Brazilian Journal of Veterinary Parasitology
ISSN 1984-2961 (Electronic)
www.cbpv.org.br/rbpv
Braz. J. Vet. Parasitol., Jaboticabal, v. 28, n. 1, p. 151-156, jan.-mar. 2019
Doi: https://doi.org/10.1590/S1984-296120180065

Short Communication

Occurrence of *Ehrlichia canis* and *Hepatozoon canis* and probable exposure to *Rickettsia amblyommatis* in dogs and cats in Natal, RN

Ocorrência de *Ehrlichia canis*, *Hepatozoon canis* e provável exposição a *Rickettsia amblyommatis* em cães e gatos de Natal, RN

Marcos Gomes Lopes[1]; Felipe da Silva Krawczak[1,2]; Julia Teresa Ribeiro de Lima[1]; Gislene Fatima da Silva Rocha Fournier[1]; Igor da Cunha Lima Acosta[1]; Diego Garcia Ramirez[1]; Arlei Marcili[1,3]; Marcelo Bahia Labruna[1]; Solange Maria Gennari[1,3]*

[1] Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo – USP, São Paulo, SP, Brasil

[2] Setor de Medicina Veterinária Preventiva, Escola de Veterinária e Zootecnia, Universidade Federal de Goiás – UFG, Goiânia, GO, Brasil

[3] Mestrado em Medicina e Bem-Estar Animal, Universidade Santo Amaro – UNISA, São Paulo, SP, Brasil

Received June 14, 2018
Accepted July 23, 2018

Abstract

Occurrence of infection or exposure to *Ehrlichia canis*, *Hepatozoon canis* and *Rickettsia* spp. was detected in feral cats living in two fragments from Atlantic rainforest, in Natal, RN, Brazil, and in dogs living around the parks. While serum samples were collected from 155 animals (53 cats living in the parks; 29 dogs living in human homes around the parks; and 73 dogs living at an animal control center - ACC), spleen samples were collected from 20 dogs that were euthanized at ACC. Serum samples were analyzed to *Rickettsia* spp. and *E. canis* antibodies using the indirect immunofluorescence assay. Seventeen of the 102 dogs (17%) had *E. canis* antibodies and 13% (20/155) of all dogs and cats (i.e. 3% (3/102) of the dogs and 32% (17/53) of the cats) were seropositive for *Rickettsia* spp. antigens. The animals were therefore been exposed to *R. amblyommatis* or by a very closely related genotype. Among the 20 dog spleen samples analyzed, eight were PCR positive for *E. canis* and two for *H. canis* (GenBank accession number MG772657 and MG772658, respectively). In none of the spleen samples were obtained amplicons for *Babesia* spp. through PCR. This study provided the first evidence that *Rickettsia* of the spotted fever group is circulating among dogs and cats in Natal.

Keywords: *R. amblyommatis*, PCR, IFA, Atlantic rainforest, Brazil.

Resumo

A ocorrência de infecção ou exposição para *Ehrlichia canis*, *Hepatozoon canis* e *Ricketts ia* spp. foi determinada em gatos ferais que viviam em dois fragmentos da Mata Atlântica, localizados em Natal, RN, Brasil e em cães que viviam em torno dos parques e em outras regiões da cidade. Enquanto amostras de soro foram coletadas de 155 animais (53 gatos que viviam nos parques, 29 cães com domicilio em torno dos parques e 73 cães do Centro de Controle de Animais -CCA), fragmentos de baço foram coletados de 20 cães eutanasiados no CCA. A detecção de anticorpos nas amostras de soros coletadas contra *Rickettsia* spp. e *E. canis* foi realizada pela Reação de Imunofluorescência Indireta. Dezessete dos 102 cães (17%) apresentaram anticorpos anti *E. canis* e 13% (20/155) de todos os cães e gatos (ou seja, 3% (3/102) dos cães e 32% (17/53) dos gatos) foram soropositivos para antígenos de *Rickettsia* spp. Os animais foram considerados expostos à *R. amblyommatis* ou a um genótipo muito relacionado. Entre as 20 amostras de baço de cães analisadas, oito foram positivas para *E. canis* e duas para *Hepatozoon canis* (números de acesso ao Genbank MG772657 e MG772658, respectivamente). Nenhuma das amostras de baço produziram amplicons de *Babesia* spp. na PCR. Observou-se, pela primeira vez, a circulação de *Rickettsia* do grupo da febre maculosa em cães e gatos em Natal, RN.

Palavras-chave: *R. amblyommatis*, PCR, RIFI, Mata Atlântica, Brasil.

***Corresponding author:** Solange Maria Gennari. Departamento de Medicina Veterinária Preventiva e Saúde Animal, Universidade de São Paulo – USP, Av. Prof. Dr. Orlando Marques de Paiva, 87, Cidade Universitária, CEP 05508-270, São Paulo, SP, Brasil. e-mail: sgennari@usp.br

Introduction

Dogs and cats are very popular as pets around the world and can be reservoirs for many microorganisms. *Ehrlichia canis*, *Hepatozoon canis* and *Rickettsia* of the spotted fever group (SFG) may cause diseases in dogs that develop either subclinical infection or severe clinical signs (HARRUS & WANER, 2011, in review; GONDIM et al., 1998; PIRANDA et al., 2008; STILES, 2000). However, the information on clinical signs showing by cats infected by tick-borne agents remains contradictory and requires further characterization (FRITZ & KJEMTRUP, 2003; SHAW et al., 2001). These agents have been reported infecting dogs in many parts of Brazil at wide ranges of occurrence and prevalence rates (COSTA et al., 2015; MIRANDA et al., 2014; KRAWCZAK et al., 2012; RAMOS et al., 2010; ROTONDANO et al., 2015, 2017; SAITO et al., 2008; SILVA et al., 2010; VIEIRA et al., 2011).

Wild carnivores inhabiting natural areas in periurban locations may be infected by the same pathogens that infect dogs and cats at higher infection rates (MILLÁN et al., 2016). Definition of the reservoirs and vectors involved in the transmission routes is an important step towards prevention of zoonoses.

In this study, we attempted to detect the occurrence of *Babesia* spp., *Ehrlichia canis*, *Hepatozoon canis* and *Rickettsia* spp. in feral cats living in two forest fragments located in the Atlantic Rainforest biome, in the municipality of Natal, state of Rio Grande do Norte (RN), Brazil, and in dogs living around the parks and in other regions of the city.

Materials and Methods

Study area and sample collection

For this study, feral cats living in two forest fragments within the Atlantic Rainforest Biome, in Natal, RN, Brazil, were selected: Parque Estadual das Dunas de Natal (Natal Dunes State Park; 5° 49' 41" S and 35° 11' 25" W) and Parque da Cidade (City Park; 5° 50' 45" S and 35° 14' 0" W). In addition, we sampled the population of dogs living in homes located around the parks and dogs at the Animal Control Center (ACC) of the city of Natal, which held dogs from several city regions.

Feral short hair cats were caught manually. These cats were found living in the parks without any care provided and in a state of uncontrolled reproduction, abandoned there by citizens. Apparently healthy dogs, living in their owners' homes around the parks, were sampled by convenience in these homes, according to the accessibility of the place. The dogs at the ACC were euthanized for a variety of reasons, and samples were collected on these occasions.

All the blood samples were collected from the cephalic or jugular vein. The blood serum from each animal was stored separately in microtubes at –20 °C until the laboratory analyses were performed. Some ticks that were found parasitizing the animals were collected and taken to the laboratory for taxonomic identification. The ticks were taxonomically identified following dichotomous keys: Martins et al. (2010) for nymphs; Barros-Battesti et al. (2006) for adults.

Serological analyses

Canine serum samples were tested individually by means of the indirect immunofluorescence assay (IFA) using *E. canis*-infected DH82 cells as the antigen. The São Paulo strain of *E. canis* was used for this, as previously described (AGUIAR et al., 2007, 2008). Reactions were performed using fluorescein-conjugated rabbit anti-dog IgG diluted 1:1000 (Sigma-Aldrich, St. Louis, MO, USA). Serum was considered to contain antibodies reactive to *E. canis* if it displayed a reaction at the dilution 1:80. Samples that reacted at the screening dilution (1:80) were then titrated using serial twofold dilutions to determine endpoint titers. The positive and negative control serum samples were derived from the study by Aguiar et al. (2007).

Feline and canine antibodies reactive to *Rickettsia* spp. were assayed by simultaneously using six *Rickettsia* isolates from Brazil: *R. bellii* strain Mogi, *R. amblyommatis* strain Ac37, *R. rhipicephali* strain HJ5, *R. rickettsii* strain Taiaçu, *R. parkeri* strain At24, and *Rickettsia felis* strain Pedreira, as previously described (LABRUNA et al., 2007a). Samples that reacted at the screening dilution (1:64) were then titrated using serial twofold dilutions to determine endpoint titers. Fluorescein isothiocyanate-labeled conjugate goat anti-cat IgG, or rabbit anti-dog IgG diluted 1:1000 (Sigma, St Louis, USA) were used. To determine the probable homologous antigens (PHA), serum showing a *Rickettsia* species reactive titer at least four-fold higher than those observed for the other *Rickettsia* species was taken to be homologous to the first *Rickettsia* species or to a very closely related genotype (LABRUNA et al., 2007b; PIRANDA et al., 2008). As control, a dog and cat serum previously shown to be non-reactive (negative controls) and known reactive serum samples (positive control) were used (HORTA et al., 2007). These samples were obtained from the serum bank of the Laboratory of Parasitic Diseases, Department of Preventive Veterinary Medicine and Animal Health (VPS), School of Veterinary Medicine and Animal Science (FMVZ), University of São Paulo (USP).

Molecular analyses

DNA was extracted from dog spleen fragments of approximately 20mg each, using the Wizard® genomic DNA purification kit (Promega Corporation, Madison, Wisconsin, USA), in accordance with the manufacturer's instructions. DNA samples were tested by Polymerase Chain Reaction (PCR) using the sets of primers described in Table 1. Reactions were performed in a final volume of 25 µl containing 10 mM of Tris-HCl (pH 8.3), 50 µM of KCl, 1.5 mM of $MgCl_2$, 0.2 mM of each desoxynucleoside triphosphate, 1.5 U of Taq DNA polymerase (Invitrogen), 11 pmol of each primer and approximately 100 ηg of canine genomic DNA. The amplified products were viewed under ultraviolet light after electrophoresis on agarose gel (1.5%) stained with SyBr gold (Invitrogen). The PCR products were purified using ExoSap (USB) and were sequenced in an automatic sequencer (model ABI Prism 310 Genetic; Applied Biosystems/Perkin Elmer, California, USA), in accordance with the manufacturer's protocol, and with the same primers as used in the PCR. The partial sequences obtained were

subjected to BLAST analyses (ALTSCHUL et al., 1990) to infer the closest similarities to sequences in GenBank.

Results

Between October 2012 and August 2013, four campaigns were conducted, and serum samples were collected from 155 animals: 53 feral cats (*Felis catus*) living in the parks; 29 dogs (*Canis familiaris*) living in human homes around the parks; and 73 dogs at the ACC. Furthermore, spleen fragments from 20 dogs that were euthanized at the ACC were also collected.

The serological results showed that 17% (17/102) of the dogs showed anti-*E. canis* antibodies and 13% (20/155) of all the animals tested, i.e. 3% (3/102) of the dogs and 32% (17/53) of the cats, were seropositive for *Rickettsia* spp. antigens (Table 2), with endpoint titers ranging from 64 to 2048 (Table 3). Except for one dog serum that only showed *R. bellii* as the PHA, and one cat serum that showed *R. parkeri* as the PHA, all the other positive animals showed seroreactivity with the highest titers for *R. amblyommatis*. At least 13 animals presented *R. amblyommatis* as the PHA, for which endpoint titers were four-fold higher than the endpoint titers shown for the other five *Rickettsia* species. These 13 animals might have been exposed to *R. amblyommatis* or a very closely related genotype (Table 3).

Parasitism by ticks of the species *Rhipicephalus sanguineus* sensu lato (s.l.) was observed in 38% (11/29) of the dogs, Park[1] 23,5% (4/17) and Park[2] 58,3% (7/12). One cat (2% [1/49]) from Parque da Cidade was found to be parasitized by one nymph stage of *Amblyomma auricularium*.

Among the 20 dog spleen samples, subjected to molecular analyses, eight were found to be positive in the PCR targeting a fragment of the 16S rRNA gene of Anaplasmataceae, and in the PCR targeting a fragment of the *dsb* gene of *Ehrlichia* spp. PCR products of 16S rRNA and *dsb* gene generated sequences 100% similarity to *E. canis*, which is found in several parts of the world, including Brazil (16S rRNA: KP642752, KP182941, KP182942; *dsb*: MG967467, KY594915, CP025749, KR920044). Furthermore, two samples were positive for *Hepatozoon canis*, which were confirmed by DNA sequences and BLAST analysis, showing to be 100% identity to sequences already reported from dogs around the world (KJ513193, KJ513198, KF621083). None of the canine spleen samples yielded amplicons in the PCR for *Babesia* spp.

DNA sequences generated in this study were deposited in GenBank under the accession numbers MH118746 for *E. canis* 16S rRNA partial sequence, MG772657 for *E. canis dsb* partial sequence, and MG772658 for *H. canis* 18S rRNA partial sequence.

Discussion

The results from the serological analyses on *Rickettsia* spp. showed that the number of seropositive dogs was low (3%), while it was relatively high (32%) among the sampled cats (Table 2). This can possibly be explained by the fact that the cats were living inside the parks and were exposed to parasitism by *A. auricularium*,

Table 1. Primer pairs used in the present study for detecting tick-borne agents.

Target agents (gene)	Primers	Primer sequences (5'-3')	(Bp)	Reference
Babesia spp. (18S rRNA)	BAB1 BAB4	GTGAACCTTATCACTTAAAGG CAACTCCTCCACGCAATCG	590	Duarte et al. (2008)
Anaplasmataceae (16S rRNA)	GE2´F2´ HE3	GTTAGTGGCAGACGGGTGAGT TATAGGTACCGTCATTATCTTCCCTAT	360	Aguiar et al. (2008)
Ehrlichia spp. (*dsb* gene)	DSB-330 DSB-728	GATGATGTCTGAAGATATGAAACAAAT CTGCTCGTCTATTTTACTTCTTAAAGT	409	Doyle et al. (2005)
Hepatozoon sp. (18S rRNA)	HEP2-169 HEP2-718	F- GGTAATTCTAGAGCTAATACATGAGC R- ACAATAAAGTAAAAAACAYTTCAAAG	574	Almeida et al. (2012)

Bp = Base Pairs.

Table 2. Distribution according to area of the numbers of dogs and cats in the state of Rio Grande do Norte, Brazil, that were analyzed for *Rickettsia* spp. and *E. canis* using the indirect immunofluorescence assay (IFA), showing the numbers of positive cases and percentages.

Animals	No. seropositive animals / No. tested (% seropositivity)		
	IFA for *Rickettsia* spp.		IFA for *E. canis*
Felis catus (Park[1])	1/4 (25)	17/53 (32%)	**Not tested**
Felis catus (Park[2])	16/49 (33)		
Canis familiaris (Park[1])	1/17 (6)	3/102 (3%)	5/17 (25)
Canis familiaris (Park[2])	2/12 (16)		3/12 (25)
Canis familiaris (ACC)	0/73 (0)		9/73 (12)
Total	20/155 (13)		17/102 (17)

[1]Parque Estadual das Dunas de Natal; [2]Parque da Cidade; ACC = animal control center.

Table 3. Results from indirect immunofluorescence assays (IFA) against six *Rickettsia* species among serum samples from dogs and cats in Natal, Rio Grande do Norte, Brazil.

Animals IDs	Endpoint titers for rickettsial antigens						
	R. ambly[1]	*R. riphi*[2]	*R. rick*[3]	*R. parke*[4]	*R. belli*[5]	*R. felis*[6]	PHA[7]
Dog 10 (park[1])	0	0	0	0	256	0	*R. belli*
Dog 23 (park[2])	1024	0	0	0	0	0	*R. ambly*
Dog 25 (park[2])	512	0	0	0	64	0	*R. ambly*
Cat 33 (park[2])	256	0	0	0	0	0	*R. ambly*
Cat 5 (park[2])	64	0	0	64	0	0	-
Cat 8 (park[2])	64	0	0	0	0	0	-
Cat 19 (park[2])	2048	512	256	256	128	64	*R. ambly*
Cat 20 (park[2])	1024	0	0	0	0	0	*R. ambly*
Cat 9 (28.2) (park[2])	512	0	0	0	0	0	*R. ambly*
Cat 6 (1.3) (park[2])	2048	256	256	0	256	0	*R. ambly*
Cat 35 (park[2])	256	0	0	0	0	0	*R. ambly*
Cat 24 (park[2])	1024	0	0	0	0	0	*R. ambly*
Cat 23 (park[2])	512	0	0	0	0	0	*R. ambly*
Cat 32 (park[2])	64	0	0	0	0	0	-
Cat 7 (park[2])	0	0	0	64	0	0	-
Cat 9 (park[2])	512	256	0	1024	0	0	-
Cat 11(park[2])	256	0	0	0	0	0	*R. ambly*
Cat 1 (1.3) (park[1])	1024	0	0	0	0	0	*R. ambly*
Cat 31(park[2])	512	0	0	0	0	0	*R. ambly*
Cat 9 (18.2) (park[2])	0	0	0	256	0	0	*R. parke*

[1]*Rickettsia amblyommatis*; [2]*R. rhipicephali*; [3]*R. rickettsii*; [4]*R. parkeri*; [5]*R. bellii*; [6]*R. felis*; [7]PHA = probable homologous antigens; park[1] = Parque Estadual das Dunas de Natal; park[2] = Parque da Cidade.

as observed on this work. It is known that this tick is a competent vector for *R. amblyommatis* (SARAIVA et al., 2013). Moreover, two dogs living in the area around the parks showed antibodies against *R. amblyommatis* while the dogs at the ACC were all negative. In a previous study in the state of São Paulo, Horta et al. (2007) showed that cats could be better rickettsial sentinels than dogs. Cats may be more exposed to ticks than other domestic animals because of their habits. In the present study, the cats abandoned in the parks had become feral, living in direct contact with the wild environment.

The importance of *R. amblyommatis* in relation to pathogenicity for humans is not yet fully elucidated. However, some Rocky Mountain spotted fever (RMSF) cases in the United States that were diagnosed as due to *R. rickettsii* may have instead been due to *R. amblyommatis* (APPERSON et al., 2008).

The IFA test showed that antibodies for *E. canis* were found in 17% (17/102) of the dogs analyzed in this study. A similar seropositivity rate was previously observed by Costa et al. (2015), who found antibodies to this agent in 14.6% of the dogs from rural and urban areas of the state of Maranhão, Brazil. Higher seropositivity rates among dogs have been reported in studies conducted in other states in northeastern Brazil: Paraíba 72.5%, 69.4%, 23% and 34% (ARAES-SANTOS et al., 2015; AZEVEDO et al., 2011; ROTONDANO et al., 2015; TANIKAWA et al., 2013); and Bahia 23.0%, 36% and 35.6% (ARAES-SANTOS et al., 2015; CARLOS et al., 2007; SOUZA et al., 2010).

Confirming the serological findings, *E. canis* DNA was detected by PCR in 40% (8/20) of the canine spleen samples. Furthermore, 75% (6/8) of these PCR-positive dogs were also positive in serological analysis for *E. canis* antibodies. Previous studies in the state of Rio Grande do Norte showed results that corroborate the findings of the present study: fragments of DNA sequences of *E. canis* and *H. canis* were detected in blood samples collected from dogs in the city of Mossoró (GONÇALVES et al., 2014). Furthermore, inclusions suggestive of *Ehrlichia* spp. were detected in 6.5% (13/198) of dogs showing clinical signs suggestive of canine monocytic ehrlichiosis in the same city (MEDEIROS & LIMA, 2004). These results revealed that *E. canis* was the main tick-borne pathogen infecting dogs in northeastern Brazil, a condition corroborated by the present study.

In Brazil, *Hepatozoon canis* infection has been reported in many regions and the occurrence rate may range from 8.6% to 100% in the southeastern region (MIRANDA et al., 2014; MUNDIM et al., 2008; O'DWYER et al., 2001; SPOLIDORIO et al., 2009) and 3.6% to 73% in the central-western region (MELO et al., 2016; MUNDIM ECS. et al., 2008; PALUDO et al., 2003; RAMOS et al., 2015), while two cases with molecular analyses has been reported in the southern region (LASTA et al., 2009; MALHEIROS et al., 2016). Studies in northeastern Brazil have shown lower occurrence rates ranging from 0.49% in Pernambuco to 9.3% in Paraiba (BERNARDINO et al., 2016; RAMOS et al., 2010; ROTONDANO et al., 2015). Gonçalves et al. (2014) reported *H. canis* infection cases in Mossoro, RN. Our results strengthen the data on circulation of this parasite in this study region through showing that 10% (2/20) of the dog spleen samples were positive for *H. canis* by means of PCR.

Rhipicephalus sanguineus s.l. was the only tick species found on the dogs evaluated in this study. This Ixodidae is a competent vector for *E. canis* (DANTAS-TORRES, 2008) corroborating with the serological and molecular results of the present study.

In conclusion, *Rickettsia* spp., represented mainly by anti-*R. amblyommatis* antibodies, are circulating among cats and dogs in the anthropized fragments of the Atlantic Rainforest biome in the municipality of Natal, RN, Brazil. Cats were shown to be an important sentinel for *Rickettsia* spp., and *E. canis* and *H. canis* are also infecting dogs in the urban area of Natal. To our knowledge, the present study provided the first evidence suggesting that *Rickettsia* of the spotted fever group is circulating among dogs and cats in the region analyzed here in the state of Rio Grande do Norte, northeastern Brazil.

Acknowledgements

This work was supported by FAPESP (2012/02556-3 Fundação de Amparo à Pesquisa do Estado de São Paulo) and CAPES.

References

Aguiar DM, Hagiwara MK, Labruna MB. *In vitro* isolation and molecular characterization of an *Ehrlichia canis* strain from São Paulo, Brazil. *Braz J Microbiol* 2008; 39(3): 489-493. http://dx.doi.org/10.1590/S1517-83822008000300014. PMid:24031251.

Aguiar DM, Saito TB, Hagiwara MK, Machado RZ, Labruna M. Diagnóstico sorológico de erliquiose canina com antígeno brasileiro de *Ehrlichia canis*. *Cienc Rural* 2007; 37(3): 796-802. http://dx.doi.org/10.1590/S0103-84782007000300030.

Almeida AP, Marcili A, Leite RC, Nieri-Bastos FA, Domingues LN, Martins JR, et al. *Coxiella* symbiont in the tick *Ornithodoros rostratus* (Acari: Argasidae). *Ticks Tick Borne Dis* 2012; 3(4): 203-206. http://dx.doi.org/10.1016/j.ttbdis.2012.02.003. PMid:22480930.

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.

Apperson CS, Engber B, Nicholson WL, Mead DG, Engel J, Yabsley MJ, et al. Tick-borne diseases in North Carolina: is "*Rickettsia amblyommii*" a possible cause of rickettsiosis reported as Rocky Mountain spotted fever? *Vector Borne Zoonotic Dis* 2008; 8(5): 597-606. http://dx.doi.org/10.1089/vbz.2007.0271. PMid:18447622.

Araes-Santos AI, Moraes-Filho J, Peixoto RM, Spolidorio MG, Azevedo SS, Costa MM, et al. Ectoparasite Infestations and Canine Infection by Rickettsiae and Ehrlichiae in a Semi-Arid Region of Northeastern Brazil. *Vector Borne Zoonotic Dis* 2015; 15(11): 645-651. http://dx.doi.org/10.1089/vbz.2015.1786. PMid:26565771.

Azevedo SS, Aguiar DM, Aquino SF, Orlandelli RO, Fernandes ARF, Uchôa ICP. Soroprevalência e fatores de risco associados à soropositividade para Ehrlichia canis em cães do semiárido da Paraíba. *Braz J Vet Res Anim Sci* 2011; 48(1): 14-18. http://dx.doi.org/10.11606/S1413-95962011000100002.

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.

Bernardino MGS, Meireles MVN, Silva EG, Xavier FJR, Satake F. Prevalência de hepatozoonose canina no município de Areia, Paraíba, Brasil. *Biotemas* 2016; 29(1): 175-179. http://dx.doi.org/10.5007/2175-7925.2016v29n1p175.

Carlos RSA, Muniz Neta ES, Spagnol FH, Oliveira LS, Brito RLL, Albuquerque GR, et al. Freqüência de anticorpos anti-*Erhlichia canis, Borrelia burgdorferi* e antígenos de *Dirofilaria immitis* em cães na microrregião Ilhéus-Itabuna, Bahia, Brasil. *Rev Bras Parasitol Vet* 2007; 16(3): 117-120. http://dx.doi.org/10.1590/S1984-29612007000300001. PMid:18078596.

Costa AP, Costa FB, Labruna MB, Silveira I, Moraes-Filho J, Soares JF, et al. A serological and molecular survey of *Babesia vogeli, Ehrlichia canis* and *Rickettsia* spp. among dogs in the state of Maranhão, northeastern Brazil. *Rev Bras Parasitol Vet* 2015; 24(1): 28-35. http://dx.doi.org/10.1590/S1984-29612015008. PMid:25909250.

Dantas-Torres F. Canine vector-borne diseases in Brazil. *Parasit Vectors* 2008; 1(1): 25. http://dx.doi.org/10.1186/1756-3305-1-25. PMid:18691408.

Doyle CK, Labruna MB, Breitschwerdt EB, Tang Y, Corstvet RE, Hegarty BC, et al. Detection of medically important *Ehrlichia* by quantitative multicolor TaqMan real-time polymerase chain reaction of the *dsb* gene. *J Mol Diagn* 2005; 7(4): 504-510. http://dx.doi.org/10.1016/S1525-1578(10)60581-8. PMid:16237220.

Duarte SC, Linhares GF, Romanowsky TN, Silveira OJ No, Borges LM. Assessment of primers designed for the subspecies-specific discrimination among *Babesia canis canis, Babesia canis vogeli* and *Babesia canis rossi* by PCR assay. *Vet Parasitol* 2008; 152(1-2): 16-20. http://dx.doi.org/10.1016/j.vetpar.2007.12.013. PMid:18242863.

Fritz CL, Kjemtrup AM. Lyme borreliosis. *J Am Vet Med Assoc* 2003; 223(9): 1261-1270. http://dx.doi.org/10.2460/javma.2003.223.1261. PMid:14621212.

Gonçalves LR, Filgueira KD, Ahid SMM, Pereira JS, Vale AM, Machado RZ, et al. Study on coinfecting vector-borne pathogens in dogs and ticks in Rio Grande do Norte, Brazil. *Rev Bras Parasitol Vet* 2014; 23(3): 407-412. http://dx.doi.org/10.1590/S1984-29612014071. PMid:25271465.

Gondim LF, Kohayagawa A, Alencar NX, Biondo AW, Takahira RK, Franco SR. Canine hepatozoonosis in Brazil: description of eight naturally occurring cases. *Vet Parasitol* 1998; 74(2-4): 319-323. http://dx.doi.org/10.1016/S0304-4017(96)01120-X. PMid:9561717.

Harrus S, Waner T. Diagnosis of canine monocytotropic ehrlichiosis (*Ehrlichia canis*): an overview. *Vet J* 2011; 187(3): 292-296. http://dx.doi.org/10.1016/j.tvjl.2010.02.001. PMid:20226700.

Horta MC, Labruna MB, Pinter A, Linardi PM, Schumaker TTS. *Rickettsia* infection in five areas of the state of São Paulo, Brazil. *Mem Inst Oswaldo Cruz* 2007; 102(7): 793-801. http://dx.doi.org/10.1590/S0074-02762007000700003. PMid:18094887.

Krawczak FS, Labruna MB, Sangioni LA, Vogel FSF, Soares JF, Lopes STA. Serological survey on *Ehrlichia* sp. among dogs in the central region of Rio Grande do Sul. *Rev Bras Parasitol Vet* 2012; 21(4): 415-417. http://dx.doi.org/10.1590/S1984-29612012005000001. PMid:23184320.

Labruna MB, Horta MC, Aguiar DM, Cavalcante GT, Pinter A, Gennari SM, et al. Prevalence of *Rickettsia* infection in dogs from the urban and rural areas of Monte Negro Municipality, Western Amazon, Brazil. *Vector Borne Zoonotic Dis* 2007b; 7(2): 249-255. http://dx.doi.org/10.1089/vbz.2006.0621. PMid:17627445.

Labruna MB, Pacheco RC, Richtzenhain LJ, Szabó MP. Isolation of *Rickettsia rhipicephali* and *Rickettsia bellii* from *Haemaphysalis juxtakochi*

Ticks in the State of São Paulo, Brazil. *Appl Environ Microbiol* 2007a; 73(3): 869-873. http://dx.doi.org/10.1128/AEM.02249-06. PMid:17142361.

Lasta CS, Santos AP, Mello FPS, Lacerda LA, Messick JB, Díaz González FH. Infecção por *Hepatozoon canis* em canino doméstico na região Sul do Brasil confirmada por técnicas moleculares. *Cienc Rural* 2009; 39(7): 2135-2140. http://dx.doi.org/10.1590/S0103-84782009005000160.

Malheiros J, Costa MM, do Amaral RB, de Sousa KCM, André MR, Machado RZ, et al. Identification of vector-borne pathogens in dogs and cats from Southern Brazil. *Ticks Tick Borne Dis* 2016; 7(5): 893-900. http://dx.doi.org/10.1016/j.ttbdis.2016.04.007. PMid:27266811.

Martins TF, Onofrio VC, Barros-Battesti DM, Labruna MB. Nymphs of the genus *Amblyomma* (Acari: Ixodidae) of Brazil: descriptions, redescriptions, and identification key. *Ticks Tick Borne Dis* 2010; 1(2): 75-99. http://dx.doi.org/10.1016/j.ttbdis.2010.03.002. PMid:21771514.

Medeiros AMM, Lima AKF. Ocorrência de ehrlichiose canina em Mossoró. *Ciênc Anim* 2004; 14(1): 53-57.

Melo ALT, Witter R, Martins TF, Pacheco TA, Alves AS, Chitarra CS, et al. A survey of tick-borne pathogens in dogs and their ticks in the Pantanal biome, Brazil. *Med Vet Entomol* 2016; 30(1): 112-116. http://dx.doi.org/10.1111/mve.12139. PMid:26467462.

Millán J, Proboste T, Fernández de Mera IG, Chirife AD, de la Fuente J, Altet L. Molecular detection of vector-borne pathogens in wild and domestic carnivores and their ticks at the human-wildlife interface. *Ticks Tick Borne Dis* 2016; 7(2): 284-290. http://dx.doi.org/10.1016/j.ttbdis.2015.11.003. PMid:26643497.

Miranda RL, O'Dwyer LH, de Castro JR, Metzger B, Rubini AS, Mundim AV, et al. Prevalence and molecular characterization of *Hepatozoon canis* in dogs from urban and rural areas in Southeast Brazil. *Res Vet Sci* 2014; 97(2): 325-328. http://dx.doi.org/10.1016/j.rvsc.2014.06.015. PMid:25039064.

Mundim AV, Morais IA, Tavares M, Cury MC, Mundim MJ. Clinical and hematological signs associated with dogs naturally infected by *Hepatozoon* sp. and with other hematozoa: a retrospective study in Uberlândia, Minas Gerais, Brazil. *Vet Parasitol* 2008; 153(1-2): 3-8. http://dx.doi.org/10.1016/j.vetpar.2008.01.018. PMid:18304739.

Mundim ECS, Silva Francisco MM, Souza JN, Alencar MA, Dias Ramalho PC. Incidência de hemoparasitoses em cães (*Canis familiares*) de rua capturados pelo Centro de Controle de Zoonoses (CCZ) da cidade de Anápolis-GO. *Ensaios Cienc: Cienc Biol. Agrária Saúde* 2008; 12(2): 107-115.

O'Dwyer LH, Massard CL, Pereira de Souza JC. *Hepatozoon canis* infection associated with dog ticks of rural areas of Rio de Janeiro State, Brazil. *Vet Parasitol* 2001; 94(3): 143-150. http://dx.doi.org/10.1016/S0304-4017(00)00378-2. PMid:11113545.

Paludo GR, Dell'Porto A, Castro e Trindade AR, McManus C, Friedman H. *Hepatozoon* spp.: report of some cases in dogs in Brasília, Brazil. *Vet Parasitol* 2003; 118(3-4): 243-248. http://dx.doi.org/10.1016/j.vetpar.2003.10.009. PMid:14729172.

Piranda EM, Faccini JL, Pinter A, Saito TB, Pacheco RC, Hagiwara MK, et al. Experimental infection of dogs with a Brazilian strain of *Rickettsia rickettsii*: clinical and laboratory findings. *Mem Inst Oswaldo Cruz* 2008; 103(7): 696-701. http://dx.doi.org/10.1590/S0074-02762008000700012. PMid:19057821.

Ramos CA, Babo-Terra VJ, Pedroso TC, Souza AF Fo, Ribeiro de Araújo F, Cleveland HP. Molecular identification of *Hepatozoon canis* in dogs from Campo Grande, Mato Grosso do Sul, Brazil. *Rev Bras Parasitol Vet* 2015; 24(2): 247-250. http://dx.doi.org/10.1590/S1984-29612015019. PMid:26154969.

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.

Rotondano TE, Almeida HK, Krawczak FS, Santana VL, Vidal IF, Labruna MB, et al. Survey of *Ehrlichia canis, Babesia* spp. and *Hepatozoon* spp. in dogs from a semiarid region of Brazil. *Rev Bras Parasitol Vet* 2015; 24(1): 52-58. http://dx.doi.org/10.1590/S1984-29612015011. PMid:25909253.

Rotondano TE, Krawczak FS, Barbosa WO, Moraes-Filho J, Bastos FN, Labruna MB, et al. *Ehrlichia canis* and *Rickettsia* spp. in dogs from urban areas in Paraiba state, northeastern Brazil. *Rev Bras Parasitol Vet* 2017; 26(2): 211-215. http://dx.doi.org/10.1590/s1984-29612017030. PMid:28658415.

Saito TB, Cunha-Filho NA, Pacheco RC, Ferreira F, Pappen FG, Farias NA, et al. Canine infection by rickettsiae and ehrlichiae in southern Brazil. *Am J Trop Med Hyg* 2008; 79(1): 102-108. PMid:18606772.

Saraiva DG, Nieri-Bastos FA, Horta MC, Soares HS, Nicola PA, Pereira LC, et al. *Rickettsia amblyommii* infecting *Amblyomma auricularium* ticks in Pernambuco, Northeastern Brazil: isolation, transovarial transmission, and transstadial perpetuation. *Vector Borne Zoonotic Dis* 2013; 13(9): 615-618. http://dx.doi.org/10.1089/vbz.2012.1223. PMid:23705586.

Shaw SE, Birtles RJ, Day MJ. Arthropod-transmitted infectious diseases of cats. *J Feline Med Surg* 2001; 3(4): 193-209. http://dx.doi.org/10.1053/jfms.2001.0149. PMid:11795958.

Silva JN, Almeida ABPF, Boa Sorte EC, Freitas AG, Santos LGF, Aguiar DM, et al. Soroprevalência de anticorpos anti-*Ehrlichia canis* em cães de Cuiabá, Mato Grosso. *Rev Bras Parasitol Vet* 2010; 19(2): 108-111. http://dx.doi.org/10.1590/S1984-29612010000200008. PMid:20624348.

Souza BMPS, Leal DC, Barboza DCPM, Uzêda RS, Alcântara ACD, Ferreira F, et al. Prevalence of ehrlichial infection among dogs and ticks in Northeastern Brazil. *Rev Bras Parasitol Vet* 2010; 19(2): 89-93. http://dx.doi.org/10.1590/S1984-29612010000200004. PMid:20624344.

Spolidorio MG, Labruna MB, Zago AM, Donatele DM, Caliari KM, Yoshinari NH. *Hepatozoon canis* infecting dogs in the State of Espírito Santo, southeastern Brazil. *Vet Parasitol* 2009; 163(4): 357-361. http://dx.doi.org/10.1016/j.vetpar.2009.05.002. PMid:19482427.

Stiles J. Canine rickettsial infections. *Vet Clin North Am Small Anim Pract* 2000; 30(5): 1135-1149. http://dx.doi.org/10.1016/S0195-5616(00)05011-7. PMid:11033879.

Tanikawa A, Labruna MB, Costa A, Aguiar DM, Justiniano SV, Mendes RS, et al. *Ehrlichia canis* in dogs in a semiarid region of Northeastern Brazil: Serology, molecular detection and associated factors. *Res Vet Sci* 2013; 94(3): 474-477. http://dx.doi.org/10.1016/j.rvsc.2012.10.007. PMid:23141416.

Vieira RFC, Biondo AW, Guimarães AMS, Santos AP, Santos RP, Dutra LH, et al. Ehrlichiosis in Brazil. *Rev Bras Parasitol Vet* 2011; 20(1): 1-12. http://dx.doi.org/10.1590/S1984-29612011000100002. PMid:21439224.