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Short Communication

Detection of a putative novel genotype of *Ehrlichia* sp. from opossums (*Didelphis aurita*) from Brazil

Detecção de um suposto novo genótipo de *Ehrlichia* sp. em gambás (*Didelphis aurita*) do Brasil

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Abstract

Erlichiosis affects humans and animals worldwide. Its distribution and prevalence depends on the presence of tick vectors and hosts in one geographic area. The aim of the present study was to investigate the occurrence of *Ehrlichia* spp. and *Anaplasma* spp. in opossums (*Didelphis* sp.) from the State of Rio de Janeiro, southeast Brazil. Blood samples from 37 animals were tested for these two pathogens using molecular methods. One animal (2.7%) was positive for *Ehrlichia* sp. by 16S rRNA-based nested PCR. In a phylogenetic analysis based on the 16S rRNA gene using the maximum likelihood method and the GTRGAMMA+I evolutionary model, we detected a novel *Ehrlichia* sp. genotype closely related to genotypes of *E. canis* previously reported in dogs from Brazil. To the authors' knowledge, this is the first molecular detection of *Ehrlichia* sp. in opossums from this State in the southeastern region of the country.

Keywords: Opossum, *Didelphis aurita*, *Ehrlichia*, ehrlichiosis, molecular characterization, Brazil.

Resumo

A erliquiose afeta seres humanos e animais em todo o mundo. Sua distribuição e prevalência dependem da presença de vetores de carrapatos e hospedeiros em uma área geográfica. O objetivo do presente estudo foi investigar a ocorrência de *Ehrlichia* sp. e *Anaplasma* sp. em gambás (*Didelphis* sp.) do Estado do Rio de Janeiro, sudeste do Brasil. Amostras de sangue de 37 animais foram testadas para estes dois patógenos usando métodos moleculares. Um animal (2,7%) foi positivo para *Ehrlichia* sp. baseado em 16S rRNA-nested PCR. Em uma análise filogenética baseada no gene 16S rRNA usando o método de máxima verossimilhança e o modelo evolutivo GTRGAMMA + I, detectamos um novo genótipo de *Ehrlichia* sp. intimamente relacionado a genótipos de *E. canis* previamente relatados em cães do Brasil. Para o conhecimento dos autores, esta é a primeira detecção molecular de *Ehrlichia* sp. em gambás deste estado na região sudeste do país.

Palavras-chave: Gambá, *Didelphis aurita*, *Ehrlichia*, erliquiose, caracterização molecular, Brasil.

Diseases transmitted by arthropod vectors represent new challenges to human and veterinary medicine. These vectors are expanding their geographical distribution mainly due to climate changes and access to new ecological niches. Many pathogens, hosts, and vectors are involved in the epidemiology of these infectious diseases (HARRUS & BANETH, 2005).

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The presence of domestic animals in wild environments has increased the translocation of arthropod-borne pathogens from wildlife reservoirs to humans and domestic animals (SHAW et al., 2001). Thus, the characterization of disease reservoirs is one of the goals in public health research globally (CASTELLAW et al., 2011).

With regard to tick-borne pathogens which are zoonotic, Anaplasmataceae comprises obligate intracellular, gram-negative, pleomorphic bacteria (DUMLER et al., 2001). Recently, new *Anaplasma* spp. and *Ehrlichia* spp. genotypes have been described in wild animals from Brazil (ANDRÉ et al., 2010, 2012; SACCHI et al., 2012; MACHADO et al., 2012; WIDMER et al.,

2011; WERTHER et al., 2017; BENEVENUTE et al., 2017; BRAGA et al., 2018; SOUSA et al., 2017).

However, to date there are few published studies on the occurrence of Anaplasmataceae in opossums. For instance, Lockhart et al. (1997) found 3 of 38 (8%) opossums (*Didelphis virginianus*) seropositive for *E. chaffeensis* in the State of Georgia, USA, with titres ranging between 64 and 512. Similarly, antibodies to *E. chaffeensis* were detected in serum samples from 3 of 19 (15.8%) opossums in the State of Mississipi, USA (CASTELLAW et al., 2011). Recently, antibodies against *Ehrlichia canis* were detected in blood specimens of 16 of 109 (14.67%) opossums from the State of São Paulo, southeast Brazil (MELO et al., 2016), and *Ehrlichia* and *Anaplasma* DNA were found in marsupials from the Brazilian Pantanal in the central-west region of the country (SOUSA et al., 2017).

The aim of the present study was to investigate the occurrence of Anaplasmataceae in opossums from the State of Rio de Janeiro, southeast Brazil, using molecular techniques including PCR.

Between January 2014 and November 2014, blood samples from 37 opossums (*Didelphis* sp.), selected by non-probability convenience collected at the Centro de Triagem de Animais Silvestres (CETAS) and at the Centro de Recuperação de Animais Silvestres from the Universidade Estácio de Sá (CRAS) located in the State of Rio de Janeiro, southeast Brazil.

This study was approved by the Committee on Animal Research and Ethics (Sisbio, n. 47791) in Brazil.

Blood samples were collected with anticoagulant by venipuncture of the ventral tail vein (JURGELSKI, 1974). DNA was extracted from 200 µL of each EDTA-whole blood sample using the ReliaPrep™ Blood gDNA Miniprep System (Promega™, Madison, Wisconsin, United States) according to the manufacturer's instructions. Ultra-pure sterile water (Invitrogen™, Carlsbad, California, United States) was used as negative controls in each batch of samples to assess DNA contamination during extraction of total DNA.

The concentration of extracted DNA samples was determined using a spectrophotometer NanoDrop 2000 (Thermo Scientific™). DNA samples were divided into aliquots and stored at -80°C for subsequent molecular analysis.

Each sample of extracted DNA was used as a template in 25µL reaction mixtures containing 10x PCR buffer, 1.0 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphate (dNTPs) mixture, 1.5U Taq DNA Polymerase (Invitrogen, Carlsbad, California, USA) with 0.5 µM of genus and species-specific primers for *Ehrlichia canis*, *E. chaffeensis* (*16S* rRNA gene) (MURPHY et al., 1998), and *Anaplasma* spp. (*16S* rRNA gene) (MASSUNG et al., 1998). For further molecular characterization, positive samples were subjected to PCR assays targeting the *omp1* for *Ehrlichia* spp. (INAYOSHI et al., 2004) and the *dsb* for *E. canis* (DOYLE et al., 2005) (Table 1).

A DNA sample used as a positive control was obtained from dogs experimentally infected with the Jaboticabal strain of *E. canis* (CASTRO et al., 2004). A DNA sample used as a positive control was obtained from a dog naturally infected with *A. platys* from the

Table 1. Description of primers, PCR product size and references used in PCR assays for *E. canis, E. chaffeensis* and *Anaplasma* spp. based on *16S rRNA*, *omp* and *dsb* genes.

Agent and Primers	Oligonucleotide Sequence	PCR Size (bp)	References
Ehrlichia spp. *(16S rRNA* gene)			
-ECC	5'- GAACGAACGCTGGCGGCAAGC-3'	478	Murphy et al. (1998)
-ECB	5'- CGTATTACCGCGGCTGCTGGCA -3'		
Nested *E. canis (16S rRNA* gene)			
-ECAN-5	5'-CAATTATTTATAGCCTCTGGCTATAGGA -3'	358	
-HE3	5'-TATAGGTACCGTCATTATCTTCCCTAT-3'		
Nested *E. chaffeensis (16S rRNA* gene)			
-CHAFF	5'- CAA TTGCTTATAACCTGGTTATAAAT-3'	410	Kocan et al. (2000)
-GAIUR	5'-GACTTTGCCGGGACTTCTTCT-3'		
Anaplasma spp. *(16S rRNA* gene)			
-gE3a	5'- CACATGCAAGTCGAACGGATTATTC-3'	932	Massung et al. (1998)
-gE10R	5'- TTCCGTTAAGAAGGATCTAATCTCC´-3'		
Nested *Anaplasma* spp. *(16S rRNA* gene)			
-gE2	5'- GGCAGTATTAAAAGCAGCTCCAGG-3'	546	
-gE9f	5'-AACGGATTATTCTTTATAGCTTGCT-3'		
Ehrlichia spp. *(omp-1* gene)			
conP28-F1	5'-AT(C/T)AGTG(G/C)AAA(A/G)TA(T/C)(A/G)T(G/A)CCAA-3'	713	Inayoshi et al. (2004)
conP28-R1	5'-TTA(G/A)AA(A/G)G(C/T)AAA(C/T)CT(T/G)CCTCC-3'		
Nested *Ehrlichia* spp. *(omp-1* gene)			
conP28-F2	5'-CAATGG(A/G)(T/A)GG(T/C)CC(A/C)AGA(AG)TAG-3'	300	
conP28-R2	5'-TTCC(T/C)TG(A/G)TA(A/G)G(A/C)AA(T/G)TTTAGG-3'		
Ehrlichia spp. (*dsb* gene)			
dsb-330	5'-GATGATGTCTGAAGATATGAAACAAAT-3'	409	Doyle et al. (2005)
dsb-728	5'-CTGCTCGTCTATTTTACTTCTTAAAGT-3'		

city of Campo Grande, State of Mato Grosso do Sul, Central-West Brazil, (DAGNONE et al., 2009). A DNA positive control for *Ehrlichia chaffeensis* was kindly provided by Prof. Dr. John Stephen Dumler (University of Maryland, Baltimore, MD, USA). Ultra-pure sterile water was used as a negative control.

In order to avoid PCR contamination, DNA extraction, reaction setup, PCR amplification, and electrophoresis were all performed in separate rooms.

The reaction products were purified using a Silica Bead DNA Gel Extraction Kit (Fermentas®, São Paulo, SP, Brazil). Purified amplified DNA fragments were submitted for sequencing in an automatic sequencer (ABI Prism 310 Genetic Analyser – Applied Biosystem/ Perkin Elmer) at the Centro de Recursos Biológicos e Biologia Genômica (FCAV/ UNESP, Jaboticabal, SP, Brazil) and used for subsequent phylogenetic analysis. Consensus sequences were obtained through the analysis of the sense and antisense sequences using the CAP3 program (HUANG & MADAN, 1999).

We compared our sequences with those deposited in GenBank using the basic local alignment search tool (BLAST). The sequences were aligned with sequences published in GenBank using Clustal/W (THOMPSON et al., 1994) and manually adjusted in Bioedit v. 7.0.5.3 (HALL, 1999). Phylogenetic inference based on maximum likelihood criterion (ML) was inferred with RAxML-HPC BlackBox 7.6.3 (STAMATAKIS et al., 2008) through the CIPRES Science Gateway.

Akaike information criterion was used with the software JModelTest on CIPRES Science Gateway in order to identify the most appropriate model of nucleotide substitution. The GTRGAMMA+I model was chosen as the most appropriate for the Maximum Likelihood analysis of the 16S rDNA alignment.

One (2.7%) out of 37 marsupials was positive for both *E. canis* and *E. chaffeensis* by nested PCR assays based on the 16S rRNA gene. The sequence was 100% identical to *Ehrlichia canis* (GenBank access number KR920044.1) by BLAST analysis.

All the blood samples tested in this survey were negative for *Anaplasma* spp. by PCR assays based on the 16S rRNA.

The only sample that was positive for *Ehrlichia* spp. by 16S rRNA-based PCR was negative in the PCR assays based on the dsb and omp-1 genes which precluded additional molecular characterization.

In the phylogenetic analysis based on the 16S rRNA gene using the maximum likelihood method and GTRGAMMA+I evolutionary model, the *Ehrlichia* sp. genotypes detected in opossums (*Didelphis* sp.) was closely related to *E. canis* and to genotypes related to *E. canis* previously been detected in dogs from Peru and Brazil (Figure 1).

The positive animal was a young female *Didelphis aurita* which weighed 363g and was captured in Vargem Grande neighborhood, Rio de Janeiro, RJ, Southeast Brazil, on March 21, 2014. This animal was transported to CRAS for rehabilitation and subsequent reintroduction into her original habitat. At the time of blood sampling on March 31, 2014, this marsupial was not infested by ticks. During treatment, the animal was kept in a cage with water provided *ad libitum* and was fed with fruits. Treatment and management were performed by a practitioner at CRAS.

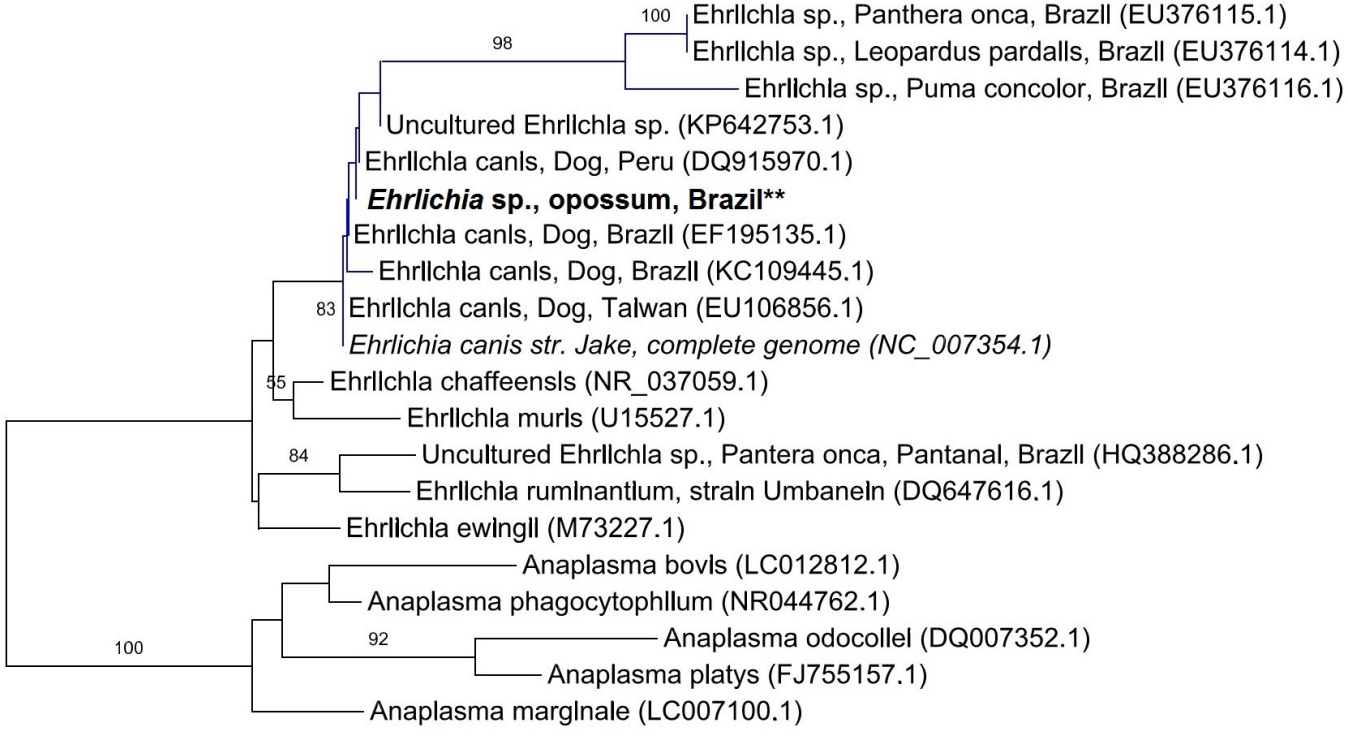

Figure 1. Phylogenetic position of *Ehrlichia* sp. isolated from a Brazilian opossum based on 16S rRNA DNA sequences (300 bp). The tree was constructed using the neighbor-joining method, and the numbers on the tree indicate bootstrap values for the branch points. Accession numbers are indicated.

Although opossums are infested with different species of ticks and can often act as amplifier hosts of a number of pathogens, there are few published studies about the detection of *Ehrlichia* spp. in these marsupials (HORTA et al., 2009).

In Brazil, antibodies against *Ehrlichia* spp. have been detected in opossums from the State of São Paulo. Authors have suggested the possibility that a unclassified *Ehrlichia* yet to be reported circulates among these animals as cross-reactivity occurs in serological assays (MELO et al., 2016; HARRUS & WANER, 2011). Recently, Sousa et al. (2017) detected *Ehrlichia* DNA in 23.3% of marsupials in the Pantanal, central-west region of the country.

Our results reinforces such findings from previous studies published elsewhere, and also highlight the fact that opossums may play a role in the epidemiology of *Ehrlichia* infection. In the present survey, the *Ehrlichia* sp. 16S rRNA sequence that was found in one female opossum belongs to *Ehrlichia* genotype closely related to *E. canis*. Unfortunately, we did not test the extracted DNA samples to a housekeeping gene. Therefore, we could not rule out the occurrence of false negative results, precluding any assumption on the prevalence of this agent in the studied population. Future studied should be conducted in order to investigate the prevalence of this putative novel genotype in opossum population from different Brazilian regions.

Among wild animals, genotypes closely related to *E. canis* have been reported in wild carnivores (ANDRÉ et al., 2010, 2012), wild birds from Brazil (MACHADO et al., 2012), Orinoco geese (*Neochen jubata*) (WERTHER et al., 2017), rodents (BENEVENUTE et al., 2017; BRAGA et al., 2018), and wild mammals (SOUSA et al., 2017) from Brazil.

Although further molecular characterization based on other genes is desirable to better assess the phylogeny of *Ehrlichia* sp. that occurs in marsupials in Brazil, the amount of DNA from animals of this study was insufficient for additional testing. In addition, attempts to make additional phylogenetic inferences based on genes other than 16S were unsuccessful. Our failed attempts corroborate the results published by Benevenute et al. (2017).

The identity at the species level of the genotypes of *Ehrlichia* that circulate in wild animals in Brazil needs to be further investigated. A larger number of marsupials and better molecular and antigenic characterization are needed in order to better assess the role of these mammals in the epidemiology of ehrlichiosis in South America.

Although the opossums tested in the present study were not infested by ectoparasites at the time of blood collection, there is a possibility that ectoparasites may have left the animal during the 10 day period of captivity.

Larvae, nymphs, and adults of *Amblyomma* spp. and *Ixodes loricatus* ticks have been reported in opossums from the State of São Paulo, Brazil (HORTA et al., 2007).

Besides, *I. loricatus* and *A. aureolatum* have been found in *Didelphis albiventris* in the Brazilian States of Mato Grosso do Sul and Rio Grande do Sul, respectively (MIZIARA et al., 2008; MULLER et al., 2005).

In conclusion, a *Ehrlichia* sp. genotype closely related to *E. canis* circulates in opossums in the state of Rio de Janeiro, southeast Brazil.

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References

André MR, Adania CH, Machado RZ, Allegretti SM, Felippe PAN, Silva KF, et al. Molecular and Serologic Detection of *Ehrlichia* spp. in Endangered Brazilian Wild Captive Felids. *J Wildl Dis* 2010; 46(3): 1017-1023. http://dx.doi.org/10.7589/0090-3558-46.3.1017. PMid:20688716.

André MR, Dumler JS, Scorpio DG, Teixeira RHF, Allegretti SM, Machado RZ. Molecular detection of tick-borne bacterial agents in Brazilian and exotic captive carnivores. *Ticks Tick Borne Dis* 2012; 3(4): 247-253. http://dx.doi.org/10.1016/j.ttbdis.2012.04.002. PMid:22749737.

Benevenute JL, Dumler JS, Ogrzewalska M, Roque ALR, Mello VVC, Sousa KCM, et al. Assessment of a quantitative 5′ nuclease real-time polymerase chain reaction using groEL gene for *Ehrlichia* and *Anaplasma* species in rodents in Brazil. *Ticks Tick Borne Dis* 2017; 8(4): 646-656. http://dx.doi.org/10.1016/j.ttbdis.2017.04.011. PMid:28457822.

Braga MD, Pereira JG, Fernandes SJ, Marques ICL, Jesus RP, Ferreira GS, et al. Molecular detection of Anaplasmataceae agents in *Dasyprocta azarae* in northeastern Brazil. *Rev Bras Parasitol Vet* 2018; 27(1): 98-104. http://dx.doi.org/10.1590/s1984-29612017071. PMid:29641788.

Castellaw AH, Chenney EF, Varela-Stokes AS. Tick-borne disease agents in various wildlife from Mississippi. *Vector Borne Zoonotic Dis* 2011; 11(4): 439-442. http://dx.doi.org/10.1089/vbz.2009.0221. PMid:20846016.

Castro MB, Machado RZ, De Aquino LP, Alessi AC, Costa MT. Experimental acute canine monocytic ehrlichiosis: clinicopathological and immunopathological findings. *Vet Parasitol* 2004; 119(1): 73-86. http://dx.doi.org/10.1016/j.vetpar.2003.10.012. PMid:15036578.

Dagnone AS, Souza AI, André MR, Machado RZ. Molecular diagnosis of Anaplasmataceae organisms in dogs with clinical and microscopical signs of ehrlichiosis. *Rev Bras Parasitol Vet* 2009; 18(4): 20-25. http://dx.doi.org/10.4322/rbpv.01804004. PMid:20040204.

Doyle CK, Labruna MB, Breitschwerdt EB, Tang YW, 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.

Dumler JS, Barbet AF, Bekker CP, Dasch GA, Palmer GH, Ray SC, et al. Reorganization of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order *Rickettsiales*: unification of some species of *Ehrlichia* with *Anaplasma, Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila. Int J Syst Evol Microbiol* 2001; 51(6): 2145-2165. http://dx.doi.org/10.1099/00207713-51-6-2145. PMid:11760958.

Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 1999; 41: 95-98.

Harrus S, Baneth G. Drivers for the emergence and re-emergence of vector-borne protozoal and bacterial diseases. *Int J Parasitol* 2005; 35(11-12): 1309-1318. http://dx.doi.org/10.1016/j.ijpara.2005.06.005. PMid:16126213.

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.

Horta MC, Moraes-Filho J, Casagrande RA, Saito TB, Rosa SC, Ogrzewalska M, et al. Experimental infection of opossums *Didelphis aurita* by *Rickettsia rickettsii* and evaluation of the transmission of the infection to ticks *Amblyomma cajennense. Vector Borne Zoonotic Dis* 2009; 9(1): 109-118. http://dx.doi.org/10.1089/vbz.2008.0114. PMid:18945194.

Huang X, Madan A. CAP3: a dna sequence assembly program. *Genome Res* 1999; 9(9): 868-877. http://dx.doi.org/10.1101/gr.9.9.868. PMid:10508846.

Inayoshi M, Naitou H, Kawamori F, Masuzawa T, Ohashi N. Characterization of *Ehrlichia* species from *Ixodes ovatus* ticks at the Foot of Mt.Fuji, Japan. *Microbiol Immunol* 2004; 48(10): 737-745. http://dx.doi.org/10.1111/j.1348-0421.2004.tb03599.x. PMid:15502406.

Jurgelski W Jr. The opossum (Didelphis virginiana Kerr) as a biomedical model. I. Research perspective, husbandry and laboratory technics. *Lab Anim Sci* 1974; 24(2): 376-403. PMid:4362893.

Kocan AA, Levesque GC, Whitworth LC, Murphy GL, Ewing SA, Barker RW. Naturally occurring *Ehrlichia chaffeensis* infection in coyotes from Oklahoma. *Emerg Infect Dis* 2000; 6(5): 477-480. http://dx.doi.org/10.3201/eid0605.000505. PMid:10998377.

Lockhart JM, Davidson WR, Stallknecht DE, Dawson JE, Little SE. Natural History of *Ehrlichia chaffeensis* (Rickettsiales: Ehrlichieae) in the Piedmont Physiographic Province of Georgia. *J Parasitol* 1997; 83(5): 887-894. http://dx.doi.org/10.2307/3284284. PMid:9379294.

Machado RZ, André MR, Werther K, Souza E, Gavioli FA, Alves JRF Jr. Migratory and Carnivorous Birds in Brazil: reservoirs for *Anaplasma* and *Ehrlichia* Species? *Vector Borne Zoonotic Dis* 2012; 12(8): 705-708. http://dx.doi.org/10.1089/vbz.2011.0803. PMid:22607070.

Massung RF, Slater K, Owens JH, Nicholson WL, Mather TN, Solberg VB, et al. Nested PCR assay for detection of granulocytic ehrlichiae. *J Clin Microbiol* 1998; 36(4): 1090-1095. PMid:9542943.

Melo ALT, Aguiar DM, Spolidorio MG, Yoshinari NH, Matushima ER, Labruna MB, et al. Serological evidence of exposure to tick-borne agents in opossums (*Didelphis* spp.) in the state of São Paulo, Brazil. *Rev Bras Parasitol Vet* 2016; 25(3): 348-352. http://dx.doi.org/10.1590/S1984-29612016028. PMid:27276663.

Miziara SR, Paiva F, Andreotti R, Koller WW, Lopes VA, Pontes NT, et al. Ocorrência de *Ixodes loricatus* Neumann, 1899 (Acari: Ixodidae) parasitando *Didelphis albiventris* (Lund, 1841), (Didelphimorphia: Didelphidae), em Campo Grande, MS. *Rev Bras Parasitol Vet* 2008; 17(3): 158-160. http://dx.doi.org/10.1590/S1984-29612008000300008. PMid:19245763.

Muller G, Brum JGW, Langone PQ, Michels GH, Sinkoc AL, Ruas JL, et al. *Didelphis albiventris* Lund, 1841, parasitado por *Ixodes loricatus* Neumann, 1899, e *Amblyomma aureolatum* (Pallas, 1772) (Acari: Ixodidae) no Rio Grande do Sul. *Arq Inst Biol* 2005; 72(3): 319-324.

Murphy GL, Ewing SA, Whitworth LC, Fox JC, Kocan AA. A molecular and serologic survey of *Ehrlichia canis, E. chaffeensis* and *E. ewingii* in dogs and ticks from Oklahoma. *Vet Parasitol* 1998; 79(4): 325-339. http://dx.doi.org/10.1016/S0304-4017(98)00179-4. PMid:9831955.

Sacchi ABV, Duarte JMB, André MR, Machado RZ. Prevalence and molecular characterization of Anaplasmataceae agents in free-ranging Brazilian marsh deer (*Blastocerus dichotomus*). *Comp Immunol Microbiol Infect Dis* 2012; 35(4): 325-334. http://dx.doi.org/10.1016/j.cimid.2012.02.001. PMid:22381686.

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.

Sousa KCM, Calchi AC, Herrera HM, Dumler JS, Barros-Battesti DM, Machado RZ, et al. Anaplasmataceae agents among wild mammals and ectoparasites in Brazil. *Epidemiol Infect* 2017; 145(16): 3424-3437. http://dx.doi.org/10.1017/S095026881700245X. PMid:29103397.

Stamatakis A, Hoover P, Rougemont J. A rapid bootstrap algorithm for the RAxML web servers. *Syst Biol* 2008; 57(5): 758-771. http://dx.doi.org/10.1080/10635150802429642. PMid:18853362.

Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994; 22(22): 4673-4680. http://dx.doi.org/10.1093/nar/22.22.4673. PMid:7984417.

Werther K, Luzzi MC, Gonçalves LR, de Oliveira JP, Alves JRF Jr, Machado RZ, et al. Arthropod-borne agents in wild Orinoco geese (*Neochen jubata*) in Brazil. *Comp Immunol Microbiol Infect Dis* 2017; 55: 30-41. http://dx.doi.org/10.1016/j.cimid.2017.09.003. PMid:29127991.

Widmer CE, Azevedo FC, Almeida AP, Ferreira F, Labruna MB. Tick-borne bacteria in free-living jaguars (*Panthera onca*) in Pantanal, Brazil. *Vector Borne Zoonotic Dis* 2011; 11(8): 1001-1005. http://dx.doi.org/10.1089/vbz.2011.0619. PMid:21612532.