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Post-mortem hemoparasite detection in free-living Brazilian brown brocket deer (*Mazama gouazoubira*, Fischer 1814)

Detecção *post-mortem* de hemoparasitos em veados catingueiro (*Mazama gouazoubira,* Fischer 1814) de vida livre no Brasil

Júlia Angélica Gonçalves da Silveira[1]; Élida Mara Leite Rabelo[1]; Paula Cristina Senra Lima[1]; Bárbara Neves Chaves[2]; Múcio Flávio Barbosa Ribeiro[1]*

[1]Departamento de Parasitologia, Instituto de Ciências Biológicas – ICB, Universidade Federal de Minas Gerais – UFMG, Belo Horizonte, MG, Brasil

[2]Departamento de Biologia Geral, Instituto de Ciências Biológicas – ICB, Universidade Federal de Minas Gerais – UFMG, Belo Horizonte, MG, Brasil

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Abstract

Tick-borne infections can result in serious health problems for wild ruminants, and some of these infectious agents can be considered zoonosis. The aim of the present study was the *post-mortem* detection of hemoparasites in free-living *Mazama gouazoubira* from Minas Gerais state, Brazil. The deer samples consisted of free-living *M. gouazoubira* (n = 9) individuals that died after capture. Necropsy examinations of the carcasses were performed to search for macroscopic alterations. Organ samples were collected for subsequent *imprint* slides, and nested PCR assays were performed to detect hemoparasite species. *Imprint* slide assays from four deer showed erythrocytes infected with Piroplasmida small trophozoites, and *A. marginale* corpuscles were observed in erythrocytes from two animals. *A. marginale* and trophozoite co-infections occurred in two deer. A nested PCR analysis of the organs showed that six of the nine samples were positive for *Theileria* sp., five were positive for *A. phagocytophilum* and three were positive for *A. marginale*, with co-infection occurring in four deer. The results of the present study demonstrate that *post-mortem* diagnostics using *imprint* slides and molecular assays are an effective method for detecting hemoparasites in organs.

Keywords: *Anaplasma* spp., Piroplasmida, molecular diagnostic, brown brocket deer, free-living, post-mortem detection, hemoparasites.

Resumo

Patógenos transmitidos por carrapatos podem resultar em sérios problemas de saúde para os ruminantes selvagens, e alguns podem ser zoonoses. O objetivo do presente estudo foi a detecção *post mortem* de hemoparasitos, em *Mazama gouazoubira* de vida livre, oriundos de Minas Gerais, através da análise de lâminas de impressão e nested PCR. Foram amostrados nove *M. gouazoubira* de vida livre, que morreram após a captura. Exames de necropsia foram realizados, e as carcaças foram examinadas para detectar alterações macroscópicas. Amostras dos órgãos foram coletadas para a realização de *imprint* em lâminas e para *nested* PCR à procura de hemoparasitos. A análise das lâminas mostrou pequenos trofozoítos de Piriplasmida nos eritrócitos de quatro dos oito animais examinados, e corpúsculos de *Anaplasma marginale* foram observados nos eritrócitos de dois dos cervídeos. A coinfecção com *A. marginale* e trofozoítos de piroplasmas ocorreu em dois animais. As análises de nPCR dos órgãos mostraram que seis dos nove animais estavam positivos para *Theileria* sp., cinco para *A. phagocytophilum* e três para *A. marginale,* sendo que a coinfecção ocorreu em quatro cervídeos. Os resultados do presente estudo demonstram que os diagnósticos *post-mortem,* pelas *imprints* em lâminas e ensaios moleculares, são métodos eficazes de detecção de hemoparasitos nos principais órgãos parasitados.

Palavras-chave: *Anaplasma* spp., Piroplasmida, diagnostico molecular, veado catingueiro, vida livre, detecção post-mortem, hemoparasitos.

***Corresponding author:** Múcio Flávio Barbosa Ribeiro
Instituto de Ciências Biológicas – ICB, Universidade Federal de Minas Gerais – UFMG, Av. Antônio Carlos, 6627, CP 486, Pampulha, CEP 31270-901, Belo Horizonte, MG, Brazil
e-mail: muciobr@icb.ufmg.br

Introduction

Tick-borne infections caused by protozoa and rickettsial organisms can result in serious problems for wild and domestic hosts, particularly when the animals have been weakened by other parasites or are undernourished or stressed (FOWLER, 1993). The brown brocket deer (*Mazama gouazoubira*) is a small forest cervid with solitary habits that can live in anthropogenic areas and in close proximity to farmers in neotropical forest areas. This behavior favors the interchange of infectious microorganisms between wild and domestic populations (DUARTE, 1997).

Babesia and *Theileria* species have been detected by molecular means in Brazilian cervids from Minas Gerais and Mato Grosso do Sul states (SILVEIRA et al., 2011, 2013). These species are known to cause clinical signs and hematological alterations in stressed animals (KREIER et al., 1962; BARKER et al., 1973). On the other hand members of the Anaplasmataceae family can infect brown brocket deer, but the consequences of the infection on deer health are not well established.

In Brazil, the molecular detections of *E. chaffeensis, A. marginale, A. bovis* and *Anaplasma* spp. have been described in wild cervids (*Blastocerus dichotomus, Ozotoceros bezoarticus* and *M. gouazoubira*) in Mato Grosso do Sul and Minas Gerais states (MACHADO et al., 2006; PICOLOTO et al., 2010; SACCHI et al., 2012; SILVEIRA et al., 2012, 2013).

Anaplasma marginale strains from deer exhibit low virulence in bovine hosts, and they have been studied as alternatives for controlling *A. marginale* infection in cattle (KUTTLER; ZAUG, 1988; KOCAN et al., 2003; SILVEIRA, 2012). *Anaplasma phagocytophilum* infects humans and a wide range of other animal hosts including rodents, ruminants, felids, horses, donkeys, dogs and birds (MADIGAN; PUSTERLA, 2000; STUEN, 2007). Capturing free-range deer is a fundamental concern for studies that require close access to the animals for sample collection and other ecological studies. However, cervid immobilization is a difficult process because these animals are easily frightened and very sensitive to capture myopathy (DUARTE, 2006). In some cases, a restrained deer can die a few days after being captured. Therefore, an alternative way to study hemoparasites from these hosts is to harvest organs from free-living deer when these animals are found injured or bowled over. The aim of the present study was the *post-mortem* detection of hemoparasites in organs from free-living *Mazama gouazoubira* in Minas Gerais state.

Material and Methods

This study was approved by the Ethical Committee on Animal Experimentation (CETEA/UFMG, Belo Horizonte, MG, Brazil) under protocol no. 142/08 and by the Brazilian Institute for Environment and Natural Renewable Resources (IBAMA, Belo Horizonte, MG, Brazil) under license no. 16064-1.

Animal samples

This study was performed from June 2007 to April 2013. The deer samples came from free-living *M. gouazoubira* (n = 24) that were captured by the Forestry Police of Minas Gerais state and transported to IBAMA. Blood from these animals was collected by puncturing the jugular vein, and the samples were transferred immediately to vials containing EDTA. Blood smears were prepared, subjected to quick Romanowsky staining and examined under an optical microscope at 100X magnification. At least 40 microscopic fields were observed for each deer blood sample. Blood sample aliquots were frozen and stored for subsequent DNA extraction.

Nine (9/24) of these animals eventually died, and necropsy examinations were performed. The animals most likely died from capture myopathy or because of erroneous immobilization. Dog attacks were confirmed in three cases (MGM specimens). The deer were identified as follows: MGI8, MGI11, MGI12 and MGM1 (adult females), MGM2 (pregnant female) MGI13 and MGI19 (young females), MGM3 (adult male) and MGI14 (young male). Their ages were determined according to the following parameters: young (1-2 months), for animals presenting white hair spots; or adult, for animals presenting mature features.

Necropsy

Necropsy examinations were performed on nine dead deer, and the carcasses were evaluated for macroscopic alterations. *Imprint* slides of lymph node, spleen, liver and brain samples were prepared, and samples of these organs were collected and frozen for subsequent DNA extraction. The *imprint* slides were subjected to quick Romanowsky staining and examined under the optical microscope at 100× magnification.

DNA extraction and PCR amplification

DNA was extracted from the cervid organ and blood samples using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA), which was employed according to the manufacturer's instructions for animal tissue and whole blood, respectively.

Nested PCR assays were performed to detect *Babesia/Theileria* species (18S rRNA region) (SILVEIRA et al., 2011), monocytic (16S rRNA region) (KAWAHARA et al., 2009) and granulocytic Anaplasmataceae agents (16S rRNA region) (MASSUNG et al., 1998) *A. phagocytophilum* (*msp4* gene) (DE LA FUENTE et al. 2005; BOWN et al., 2007) and *A. marginale* (*msp4* gene) (DE LA FUENTE et al. 2008; SILVEIRA et al. 2012) (Table 1). Double-distilled water was used as the negative control (with no DNA). DNA was extracted from 300 µl of whole blood from a calf that had been experimentally infected with *A. marginale* (strain UFMG1) (BASTOS et al., 2010) and another that was experimentally infected with both *B. bovis* (strain BbovMG) and *B. bigemina* (strain BbigMG) (COSTA et al., 2013) for the positive control. DNA from IDE8 cell cultures infected with *A. phagocytophilum* (as isolated from a German dog and kindly donated by Dr. Erich Zweygarth-Institut für Vergleichende Tropenmedizin und Parasitologie, Ludwig Maximilians Universität München), was used for granulocytic Anaplasmataceae agents and *A. phagocytophilum* tests, or a dog infected with *E. canis* (Jaboticabal strain) (MOREIRA, 2001) was used for monocytic ehrlichiosis tests.

Table 1. Specific primers used for the detection of hemoparasites.

Specificity	Primer sequence (5'- 3')	Target	Name	Product size (bp)	References
A. marginale/A. ovis 1st reaction	GGGAGCTCCTATGAATTACAGAGAATTGTTTAC CCGGATCCTTAGCTGAACAGGAATCTTGC	*msp4*	MSP45 MSP43	872	de la Fuente et al. (2008)
2nd reaction	CGCCAGCAAACTTTTCCAAA ATATGGGGACACAGGCAAAT	*msp4*	AnapF AnapR	294	Silveira et al. (2012)
Babesia/Theileria 1st reaction	CGGGATCCAACCTGGTTGATCCTGC CCGAATTCCTTGTTACGACTTCTC	18S rRNA	RIB-19 RIB-20	1700	Zahler et al. (2000)
2nd reaction	ACCTCACCAGGTCCAGACAG GTACAAAGGGCAGGGACGTA	18S rRNA	BAB-rumF BAB-rumR	430	Silveira et al. (2011)
Anaplasma phagocytophilum 1st reaction	ATGAATTACAGAGAATTGCTTGTAGG TTAATTGAAAGCAAATCTTGCTCCTATG	*msp4*	MSP4AP5 MSP4AP3	no data	de la Fuente et al. (2005)
2nd reaction	CTATTGGYGGNGCYAGAGT GTTCATCGAAAATTCCGTGGTA	*msp4*	msp4F msp4R	450	Bown et al. (2007)
***Anaplasma* spp.** (*A. phagocytophilum, A. bovis, A. platys)* 1st reaction	CACATGCAAGTCGAACGGATTATTC TTCCGTTAAGAAGGATCTAATCTCC	16S rRNA	GE3a GE10r	932	Massung et al. (1998)
2nd reaction	AACGGATTATTCTTTATAGCT TGCT GGCAGTATTAAAAGCAGCTCCAGG	16S rRNA	GE9f GE2	546	Massung et al. (1998)
***Ehrlichia* spp. (*E. chaffeensis, E. canis*)** 1st reaction	ACGGACAATTGCTTATAGCCTT ACAACTTTTATGGATTAGCTAAAT	16S rRNA	NS16SCH1F NS16SCH1R	1195	Kawahara et al. (2009)
2nd reaction	GGGCACGTAGGTGGACTAG CCTGTTAGGAGGGATACGAC	16S rRNA	NS16SCH2F NS16SCH2R	443	Kawahara et al. (2009)

The first reaction mixture contained 1.2 µl of dNTPs (2.5 mM), 0.15 µl of Taq polymerase (Phoneutria, Belo Horizonte, MG, Brazil) (0.05 U), 1.5 µl of reaction buffer IB (Phoneutria, Belo Horizonte, MG, Brazil), 0.6 µl of a solution containing the mixed primers (10 µM) and 10.05 µl of sterile ultra-pure water. A 1.5 µl aliquot of the DNA template was added to the reaction mixture to achieve a final volume of 15 µl.

The second reaction mixture contained 2.0 µl of dNTPs (2.5 mM), 0.25 µl of Taq polymerase, 2.5 µl of reaction buffer IB, 1.0 µl of a solution containing the mixed primers (10 µM) and 16.75 µl of sterile ultra-pure water. An aliquot (2.5 µl) of amplicon that was obtained after the first reaction was added to the reaction mixture to achieve a final volume of 25 µl.

Amplification was performed with an Eppendorf Mastercycler (Eppendorf, São Paulo, SP, Brazil) thermocycler. The amplification program used in all nPCR assays was as follows: 94 °C for 5 min (initial denaturation step); 30 cycles of 92 °C for 1 min (denaturation), 54 °C for 1 min (annealing) and 72 °C for 2 min (extension), followed by 72 °C for 8 min (final extension step). After the amplification, the reaction mixtures were maintained at 12 °C. The PCR amplicons were separated by electrophoresis on 1% agarose gel (40 min, 100 V), which was stained with GelRed (Biotium, Hayward, CA, USA) and visualized under ultraviolet light.

The positive products of the second-round PCRs were purified using a QIAquick PCR Purification Kit (Qiagen Biotecnologia Brazil, São Paulo, Brazil), according to the manufacturer's instructions. The purified amplicons were sequenced by using ABI3130 with POP7 polymer and BigDye v3.1 (Myleus Biotechnology, Belo Horizonte, MG, Brazil) with the second reaction primers. The sequences were aligned, edited and analyzed at the URL http://asparagin.cenargen.embrapa.br/phph/ and using the MEGA 6.0 software (TAMURA et al., 2013). The identity of each sequence was confirmed through a comparison with the sequences available at GenBank using the BLAST software (ALTSCHUL et al., 1990). Phylogenetic analyses were conducted using the MEGA 6.0 software, and the phylogenetic position of the pathogens was inferred using the neighbor-joining method. The combination of phylogenetic clusters was assessed by bootstrap tests with 1000 replicates, and a Kimura 2-parameter model was used to test the stability of the trees.

Results

No visible macroscopic lesions were observed during the necropsy examinations of nine brown brocket deer, which led to the conclusion that the animal deaths were most likely caused by stress from animal handling. Imprint slides from deer MGI19 could not be analyzed because of the low quality of the smears; thus, slides from eight of the nine deer were analyzed. Protozoa in the form of Piroplasmida small trophozoites (<2 µm) were

found in the erythrocytes of 50% (4/8) of the analyzed animals, and *A. marginale* corpuscles were observed in the erythrocytes of 25% (2/8) of the deer. Intraerythrocytic Piroplasmida trophozoites were detected in three spleen samples, three liver samples and one brain sample. A co-infection with *A. marginale* corpuscles and piroplasmid trophozoites was observed in two animals using spleen and liver slides (Table 2). The liver and spleen were the most parasitized organs, and no infected erythrocytes were observed in lymph nodes.

A molecular examination of whole blood that was published in Silveira et al. (2011, 2012) revealed that 55.5% (5/9) of the samples were infected with hemoparasites; five were infected with *A. marginale*, four with *T. cervi* and one with *Anaplasma* sp. (which is closely related to *A. platys*/*A. phagocytophilum*). Co-infection was present in 44.4% (4/9) of the samples; three had *T. cervi* and *A. marginale*, and one had *T. cervi, Anaplasma* sp. and *A. marginale* (Table 2).

The nucleotide sequences amplified from parasites in the whole blood have been deposited in GenBank under the following accession numbers: *Anaplasma marginale*, JN022561.1, JN022563.1, JN022564.1 and JN022568.1; *Theileria* sp., HM466920.1; and *T. cervi*, HM466923.1 and HM466925.1 (SILVEIRA et al., 2011, 2012). The organ sample sequences have been deposited under the following accession numbers: *Anaplasma phagocytophilum*, KF790914-KF790918 (*msp4* gene) and KF790919- KF790920 (16S rRNA gene); *Theileria* sp. (18S rRNA gene), KF790921 - KF790922 (Table 2).

According to the nPCR assays of the organ samples, none of the animals were positive for monocytic Anaplasmataceae, 55.5% (6/9) of the samples were positive for 18S rRNA *Theileria* species (sharing 96-99% of their identity with *Theileria* strains deposited in GenBank with accession numbers GU946217.1 and AY735137.1), 55.5% (5/9) were positive for msp4 from *A. phagocytophilum* (98-100% shared identity, accession numbers HQ661162.1, AY829455.1, DQ104442.1 and KF111754.1), 22.2% (2/9) were positive for the 16S rRNA gene from granulocytic Anaplasmataceae agents (99% shared identity with *A. phagocytophilum*, accession numbers DQ104442.1, KF481943.1 and KF481930.1) and 33.3% (3/9) were positive for msp4 from *A. marginale* (Figures 1-3). Samples from the *A. marginale*-positive organs were not successfully sequenced because of their low amplified DNA concentration. The brain and liver were the most parasitized organs based on the results of nPCR assays at 3/5 (60%) and 5/9 (55.5%), respectively.

A phylogenetic analysis of the partial 16S rRNA and the *msp4* gene showed that the phylogenetic topology generally placed samples from this study in close proximity with other *A. phagocytophilum* isolates, including isolates from humans and wild ruminants, reinforcing that these isolates are from *A. phagocytophilum* (Figures 1 and 2). The 18S rRNA from the Piroplasmida phylogenetic tree showed a cluster containing *T. cervi* isolates from other deer, including one sequence (gb HM466930.1, Silveira et al. (2012)) from *M. gouazoubira* in Minas Gerais, Brazil (Figure 3).

No hemoparasites were detected in organ samples from MGI12, although the infection was detected in whole blood. However, MGI13 was not positive for any parasites in the whole blood, but it was positive for *Babesia*/*Theileria* species in the lymph node sample. Half of the lymph nodes tested (3/6) were positive for hemoparasites, and the liver samples from five animals were positive. Co-infection was most common in whole blood (4/9) compared with organ samples; however, one animal (MGI14) presented co-infection only in the organ samples (Table 2). None of the young animals were positive for hemoparasites according to the imprint slides analysis, but nPCR assays revealed that co-infection occurred in two of these animals (2/3).

Discussion

Free-ranging wild animals may be considered reservoirs of an increasing number of pathogens with zoonotic potential. When some parasites infect both susceptible wild and domestic species that share the same habitats, both present enzootic importance and must be monitored (KUIKEN et al., 2005). Furthermore, some hemoparasites are a serious problem for domestic ruminants and humans, and both can be in close contact with wild animals, particularly deer, in South America (DUARTE et al., 2001).

According to Warns-Petit et al. (2010), *post-mortem* findings are the primary data collected for general disease surveillance. No macroscopic lesions were observed during the necropsy examinations of the nine brown brocket deer that were evaluated in the present study. Death caused by stressful handling can be acute, and *post-mortem* macroscopic alterations may not be evident, as found in this study. However, deer are often capable of harboring some hemoparasite infections in a latent asymptomatic stage for a long time (BARKER et al., 1973; KUTTLER; ZAUG, 1988; DAWSON et al., 1994; CANTU et al., 2007; DAVIDSON et al., 2001).

The blood smear is not reliable for distinguishing between some hemoparasites, such as *Babesia* and *Theileria* species, especially when both species occur together in endemic areas (SILVEIRA et al., 2011). Although the diagnosis of a hemoparasite infection is normally achieved through the examination of blood smears, this method exhibits poor sensitivity when there is a low level of parasitemia.

Nested PCR assays have been shown to provide more consistent data for mapping the distribution of hemoparasites that affect the wild fauna of Brazil. Whole blood analysis is a useful way to detect hemoparasites by using molecular techniques, and it is more amenable to DNA extraction compared with using organ samples. However, organ samples were found to be a suitable alternative to *post-mortem* detection. The present study reinforces the importance and utility of molecular techniques for *post-mortem* diagnostics and organ samples were found to be a suitable alternative to detection in necropsy. Erythrocytes in lymph node *imprint* slides have not shown infected, but half of the lymph node samples tested by nPCR were positive for hemoparasites.

No hemoparasites were detected in the whole blood of MGI13 and MGM3 deer; however, lymph nodes from MGI13 and the liver and brain of MGM3 were positive for *Theileria* species. Furthermore, none of the young were positive for hemoparasites in their imprint slides, but nPCR assays of blood or organ samples revealed that co-infection occurred in these animals. These facts

Table 2. Hemoparasite detection in organs and whole blood from nine free-living brown brocket deer in the state of Minas Gerais, Brazil, as determined by imprint slides and nested PCR.

Deer	Imprint slides				nPCR				
	Spleen	**Liver**	**Lymph node**	**Brain**	**Spleen**	**Liver**	**Lymph node**	**Brain**	**Whole Blood (nucleotide sequence)**
MGI8 (adult female)	x	+ T	x	+ T	x	*Theileria* sp. (KF790921), *A. phagocytophilum* (msp4: KF790914; 16S rRNA: KF790919)	x	*Theileria* sp. (KF790922), *A. phagocytophilum* (msp4: KF790915; 16S rRNA: KF790920)	*T. cervi* (HM466925.1), *A. marginale* (JN022561.1)
MGI11 (adult female)	+A, +T	+A, +T	x	x	-	+ B/T	x	x	*Theileria* sp. (HM466920.1), *A. marginale* (JN022563.1), *Anaplasma sp.*
MGI12 (adult female)	+A, +T	+A, +T	x	x	-	-	x	x	*T. cervi* (HM466923.1), *A. marginale* (JN022564.1)
MGM1 (adult female)	-	-	-	-	-	-	-	-	-
MGM2 (pregnant female)	-	-	-	-	-	-	-	-	-
MGI13 (young female)	-	-	-	-	-	-	+ B/T	x	-
MGI14 (young male)	-	-	-	-	+B/T,+ A, *A. phagocytophilum* (msp4: KF790916)	+ B/T, *A. marginale*	+ B/T, *A. marginale*	-	*A. marginale*
MGI19 (young female)	x	x	x	x	+ B/T, + A, *A. phagocytophilum* (msp4: KF790917)	+ B/T	+ B/T	+ B/T	*T. cervi, A. marginale* (JN022568.1)
MGM3 (adult male)	+T	-	-	-	+ B/T, *A. phagocytophilum* (msp4: KF790918)	+ B/T	-	+ B/T	-

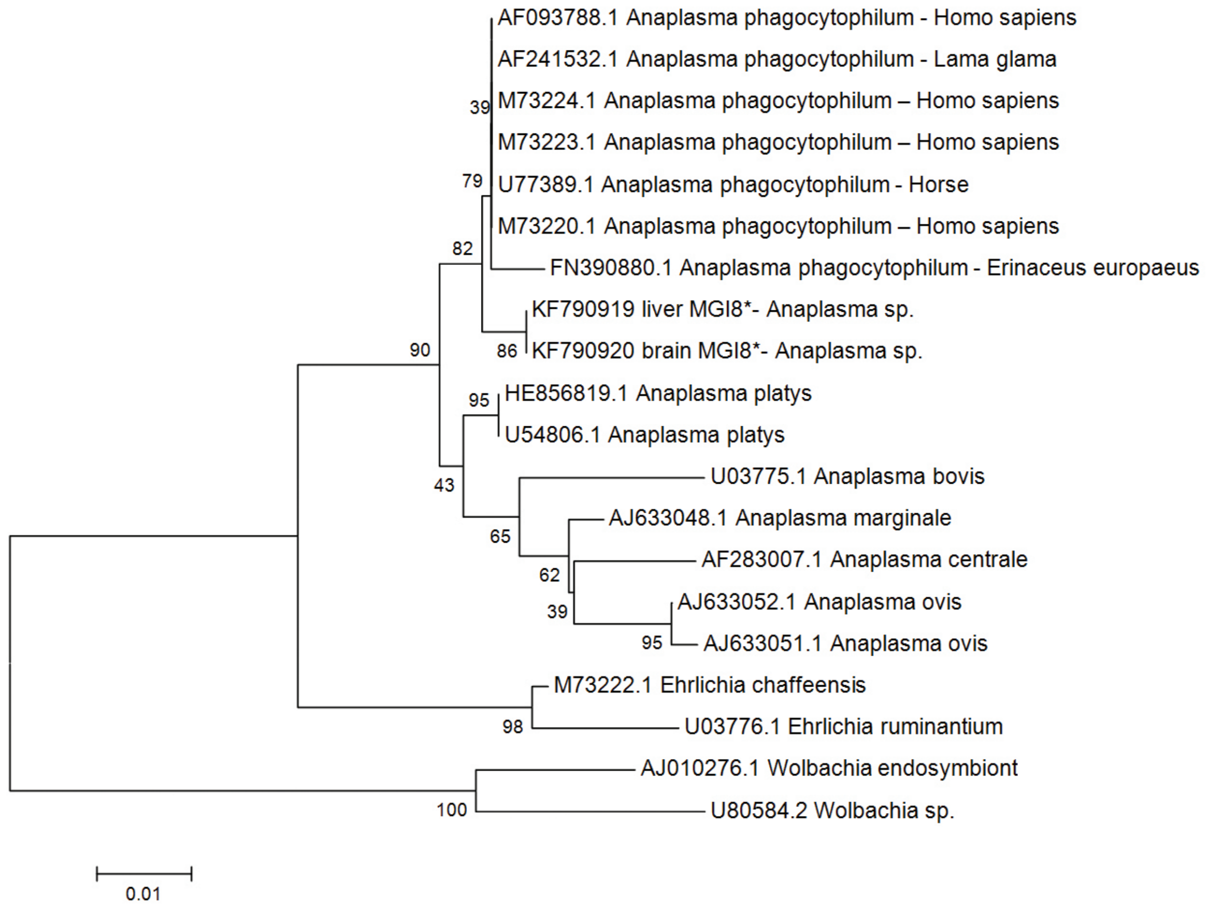

Figure 1. The phylogenetic tree of *Anaplasma phagocytophilum* as isolated from *M. gouazoubira* MGI8 organs and based on a partial sequence of the 16S rRNA gene. The GenBank accession numbers are shown. The tree was constructed using the neighbor-joining method, and the numbers above the internal nodes indicate the percentages of 1,000 bootstrap replicates that supported the branch. Outgroup: *Wolbachia* spp. *samples from this study.

highlight the importance of examining the lymphoid organs, liver and brain by using nPCR assays.

In relation to hemoparasites finding in this study, deer are important natural reservoir hosts of Anaplasmataceae, and they are capable of harboring latent *A. marginale* and *A. phagocytophilum* infections (CHRISTENSEN et al., 1958). Natural infection with *A. phagocytophilum* has been reported in wild deer, and these animals are considered reservoirs in Europe, Asia and North America (revised by Stuen et al. (2013)). Pathogenic and apathogenic genetic variants have been found in these animals (MASSUNG et al., 2005; SILAGHI et al., 2011; OVERZIER et al., 2013).

In Brazil, *A. phagocytophilum* has been molecularly detected in dogs, carnivorous birds, *Rhipicephalus sanguineus* and *Amblyomma cajennense* ticks (SANTOS et al., 2006, 2013; MACHADO et al., 2012). *Amblyomma cajennense* was found in MGI14 and MGI19 brown brocket deer, which were positive for *A. phagocytophilum*, by employing msp4 gene amplification (SILVEIRA, 2012). However, future studies must investigate which vectors and hosts are responsible for maintaining *A. phagocytophilum* in nature.

According to a BLASTn analysis of the 16S rRNA gene, the MGI8 samples shared a high percentage of their identity (100%) with an *A. phagocytophilum* isolate from a person from Poland (gb KF111754.1) and an isolate from white-tailed deer in the USA (gb DQ104442.1). The *msp4* gene analysis showed a high shared identity (98-100%) with European samples isolated from ovine, horses and *Ixodes ricinus* ticks. A phylogenetic tree analysis showed that the *Anaplasma* agent found in the present study is closely related to *A. phagocytophilum* at one site that is specific to this species, namely the *msp4* gene, and in another site in hypervariable regions that can provide a species-specific signature, namely the 16S rRNA gene, reinforcing that these isolates are *A. phagocytophilum* (Figures 1 and 2). To the best of our knowledge, this is the first molecular evidence of closely related *A. phagocytophilum* species in Brazilian deer, and the zoonotic role of this agent is still not understood.

Liver and brain samples from the MGI8 animal were positive for *A. phagocytophilum* according to a PCR of 16S rRNA and msp4 targets, reinforcing the identity of the isolate. Additionally, this agent was detected in the spleens of other cervids (MGM3,

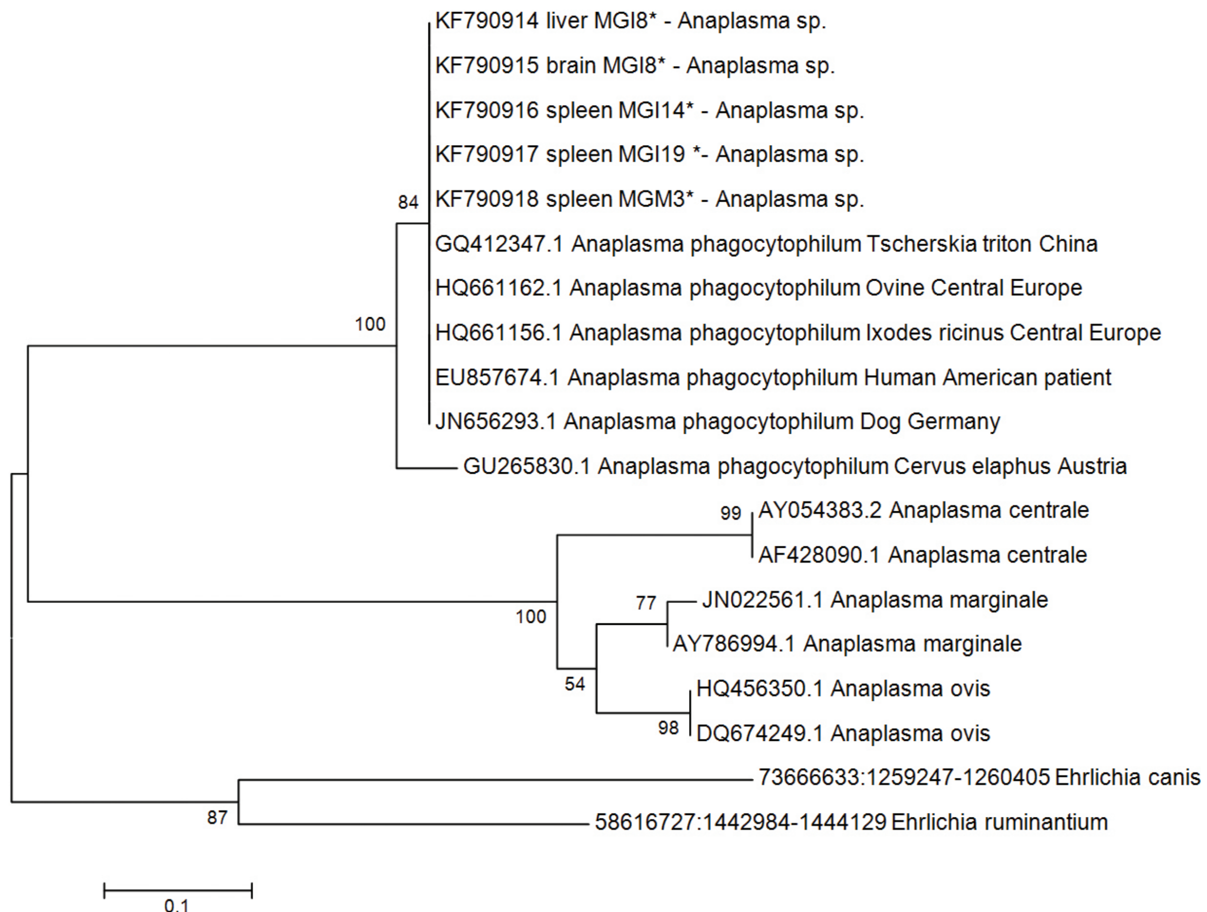

Figure 2. The phylogenetic tree of *Anaplasma phagocytophilum* as isolated from *M. gouazoubira* organ samples based on a partial sequence of the *msp4* gene. GenBank accession numbers are shown. The tree was constructed using the neighbor-joining method, and the numbers above the internal nodes indicate the percentages of 1,000 bootstrap replicates that supported the branch. Other Anaplasmataceae were used as outgroups. *samples from this study.

MGI14 and MGI19). Previous reports have shown that various tissues and cells are susceptible to infection by *A. phagocytophilum* (KLEIN et al., 1997; MUNDERLOH et al., 2004). The pathological change described during *A. phagocytophilum* infection in deer was an enlarged spleen with subcapsular bleeding in European roe deer (*Capreolus capreolus*) and reindeer (*Rangifer tarandus*) (STUEN, 2003).

The prevalence of *A. marginale* infection in Brazilian cervids has been reported at different values, specifically 6.3% and 48% in *O. bezoarticus* from the Pantanal region according to Picoloto et al. (2010) and Silveira et al. (2013), respectively, and 79.3% in *M. gouzoubira* from Minas Gerais state according to Silveira et al. (2012). However, the consequences of infection for deer health are not clearly understood. Nevertheless, anaplasmosis is known to be responsible for great economic losses among domestic ruminants in tropical and subtropical areas because of the intravascular anemia evident in infected animals (DUARTE et al., 2001).

Theileria spp. infections in Brazilian cervids were first described by our research group. The prevalence of infections in *M. gouzoubira* and *B. dichotomus* from Minas Gerais was 47.6% (SILVEIRA et al., 2012), and it was 20% in *O. bezoarticus* from Pantanal (SILVEIRA et al., 2013). *Theileria cervi* infections in MGI8 and MGI19 were confirmed by nucleotide sequencing (SILVEIRA et al., 2011), and the 18S rRNA in the Piroplasmida phylogenetic tree of the present study showed a cluster containing *T. cervi* isolates from other deer, including one sequence (gb HM466930.1, Silveira et al. (2012)) from *M. gouazoubira* in Minas Gerais, Brazil (Figure 3).

The pathogenesis of *T. cervi* infection is poorly understood, but in immunosuppressed and splenectomized deer, it can result in severe microcytic anemia (KREIER et al., 1962; FOWLER, 1993; YABSLEY et al., 2005). A high percentage of parasitized red cells in the capillaries of the brain can be found in some infections with intraerythrocytic piroplasms (RISTIC, 1988). Although the consequence may be cerebral ischemia, the magnitude of this parasitism in deer is unknown.The present study detected hemoprotozoa in the brain samples of these deer; however, whether brain parasitism can cause nervous symptoms, especially in immunosuppressed animals, remains unclear.

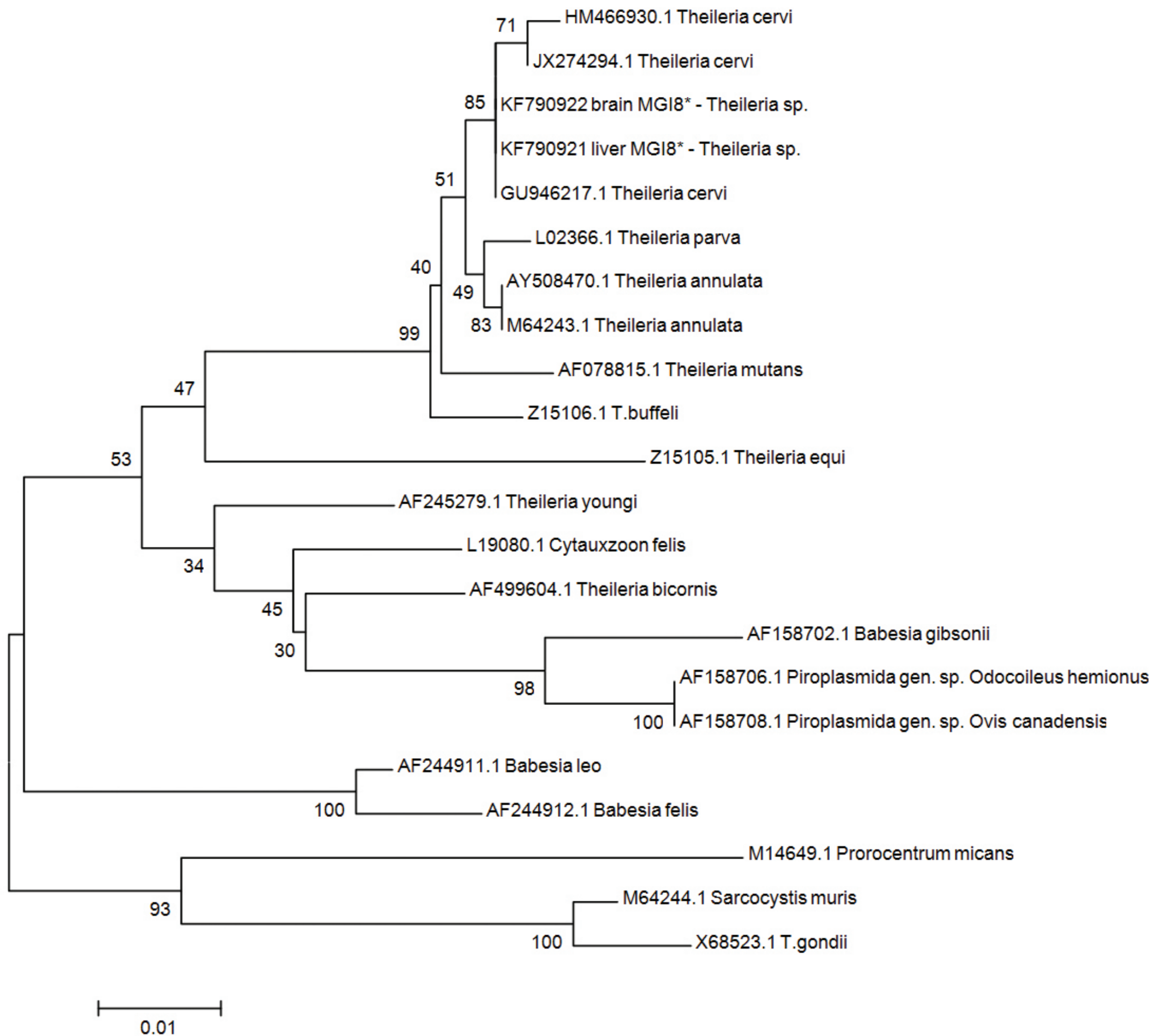

Figure 3. The phylogenetic tree of *Theileria* as isolated from *M. gouazoubira* MGI8 organs and based on the partial sequence of the 18S rRNA gene. The GenBank accession numbers are shown. The tree was constructed using the neighbor-joining method, and the numbers above the internal nodes indicate the percentages of 1,000 bootstrap replicates that supported the branch. *Toxoplasma gondii, P. micans* and *S. muiris* were used as outgroups. *samples from this study.

Conclusion

The results of the present study showed that *post-mortem* diagnostics using *imprint* slides and molecular assays were effective for detecting hemoparasites in deer organs.

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