avoid this problem, water or wet food should be provided after administration of the drug, in order to reduce the time of contact of the drug with the esophageal mucosa [26].

4. CONCLUSION

From this report, it is evident the importance of researching hemoparasites in the feline clinical practice is crucial, as the confirmation of the causative agent through complementary exams is of paramount importance for the ideal treatment to be advocated. And for this, molecular tests are the most suitable for the diagnosis, as they have high sensitivity and specificity.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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