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Serological Survey of *Ehrlichia canis*, *Babesia canis* and *Leishmania infantum* in a Brazilian Canine Population

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ABSTRACT

This study evaluated occurrences of babesiosis, ehrlichiosis and leishmaniasis, and the presence of coinfection due to these diseases among dogs in the municipality of Concordia, in the west of the state of Santa Catarina (SC), Brazil. Blood was collected from 424 dogs that were attended at the Clinical Practice Center of the Federal Institute of Santa Catarina, Concordia, and also in clinics in the city and its rural zone. The presence of antibodies against *L. infantum* was investigated using the Indirect Immunofluorescence reaction and against *Babesia canis* and *Ehrlichia canis* using the Enzyme-Linked Immunosorbent Assay. The chi-square test at the significance level of 5% was used to compare the positive and negative animals in the sampled population. Out of the 424 samples evaluated, 170 (40.09%) were positive for ehrlichiosis, 178 (41.98%) were positive for babesiosis, and 59 (13.91%) were positive for leishmaniasis. There were statistical differences regarding ehrlichiosis in relation to the variables of area, street access and breed. Moreover, among the positive samples, there were coinfection reactions. From these results, it can be suggested that these three diseases are present in the municipality of Concordia, SC.

Introduction

Canine ehrlichiosis, babesiosis and leishmaniasis are diseases transmitted through vectors that have a high incidence in many countries around the world (Greene, 2012). The diseases unleashed by these hemoparasites can trigger similar clinical-laboratory alterations in their hosts, and this may lead to conflicting diagnoses (Miró *et al.*, 2013; Maggi *et al.*, 2014).

The agent that causes canine ehrlichiosis is *Ehrlichia canis* (Dumler *et al.*, 2001), which is mainly transmitted by the tick *Rhipicephalus sanguineus* (Lewis *et al.*, 1977). In its subclinical phase, antibody titers remain in serum for periods greater than six months, thus indicating that the duration of infection is prolonged and that antigen stimulation occurs (Waner *et al.*, 1997; Harrus *et al.*, 1998). Thus, dogs are left incapable of setting up an effective immune response and remain chronically infected (Harrus *et al.*, 1998). For these cases, Enzyme-Linked Immunosorbent Assay (ELISA) is one of the serological methods that mostly used for diagnosis of the disease (Waner *et al.*, 2001).

Canine babesiosis is caused by the protozoa *Babesia canis* and *Babesia gibsoni*, and it is transmitted by the tick *R. sanguineus* (Ayoob *et al.*, 2010). In babesiosis, some animals may remain in the subclinical phase (Macintire *et al.*, 2002), which makes the ELISA test useful in these cases as well. Moreover, this test also makes it possible to diagnose babesiosis in situations of low parasitemia in both the chronic and the subacute phase (Olicheski, 2003).

The vectors involved in the transmission of leishmaniasis that are found in Brazil are *Lutzomyia longipalpis* and *Lutzomyia cruzi* (Santos *et al.*, 1998; Lainson and Shaw, 1987). Domestic dogs are the main reservoir for *L. infantum*, and they play an essential role in the transmission of this disease (Castro-Junior *et al.*, 2014), both because of their proximity to humans and because many dogs are positive but asymptomatic. Domestic dogs act as carriers of the parasite and form a source of contamination for the vectors that transmit the disease (Marzochi *et al.*, 2009). Serological tests like Indirect Immunofluorescence (IIF) are frequently used to detect this disease in epidemiological surveys (Alves and Bevilacqua, 2004).

In this light, the present study had the aim of evaluating occurrences of *E. canis*, *B. canis* and *L. infantum*, in dogs in the

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municipality of Concordia, in the west of the state of Santa Catarina, and also the presence of coinfection among the hemoparasites.

Materials and methods

Experimental animals

Serum from 424 dogs (*Canis familiaris*) was used in this study. All of these samples were used in evaluating occurrences of ehrlichiosis, leishmaniasis and babesiosis. The dogs selected could either be presenting clinical alterations compatible with hemoparasites, or could be asymptomatic. All of them were attended either at the Clinical Practice Center of the Federal Institute of Santa Catarina, Concordia or at private clinics in the city or on farms in this municipality's rural zone. From each dog, 5 mL of total blood was collected. The serum was then separated and was used to detect anti-*E. canis*, anti-*B. canis* and anti-*L. infantum* antibodies. From the animals that were sampled, information on sex, access to the streets, place of residence, age and breed was compiled. The samples collected were stored at -20 °C until the time of processing. This study was approved by the Ethics Committee for the use of animals, at the Federal Institute of Santa Catarina, under protocol number 31/2014.

ELISA for the detection of anti-E. canis and anti-Babesia canis vogeli antibodies

To detect anti-*E. canis* and anti-*Babesia canis* vogeli antibodies, the ELISA test was used, through kits marketed by the Imunodot laboratory (Imunotest, Brazil). A sample dilution of 1:200 µL was used, and the reaction was conducted following the manufacturer's instructions. In the end, the plates were read in an ELISA reader with a filter of 405 nm (Mindray MR-96A). To interpret the results, a cutoff index was calculated (average optical density of the negative control serum, multiplied by 2.5). The samples that presented intense yellowish coloration and an optical density greater than or equal to the cutoff index were considered to be positive for *E. canis* and *B. canis*.

Indirect immunofluorescence (IIF) test for the detection of anti-L. infantum antibodies

To perform the IIF test, kits marketed by Imunodot (Imunotest, Brazil) for *L. infantum* were acquired. The tests were run using a sample dilution of 1:40 µL. The readings were performed under an optical microscope using inverted light/fluorescence (BEL Photonics- FLUO 3). The samples were considered positive if greenish fluorescence from the parasites was present across the entire surface, and negative if there was no fluorescence.

Statistical analysis

The chi-square test at the significance level of 5% was used to compare the variables (sex, street access, place of residence, age and breed of the animals) between the positive and negative animals of the sampled population, using Minitab 19 statistical software.

Results

Out of the 424 samples evaluated, 170 (40.09%) were positive for ehrlichiosis, 178 (41.98%) for babesiosis and 59 (13.91%) for leishmaniasis, as displayed in Tables 1, 2 and 3, respectively. Only for ehrlichiosis were there statistically significant differences ($P \leq 0.05$) in relation to the place of residence, street access and breed ($P = 0.0001$, 0.0010 and 0.0081 respectively). Moreover, among the 424 animals, 10 (2.36%) presented co-infection among the three diseases: 69 (16.27%) between babesiosis and ehrlichiosis, 19 (4.48%) between ehrlichiosis and leishmaniasis and 17 (4.00%) between babesiosis and leishmaniasis.

Table 1. Seropositivity for anti-*E. canis* antibodies, according to ELISA, among dogs in the municipality of Concordia, Santa Catarina.

Variables		Dogs	
		Positive (n/%)	Negative (n/%)
Sex	Female[a]	100/170 (58.82)	169/254 (66.53)
	Male[a]	70/170 (41.18)	85/254 (33.46)
Location	Urban area[a]	113/170 (66.47)	223/254 (87.80)
	Rural area[b]	57/170 (33.53)	31/254 (12.20)
Street access	With street access[a]	86/170 (50.59)	88/254 (34.64)
	Without street access[b]	84/170 (49.41)	166/254 (65.35)
Breed	Mongrel[a]	96/170 (56.47)	125/254 (49.21)
	Poodle[b]	10/170 (5.88)	38/254 (14.96)
	German Shepherd[b]	8/170 (4.70)	2/254 (0.79)
	Pinscher[b]	6/170 (3.53)	21/254 (8.27)
	Other breeds[a]	50/170 (29.41)	68/254 (26.77)
Age	Less than 1 year[a]	8/170 (4.70)	15/254 (5.90)
	Between 1 and 5 years[a]	67/170 (39.41)	78/254 (30.70)
	Between 5 and 10 years[a]	49/170 (28.82)	74/254 (29.13)
	Over 10 years[a]	35/170 (20.59)	74/254 (29.13)
	Not informed[a]	11/170 (6.47)	13/254 (5.12)

[a,b] Different lowercase letters indicate the presence of a statistically significant difference, with a significance level of 5% ($P < 0.05$).

Table 2. Seropositivity for anti-*B. canis* antibodies, according to ELISA, among dogs in the municipality of Concordia, Santa Catarina.

Variables		Dogs	
		Positive (n/%)	Negative (n/%)
Sex	Female	117/178 (65.73)	151/246 (61.38)
	Male	61/178 (34.27)	95/246 (38.61)
Location	Urban area	146/178 (82.02)	190/246 (77.23)
	Rural area	32/178 (17.98)	56/246 (22.76)
Street access	With street access	71/178 (39.89)	104/246 (42.28)
	Without street access	107/178 (60.11)	142/246 (57.72)
Breed	Mongrel	91/178 (51.12)	130/246 (52.84)
	Poodle	17/178 (9.55)	31/246 (12.60)
	Pinscher	8/178 (4.49)	20/246 (8.13)
	Other breeds	62/178 (34.83)	65/246 (26.42)
Age	Less than 1 year	5/178 (2.81)	19/246 (7.72)
	Between 1 and 5 years	64/178 (35.95)	81/246 (32.93)
	Between 5 and 10 years	56/178 (31.46)	73/246 (29.67)
	Over 10 years	39/178 (21.91)	63/246 (25.61)
	Not informed	14/178 (7.86)	10/246 (4.06)

Table 3. Seropositivity for anti-*L. infantum* antibodies, according to the Indirect Immunofluorescence (IIF) reaction, among dogs in the municipality of Concordia, Santa Catarina.

Variables		Dogs	
		Positive (n/%)	Negative (n/%)
Sex	Female	37/59 (62.71)	232/365 (63.56)
	Male	22/59 (37.29)	133/365 (36.44)
Location	Urban area	48/59 (81.35)	288/365 (78.90)
	Rural area	11/59 (18.65)	77/365 (21.10)
Street access	With street access	19/59 (32.20)	156/365 (42.74)
	Without street access	40/59 (67.80)	209/365 (57.26)
Breed	Mongrel	36/59 (61.02)	185/365 (50.68)
	Poodle	5/59 (8.47)	43/365 (11.78)
	Pinscher	4/59 (6.77)	25/365 (6.85)
	Other breeds	14/59 (23.73)	112/365 (30.68)
Age	Less than 1 year	-	22/365 (6.03)
	Between 1 and 5 years	23/59 (38.98)	122/365 (33.42)
	Between 5 and 10 years	17/59 (28.81)	114/365 (31.23)
	Over 10 years	17/59 (28.81)	85/365 (23.29)
	Not informed	2/59 (3.39)	22/365 (6.03)

Discussion

Studies conducted in Brazil have shown that the seroprevalence of ehrlichiosis in dogs is similar among the regions, for example: 31.2% in northern Brazil, 42.5% in the central region, 35.6% in the northeast and 44.7% in the southeast (Aguiar *et al.*, 2007; Costa *et al.*, 2007; Saito *et al.*, 2008; Silva *et al.*, 2010; Souza *et al.*, 2010). However, in one study conducted in the southern region by Saito *et al.* (2008), among the 389 dogs surveyed, only 19 (4.8%) were positive for *E. canis*. Another study developed by Labarthe *et al.* (2003), also showed a low prevalence of *E. canis* in the southern region, comprising 4.7% in Parana, 1.7% in Rio Grande do Sul and 0.7% in Santa Catarina. The present study is divergent from theirs, in that we observed seroprevalence of 40.09% among the animals. Thus, the prevalence found in the municipality of Concordia is similar to that of other regions of Brazil. This may suggest that the disease is occurring in an endemic form.

Regarding the seropositivity for babesiosis that was found in this study, it was observed that this finding corroborates the results from studies conducted in other regions, such as 42.2% in Sao Paulo, 51.4% in Minas Gerais and 35.7% on Paraná. In all of these studies, the IIF technique was used (Dell' Porto *et al.*, 1993; Milken *et al.*, 2004; Trapp *et al.*, 2006). Based on these results, it can be suggested that this disease is en-

demic in this municipality.

In the present study, it was observed that 59 (13.91%) of the animals tested showed antibodies for *L. infantum*. This result allows us to suggest the disease is occurring in Concordia, and this serves as a warning: if a focus of the transmitting agent is present, this municipality will be in a situation of risk regarding the transmission of this disease between dogs and humans.

Most animals that are seropositive for leishmaniasis are asymptomatic (Barata *et al*., 2013; Penaforte *et al*., 2013). This emphasizes the importance of dogs as domestic reservoirs for *L. infantum* (Silva, 2007). The data of the present study reinforce this, since not all the positive animals showed clinical signs consistent with the disease.

Maziero *et al*. (2014) conducted a study in the west of the state of Santa Catarina on 252 animals and found that, among them, 43 showed seropositivity for *L. infantum* through the IIF test. These authors also suggested this region is exposed to the agent, which was thus in line with the findings of the present study. Another occurrence that reinforces the suggestion that the western region is exposed to the agent was the leishmaniasis case that was confirmed in the municipality of Chapeco.

Coinfection between babesiosis and ehrlichiosis was found in 69 (16.27%) of the animals, and this rate was higher than what has been reported in the literature. In a study conducted by Krawczak *et al*. (2015), nine (9.37%) out of 96 animals showed coinfection, using the IIF test. Fonseca *et al*. (2017) detected a rate of 5.6% in their study, using the same serological test. Krawczak *et al*. (2015) also observed (via the IIF and ELISA tests) that 4.3% of the dogs examined were positive for ehrlichiosis and leishmaniasis. The findings of the present study were in line with theirs, since coinfection between ehrlichiosis and leishmaniasis occurred in 4.48% (19/424) of our animals.

In Campo Grande, Mato Grosso do Sul, a study conducted by Sousa (2012), demonstrated that some dogs that were serologically positive for leishmaniasis also showed antibodies against babesiosis and ehrlichiosis, among which 39 (65%) were co-infected with ehrlichiosis, 26 (43.33%) with babesiosis and 20 (33.33%) with both of these. In the present study, considering the same perspective, the percentage of the dogs that were serologically positive for leishmaniasis and presented coinfection was lower, such that 32.2% (19/59) were coinfected with ehrlichiosis, 28.81% (17/59) with babesiosis and 16.95% (10/59) with both of these diseases. This was possibly due to the favorable climatic conditions in the Campo Grande region for vector development (Salgado, 2006). Moreover, the situation regarding leishmaniasis in the state of Mato Grosso do Sul is "worrisome", according to this state's health department (Sousa, 2012).

The existence of cross-reactions among antibodies is questionable in relation to diagnosing blood parasitoses. Ferreira *et al*. (2007) reported that cross-reactivity due to *E. canis* occurred in animals that were seropositive for leishmaniasis. Mettler *et al*. (2005) identified reactivity due to *B. canis* in relation to the same disease. However, studies conducted in endemic areas have found through serological tests that there are no cross-reactions between *E. canis*, *B. canis* and *L. infantum* but, rather, occurrences of coinfection (Oliveira *et al*., 2000, Krawczak *et al*., 2015). Because of these discrepancies in the results, additional studies on the samples from Concordia are needed. Such studies would use techniques like molecular biology to identify the DNA of these agents in the samples obtained through the present study. Only in this manner will it become possible to ascertain how disseminated these diseases are and how many of these seropositive samples might have resulted from cross-reactions with other pathogens.

Conclusion

From the present study, it can be suggested that canine visceral leishmaniasis is a disease that is present in the municipality of Concordia. Given that canine cases precede human cases, there is a need to study the presence of *Phlebotominae* in this city. Regarding babesiosis and ehrlichiosis, it can be suggested that both of these diseases are endemic in this municipality. Lastly, studies using molecular biology techniques are needed in order to evaluate the possibility of cross-reactions with other pathogens and thus ascertain the real seropositivity.

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Conflict of interest

Authors declared no conflict of interests exists.

References

Aguiar, D.M., Cavalcante, G.T., Pinter, A., Gennari, S.M., Camargo, L.M.A., Labruna, M.B., 2007. Prevalence of *Ehrlichia canis* (Rickettsiales: Anaplasmataceae) in dogs and *Rhipicephalus sanguineus* (Acari: Ixodidae) ticks from Brazil. J. Med. Entomol. 44, 126–132.

Alves, W.A., Bevilacqua, P.D., 2004. Reflexões sobre a qualidade do diagnóstico da leishmaniose visceral canina em inquéritos epidemiológicos: o caso da epidemia de Belo Horizonte, Minas Gerais, Brasil, 1993-1997. Cad. Saúde Pública 20, 259-265.

Ayoob, A.L., 2010. Clinical management of canine babesiosis. J. Vet. Emerg. Crit. Care 20, 77-89.

Barata, R.A., Peixoto, J.C, Tanure, A., Gomes, M.E., Apolinario, E.C., Bodevan, E.C., Araújo, H.S., Dias, E.S., Pinheiro, A.C., 2013. Epidemiology of Visceral Leishmaniasis in a Reemerging Focus of Intense Transmission in Minas Gerais State, Brazil. Biomed. Res. Int. 2013, 1-6.

Castro-Júnior, J.G., Freire, M.L., Campos, S.P.S., Scopel, K.K.G., Porrozzi, R., Silva, E.D., Colombo, F.A., Silveira, R.C.V., Marques, M.J., Coimbra, E.S., 2014. Evidence of *Leishmania (Leishmania) Infantum* Infection in Dogs from Juiz De Fora, Minas Gerais State, Brazil, Based On Immunochromatographic Dual-Path Platform (Dpp%) And Pcr Assays. Ver. Ins. Med. Trop. 56, 225-229.

Costa, L.M.Jr., Rembeck, K., Ribeiro, M.F., Beelitz, P., Pfister, K., Passos, L.M., 2007. Seroprevalence and risk indicators for canine ehrlichiosis in three rural areas of Brazil. Vet. J. 174, 673–676.

Dell' Porto, A., Oliveira, M.R., Miguel, O., 1993. *Babesia canis* in stray dogs of the city of Sao Paulo. Comparative studies between the clinical and hematological aspects and the indirect fluorescent antibody test. Rev. Bras. Parasitol. Vet. 2, 37-40.

Dumler, J.S., Barbet, A.F., Bekker, C.P.J., Dasch, G.A., Palmer, G.H., Ray, S.C., Rikihisa, Y., Rurangirwa, F.R., 2001. Reorganization of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order *Rickettsiales*: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of Ehrlichia equi and HGE agent as subjective synonyms of *Ehrlichia phagocytophila*. Int. J. Syst. Evolut. Microbiol. 51, 2145-2165.

Ferreira, E.C., Lana, M., Carneiro, M., Reis, A.B., Paes, D.V., Silva, E.S., Shallig, H., Gontijo, C.M., 2007. Comparison of serological assays for the diagnosis of canine visceral leishmaniasis in animals presenting different clinical manifestations. Vet. Parasitol. 146, 235–241.

Fonseca, J.P., Bruhn, F.R.P., Ribeiro, M.J.M., Hirsch, C., Rocha, C.M.B.M.,

Guedes, E., Guimaraes, A.M., 2017. Hematological Parameters and Seroprevalence of *Ehrlichia canis* and *Babesia vogeli* in Dogs. Ciênc. Anim. Bras. 18, 1-9.

Greene, C.E., 2012. Infectious Diseases of the dog and cat, Fourth Ed. Saunders Elsevier, St. Louis, MO, USA, pp. 1383.

Harrus, S., Waner, T., Aizenberg, I., Foley, J.E., Poland, A.M., Bark, H., 1998. Amplification of ehrlichial DNA from dogs 34 month safter infection with *Ehrlichia canis*. J. Clin. Microbiol. 36, 73-76.

Krawczak, F.S., Reis, I.A., Silveira, J.A., Avelar, D.M., Marcelino, A.P., Werneck, G.L., Labruna, M.B., Paz, G.F., 2015. *Leishmania, Babesia* and *Ehrlichia* in urban pet dogs: co-infection or cross-reaction in serological methods? Ver. Soc. Bras. Med. Trop. 48, 64-68.

Labarthe, N., Pereira, M.C., Barbarini, O., McKee, W., Coimbra, C.A., Hoskins, J., 2003. Serologic Prevalence of *Dirofilaria immitis*, *Ehrlichia canis*, and *Borrelia burgdorferi* Infections in Brazil. Vet. Ther. 4, 67-75.

Lainson, R., Shaw, J.J., 1987. Evolution, classification and geographical distribution. In: The Leishmaniasis in Biology and Medicine. Academic Press, London, Eng, pp. 1-120.

Lewis G.E., 1977. The Brown dog tick *Rhipicephalus sanguineus* and the dog as experimental hosts of *Ehrlichia canis*. Am. J. Vet. Res. 32, 1953-1955.

Macintire, D.K., Boudreaux, M.K., West, G.D., Bourne, C., Wright, J.C., Conrad PA. 2002. *Babesia gibsoni* infection among dogs in the southeastern United States. J. Am. Vet. Med. Assoc. 220, 325–329.Maggi, R.G., Birkenheuer, A.J., Hegarty, B.C., Bradley, J.M., Levy M.G., Breitschwerdt E.B., 2014. Comparison of serological and molecular panels for diagnosis of vector-borne diseases in dogs. Parasit. Vectors. 7, 2–9.

Marzochi, M.C.A., Fagundes, A., Andrade, M.V., Souza, M.B., Madeira, M.F., Mouta-Confort, E., Schubach, A.O., Marzochi, K.B.F., 2009. Visceral leishmaniasis in Rio de Janeiro, Brazil: eco-epidemiological aspects and control. Rev. Soc. Bras. Med. Trop. 42, 570-580.

Maziero, N., Thomaz-Soccol, V., Steindel, M., Link, J.S., Rossini, D., Alban, S.M., Nascimento, A.J., 2014. Rural-urban focus of canine visceral leishmaniosis in the far werten region of Santa Catarina State, Brazil. Vet. Parasitol. 205, 92–95.

Mettler, M., Grimm, F., Capelli, G., Camp, H., Deplazes, P., 2005. Evaluation of Enzyme-Linked Immunosorbent Assays, an Immunofluorescent-Antibody Test, and Two Rapid Tests (Immunochromatographic-Dipstick and Gel Tests) for Serological Diagnosis of Symptomatic and Asymptomatic Leishmania Infections in Dogs. J. Clin. Microbiol. 43, 5515–5519.

Milken, V.M.F., Cabral, D.D., Figueiredo, J.F., Gonçalves, C.L., 2004. Ocorrência de babesiose canina no município de Uberlândia, Minas Gerais. Arq. Ciênc. Vet. Zool. 7, 19-22.

Miró, G., Montoya, A., Roura, X., Gálvez, R., Sainz, A., 2013. Seropositivity rates for agents of canine vector-borne diseases in Spain: a multicentre study. Parasit. Vectors. 6, 1–9.

Olicheski, A.T., 2003. Comparação entre os métodos de coloração panótico rápido e giemsa para o diagnóstico de protozoários do gênero Babesia (Starcovici, 1893) e de riquétsias do gênero *Ehrlichia* (Ehrlich, 1888) em cães (Canis familiaris) no município de Porto Alegre, RS, Brasil. Thesis, Master of Veterinary Medicine, Universidade Federal do Rio Grande do Sul.

Oliveira, D., Tie Nishimori, C., Costa, M.T., Machado, R.Z., Castro M.B., 2000. Anti-*Ehrlichia canis* antibodies detection by "Dot-ELISA" in naturally infected dogs. Rev. Bras. Parasitol. Vet. 9, 1-5.

Penaforte, K.M., Belo, V.S., Teixeira-Neto, R.G., Ribeiro, R.A.N., Oliveira, R.B., Schettini D.A., Silva, ES., 2013. Leishmania infection in a population of dogs: na epidemiological investigation relating to visceral leishmaniasis control. Rev, Bras. Parasitol. Vet. 22, 592-596.

Saito, T.B., Cunha-filho, N.A., Pacheco, R.C., Ferreira, F., Pappen, F.G., Farias, N.A.R., Larsson, C.E., Labruna, M.B., 2008. Canine Infection by *Rickettsiae* and *Ehrlichiae* in Southern Brazil. Am. J. Trop. Med. Hyg. 79, 102–108.

Salgado, F.P., 2006. Identificação de hemoparasitos e carrapatos de cães procedentes do Centro de Controle de Zoonoses de Campo Grande Estado do Mato Grosso do Sul, Brasil. Thesis, Master of Veterinary Medicine, Universidade Federal de Mato Grosso do Sul.

Santos, S.O., Arias, J., Ribeiro, A.A., Paiva, H.M., Freitas, R.A., Malacco, M.A., 1998. Incrimination of *Lutzomyia cruzi* as a vector of American visceral leishmaniasis. Med. Vet. Entomol. 12, 315–317.

Silva, F.S., 2007. Patologia e patogênese da leishmaniose visceral canina. R. Tróp. Ci. Agr. Biol. 1, 20-31.

Silva, J.N., Almeida, A.B.P.F., Sorte E.C.B., Freitas, A.G., Santos, L.G., Aguiar, D.M., Sousa, V.R.F., 2010. Soroprevalência de anticorpos anti-*Ehrlichia canis* em cães de Cuiabá, Mato Grosso. Ver. Bras. Parasitol. Vet. 19, 34–37.

Sousa, K.C.M., 2012. Coinfecção por *Ehrlichia canis*, *Leishmania chagasi* e *Babesia canis* em cães naturalmente infectados em Campo Grande, Mato Grosso do Sul. Thesis, Master of Veterinary Medicine, Faculdade de Ciências Agrárias e Veterinárias.

Souza, B.M.P.S., Leal, D.C., Barboza, D.C.M., Uzêda, R.S., De Alcântara, A.C., Ferreira, F., Labruna, M.B., Gondim, L.F.P., Franke C.R., 2010. Prevalence of ehrlichial infection among dogs and ticks in northeastern Brazil. Ver. Bras. Parasitol. Vet. 19, 15–19.

Trapp, S.M., Dagnone, A.S., Vidotto, O., Freire, R.L., Amude, A.M., Morais, H.S.A., 2006. Seroepidemiology of canine babesiosis and ehrlichiosis in a hospital population. Vet. Parasitol. 140, 223-230.

Waner, T., Harrus, S., Bark, H., Bogin, E., Avidar, Y., Keysary, A., 1997. Characterization of subclinical phase of canine ehrlichiosis in experimentally infected beagles dogs. Vet. Parasitol. 69, 307-317.

Waner, T., Harrus, S., Jongejan, F., Bark, H., Keysary, A., Cornelissen, A.W., 2001. Significance of serological testing for ehrlichial diseases in dogs with special emphasis of canine monocytic ehrlichiosis caused by *Ehrlichia canis*. Vet. Parasitol. 95, 1–15.