Research Note

Braz. J. Vet. Parasitol., Jaboticabal, v. 25, n. 3, p. 359-363, jul.-set. 2016
ISSN 0103-846X (Print) / ISSN 1984-2961 (Electronic)
Doi: http://dx.doi.org/10.1590/S1984-29612016043

Occurrence of *Mycoplasma haemocanis* in dogs infested by ticks in Campo Grande, Mato Grosso do Sul, Brazil

Ocorrência de *Mycoplasma haemocanis* em cães infestados por carrapatos em Campo Grande, Mato Grosso do Sul, Brasil

Rodrigo Leite Soares[1]; Jessica Teles Echeverria[1]; Giovana Pazzuti[1]; Herbert Patric Kellerman Cleveland[2]; Verônica Jorge Babo-Terra[1]; Elisabete Friozi[3]; Carlos Alberto do Nascimento Ramos[1,2]

[1] Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul – UFMS, Campo Grande, MS, Brasil

[2] Laboratório de Biologia Molecular, Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul – UFMS, Campo Grande, MS, Brasil

[3] Centro de Controle de Zoonoses, Campo Grande, MS, Brasil

Received November 13, 2015
Accepted March 2, 2016

Abstract

Hemotropic mycoplasmas in dogs, such as *Mycoplasma haemocanis*, have been described worldwide. Recently, these pathogens have been reported to be causative agent of zoonosis. It is known that its transmission may occur through the action of blood-sucking arthropods (e.g. ticks or fleas), through blood transfusion, contaminated fomites and/or transplacentally. In Brazil, *M. haemocanis* is present in practically all regions and the tick *Rhipicephalus sanguineus* sensu lato is suspected the main vector. In the municipality of Campo Grande, state of Mato Grosso do Sul, there is little information about infection of dogs by *M. haemocanis*, or on the main epidemiological features associated with it. Thus, the aim of the present study was to determine the occurrence of *M. haemocanis* among dogs infested by ticks and to assess possible associations with some epidemiological factors. The polymerase chain reaction (PCR) and DNA sequencing were used to analyze dog blood samples (n = 94). DNA from *M. haemocanis* was detected in four samples. No significant associations were observed with any epidemiological parameter analyzed here. However, the results from this study confirm that this pathogen is circulating in this region and should be considered in the differential diagnosis of diseases among anemic dogs.

Keywords: Canine hemoplasmas, molecular diagnosis, central western Brazil.

Resumo

Micoplasmas hemotrópicos de cães, como *Mycoplasma haemocanis*, já foram descritos em todo o mundo. Recentemente, esses patógenos têm sido apontados como causadores de zoonoses. É sabido que a transmissão pode ocorrer devido à ação de artrópodes sugadores de sangue (carrapatos, pulgas), transfusão sanguínea e/ou fômites contaminados e por via transplacentária. No Brasil, *Mycoplasma haemocanis* está presente em praticamente todas as regiões, e o carrapato *Rhipicephalus sanguineus sensu lato* é suspeito como principal vetor. No município de Campo Grande, Estado de Mato Grosso do Sul, Brasil não existem muitas informações acerca de infecções de cães por *M. haemocanis,* assim como quais são os principais aspectos epidemiológicos associados a este patógeno. Assim, o objetivo, no presente estudo, foi determinar a ocorrência de *M. haemocanis* em cães infestados por carrapatos e analisar possíveis associações com alguns fatores epidemiológicos. A Reação em Cadeia da Polimerase (PCR) e o sequenciamento de DNA foram utilizados para analisar amostras de sangue de cães (n = 94). DNA de *M. haemocanis* foi identificado em quatro amostras. Não foram observadas associações significativas com qualquer parâmetro epidemiológico analisado. No entanto, os resultados deste estudo confirmam que esse patógeno está circulando na região e deve ser considerado no diagnóstico diferencial de causas de anemia em cães.

Palavras-chave: Hemoplasma caninos, diagnóstico molecular, Centro-Oeste do Brasil.

***Corresponding author:** Carlos Alberto do Nascimento Ramos. Universidade Federal de Mato Grosso do Sul – UFMS, Avenida Senador Filinto Muller, 2443, Vila Ipiranga, CEP 79070-900, Campo Grande, MS, Brasil.
e-mail: carlos.nascimento@ufms.br

Introduction

The genus *Mycoplasma* comprises a group of bacteria without a cell wall and with a diminutive genome that makes them strictly dependent on the host cell. These bacteria may infect several species of animals and are associated with apathy, arthritis, fever, hemolytic anemia, infertility, lethargy, respiratory disorders, lymphadenopathy, pale mucosae, urogenital disease and weight loss (CHALKER, 2005; WILLI et al., 2007; COSTA, 2011; SILVA-SANTOS et al., 2014).

Hemoplasma species that affect dogs, as *Mycoplasma haemocanis* and "*Candidatus* Mycoplasma haematoparvum", have been described throughout the world (BARKER et al., 2010; ROURA et al., 2010; RANI et al., 2011; COMPTON et al., 2012; SILVA-SANTOS et al., 2014; HII et al., 2015). Indeed, it has been demonstrated that transmission may occur mainly through blood-sucking arthropods, blood transfusion, contaminated fomites and/or transplacentally. The tick *Rhipicephalus sanguineus* sensu lato (s.l.) has been considered to be the main vector of *M. haemocanis* among dogs (SENEVIRATNA et al., 1973; MESSICK, 2003).

Most of infected dogs show chronic asymptomatic infection (HOSKINS, 1991; CHALKER, 2005; COSTA, 2011). In fact, disease manifestations in animals are most frequently reported in special cases, for example in association with drugs or retrovirus-induced immunosuppression, poor nutrition, pregnancy, lactation and other concomitant diseases (MACIEIRA et al., 2008).

In Brazil, canine hemotropic mycoplasmas has been reported in the northeastern region (RAMOS et al., 2010; SILVA-SANTOS et al., 2014), southeastern region (O'DWYER et al., 1997; ALVES et al., 2014), southern region (VALLE et al., 2014) and central-western regions (COSTA, 2011). In the state of Mato Grosso do Sul (MS), which is located in the central-western region of Brazil, only one survey of hemoplasmas mycoplasmas has been conducted so far, among cats (SANTIS et al., 2014). Therefore, the aim of the present study was to assess the occurrence of hemotropic mycoplasmas among dogs in Campo Grande, MS, Brazil, and to identify the main epidemiological features associated with the positivity to these pathogens.

Materials and Methods

The present study was approved by the Ethics Committee for Animal Experimentation of the Federal University of Mato Grosso do Sul (protocol number 592/2014), from August 2014 to April 2015. A convenience sampling was conducted by collecting 94 blood samples (n = 94) from dogs at the Zoonosis Control Center of the municipality of Campo Grande, state of Mato Grosso do Sul, Brazil. Samples were collected by puncturing cephalic vein and the material obtained was stored in sterile tubes containing EDTA (ethylenediaminetetraacetic acid).

Sampling was performed only from dogs infested by ticks, with no restrictions regarding gender, breed, age or origin. Clinical and epidemiological information on all animals was obtained (i.e. breed, gender, contact with fleas, outdoor access and urban or rural origin). Ticks were collected and stored in 70% ethanol for further identification using the identification key modified by Barros-Battesti et al. (2006).

DNA samples were extracted from 350µl of blood using the methodology described by Araújo et al. (2009). The concentration and integrity of the extracted samples were evaluated by means of spectrophotometry (BioPhotometer Plus; Eppendorf®) and agarose gel electrophoresis (1%), respectively. In order to check for the absence of PCR inhibitors, all samples were subjected to PCR targeting the β-actin gene (WANG et al., 2007).

Afterwards, the samples were analyzed through conventional PCR for *Mycoplasma* sp., using a primer set (fHf5 and rHf6) previously described to identify *Mycoplasma haemofelis* in cats (MESSICK et al., 1998) and later successfully used to detect *M. haemocanis* in a dog (BRINSON & MESSICK, 2001).

The PCR reactions were performed in a final volume of 25 µL, containing 10 mM Tris-HCL (pH 8.3), 50 mM of KCl, 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide (dNTP), 1.25 U of Taq DNA polymerase (Ludwig Biotec), 11 pmol of each primer, and approximately 100 ηg of genomic DNA. The cycling condition were: 94 °C for three minutes, followed by 30 cycles at 94 °C for one minute, annealing at 51 °C for 30 seconds and extension at 72 °C for 40 seconds. A final extension step at 72 °C for three minutes was performed.

Mycoplasma haemocanis positive sample used as the positive control was obtained in a previous study, and its DNA sequence is deposited in GenBank under accession number FJ911910 (RAMOS et al., 2010). Nuclease-free water was used as the negative control.

The amplification products were viewed under ultraviolet light after electrophoresis on agarose gel (2%) stained with GelRed (Biotium) according to manufacturer's instructions.

Positive amplicon bands were cut from gels and purified using a commercial kit (Qiaex II; Qiagen), in accordance with the manufacturer's instructions. The samples were sequenced in both directions using an automated sequencer (3130 ABI; Applied Biosystems). The chromatograms were evaluated and edited using BioEdit v.7.2.5 software (HALL et al., 1999), and consensus sequences were subjected to BLASTn search (ALTSCHUL et al., 1990), in order to determine the sequence identity by comparison with orthologous sequences available in the GenBank database.

Results and Discussion

All the DNA samples showed amplification for the constitutive gene (β-actin), indicating thus the absence of PCR inhibitors.

Four (4.25%) out of the 94 blood samples analyzed revealed amplicons (650bp) compatible with *Mycoplasma* sp., in accordance with Messick et al. (1998). Through DNA sequence analysis, it was possible to identify the species *Mycoplasma haemocanis* (99% identicalness with sequences available in the GenBank database). Considering that all the DNA sequences obtained in the presented study showed 100% of identity with each other, only one sequence was deposited in GenBank, under the accession number KT163241.

A total of 345 ticks were collected from the animals sampled, and all were identified as *R. sanguineus* s.l.

Studies on frequency using PCR to detect *M. haemocanis* have been conducted in several regions of Brazil, and have presented similar results (RAMOS et al., 2010; COSTA, 2011; ALVES et al., 2014). In the present study, only samples from dogs parasitized by *R. sanguineus* s.l. were used.

In Brazil, despite the high rate of infestation by *R. sanguineus* s.l. among dogs (LABRUNA & PEREIRA, 2001; ALMEIDA et al., 2013), the frequency of hemotropic mycoplasmas is generally low, especially when compared with other tick-borne pathogens such as *Ehrlichia canis*, *Anaplasma platys* and *Babesia vogeli*, also transmitted by ticks (RAMOS et al., 2010; COSTA, 2011). This observation may raise questions about role of *R. sanguineus* s.l. as the main vector of *Mycoplasma*, especially because there is only one study that has proven the capacity of *R. sanguineus* in transmitting *M. haemocanis* (SENEVIRATNA et al., 1973). In fact, studies have correlated the frequency of infection and presence of ticks in dogs. However, significant associations have not always been observed (BARKER et al., 2010). Moreover, the action of other arthropod vectors should be considered, since the same climatic conditions that favor tick development may also favor them. Thus, when a positive correlation between ticks and hemoplasmas is found, probably it is also found for these other arthropods. In addition, other transmission routes in dogs, including the participation of other arthropods should be investigated.

Several arthropods (e.g. ticks, fleas, lice, mites, flies and mosquitoes) have been identified as vectors of numerous species of hemoplasmas in different vertebrate hosts (Table 1). Most of them are known to infest different species and represent a possible interspecific transmission vector, as probably occurred in animal-origin human mycoplasmosis (SANTOS et al., 2008; MAGGI et al., 2013). Therefore, it is mandatory to investigate the vector competence of other arthropods in relation to hemotropic mycoplasmas transmission to dogs, since the importance of the *R. sanguineus* tick remains unclear.

In the present study, was not possible to perform an association study between *M. haemocanis* PCR positive results and epidemiological parameters (i.e. breed, gender, contact with fleas, outdoor access and urban or rural area) due to the low number of PCR positive samples. However, no significant associations were found in studies carried out in France (KENNY et al., 2004), Switzerland (WENGI et al., 2008), and Brazil (ALVES et al., 2014). Although association between contact with fleas and infection by *M. haemocanis* was not evaluated in the present study, three of the four infected animals had a history of flea infestation.

Interestingly, in southern Europe, Novacco et al. (2010) observed that young mongrel dogs living in shelters were significantly more predisposed towards infection by hemoplasmas. On the other hand, in Tanzania and Trinidad and Tobago (BARKER et al., 2010), male dogs with free access to outdoor environments were significantly more likely to present infection by hemoplasmas. In these cases, contact with infected blood during fights would probably favor infection, since no association with tick infestation was observed. Although contact with saliva was not confirmed as a risk factor for infection, a study by Sazaki et al. (2008) showed that dogs of the Tosa breed, which are usually known as fighting dogs, are more prone to infection in Japan.

Table 1. Hemoplasma species and their known vectors for some vertebrate hosts.

Host	Vector	Vector species	Agent	Reference
Dogs	Ticks	*R. sanguineus*	*Mycoplasma haemocanis* *"C.*M. haematoparvum"	Seneviratna et al. (1973)
Cats	Fleas	*Ctenocephalides felis*	*M. haemofelis* *"C.*M. haemominutum"	Woods et al. (2005)
Mice	Lice	*Polyplax serrata* *P. spinulosa*	*M. coccoides*	Berkenkamp & Wescott (1988)
Cattle	Flies	*Stomoxys calcitrans* *Haematobia irritans* *Tabanus bromius* *T. bovinus*	*M. alkalescens* *M. arginini* *M. bovirhinis* *M. bovis* *M. mycoides mycoides*	Santos et al. (2012)
	Mites	*Raillietia auris* *R. flechtmanni*	*M. conjutiviae* *M. capricolum*	
Pig	Mosquitoes Flies	*Aedes aegypti* *S. calcitrans*	*M. suis*	Prullage et al. (1993)
Birds	Mosquitoes	*Culex pipiens pipiens*	*M. gallisepticum*	Darbro et al. (2007)
Human	not determined	not determined	*M. haemofelis* *M. ovis* *M. haematoparvum*	Santos et al. (2008) Sykes et al. (2010) Maggi et al. (2013)

Immunosuppression and weakness caused by other concomitant diseases have been identified as important factors that favor infection by these agents (PRYOR & BRADBURY, 1975; KRAKOWKA, 1977; GRETILLAT, 1981; NOVACCO et al., 2010; ROURA et al., 2010). In Brazil, coinfections by *M. haemocanis* and other tick-borne pathogens in dogs have also been reported (TRAPP et al., 2006; RAMOS et al., 2010). In the present study, two dogs were positive for *M. haemocanis* and sero positive for *Leishmania* sp., but it was not possible to assure the correlation between the pathogens (i.e. *M. haemocanis* and *Leishmania sp.*). Because of low sanitary conditions of these animals, other pathogens might also be present, but detection of microorganisms other than hemoplasmas was outside the scope of this study.

Despite the low frequency of *M. haemocanis* infection in dogs in Campo Grande (4/94), the results from this study confirm that this pathogen is circulating in this region and should be considered in the differential diagnosis of diseases among anemic dogs.

References

Almeida RCF, Garcia MV, Cunha RC, Matias J, Silva EA, Matos MFC, et al. Ixodid fauna and zoonotic agents in ticks from dogs: first report of *Rickettsia rickettsii* in *Rhipicephalus sanguineus* in the state of Mato Grosso do Sul, mid-western Brazil. *Exp Appl Acarol* 2013; 60(1): 63-72. http://dx.doi.org/10.1007/s10493-012-9641-y. PMid:23229491.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol* 1990; 215(3): 403-410. http://dx.doi.org/10.1016/S0022-2836(05)80360-2. PMid:2231712.

Alves TB, Faggion SA, Santos EV, Roberto PG, França SC, Fachin AL, et al. Real-time PCR-based study of haemotrophic mycoplasmas in dogs from Ribeirão Preto, Brazil. *Arch Med Vet* 2014; 46(2): 333-336. http://dx.doi.org/10.4067/S0301-732X2014000200021.

Araujo FR, Ramos CAN, Luiz HL, Peres IAHFS, Oliveira RHM, Souza IIF, et al. *Avaliação de um protocolo de extração de DNA genômico a partir de sangue total.* Campo Grande: Embrapa Gado de Corte; 2009. 5 p. 120 Comunicado Técnico.

Barker EN, Tasker S, Day MJ, Warman SM, Woolley K, Birtles R, et al. Development and use of real-time PCR to detect and quantify *Mycoplasma haemocanis* and "*Candidatus* haematoparvum" in dogs. *Vet Microbiol* 2010; 140(1-2): 167-170. http://dx.doi.org/10.1016/j.vetmic.2009.07.006. PMid:19646827.

Barros-Battesti DM, Arzua M, Bechara GH. *Carrapatos de importância médico-veterinária da região neotropical: um guia ilustrado para identificação de espécies*. São Paulo: Vox/ICTTD-3/Butantan; 2006. 223 p.

Berkenkamp SD, Wescott RB. Arthropod transmission of *Eperythrozoon coccoides* in mice. *Lab Anim Sci* 1988; 38(4): 398-401. PMid:3184845.

Brinson JJ, Messick JB. Use of a polymerase chain reaction assay for detection of *Haemobartonella canis* in a dog. *J Am Vet Med Assoc* 2001; 218(12): 1943-1945, 1936. http://dx.doi.org/10.2460/javma.2001.218.1943. PMid:11417738.

Chalker VJ. Canine mycoplasmas. *Res Vet Sci* 2005; 79(1): 1-8. http://dx.doi.org/10.1016/j.rvsc.2004.10.002. PMid:15894017.

Compton SM, Maggi RG, Breitschwerdt EB. *Candidatus* Mycoplasma haematoparvum and *Mycoplasma haemocanis* infections in dogs from the United States. *Comp Immunol Microbiol Infect Dis* 2012; 35(6): 557-562. http://dx.doi.org/10.1016/j.cimid.2012.06.004. PMid:22819621.

Costa HX. *Interação de hemoparasitos e hemoparasitoses em casos clínicos de trombocitopenia em cães no município de Goiânia* [Dissertação]. Goiânia: Escola de Veterinária, Universidade Federal de Goiás; 2011.

Darbro JM, Dhondt AA, Vermeylen FM, Harrington LC. *Mycoplasma gallisepticum* infection in house finches (*Carpodacus mexicanus*) affects mosquito blood feeding patterns. *Am J Trop Med Hyg* 2007; 77(3): 488-494. PMid:17827365.

Gretillat S. *Haemobartonella canis* (Kiküth, 1928) in the blood of dogs with parvovirus disease. *J Small Anim Pract* 1981; 22(10): 647-653. http://dx.doi.org/10.1111/j.1748-5827.1981.tb00568.x. PMid:7334796.

Hall RE, Kestler DP, Agarwal S, Goldstein KM. Expression of the monocytic differentiation/activation factor P48 in *Mycoplasma* species. *Microb Pathog* 1999; 27(3): 145-153. http://dx.doi.org/10.1006/mpat.1999.0293. PMid:10455005.

Hii SF, Traub RJ, Thompson MF, Henning J, O'Leary CA, Burleigh A, et al. Canine tick-borne pathogens and associated risk factors in dogs presenting with and without clinical signs consistent with tick-borne diseases in northern Australia. *Aust Vet J* 2015; 93(3): 58-66. http://dx.doi.org/10.1111/avj.12293. PMid:25708788.

Hoskins JD. Canine haemobartonellosis, canine hepatozoonosis and feline cytauxzoonosis. *Vet Clin North Am Small Anim Pract* 1991; 21(1): 129-140. http://dx.doi.org/10.1016/S0195-5616(91)50013-9. PMid:2014617.

Kenny MJ, Shaw SE, Beugnet F, Tasker S. Demonstration of two distinct hemotropic Mycoplasmas in French dogs. *J Clin Microbiol* 2004; 42(11): 5397-5399. http://dx.doi.org/10.1128/JCM.42.11.5397-5399.2004. PMid:15528754.

Krakowka S. Transplacentally acquired microbial and parasitic disease of dogs. *J Am Vet Med Assoc* 1977; 171(8): 750-753. PMid:200592.

Labruna MB, Pereira MC. Carrapato em cães no Brasil. *Clin Vet (Milano)* 2001; 6(30): 24-32.

Macieira DB, Menezes RCAA, Damico CB, Almosny NRP, McLane HL, Daggy JK, et al. Prevalence and risk factors for hemoplasmas in domestic cats naturally infected with feline immunodeficiency virus and/or feline leucemia virus in Rio de Janeiro – Brazil. *J Feline Med Surg* 2008; 10(2): 120-129. http://dx.doi.org/10.1016/j.jfms.2007.08.002. PMid:17905624.

Maggi RG, Mascarelli PE, Havenga LN, Naidoo V, Breitschwerdt EB. Co-infection with *Anaplasma platys, Bartonella henselae* and *Candidatus* Mycoplasma haematoparvum in a veterinarian. *Parasit Vectors* 2013; 6(1): 103. http://dx.doi.org/10.1186/1756-3305-6-103. PMid:23587235.

Messick JB, Berent LM, Cooper SK. 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. *J Clin Microbiol* 1998; 36(2): 462-466. PMid:9466759.

Messick JB. New perspectives about *Hemotrophic mycoplasma* (formely, *Haemobartonella* and *Eperythrozoon* species) infections in dogs and cats. *Vet Clin North Am Small Anim Pract* 2003; 33(6): 1453-1465. http://dx.doi.org/10.1016/j.cvsm.2003.08.002. PMid:14664208.

Novacco M, Meli ML, Gentilini F, Marsilio E, Ceci C, Pennisi MG, et al. Prevalence and geographical distribution of canine hemotropic mycoplasma infections in Mediterranean countries and analysis of risk factors for infection. *Vet Microbiol* 2010; 142(3-4): 276-284. http://dx.doi.org/10.1016/j.vetmic.2009.09.069. PMid:19931320.

O'Dwyer LH, Guimarães L, Massard CL. Occurence of multiple infection with *Babesia canis, Hepatozoon canis* and *Haemobartonella canis* in a splenectomized dog. *Rev Bras Cienc Vet* 1997; 4(2): 83-84.

Pryor WH Jr, Bradbury RP. *Haemobartonella canis* infection in research dogs. *Lab Anim Sci* 1975; 25(5): 566-569. PMid:1242503.

Prullage JB, Williams RE, Gaafar SM. On the transmissibility of *Eperythrozoon suis* by *Stomoxys calcitrans* and *Aedes aegypti. Vet Parasitol* 1993; 50(1-2): 125-135. http://dx.doi.org/10.1016/0304-4017(93)90013-D. PMid:8291187.

Ramos R, Ramos C, Araújo F, Oliveira R, Souza I, Pimentel D, et al. Molecular survey and genetic characterization of tick-borne pathogens in dogs in metropolitan Recife (north-eastern Brazil). *Parasitol Res* 2010; 107(5): 1115-1120. http://dx.doi.org/10.1007/s00436-010-1979-7. PMid:20680344.

Rani PAMA, Irwin PJ, Coleman GT, Gatne M, Traub RJ. A survey of canine tick-borne diseases in India. *Parasit Vectors* 2011; 4(1): 141. http://dx.doi.org/10.1186/1756-3305-4-141. PMid:21771313.

Roura X, Peters RI, Altet L, Tabar MD, Barker EN, Planellas M, et al. Prevalence of hemotropic mycoplasmas in healthy and unhealthy cats and dogs in Spain. *J Vet Diagn Invest* 2010; 22(2): 270-274. http://dx.doi.org/10.1177/104063871002200219. PMid:20224091.

Santis ACGA, Herrera HM, Sousa KCM, Gonçalves LR, Denardi NCB, Domingos IH, et al. Molecular detection of hemotrophic mycoplasmas among domiciled and free-roaming cats in Campo Grande, state of Mato Grosso do Sul, Brazil. *Rev Bras Parasitol Vet* 2014; 23(2): 231-236. http://dx.doi.org/10.1590/S1984-29612014039. PMid:25054503.

Santos AP, Santos RP, Biondo AW, Dora JM, Goldani LZ, Oliveira ST, et al. Hemoplasma infection in HIV-positive patient, Brazil. *Emerg Infect Dis* 2008; 14(12): 1922-1924. http://dx.doi.org/10.3201/eid1412.080964. PMid:19046522.

Santos SB, Nascimento ER, Faccini JLH, Barreto ML, Mota RA. Associação entre *Mycoplasma* spp. e ácaros do conduto auditivo de bovinos. *Pesqui Vet Bras* 2012; 32(4): 293-296. http://dx.doi.org/10.1590/S0100-736X2012000400004.

Sazaki M, Ohta K, Matsuu A, Hirata H, Ikadai H, Oyamada T. A molecular survey of *Mycoplasma haemocanis* in dogs and foxes in Aomori Prefecture, Japan. *J Protozool Res* 2008; 18(2): 57-60.

Seneviratna P, Weerasinghe N, Ariyadasa S. Transmission of *Haemobartonella canis* by the dog tick, *Rhipicephalus sanguineus. Res Vet Sci* 1973; 14(1): 112-114. PMid:4736045.

Silva-Santos M, Matos PT, Lima VFS, Meira-Santos PO, Rocha LB. Micoplasmose em cães: relato de 4 casos. *Enciclopédia Biosfera* 2014; 10(19): 1298-1306.

Sykes JE, Lindsay LL, Maggi RG, Breitschwerdt EB. Human coinfection with *Bartonella henselae* and two hemotropic Mycoplasma variants resembling *Mycoplasma ovis. J Clin Microbiol* 2010; 48(10): 3782-3785. http://dx.doi.org/10.1128/JCM.01029-10. PMid:20702675.

Trapp SM, Messick JB, Vidotto O, Jojima FS, de Morais HSA. *Babesia gibsoni* genotype Asia in dogs from Brazil. *Vet Parasitol* 2006; 141(1-2): 177-180. http://dx.doi.org/10.1016/j.vetpar.2006.04.036. PMid:16765518.

Valle ST, Messick JB, Santos AP, Kreutz LC, Duda NCD, Machado G, et al. Identification, occurence and clinical findings of canine hemoplasmas in southern Brazil. *Comp Immunol Microbiol Infect Dis* 2014; 37(4): 259-265. http://dx.doi.org/10.1016/j.cimid.2014.08.001. PMid:25159996.

Wang YS, Chi KH, Chu RM. Cytokine profiles of canine monocyte-derived dendritic cells as a function of lipopolysaccharide or tumor necrosis factor-alpha-induced maturation. *Vet Immunol Immunopathol* 2007; 118(3-4): 186-198. http://dx.doi.org/10.1016/j.vetimm.2007.05.010. PMid:17617471.

Wengi N, Willi B, Boretti FS, Cattori V, Riond B, Meli ML, et al. Real-time PCR-based prevalence study, infection follow-up and molecular characterization of canine hemotropic mycoplasmas. *Vet Microbiol* 2008; 126(1-3): 132-141. http://dx.doi.org/10.1016/j.vetmic.2007.06.018. PMid:17656047.

Willi B, Boretti FS, Tasker S, Meli ML, Wengi N, Reusch CE, et al. From *Haemobartonella* to hemoplasma: molecular methods provide new insights. *Vet Microbiol* 2007; 125(3-4): 197-209. http://dx.doi.org/10.1016/j.vetmic.2007.06.027. PMid:17706380.

Woods JE, Brewer MM, Hawley JR, Wisnewski N, Lappin MR. Evaluation of experimental transmission of *Candidatus Mycoplasma haemominutum* and *Mycoplasma haemofelis* by *Ctenocephalides felis* to cats. *Am J Vet Res* 2005; 66(6): 1008-1012. http://dx.doi.org/10.2460/ajvr.2005.66.1008. PMid:16008224.