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RESEARCH ARTICLE OPEN ACCESS

OCCURRENCE OF HEMOTROPIC MYCOPLASMAS IN DOMESTIC CATS (*FELIS CATUS*) FROM BELÉM, BRAZIL

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ABSTRACT

Mycoplasma sp. is a pathogenic bacterium that causes feline mycoplasmosis or even feline infectious anemia, which in most cases is subclinical, but in some cases takes on an acute form resulting in hemolytic anemia. This study had as the objective of to detect the presence of hemotropic mycoplasmas (hemoplasmas) in blood samples from cats using PCR assays, and to association sex, age and hematologic changes. 100 samples were collected for blood count and PCR. Of the samples analyzed, 44% were positive for hemoplasmas. Of the animals testing positive, 42% were males and 45% females, with most animals being over one year old. In hematological alterations there were no relevant differences when PCR-positive and -negative animals were compared. Hemoplasmas infection in felines is frequent in both sexes, and occurs mainly in adult animals. There are no significant hematological changes in infected animals, making molecular examination an extremely important tool in the veterinary clinical routine. Hemoplasmas are of, as they cause infectious diseases that can affect several species of animals.

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INTRODUCTION

Hemotropic mycoplasmas (hemoplasmas) are bacteria with the ability to infect mammalian erythrocytes, leading to clinical disease in infected animals and hematophagous arthropods have been suggested as natural vectors of hemoplasmosis. This pathogen can affect farm and companion animals such as cats. (Sykes, 2010; WillI *et al*., 2007). The main infectious species in cats have been identified in other authors as *Mycoplasma haemofelis* and '*Candidatus* Mycoplasma haemominutum', *Candidatus* Mycoplasma haematoparvum and '*Candidatus* Mycoplasma turicensis' (Willi *et al*., 2006; Neimark *et al*., 2001; Barker & Tasker, 2013). The clinical signs depend on the pathogenicity of the haemoplasma species, and host factors, therefore some cats the infection may be asymptomatic, with mild anemia, or have the following clinical signs observed, anemia accompanied by tachycardia and tachypnea, apathy, inappetence, dehydration, pyrexia, weakness, anorexia, weight loss and mucosal pallor (Tasker, 2010). The diagnosis by blood smears microscopy is a widely used technique (Compton *et al*., 2012).

However, the polymerase chain reaction (PCR)-based assay is considered an effective reference method for diagnosis, as it is a highly sensitive and specific technique that, starting from target sequences (mainly 16S rRNA), promotes exponential amplification of a bacterial DNA fragment, allowing diagnosis of acute and chronic infections (Messick *et al*., 2011). The objective of this study was to detect the presence of hemoplasmas in domestics felines and to identify the associations between hemoplasmosis and sex, age, and hematological changes.

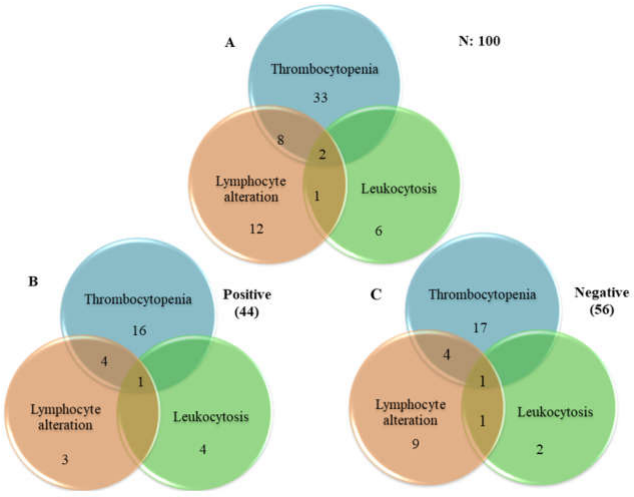
MATERIALS AND METHODS

All samples used in this study were collected in full compliance approved by the Comitê de Ética em Pesquisa com Animais de Experimentação da Universidade Federal rural da Amazônia, protocol nº 048/2017 (CEUA) and 23084.016900/2017-48 (UFRA). Hundred blood samples were collected from domestic cats (*Felis catus*) from the "worthy life" castration program of the UFRA, with owners' permission, between November and December 2017. The cats selected were different ages, breed and sex. Blood samples were

collected by venipuncture of the jugular aseptically to store 1-2 mL of whole blood in a sterile tube containing anticoagulant ethylenediaminetetraacetic acid (EDTA) for immediate blood count, and then stored in an ultra-freezer at -80ºC until the moment of DNA extraction. Erythrogram, leukogram, and platelet analyses were performed with a compact automatic hematology analyzer (BC-2800 Vet®, Mindray, China), configured for the feline species. The reference values used were: red blood cells (5.0-10.0 × 10□ cells / mm^3); hemoglobin (8-15 g / dL); total leukocytes (5,500-19,500); lymphocytes (1,500-7,000 cells / mm3); platelets (300,000-800,000 × 10□ cells / mm^3) (Weiss & Wardrop, 2010). The DNA from the samples was extracted using a commercial kit (Pure Link® Genomic DNA Mini Kit, invitrogen ™, Carlsbad, CA) according to the manufacturer's recommendations. The DNA was eluted in 50 µl of elution buffer and then the samples were stored in the ultra-freezer at -80ºC. The reactions were performed according to Willi *et al*. (2010). For 16S rRNA gene fragment amplification, the following universal primers were used to detect hemotropic mycoplasmas that amplify 150 base pair sequences (pb): Primer forward Myco-LF (5'-AGC AAT RCC ATG TGA ACG ATG AA-3'), Primer reverse Myco-LR1 (5'-TGG CAC ATA GTT TGC TG T CAC TT-3'), and Myco-LR2 (5'-GCT GGC ACA TAG TTA GCT GTC ACT-3'). As a positive control, a sample previously identified as positive for hemoplasmosis was used, and as a negative control sterile double-distilled water was used. The obtained amplicons were analyzed with 3% agarose gel electrophoresis, running in 0.5% TBE buffer at 100 V for 50 minutes. Afterwards, a reading was obtained using an ultraviolet light transilluminator. Amplified fragment size (150 bp) was estimated by comparing with 100 bp and / or 1 Kb DNA ladder molecular weight markers (Invitrogen™, Carlsbad, CA). For analysis of $\chi 2$ test was used BioEstat® version 5.3 software, assuming a significance level of 5% ($p < 0.05$).

RESULTS

Among the domestic cat population tested, 44% (44/100) were positive for hemotropic mycoplasmas (hemoplasmas). Regarding the sex of the positive animals, 42% (14/33) were males and 45% (30/67) were females, with no significant difference between males and females. Regarding the age group, 64% (64/100) were animals with one year old, 26% (26/100) were two years old and 10% (10/100) were three to four years old. In this context, the results for PCR showed that 39% (25/64) of the positive results were between six months to one year old ($p = 0.0287$), 50% (13/26) were older than one year to two years years of age ($p = 1$) and 60% (6/10) were older than two years of age up to four years of age ($p = 0.0455$). In this case, it is possible to observe a higher incidence among older animals, since most of the positive animals in this study were adults. In the hematological analysis, 40% (40/100) did not present any hematological alteration and 60% (60/100) of the samples presented some alteration, 23 of which were lymphocyte alterations, 43 with thrombocytopenia and nine with leukocytosis, with thrombocytopenia being the alteration most prevalent (Chi-square 5,063, $p = 0.0244$). Of the total samples analyzed, none showed anemia. The results of the molecular analysis showed that 56% (56/100) were negative and 44% (44/100) positive, with no statistical difference (Chi-square 1.44, $p = 0.2301$). The split and overlaps of changes can be seen in the figure below (Figure 1). When evaluating platelets separately, it was observed that 43 felines presented platel*et al*teration with thrombocytopenia, of which 49% (21/43) were positive for mycoplasma CRP and 51% (22/43) were negative. The remaining 57 cats showed no change in platelet numbers, although 40% (23/57) were positive by PCR. Regarding lymphocytes, it was observed that only 23 felines showed lymphocyte alteration, in which 35% (8/23) were positive in PCR and 65% (15/23) were negative, however, 77 felines presented normal lymphocyte series, of which 47% (36/77) were positive by PCR. Leukocytes, on the other hand, were observed that only nine cats showed alteration, leukocytosis, of these 56% (5/9) were positive by PCR and 44% (4/9) negative; the remaining 91 cats did not show changes in leukocyte numbers, of which 43% (39/91) were positive by PCR.



Figure[1]. (a) Hematological changes by the total number of samples examined (b) hematological changes by the total of positive samples (c) hematological changes by the total number of negative samples

DISCUSSION

Until now, these microorganisms have been reported in different cat populations in Brazil. SANTIS *et al*. (2014) in the state of Mato Grosso do Sul, observed an infection rate of 36.4% (55/151), corroborating the results of this study. In other Brazilian states, the infection rate was lower than observed in this study. For example, MACIEIRA *et al*. (2007) in Rio de Janeiro report 11.7% infection (18/149), Braga *et al* (2012) in Maranhão reported 12% (29/200), BORTOLI *et al*. (2012) in São Paulo reported 6.5% (3/46), MICELI *et al*. (2013) in Mato Grosso reported 8.4% (15/178), Aragão de Sousa *et al*. (2013) in Belém reported 19.9% (40/201) and PETRY (2013) in Rio Grande do Sul reported 14.6% infection. According to Willi *et al*. (2009), understanding the prevalence of hemoplasmosis is problematic when comparing different studies, because the populations studied are often quite different. In some studies, the sample consists of anemic cats, while others have clinical conditions or even regardless of their health. In addition, other factors can influence infection rates, such as the climate and geography of different regions, and the frequency of exposure to ectoparasites. These results show higher infection rates to those found by Ferreira & Alves (2018) in stray cats on the island of Faro, Portugal, where DNA from *Mycoplasma* spp. was detected using PCR in 20.4% (32/157) of the animals. In contrast to the current study, 73.1% of animals infected were male and 26.9% were female. Similarly, Aquino *et al*. (2014) found that male cats had a significantly higher overall prevalence of hemoplasmas infection ($p = 0.01$) and were more likely to be positive for *Mycoplasma haemofelis* ($p = 0.004$), positive for '*Candidatus* Mycoplasma haemominutum' ($p = 0.009$), and positive for '*Candidatus* Mycoplasma turicensis' ($p = 0.03$) than females were.

According to Tasker *et al*. (2002) and Sykes *et al*. (2010) this higher occurrence of infection in males may be linked to behavioral patterns. Male cats are more susceptible than females, probably due to more aggressive behavior and social interaction through fights, ultimately exposing the animals to the organisms responsible for the infection. Ferreira & Alves (2018) reported that 95.7% of infected animals were adults, and 4.3% were less than one year old. Corroborating the results obtained in this study, Bauer *et al*. (2008) also described this relationship between older animals, where they remain a carrier state for several years, but this result differs from that observed in Marques (2013) and Aquino (2015) research where there was a greater predisposition to infection in young felines. Unlike this study, Vicente (2015) did not report a significant difference in infection rates between animals older or younger than seven years. Regarding hematological analyses, the results corroborate Firmino *et al*. (2016) who did not reveal significant differences. This is likely due to the

mild effects of '*Candidatus* Mycoplasma haemominutum' alone or on co-infection with *Mycoplasma haemofelis*. Although anemia was not present in any of the samples analyzed, in a study by FIRMINO *et al*. (2016) cats infected with *M. haemofelis* had normochromic normocytic anemia, whereas cats infected with '*Candidatus* Mycoplasma haemominutum' or both species did not. And according to Tasker *et al*. (2010) cats can be affected by different degrees of anemia. Meanwhile Duarte *et al*. (2015) and Santos *et al*. (2014) explain that the absence of anemia may indicate a long duration of exposure to hemoplasmosis, resulting in a host-parasite balance. In accordance with this study, Raimundo *et al*. (2016) observed that among the 197 cats analyzed at veterinary clinics in Rio de Janeiro, PCR assay results for *Mycoplasma haemofelis* were 4.6% positive and 95.4% negative, and for positive hematological values 16.2% showed anemia, 7.8% thrombocytopenia, 14.8% lymphocytosis and 6% showed the presence of activated monocytes. No hematological changes were associated with infection. The hematological alterations of the cats analyzed, when related to the PCR results, did not have a significant difference, as observed in Aquino (2015) research. According to SANTOS *et al*. (2014) hematocrit in asymptomatic cats can vary over time from normal or mild to moderately decreased. Likewise, the percentage of leukocytes is very variable and of little help in diagnosis, and in platelet counts the values are usually between the reference values. According to Firmino *et al*. (2016) leukogram analysis often does not show significant alterations, and no correlation between the phase of the disease and the percentage of neutrophils and lymphocytes. In this study, despite the leukocyte and lymphocyte alterations, they did not have significant values when compared to the PCR results.

CONCLUSION

Infection by hemoplasmas in clinically healthy felines was found mainly in adult animals of both sexes equally, the prevalence is higher with age and often unassociated with hematological changes. This makes the use of more advanced techniques, such as PCR, essential as a diagnostic tool in the detection of hemoplasmas. In addition, healthy cats may be asymptomatic carriers of this agent, emphasizing the importance of screening tests in blood donor animals. Moreover, caution is advised when subjecting these animals to procedures that may cause immune suppression, as the reactivation of infection could lead to clinical disease.

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