Frequency of *Mycoplasma haemocanis* infection in dogs of Cuiaba, Mato Grosso, Brazil

Frequência da infecção por *Mycoplasma haemocanis* em cães de Cuiabá, Mato Grosso, Brasil

Débora Ferreira dos Santos[1]; Arleana do Bom Parto Ferreira de Almeida[2]; Mayara Aparecida Araújo Cayuela[3]; Vanessa Danielle de Freitas[1]; Maíra Fernanda Gonçalves Koiyama[3]; Valéria Dutra[2]; Valéria Régia Franco Sousa[2*]

Abstract

Haemotropic mycoplasmas are pleomorphic, epicellular, gram-negative bacteria, which infect red blood cells. In dogs, the acute phase of this infection may lead to haemolytic anaemia potentially precipitated by splenectomy, or by immunosuppressive drugs or diseases; however, in cases of chronic infection, the signs are inapparent. Molecular techniques and microscopic observation are the most commonly used methods to diagnose mycoplasma infection, though studies about haemotropic mycoplasma infections in dogs from Cuiabá, capital of Mato Grosso, Brazil are scarce. The objective of this study was to evaluate the frequency of mycoplasma infection, the *Mycoplasma* species present, and factors associated with infection in 334 dogs diagnosed using polymerase chain reaction (PCR). The frequency of infection was 5.4%, and *M. haemocanis* was the only species identified. No epidemiological factor investigated showed a statistically significant association with infection, including tick infestation.
Key words: Epidemiological survey. Mycoplasmosis. PCR. Sequencing.

Resumo

Micoplasmas hemotróficos são bactérias pleomórficas, epicelulares, gram negativas, que infectam eritrócitos**.** A infecção em cães pode causar anemia hemolítica na fase aguda que pode ser precipitada por esplenectomia, ou por drogas e/ou doenças imunossupressoras, porém na doença crônica os sinais são inaparentes. Além da observação microscópica, as técnicas moleculares são os métodos diagnósticos mais empregados. No município de Cuiabá, Mato Grosso, Brasil, ainda são escassos os estudos sobre a infecção por micoplasmas hemotróficos em cães. Diante do exposto, o objetivo deste estudo foi determinar a frequência de *Mycoplasma* spp., assim como os fatores associados à infecção em 334 cães avaliados pela Reação em Cadeia da Polimerase (PCR). A frequência da infecção por *Mycoplasma* spp. foi de 5,4% e *M. haemocanis* foi a única espécie identificada. Nenhum fator epidemiológico investigado apresentou associação estatisticamente significativa com a infecção, mesmo a infestação por carrapatos.
Palavras-chave: Inquérito epidemiológico. Micoplasmose. PCR. Sequenciamento.

[1] Médicas Veterinárias Residentes. Programa de Residência Uniprofissional em Medicina Veterinária, PRUMV, Universidade Federal de Mato Grosso, UFMT, Cuiabá, MT, Brasil. E-mail: ferreira01.debora@gmail.com; vanessa.danielle@hotmail.com

[2] Profs., Faculdade de Medicina Veterinária, FAVET, UFMT, Cuiabá, MT, Brasil. E-mail: arleferreira@gmail.com; valeriadutra. dutra@gmail.com; valeriaregia27@gmail.com

[3] Discentes, Graduação em Medicina Veterinária, UFMT, Cuiabá, MT, Brasil. E-mail: mayaraaacayuela@gmail.com; mairakoiyam@hotmail.com

* Author for correspondence

Haemotropic mycoplasmas are pleomorphic non-cultured, cell wall-free bacteria in the class Mollicutes and genus *Mycoplasma* which infect the erythrocytes of various mammals. *Candidatus* Mycoplasma haematoparvum and *Mycoplasma haemocanis* are the main species of haemotropic mycoplasmas which infect domestic dogs (VALLE et al., 2014).

Studies related to the transmission pathways of haemotropic mycoplasmas are scarce (BIONDO et al., 2009). However, they are believed to be transmitted by the tick *Rhipicephalus sanguineus* sensu lato (NOVACCO et al., 2010), either by direct inoculation or transplacental transmission (COMPTON et al., 2012).

Many dogs do not present clinical signs, but one to two weeks after infection or activation fever, weight loss, anorexia, lethargy, and pallor of mucous membranes may occur (BIONDO et al., 2009) in conjunction with laboratory findings such as severe anaemia, reticulocytosis and polychromasia as a result of haemolysis (SHARIFIYAZDI et al., 2014).

The diagnostic technique using microscopic detection of haemotrophic mycoplasmas in blood smears has low sensitivity and specificity (KEMMING et al., 2004). However, the detection of *Mycoplasma* DNA with conventional PCR or real-time PCR has been widely used in prevalence studies in various regions (NOVACCO et al., 2010).

The objective of this study was to use PCR to verify the frequency and species of *Mycoplasma* sp. as well as factors associated with infection in domestic canines living in the city of Cuiabá, Mato Grosso, Brazil.

Considering the estimated population of 2295 dogs from the 2007 canine census (Municipal Health Department, 2007 unpublished data) and assuming a prevalence of 50% and acceptable error rate of 5%, the minimum sample was determined to be 329 dogs. The blood samples were collected previously in a serological survey for visceral leishmaniasis (ALMEIDA et al., 2012) with consent of the owners and completion of an epidemiological questionnaire and individual clinical record. Aliquots of blood were stored (-20 ° C) in microtubes with ethylenediaminetetraacetic anticoagulant (EDTA). Dogs of different ages, sexes, and breeds from the city of Cuiabá (15 ° 35'56.10 "S and 56 ° 05 '41.62" W), capital of the State of Mato Grosso, were included. Dogs were collected from urban areas in the neighbourhoods of Osmar Cabral, Bela Vista and Jardim União (240 dogs) and rural areas in the neighbourhoods of Barreiro Branco and Coxipó do Ouro (94 dogs).

The extraction of DNA from the blood samples was performed with the phenol-chloroform method and precipitation by isopropanol (SAMBROOK; RUSSELL, 2001). To ensure the quality and integrity of the DNA, the canine endogenous gene β-globin was evaluated in 10% of the samples by PCR following the protocol of Quaresma et al. (2009).

PCR was performed using universal primers for *Mycoplasma* sp.: forward (HBT-F) = ATACGGCCCATATTCCTACG (positions 313-332 in AF-178677); reverse (HBT-R) = TGCTCCACCACTTGTTCA (positions 889-908 in AF-178677), delimiting a 595 bp product. The thermal cycling profile consisted of initial heating for 10 min at 94 ° C followed by 40 cycles of 30 sec at 94 ° C, 30 sec at 56 ° C, and 30 sec at 72 ° C, and a final extension of 10 min at 72 ° C (CRIADO-FORNELIO et al., 2003). The amplification products were analysed on 1.5% agarose gel via electrophoresis with a molecular weight marker of 100 bp, stained with GelRed ™ (Biotium®), and observed in a Photodocumentator Chemidoc ™ XRS using Image Lab software. In each reaction, negative (DNA free reaction) and positive (KY863524) control samples were used.

After purification of the PCR product with GFX PCR DNA purification kit (GE Healthcare®), all samples positive for *Mycoplasma* were sequenced with an ABI-PRISM 3500 Genetic

Analyzer (Applied Biosystems®) according to the manufacturer's recommendations.

To examine the association between the factors investigated and infection by *Mycoplasma*, non-parametric chi-square or Fisher's exact tests were applied.

Of the 334 dogs evaluated, detection via DNA amplification of *M. haemocanis* occurred in 18 dogs (5.4%). The DNA sequences obtained in the present study were identified after alignment with DNA sequences deposited with GenBank with the aid of the Blast program. The sequences showed 99% homologous identity with the sequence KY117659.1, 98% homology with the sequence KY002678.1 and 97% homology with sequence KY002679.1.

The low prevalence of infection found is similar to that found in Campo Grande, Mato Grosso do Sul: a 4.25% infection rate among 94 dogs tested (SOARES et al., 2016). However, the infection rate in this study was higher than the observed prevalence of *M. haemocanis* (1.9%) and *Candidatus* M. haematoparvum (0.6%) in 154 dogs from Ribeirão Preto (ALVES et al., 2014). The difference between the prevalences may vary according to the population sampled, the distribution of *R. sanguineus* (a probable vector), or the sensitivity of the diagnostic technique employed (AQUINO et al., 2016). Table 1 shows factors investigated for association with *M. haemocanis* infection.

Table 1. Univariate analysis of the factors associated with *Mycoplasma haemocanis* infection in 18 dogs living in Cuiabá, Mato Grosso, Brazil in 2018.

Variables	Positive	(%)	P
Sex			
Male	10	55,2%	0,78
Female	8	47,8%	
Breed			
SRD	15	83,3%	0,85
Purebred	3	16,7%	
Mucous			
Pales	3	16,7%	0,93
Normocoradas	15	83,3%	
Hyperemic	0	0%	
Ectoparasites			
Yes	17	94,4%	0,22
No	1	5,5%	
Access on street			
Yes	14	77,8%	0,25
No	4	22,2%	
Place of residence			
Urban	12	66,7%	0,62
Rural	6	33,3%	

Of dogs with the infection, 7/18 (38.9%) were aged between 1 and 3 years old, a greater percentage than in other age groups; 3/18 (16.7%) animals were aged less than 1 year, 4/18 (22.2%) were 3-6 years of age, and the remaining 4 (22%) were >6 years of age. There was no apparent bias of infection by sex or breed. Of those infected, 10/18 (55.6%) were male dogs while 8/18 (44.4%) were females. Approximately 83.3% of the dogs were mixed-breed, while 3/18 were purebred. No dog had a history of splenectomy. In a previous study, Aquino et al. (2016) did not identify any significant age or breed predisposition despite detecting *M. haemocanis* infection more in males (differing from that observed in this study).

In Brazil, the rate of infestation by *R. sanguineus* ticks in dogs is high (SILVA et al., 2017), especially in urban areas; therefore, it logically follows that there would be a higher potential for mycoplasmosis. However, the incidence of the disease is generally low when compared to other pathogens transmitted by ticks such as *Anaplasma platys*, *Babesia vogeli,* and *Ehrlichia canis* (RAMOS et al., 2010). Seventeen dogs with *M. haemocanis* infections were infested with *R. sanguineus*, but this factor was not statistically significant (p >0.05); this induces scepticism regarding whether the tick is the main vector or if other arthropods participate in the transmission of M. haemocanis (SOARES et al., 2016).

Eleven of the 18 dogs (61.1%) infected with *M. haemocanis* were had free access to the street, while the 7 others were confined to homes; street dogs were not significantly more likely to have the disease. In comparison, in southern Europe it was found that crossbred dogs living in shelters who were previously free-roaming were significantly more predisposed to hemoplasma infection (NOVACCO et al., 2010).

Of the infected dogs, 13 (72.2%) were determined to have a good body condition, while five (27.8%) had average body conditions; this is likely related to the fact that in most cases in dogs, *Mycoplasma* infection is chronic and asymptomatic (VALLE et al., 2014). Among clinical signs observed in dogs with *M. haemocanis* infection, pale mucous membranes were observed in just 3/18 dogs (16.7%). A previous study in Switzerland found no relationship between *M. haemocanis* infection and anaemia (WENGI et al., 2008).

It can be concluded that the frequency of *Mycoplasma* infection in dogs living in Cuiabá is relatively low, and the variables analysed in this study were not predisposing factors to infection. *Mycoplasma haemocanis* was the only species identified in this study.

Ethics Committee

This study was approved by the Ethics Committee for the Use of Animals (CEUA) of the Federal University of Mato Grosso under protocol number 23108019868 / 099.1.

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