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Molecular, serological, and parasitological detection of *Babesia vogeli* in dogs in the state of Piauí, Brazil

Detecção molecular, sorológica e parasitológica de *Babesia vogeli* em cães do estado do Piauí, Brasil

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Abstract

Studies on canine babesiosis in northeastern Brazil are scarce, although the weather conditions in this region are favorable for the development of the tick vector. This study determined the prevalence of *Babesia vogeli* in dogs sampled in Teresina, state of Piauí, northeast Brazil, using direct and indirect diagnostic methods and performed a phylogenetic analysis of 18S rRNA sequences. A total of 315 dogs were screened during routine care regardless of clinical suspicion. Blood was collected by jugular venipuncture to perform indirect immunofluorescence assay (IFA) and polymerase chain reaction (PCR) and for parasite screening in peripheral blood smears. Positivity was 2.2% (7/315) by microscopy, 4.8% (15/315) by PCR, and 48.6% (153/315) by IFA. PCR amplified a 602-bp fragment of the piroplasmid 18S rRNA gene, and sequence alignment and analysis revealed 99% homology with *B. vogeli* isolates from other regions of Brazil and other countries. In addition, there was high variability among sequences from other northeast states of Brazil. This study is the first to perform the molecular analysis of *B. vogeli* in Piauí. The results demonstrate that canine babesiosis is endemic in dogs sampled in Teresina and that PCR may be the method of choice to perform parasite screening in this region.
Key words: Molecular analysis. Babesiosis. Blood parasites. PCR. IFA.

Resumo

Estudos sobre a babesiose canina são escassos no Nordeste do Brasil, apesar das condições climáticas favoráveis ao desenvolvimento do carrapato vetor. Esta pesquisa objetivou determinar a ocorrência de *Babesia vogeli* em cães amostrados em Teresina, estado do Piauí, região Meio Norte do Brasil, através de métodos diretos e indiretos de diagnóstico, além de realizar análise filogenética das sequências 18S rRNA de piroplasmídeos obtidas no estudo. Foram avaliados 315 cães atendidos em clínicas

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veterinárias, sob qualquer suspeita clínica. Desses animais, foi colhido sangue por venopunção jugular para Reação de Imunofluorescência Indireta (RIFI) e Reação em Cadeia pela Polimerase (PCR). Além disso, esfregaços de sangue periférico foram realizados para pesquisa direta do parasita. A positividade dos animais foi de 2,2% (7/315) ao esfregaço sanguíneo, 4,8% (15/315) à PCR e 48,6% (153/315) à RIFI. O sequenciamento de amostras positivas à PCR resultou em um fragmento de 602 pb do gene 18S rRNA de piroplasmídeos, cujo alinhamento e análise da sequência revelaram 99% de homologia com isolados de *B. vogeli* de outras regiões do Brasil, além de outros países. É interessante ressaltar que, comparando isolados em diferentes estados do Nordeste, a homologia pode ser bastante variável. Esses são os primeiros resultados sobre a análise molecular de *B. vogeli* no Estado do Piauí. Além disso, este estudo demonstra que a babesiose canina é endêmica em cães de Teresina, Nordeste do Brasil, e que a PCR pode ser o método de escolha para diagnóstico da doença nessas áreas.
Palavras-chave: Análise molecular. Babesiose. Hemoparasitas. PCR. RIFI.

Introduction

Canine babesiosis is an emerging disease worldwide (SCHNITTGER et al., 2012; PETRA et al., 2018) caused by intraerythrocytic protozoa of the genus *Babesia* (order Piroplasmida, phylum Apicomplexa) and transmitted by ixodid ticks of different genera. *B. gibsoni* and *B. canis* are the main etiological agents in dogs (DANTAS-TORRES; FIGUEREDO, 2006). The *Babesia* species that infect dogs are found in different regions of the world, and identification is based on geographic distribution, vector specificity, pathogenicity, and genetic characteristics (SCHNITTGER et al., 2012). For instance, *Babesia vogeli* and *B. gibsoni* have a global distribution, whereas *B. rossi* and *B. canis* occur predominantly in Africa and Europe, respectively (PETRA et al., 2018).

The clinical signs of babesiosis include anemia, hemoglobinuria, lymphadenomegaly, splenomegaly, lethargy, anorexia (BOURDOISEAU, 2006; CARLI et al., 2009), and jaundice (FURLANELLO et al., 2005). However, these signs vary depending on the protozoal species or isolate, host immunity and age, concomitant diseases, and geographic location (UILENBERG, 2006; GOPEGUI et al., 2007). In Brazil, canine babesiosis is relatively mild (CACCIÒ et al., 2002) and is found throughout the country; it is caused by *B. vogeli* (DANTAS-TORRES, 2008a), and the known vector is the tick *Rhipicephalus sanguineu*s sensu lato (DANTAS-TORRES, 2008b). However, further research on the role of domestic dogs as reservoirs for *B. vogeli* is critical (SOUSA et al., 2018).

Diagnosis is established by the size and morphology of intraerythrocytic forms in peripheral blood smears (PASSOS et al., 2005), and these forms are more commonly observed during infection, especially in febrile dogs (GUIMARÃES et al., 2004). Serological tests are useful for identifying subclinical and chronic infections, in which parasitemia may be low and undetectable in peripheral blood smears. However, dogs may remain seropositive even after eliminating the etiological agent (DANTAS-TORRES; FIGUEREDO, 2006), indicating that parasite DNA detection is more reliable since it demonstrates parasite presence and enables differentiation among parasite species (MARTIN et al., 2006).

The city of Teresina is the capital of the state of Piauí, located in northeast Brazil. The climate of the city is tropical with favorable conditions for the development of the ixodid tick vector (MEDEIROS, 2004). Nevertheless, studies on canine babesiosis in Teresina are scarce, although a study found that the percentage of dogs from a hospital population diagnosed with *Ehrlichia canis* and *Anaplasma platys*, which are transmitted by the same tick vector, was high (SILVA, 2010).

This study determined the prevalence of *Babesia* spp. in Teresina using direct and indirect detection methods and evaluated the phylogenetic relationship of *B. vogeli* sequences.

Materials and Methods

Animals

A total of 315 dogs were screened during routine care at the Veterinary Hospital of the Center for Agrarian Sciences of the Federal University of Piauí (Centro de Ciências Agrárias da Universidade Federal do Piauí-CCA/UFPI), Brazil, and private veterinary clinics of Teresina, Piauí, regardless of clinical suspicion, gender, breed, or age. The minimum sample size was calculated using the formula defined by the Centro Panamericano de Zoonosis (1973): $N = p\,(100\text{-}p) \times Z^2 / (d \times p / 100)^2$, where N is the number of samples, p is the expected prevalence, Z is the confidence level, and d is the error margin. In this study, we considered an expected prevalence of 55% according to a previous study (unpublished data), a confidence interval of 95%, and an error margin of 10%. This study was approved by the Animal Research Ethics Committee of the UFPI under Protocol No. 048/2010.

Blood samples

Blood samples were collected by jugular venipuncture and used for DNA extraction, polymerase chain reaction (PCR), and indirect immunofluorescence assay (IFA). In addition, capillary blood was obtained by ear (pinna) venipuncture for detecting *Babesia* spp. in blood smears. The slides were fixed in methyl alcohol, stained with Giemsa (Laborclin, Pinhais, Brazil), visualized at 1000× magnification, and analyzed in a computerized image analyzer version 4.1 (Leica Qwin D-1000; Cambridge, United Kingdom). In addition, tick-infested dogs were identified during clinical examination.

Detection of anti-Babesia vogeli antibodies

Anti-*Babesia vogeli* antibodies were detected using IFA as described by IICA (1987). For this purpose, slides sensitized with *B. vogeli* antigens prepared from the venous blood of dogs experimentally infected with the parasite. The antigens were produced at the Protozoology Laboratory of the Institute of Biological Sciences of the Federal University of Minas Gerais (Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais-ICB/UFMG) and kindly provided by Professor Múcio Flávio Barbosa Ribeiro. Antigen slides were removed from the freezer immediately before use, dried at 37 °C for 5 min, and marked with circles. The slides were incubated with test sera (1:40 dilution), sera from dogs known to be positive for *B. vogeli* (positive control, kindly provided by Professor Múcio Flávio B. Ribeiro), and phosphate-saline buffer (negative control). The slides were washed, dried, and incubated with a fluorescein isothiocyanate-conjugated anti-dog antibody (Sigma-Aldrich®) diluted 1:100 in Evans blue (diluted 1:50 in PBS). The slides were washed, dried, incubated with buffered glycerin, covered with a coverslip, and examined under an epifluorescence microscope (BX41 Laboratory, Olympus®) at a 400× magnification. The reactions in which fluorescent parasites were detected at a 1:40 dilution were considered positive.

Detection of Babesia vogeli DNA

DNA from blood samples was extracted using the Illustra blood genomicPrep Mini Spin Kit (GE Healthcare) according to the manufacturer's recommendations. The amount and purity of genomic DNA were determined by optical density in a spectrophotometer (NanoDrop 2000/2000c, Thermo Scientific). DNA samples with a 260/280 ratio of 1.8-2.0 were analyzed by PCR. The following *B. vogeli*-specific primers were used in PCR: BAB1 forward, 5'-GTGAACCTTATCACTTAAAGG-3'; and BAB4 reverse, 5'-CAACTCCTCCACGCAATCG-3'. The forward primer was designed based on the sequence of a conserved 3' end region of the 18S rRNA gene of the *Babesia* genus, and the reverse primer was

designed based on the sequence of the *B. vogeli* 5.8S ribosome subunit, as previously described (DUARTE et al., 2008).

PCR was performed in a reaction volume of 25 µL containing ultrapure water, 0.2 mM of each dNTP (Promega), 1.0 mM $MgCl_2$, 0.4 µM of each primer, 1.5 U Taq DNA polymerase (Promega), and 200 ng of sample DNA. DNA amplification was performed in a thermocycler (Mastercycler Personal, Eppendorf) with an initial denaturation cycle at 94 °C for 2 min, followed by 35 amplification cycles at 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min, and final extension at 72 °C for 5 min. The amplification products were stained with ethidium bromide, electrophoresed on 1.2% agarose gel, and visualized on a UV transilluminator (MiniBis Pro; DNR Bio-Imaging Systems). The products with approximately 603 base pairs (bp) were considered positive.

DNA sequencing and phylogenetic analysis

Eight PCR-positive samples were randomly selected for sequencing. Amplification products were purified from the agarose gel using the QIAquick Gel Extraction kit (Qiagen®) and ligated into the pGEM-T vector (Promega®) as directed by the manufacturer. After that, 10 µL of the ligation reaction was used to transform competent *Escherichia coli* DH5α cells by thermal shock to obtain recombinant clones. Transformed cells were seeded and incubated in LB liquid medium (Luria Bertani) for 2 h at 37 °C under agitation. After incubation, 100 µL of the culture was seeded overnight in Petri dishes containing LB agar medium supplemented with ampicillin (100 µg/mL), 0.5 mM IPTG (isopropyl-β-D-thiogalactoside), and 80 µg/mL X-Gal (5-bromo-4-chloro-3-indolyl β-D-galactoside). The colonies with recombinant clones were isolated and grown in liquid LB medium supplemented with 100 µg/mL ampicillin.

The recombinant plasmids were extracted using the QIAprep Spin Miniprep kit (Qiagen®) according to the manufacturer's instructions, digested with restriction enzymes ApaI and NotI, and the presence of the insert was confirmed on a 1.0% agarose gel. The recombinant clones (N = 4) were sequenced according to Sanger's method on an ABI 3100 automated DNA sequencer (Applied Biosystems, USA) using the APBiotech DYEnamic ET Dye Terminator Cycle Sequencing kit. The obtained sequences were analyzed in Chromas Lite® software version 2.01 and compared to GenBank sequences using the BLASTN program.

The sequences were aligned using the ClustalW software (THOMPSON et al., 1994) in BioEdit version 7.2 using standard parameters. Phylogenetic analysis was performed using the Molecular Evolutionary Genetics Analysis software (MEGA) version 6.0 (TAMURA et al., 2013). The maximum-likelihood method was used to construct the dendrogram (SAITOU; NEI, 1987), and 1000 repetitions were applied to estimate the branching patterns of the tree. Nodes with a bootstrap value of less than 50% are not shown because they are not supported by the test.

The isolates and GenBank accession numbers used to construct the phylogenetic tree are shown in Results section.

Results

Blood smears

Pyroplasmid trophozoites and/or merozoites were detected in 2.2% (7/315) of the samples, of which 71.4% (5/7) contained parasite DNA on PCR. In addition, anti-*B. vogeli* antibodies and parasite DNA were detected by IFA and PCR, respectively, in 57.1% (4/7) of the animals with intraerythrocytic inclusions compatible with the etiological agent.

Indirect immunofluorescence assay

Anti-*B. vogeli* antibodies were detected by IFA in 48.6% (153/315) of the sera (1:40 dilution). However, piroplasmids were detected in blood smears and parasite DNA was detected by PCR in 2.6% (4/153) and 6.5% (10/153) of seropositive animals, respectively.

Polymerase chain reaction

B. vogeli DNA was identified in 4.8% (15/315) of the blood samples. Parasites were detected by both microscopy and PCR in 1.6% (5/315) of the samples, revealing that parasites were detected by microscopy in 33.3% (5/15) of PCR-positive samples. In addition, 3.2% (10/315) of the dogs were positive by both molecular and serological tests, indicating that anti-*B. vogeli* antibodies were detected in 66.7% (10/15) of PCR-positive samples.

Molecular characterization of B. vogeli

The sequencing of eight *B. vogeli* PCR products yielded 602-bp fragments, and the gene sequence was deposited in GenBank under accession number JX535812.1. Sequence alignment and analysis using BLASTN showed that these fragments were identical and had 99% homology with *B. vogeli* isolates from different geographical regions, including Recife, Brazil (FJ588003 and KP683155, from dogs and cats, respectively), Colombia (KT946903), United States (EU084675), France (KF953983), Tunisia (KT445941), Cape Verde (GC395377), India (KR149261), Taiwan (EF180054), and Malaysia (KU510312) (Figure 1).

Figure 1. Dendrogram based on the analysis of the secondary structure of the 18S rRNA gene from *Babesia canis vogeli*. The tree was constructed using the neighbor-joining method with 11 species/isolates of *Babesia* sp. and *B. canis vogeli*. *Ehrlichia canis* was chosen as an outgroup. The numbers on nodes indicate bootstrap values for branch points. The host species and country of origin of each isolate are shown next to the respective access.



Ectoparasitism

The results of the physical examination revealed that 51.4% (161/313) of the animals were infested with ticks at the time of sample collection.

Discussion

In this study, the employed diagnostic techniques confirmed the presence of *B. vogeli* in the state of Piauí, northeast Brazil. *Babesia* spp. parasites were detected in blood smears in a small percentage of dogs and were not detected by microscopy in most PCR-positive samples. Similar results were observed in a study with dogs from rural areas of the state of São Paulo, where the rate of detection of *Babesia* sp. by microscopy was lower than that by PCR (O'DWYER et al., 2009). In addition to the high sensitivity of PCR, the lower rate of positivity in blood smears relative to PCR is because, although parasite detection in capillary blood is usually higher than that in venous blood (BICALHO et al., 2002), the low parasitemia in animals after the acute phase of the disease (TODOROVIC, 1975) limits detecting parasitized red blood cells (BICALHO et al., 2002). Assis (1993) has shown that peak parasitemia occurs seven days before the appearance of clinical signs, which limits parasite detection in blood smears. Moreover, the samples were obtained from dogs from a hospital population, in which peak parasitemia might have occurred before the molecular tests were performed. In some situations, although dogs have high antibody titers, parasites may be undetectable in blood smears after the acute phase of the disease (BREITSCHWERDT et al., 1983).

Approximately 50% of the examined dogs were seropositive using IFA. Similarly, Furuta et al. (2009) found that the seroprevalence of apparently healthy dogs screened during an anti-rabies vaccination campaign in the state of São Paulo was 59.4% (146/246). Ribeiro et al. (1990) and Spiewak (1992) examined dogs in Belo Horizonte, state of Minas Gerais, Brazil, during routine care in hospitals and clinics and stray dogs and found that seropositivity was higher than that reported in the present study. In contrast, Trapp et al. (2006) detected anti-*B vogeli* antibodies in 36% of the dogs from a hospital population in Londrina, Brazil. The high seropositivity in dogs in our sample may be because of the tropical climatic conditions in Teresina (MEDEIROS, 2004), which favors the development of the tick vector, that parasitizes dogs throughout the year, as confirmed in our samples.

The rate of detection of *B. vogeli* DNA in dogs by PCR in the study population was low, although most animals were infested with ticks at the time of sample collection. Similar results were found by M'ghirbi and Bouattour (2008), wherein the rate of detection of *B. vogeli* DNA by PCR was low (6.7%, 12/180), although most animals were infested with *R. sanguineus* at the time of blood collection. It is worth stressing that the impact of the disease is low in endemic areas in which the number of infected vector ticks is high because young animals acquire immunity and vertebrate hosts acquire age-dependent tolerance to the disease by exposure to infected ticks throughout life (UILENBERG, 2006). This result suggests that *B. vogeli* PCR positive dogs were referred to clinical care for reasons other than parasite infection, and the detection of parasite DNA was occasional, which was also suggested by Cardoso et al. (2008).

The difference in the rate of positivity between immunofluorescence and PCR in the study population may reflect previous exposure to piroplasmids, with maintenance of antibody titers without PCR detection of parasites or because of the low sensitivity of PCR in samples collected from *Babesia*-infected asymptomatic dogs or dogs in the chronic phase of the disease (BOOZER; MACINTIRE, 2003). Although conventional PCR is highly sensitive and specific, the use of alternative techniques such as nested-PCR and real-time PCR (TROSKIE et al., 2018) may increase sensitivity. PCR may be particularly useful in areas endemic to *Leishmania infantum*, where DNA from other vector-

borne pathogens, including *B. vogeli*, is detected in *L. infantum*-seropositive dogs, reinforcing the importance of molecular tests for detecting the causative agents of vector-borne diseases in these areas (SOUSA et al., 2013), including Teresina, which is endemic for canine visceral leishmaniasis (DRUMOND; COSTA, 2011).

Sequence analysis of the *B. vogeli* 18S gene is essential in epidemiological studies of canine babesiosis because the coding regions of this gene are highly conserved (ZAHLER et al., 1998). The present results demonstrated the genetic similarity between *B. vogeli* isolates from Piauí, other Brazilian states such as Pernambuco, and other countries. However, data on the molecular characterization of this parasite are scarce and underscore the need for further epidemiological and pathogenicity studies on canine babesiosis in Brazil to assess the effect of genetic and geographical differences on clinical-pathological aspects of the disease and assist in clinical diagnosis and therapeutic management of infected animals.

Conclusions

The results confirmed the presence of *B. vogeli* in dogs sampled in the state of Piauí, Brazil. The high percentage of seropositive animals combined with parasite detection by microscopy and PCR demonstrates that babesiosis is endemic in dogs in Teresina; nonetheless, the disease does not appear to impair the health of infected dogs in this region. Furthermore, PCR detection of *B. vogeli* DNA is fundamental in endemic areas because the sensitivity of microscopic detection is low, and antibody positivity does not confirm infection.

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References

ASSIS, C. B. *Estudo de aspectos hematológico, bioquímico e imunológico em cães não esplenectomizados e esplenectomizados inoculados com Babesia canis*. 1993. Tese (Doutorado em Medicina Veterinária) - Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, UNESP, Botucatu.

BICALHO, K. A.; PASSOS, L. M. F.; RIBEIRO, M. F. B. Infecção experimental de cães com amostras de *Babesia canis* isoladas em Minas Gerais. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 54, n. 5, p. 546-548, 2002. DOI: 10.1590/S0102-09352002000500016

BOOZER, A. L.; MACINTIRE, D. K. Canine babesiosis. *Veterinary Clinics of North America-Small Animal Practice*, London, v. 33, n. 4, p. 885-904, 2003. DOI: 10.1016/S0195-5616(03)00039-1

BOURDOISEAU, G. Canine babesiosis in France. *Veterinary Parasitology*, Amsterdam, v. 138, n. 1-2, p. 118-125, 2006. DOI: 10.1016/j.vetpar.2006.01.046

BREITSCHWERDT, E. B.; MALONE, J. B.; MACWILLIANS, P.; LEVY, M. G.; QUALLS JUNIOR, C. W.; PRUDICH, M. J. Babesiosis in the greyhound. *Journal of the American Veterinary Medical Association*, Ithaca, v. 182, n. 9, p. 978-982, 1983.

CACCIÒ, S. M.; ANTUNOVIC, B.; MORETTI, A.; MANGILI, V.; MARINCULIC, A.; BARIC, R. R.; SLEMENDA, S. B.; PIENIAZEK, N. J. Molecular characterization of *Babesia canis canis* and *Babesia canis vogeli* from naturally infected European dogs. *Veterinary Parasitology*, Amsterdam, v. 106, n. 4, p. 285-292, 2002. DOI: 10.1016/S0304-4017(02)00112-7

CARDOSO, L.; COSTA, A.; TUNA, J.; VIEIRA, L.; EYAL, O.; YISASCHAR-MEKUZAS, Y.; BANETH, G. *Babesia canis canis* and *Babesia canis vogeli* infections in dogs from northern Portugal. *Veterinary Parasitology*, Amsterdam, v. 156, n. 3-4, p. 199-204, 2008. DOI: 10.1016/j.vetpar.2008.05.027

CARLI, E.; TASCA, S.; TROTTA, M.; FURLANELLO, T.; CALDIN, M.; SOLANO-GALLEGO, L. Detection of erythrocyte binding IgM and IgG by flow cytometry in sick dogs with *Babesia canis canis* or *Babesia canis vogeli* infection. *Veterinary Parasitology*, Amsterdam, v. 162, n. 1-2, p. 51-57, 2009. DOI: 10.1016/j.vetpar.2009.02.002

CENTRO PANAMERICANO DE ZOONOSIS - Procedimentos para estudios de prevalencia de enfermidades cronicas en el ganado. Buenos Aires: Ramos Mejia, 1973. 33 p. (Nota técnica, 18).

DANTAS-TORRES, F. Canine vector-borne diseases in Brazil. *Parasites & Vectors*, Londres, v. 1, n. 25, p. 1-17, 2008b. DOI: 10.1186/1756-3305-1-25

DANTAS-TORRES, F. Causative agents of canine babesiosis in Brazil. *Preventive Veterinary Medicine*, Amsterdam, v. 83, n. 2, p. 210-211, 2008a. DOI: 10.1016/j.prevetmed.2007.03.008

DANTAS-TORRES, F.; FIGUEREDO, L. A. Canine babesiosis: a Brazilian perspective. *Veterinary Parasitology*, Amsterdam, v. 141, n. 3-4, p. 197-203, 2006. DOI: 10.1016/j.vetpar.2006.07.030

DRUMOND, K. O.; COSTA, F. A. L. Forty years of visceral leishmaniasis in the state of Piauí: a review. *Revista do Instituto de Medicina Tropical de São Paulo*, São Paulo, v. 53, n. 1, p. 3-11, 2011. DOI: 10.1590/S0036-46652011000100002

DUARTE, S. C.; LINHARES, G. F. C.; ROMANOWSKY, T. N.; SILVEIRA NETO, O. J.; BORGES, L. M. F. Assessment of primers designed for the subspecies-specific discrimination among *Babesia canis canis*, *Babesia canis vogeli* and *Babesia canis rossi* by PCR assay. *Veterinary Parasitology*, Amsterdam, v. 152, n. 1-2, p. 16-20, 2008. DOI: 10.1016/j.vetpar.2007.12.013

FURLANELLO, T.; FIORIO, F.; CALDIN, M.; LUBAS, G.; SOLANO-GALLEGO, L. Clinicopathological findings in naturally occurring cases of babesiosis caused by large form Babesia from dogs of northeastern Italy. *Veterinary Parasitology*, Amsterdam, v. 134, n. 1-2, p. 77-85, 2005. DOI: 10.1016/j.vetpar.2005.07.016

FURUTA, P. I.; OLIVEIRA, T. M.; THEIXEIRA, M. C.; ROCHA, A. G.; MACHADO, R. Z.; TINUCCI-COSTA, M. G. Comparison between a soluble antigen-based ELISA and IFAT in detecting antibodies against *Babesia canis* in dogs. *Revista Brasileira de Parasitologia Veterinária*, Jaboticabal, v. 18, n. 3, p. 41-45, 2009. DOI: 10.4322/rbpv.01803007

GOPEGUI, R. R.; PENALBA, B.; GOICOA, A.; ESPADA, Y.; FIDALGO, L. E.; ESPINO, L. Clinicopathological findings and coagulation disorders in 45 cases of canine babesiosis in Spain. *The Veterinary Journal*, Londres, v. 174, n. 1, p. 129-132, 2007. DOI: 10.1016/j.tvjl.2006.05.017

GUIMARÃES, J. C.; ALBERNAZ, A. P.; MACHADO, J. A.; MELO JÚNIOR, O. A.; GARCIA, L. N. N. Aspectos clínico-laboratoriais da babesiose canina na cidade de Campos do Goytacazes, RJ. *Revista Brasileira de Parasitologia Veterinária*, Jaboticabal, v. 13, p. 229, 2004. Supplement 1.

INSTITUTO INTERAMERICANO DE COOPERACIÓN PARA LA AGRICULTURA - IICA. Técnicas para el diagnóstico de babesioses y anaplasmosis bovina. San José: IICA, 1987. 79 p.

M'GHIRBI, Y.; BOUATTOUR, A. Detection and molecular characterization of *Babesia canis vogeli* from naturally infected dogs and *Rhipicephalus sanguineus* ticks in Tunisia. *Veterinary Parasitology*, Amsterdam, v. 152, n. 1-2, p. 1-7, 2008. DOI: 10.1016/j.vetpar.2007.12.018

MARTIN, A. R.; DUNSTAN, R. H.; ROBERTS, T. K.; BROWN, G. K. *Babesia canis vogeli*: a novel PCR for its detection in dogs in Australia. *Experimental Parasitology*, Orlando, v. 112, n. 1, p. 63-65, 2006. DOI: 10.1016/j.exppara.2005.09.001

MEDEIROS, R. M. *Estudo Agrometeorológico para o Estado do Piauí.* Teresina: Secretaria do Meio Ambiente e Recursos Hídricos do Estado do Piauí, 2004. 113 p.

O'DWYER, L. H.; LOPES, V. V. A.; RUBINI, A. S.; PADUAN, K. S.; RIBOLLA, P. E. M. *Babesia* spp. infection in dogs from rural areas of São Paulo State, Brazil. *Revista Brasileira de Parasitologia Veterinária*, Jaboticabal, v. 18, n. 2, p. 23-26, 2009. DOI: 10.4322/rbpv.01802005

PASSOS, L. M.; GEIGER, S. M.; RIBEIRO, M. F. B.; PfiSTER, K.; ZAHLER-RINDER, M. First molecular detection of *Babesia vogeli* in dogs from Brazil. *Veterinary Parasitology*, Amsterdam, v. 127, n. 1, p. 81-85, 2005. DOI: 10.1016/j.vetpar.2004.07.028

PETRA, B.; JOSIPA, K.; RENATA, B. R.; VLADIMIR, M. Canine babesiosis: where do we stand? *A Acta Veterinaria-Beograd*, Belgrade, v. 68, n. 2, p. 127-160, 2018. DOI: 10.2478/acve-2018-0011

RIBEIRO, M. F. B.; PASSOS, L. M. F.; LIMA, J. D.; GUIMARÃES, A. M. Frequency of anti-*Babesia canis* antibodies in dogs, in Belo Horizonte, Minas Gerais. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 42, n. 6, p. 511-517, 1990.

SAITOU, N.; NEI, M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, New York, v. 4, n. 4, p. 406-425, 1987. DOI: 10.1093/oxfordjournals.molbev.a040454

SCHNITTGER, L.; RODRIGUEZ, A. E.; FLORIN-CHRISTENSEN, M.; MORRISON, D. A. *Babesia*: a world emerging. *Infection, Genetics and Evolution*, Amsterdam, v. 12, n. 8, p. 1788-809, 2012. DOI: 10.1016/j.meegid.2012.07.004

SILVA, L. S. *Erliquiose e anaplasmose canina em Teresina, Piauí*. 2010. Dissertação (Mestrado em Ciência Animal) - Universidade Federal do Piauí, Teresina.

SOUSA, K. C. M.; FERNANDES, M. P.; HERRERA, H. M.; FRESCHI, C. R.; MACHADO, R. Z.; ANDRÉ, M. R. Diversity of piroplasmids among wild and domestic mammals and ectoparasites in Pantanal wetland, Brazil. *Ticks and Tick-Borne Diseases*, Amsterdam, v. 9, n. 2, p. 245-253, 2018. DOI: 10.1016/j.ttbdis.2017.09.010

SOUSA, K. C. M.; ANDRÉ, M. R.; HERRERA, H. M.; ANDRADE, G. B.; JUSI, M. M.; SANTOS, L. L.; BARRETO, W. T.; MACHADO, R. Z.; OLIVEIRA, G. P. Molecular and serological detection of tick-borne pathogens in dogs from an area endemic for *Leishmania infantum* in Mato Grosso do Sul, Brazil. *Revista Brasileira de Parasitologia Veterinária*, Jaboticabal, v. 22, n. 4, p. 525-531, 2013. DOI: 10.1590/S1984-29612013000400012

SPIEWAK, G. *Aspectos epidemiológicos, clínicos e de diagnóstico da infecção por* Babesia canis *em cães atendidos em clínicas veterinárias em Belo Horizonte*. 1992. Dissertação (Mestrado em Medicina Veterinária) - Escola de Veterinária da Universidade Federal de Minas Gerais, Belo Horizonte.

TAMURA, K.; STECHER. G.; PETERSON, D.; FILIPSKI, A.; KUMAR, S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, New York, v. 30, n. 12, p. 2725-2729, 2013. DOI: 10.1093/molbev/mst197

THOMPSON, J. D.; HIGGINS, D. G.; GIBSON, T. J. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic acids research*, Oxford, v. 22, n. 22, p. 4673-4680, 1994. DOI: 10.1093/nar/22.22.4673

TODOROVIC, R. A. Serological diagnosis of babesiosis: a review. *Tropical Animal Health and Production*, Heidelberg, v. 7, n. 1, p. 1-14, 1975. DOI: 10.1007/BF02383236

TRAPP, S. M.; DAGNONE, A. S.; VIDOTTO, O.; FREIRE, R. L.; AMUDE, A. M.; MORAIS, H. S. Seroepidemiology of canine babesiosis and ehrlichiosis in a Hospital population. *Veterinary Parasitology*, Amsterdam, v. 140, n. 3-4, p. 223-230, 2006. DOI: 10.1016/j.vetpar.2006.03.030

TROSKIE, M.; VILLIERSB, L.; LEISEWITZB, A.; OOSTHUIZENA, M. C.; QUANA, M. Development and validation of a multiplex, real-time PCR assay for *Babesia rossi* and *Babesia vogeli*. *Ticks and Tick-Borne Diseases*, Amsterdam, v. 10, n. 2, p. 421-432, 2018. DOI: 10.1016/j.ttbdis.2018.12.004

UILENBERG, G. Babesia - a historical overview. *Veterinary Parasitology*, Amsterdam, v. 138, n. 1-2, p. 3-10, 2006. DOI: 10.1016/j.vetpar.2006.01.035

ZAHLER, M.; SCHEIN, E.; RINDER, H.; GOTHE, R. Characteristic genotypes discriminate between *Babesia canis* isolates of differing vector specificity and pathogenicity to dogs. *Parasitology Research*, Berlin, v. 84, n. 7, p. 544-548, 1998. DOI: 10.1007/s004360050