

Communication

Page 1 a 7

[Comunicação]

A putative novel *Ehrlichia* genotype in a cat from Rio de Janeiro, Brazil[Provável novo genótipo de *Ehrlichia* em um gato do Rio de Janeiro, Brasil]A. Guimarães¹ , J.M. Raimundo¹ , H.J.M. Souza¹ , M.R. André² , C.D. Baldani¹ ,¹Instituto de Medicina Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brasil²Universidade Estadual Paulista (Unesp), Jaboticabal, São Paulo, Brasil

Ehrlichiosis and anaplasmosis are tick-borne diseases caused by Gram-negative obligatory intracellular α -proteobacteria belonging to Anaplasmataceae family (order Rickettsiales) with a tropism for hematological cells, such as monocytes and macrophages. Even though it has been assumed that feline ehrlichiosis is a tick-borne disease, it is not known for sure which are the vectors in Brazil. Transmission routes of natural infections are likely to hinder participation of *Rhipicephalus sanguineus* sensu lato, following what has been proved for in canine ehrlichiosis (Little, 2010; Dryden and Payne, 2004). Indeed, cats residing in homes with established populations of *R. sanguineus* (s.l.) may be at higher risk of infection (Little, 2010).

Occurrence of feline ehrlichiosis in the world varies widely according to the region studied and the technique used for diagnosis. In Brazil, based on Immunofluorescence Antibody Test (IFAT) for *Ehrlichia* spp., seropositive rates ranging from 0 to 41.5% in cats have been reported (Braga *et al.*, 2012, 2013; Guimarães *et al.*, 2019a), while by Polymerase Chain Reaction (PCR), the infection rate usually is less than 10% when apparently healthy populations are evaluated (Braga *et al.*, 2012, 2013; Guimarães *et al.*, 2019a; André *et al.*, 2015, 2022). *Ehrlichia canis* has been the main Anaplasmataceae agent in cats from Brazil (Braga *et al.*, 2012, 2017; Guimarães *et al.*, 2019a; André *et al.*, 2015). Interestingly, a 16S rRNA genotype closely related to *E. chaffeensis* was identified in cat from São Luís, state of Maranhão, northeastern Brazil (Braga *et al.*, 2012), demonstrating that

there is still much to be known about these pathogens in the feline population. Hematological alterations usually described in feline ehrlichiosis are anemia, thrombocytopenia, lymphopenia, monocytosis, leukocytosis, left shift neutrophil and hyperproteinemia, which have also been observed in *E. canis* PCR positive cats (Braga *et al.*, 2013; Guimarães *et al.*, 2019a).

On the other hand, there are even fewer studies regarding feline anaplasmosis in cats from Brazil. *Anaplasma* sp. closely related to *Anaplasma phagocytophilum* was detected in blood samples from cats from the states of São Paulo, Rio Grande do Norte, and Santa Catarina (André *et al.*, 2014, 2017). Additionally, *Anaplasma platys* infection rates in cats seems to be low in cats from Brazil (Guimarães *et al.*, 2019b; André *et al.*, 2022). In cats, although *A. platys* might not be pathogenic and not cause any clinical disorders other than thrombocytopenia, further studies will be required to determine if disease associations exist with this agent in cats (Zobba *et al.*, 2015; Lappin, 2018). Recently, a 16S rRNA genotype closely related to ‘*Candidatus Anaplasma amazonensis*’, a putative novel *Anaplasma* genotype previously detected in sloths from Amazon (Calchi *et al.*, 2020), was detected in cats from Minas Gerais state, southeastern Brazil (André *et al.*, 2022).

The aim of the present study was to report a putative novel *Ehrlichia* genotype in a cat from Rio de Janeiro, southeastern Brazil, and to evaluate the hematologic changes associated with this infection.

The study was conducted at a feline specialized Veterinary Clinic, located in Rio de Janeiro city, southeastern region of Brazil. In total, 178 cats were randomly selected, between the years of 2014 and 2015. Healthy cats (from routine examination or surgery for elective castration) and sick cats were included in this study, without discrimination of age, sex, or breed.

At time of blood collection, information related to sampled animals (gender, breed, and age) was recorded, aiming to evaluate possible associations with positivity in PCR tests. From each animal, blood samples were collected in tubes with ethylenediaminetetraacetic acid (EDTA) anticoagulant through cephalic or jugular venipuncture. EDTA-blood samples were used in hematological analyses using Poch-100iv (Roche, USA) and Diff Quick (Laborclin) stained-blood. Aliquots of blood sample were stored at -80°C for use in molecular assays.

DNA was extracted from 200 µL of EDTA-whole blood samples using ReliaPrep™ Blood gDNA Miniprep System (Promega™, Madison, Wisconsin, United States) according to manufacturer's instructions. Ultra-pure sterile water (Invitrogen™, Carlsbad, California, United States) was used as negative control in each battery samples for monitoring contaminant DNA during total DNA extraction process. Concentration of extracted DNA samples was determined by NanoDrop 2000 (Thermo Scientific™) spectrophotometer. DNA samples were separated into aliquots and stored at -80°C until the time of molecular assays.

Each extracted DNA sample was used as a template in a 25 µL nested PCR reaction mixture containing 10X PCR buffer, 1.0 mM MgCl₂, 0.2 mM deoxynucleotide triphosphate (dNTPs) mixture, 1.5 U Taq DNA Polymerase (Invitrogen, Carlsbad, California, USA), and 0,5 µM of *Ehrlichia* spp. genus primers ECC and ECB in first reaction, and ECAN-5 and HE-3 in second reaction (Murphy *et al.*, 1998) for *E. canis*, based on sequence of the 16S rRNA gene. *Ehrlichia canis* positive control DNA sample was obtained from an experimentally infected dog with Jaboticabal strain-*E. canis* (Castro *et al.*, 2004). To detect *E. chaffeensis* based on sequence of the 16S rRNA gene, reactions with ECC and ECB primers were performed as in previous test. The second reaction used primers

GAIUR and CHAFF (Persing, 1996; Kocan *et al.*, 2000). *Ehrlichia chaffeensis* positive control DNA sample was obtained from Arkansas strain.

The presence of *Anaplasma* DNA was investigated by a nested PCR targeting part of the 16S rRNA gene when first reaction was with gE3a and gE10R primers and the second with gE2 and gE9F (Massung *et al.*, 1998). Positive control samples of *E. chaffeensis* and *Anaplasma phagocytophilum* were kindly donated by Prof. Dr. John Stephen Dumler (Uniformed Services University of the Health Sciences, Bethesda, MD). To prevent PCR contamination, DNA extraction, reaction setup, PCR amplification and electrophoresis were performed in separated rooms. All reaction products were purified using Wizard™ SV Gel and PCR Clean-Up System (Promega™, Madison, Wisconsin, United States). Purified amplified DNA fragments were submitted for sequence confirmation in an automatic sequencer (ABI Prism 310 Genetic Analyser Applied Biosystem/ Perkin Elmer) and used for subsequent phylogenetic analysis.

The sequence was aligned with those published in GenBank using Clustal/W (Thompson *et al.*, 1994) and manually adjusted in Bioedit v. 7.0.5.3 (Hall, 1999). Phylogenetic reconstruction was performed using the Neighbor-Joining method. Nucleotide substitution models were selected based on Akaike information criterion (AIC) in Mega X software (Kumar *et al.*, 2018). The Kimura 2-parameter model was used to calculate evolutionary distances. The combination of phylogenetic clusters was assessed using a bootstrap test with 1000 replicates to test different phylogenetic reconstructions. Phylogenetic valuation was conducted using the Mega X software (Kumar *et al.*, 2018).

There was no positive animal for *Anaplasma* spp. or for *Ehrlichia canis* in the population studied. Only one (0,56%) cat was positive in the nested PCR assay for *E. chaffeensis* based on the 16S rRNA. The animal was a male, neutered, undefined breed, 16 years old, resident in Ramos neighborhood, Rio de Janeiro city. Fleas were observed at sampling time, but no ticks. During clinical examination it presented a heart rate of 200 beats per minute, pulmonary auscultation without changes, body temperature of 38.8 C and reduced skin turgor. The owner reported anorexia and vomiting. The animal had a

A putative novel...

previous history of Feline viral respiratory complex. Hemogram showed no alterations, with all parameters within the reference limits.

Amplified product (a 410bp fragment of 16S rRNA gene) was purified and submitted to sequencing. The partial sequence showed 99-100% of identity to isolates of *Ehrlichia chaffeensis* by BLAST analysis (GenBank numbers NR074500.1, MN368552.1, MK611628., KY644147.1, CP007479.1, CP007478., CP007477.1) A minimum identity of

97%, coverage of 98% and e-value of $1e^{-100}$ was considered to define the *Ehrlichia* species (Schloss and Handelsman, 2005).

The resulting phylogenetic tree grouped *Ehrlichia* sp. of the cat from Rio de Janeiro, Brazil, with other sequences for Anaplasmataceae agents from various regions of the world (Fig. 1). The sequence obtained in the present study was published in GenBank with the accession number ON239604.

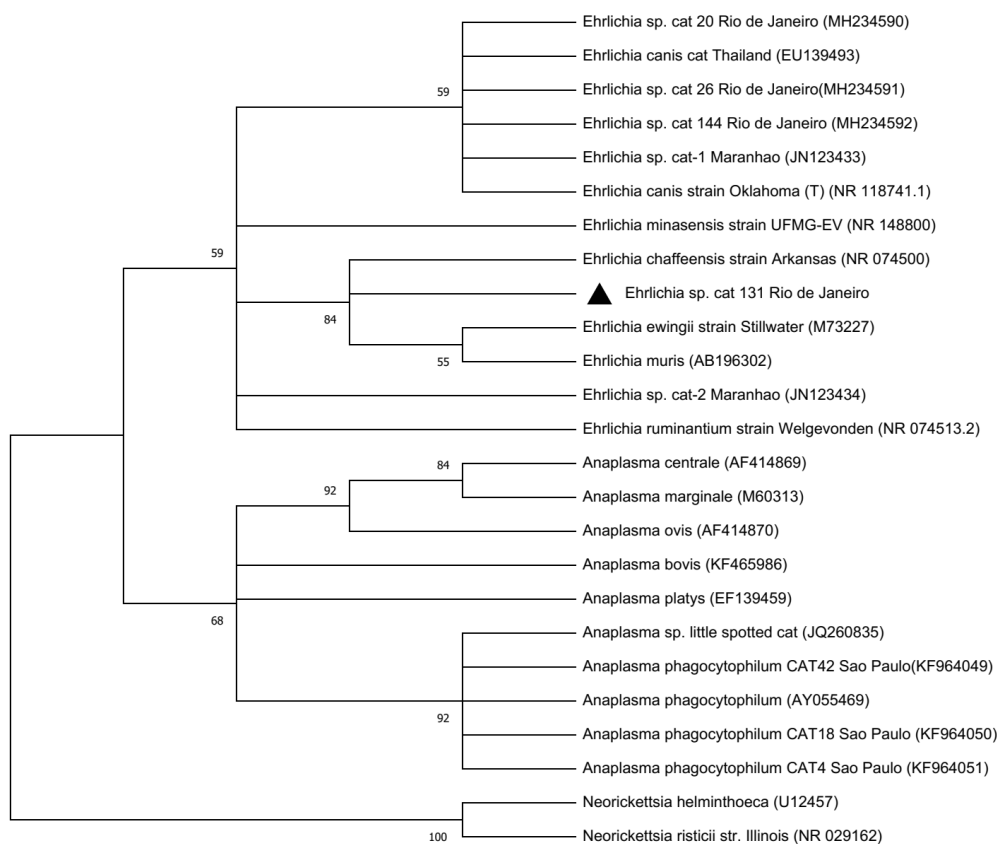


Figure 1. Phylogenetic position of *Ehrlichia* sp. isolate from Brazilian domestic cat from Rio de Janeiro, Brazil, based on 16S rRNA sequences (300 bp). The tree was constructed using the neighbor-joining method and the numbers on the tree indicate bootstrap values for the branch points. Accession numbers and place of origin of the isolates are shown beside the sequence names.

In Brazil, antibodies anti-*Ehrlichia chaffeensis* were detected in serum samples from dogs and humans in Minas Gerais state (Costa *et al.*, 2006), emphasizing the importance of future studies regarding the real occurrence of this agent as a causative agent of zoonotic disease.

Indeed, 16S rRNA and *dsb* genotypes closely related to *E. chaffeensis* were also detected Brazilian marsh deer (*Blastocerus dichotomus*) (Machado *et al.*, 2006; Sacchi *et al.*, 2012), birds (Machado *et al.*, 2012; Werther *et al.*, 2017) and in a cat from São Luis, Maranhão state (Braga *et*

al., 2012). Recently, a study provided the first report of anti-*E. canis*-specific antibodies in domestic cats and the possible existence of a new *E. canis* genotype infecting felines (Braga *et al.*, 2021), reinforcing the existence of different *Ehrlichia* spp. genotypes in the feline population. The present study consists in the first molecular detection of a genotype closely related to *E. chaffeensis* in cats from Rio de Janeiro state, Brazil, and brings rise to further studies considering zoonotic agents transmitted by cats.

There are few and recent studies investigating *Ehrlichia* in felines, especially in Brazil. The first molecular detection of *E. canis* was in 2009, in the state of Minas Gerais in 3 of 15 cats studied (Oliveira *et al.*, 2009). In 2010, André and collaborators observed *Ehrlichia* DNA in 11 wild felines from zoos of São Paulo and Brasília. Few studies have been carried out on feline ehrlichiosis in the last decade (Braga *et al.*, 2012, 2013, 2017; Fontalvo *et al.*, 2016; Malheiros *et al.*, 2016; André *et al.*, 2015, 2017, 2022; Pedrassani *et al.*, 2019; Guimarães *et al.*, 2019a) with most studies in the Southeast region, few in the Midwest and Northeast, one in the South and one in North of the country. Thus, there is much to be understood about the distribution and pathogenesis of these agents in our country, which has continental proportions.

Clinical signs and laboratory determinations of felines infected with *Ehrlichia* sp. are variable and include fever, apathy, anorexia, mucosal pallor, lymphadenomegaly, splenomegaly, anemia, thrombocytopenia, leukopenia, increased serum aminotransferase activity and alkaline phosphatase and hyperglobulinemia (Almosny *et al.*, 1998; Almosny and Massard, 1999; Stubbs *et al.*, 2000). Hematological alterations such as low erythrocyte count, thrombocytopenia, lymphopenia and monocytosis were observed in *E. canis* PCR positive cats (Braga *et al.*, 2013). However, the animal studied did not show hematological alterations. At sampling time, clinical changes observed were dehydration and apathy. The animal's history of vomiting, anorexia, Feline viral respiratory complex, and advanced age may have contributed to immunosuppression, resulting in increased parasitemia and a diagnosis of hemoparasitosis.

The positive cat was a male, elderly, neutered, undefined breed with outdoor access. Previous

studies have shown no correlation between age and sex, suggesting that infection in cats does not have well established associated factors (Braga *et al.*, 2013). But it is possible that outdoor access facilitates contact with the vector.

The vector of canine ehrlichiosis is *Rhipicephalus sanguineus* sensu lato and is possibly also related to the transmission of feline ehrlichiosis. In addition, it has been proven experimentally that *Dermacentor* sp. can also transmit *E. canis* and readily feed on cats (Johnson *et al.*, 1998; Little, 2010). Adult *Amblyomma americanum* also are commonly found on cats but reports of molecular evidence of infection with *E. chaffeensis* or *E. ewingii* in cats are lacking (Little, 2010). *Ixodes scapularis* readily feeds on cats as both a nymph and adult (Dryden and Payne, 2004). *Amblyomma americanum* is considered the primary vector of *E. chaffeensis* in the USA. However, based on molecular detections, it is possible that other *Amblyomma* species, and *Ixodes* and *Dermacentor* species may play at least a minor role in the transmission of *E. chaffeensis* (Yabsley, 2010). Thus, is still not known if the vector of feline ehrlichiosis and transmission routes on natural infection are uncertain, and it is probable that the exposure to arthropods, especially ticks, are the main causes of feline infection (Little, 2010). In the present study, the cat had fleas at sampling time, but no ticks and, unfortunately, fleas were not collected for molecular testing. A limitation of the present study is that the phylogeny was based on a very small fragment of the 16S rRNA, a conserved gene, which precluded additional phylogenetic inferences. It would be important to characterize the genotype by analyzing other DNA fragments, however, it is not feasible to conduct additional molecular tests, as the sample has undergone multiple analyses, exhausting the available DNA. Moreover, the animal was sampled in 2014, making it impossible to obtain new blood material.

Information related to geographical distribution, pathological findings and zoonotic potential of feline ehrlichiosis has expanded worldwide in recent years. Identification of species that affect domestic animals, especially domestic cats, is important for development of control measures for this disease, either in animals or in humans, due to close interaction between them.

Combination of diagnostic techniques in the identification should be employed to characterize infection by these agents.

A putative novel *Ehrlichia* genotype circulates in cat population of Rio de Janeiro. Further studies

are needed to characterize vectors, transmission routes and pathogenesis in feline infection.

Keywords: vector-borne diseases, feline, Anaplasmataceae, ehrlichiosis, clinical, hematological changes

RESUMO

A ocorrência da erliquiose felina no mundo varia muito de acordo com a região estudada e a técnica utilizada para diagnóstico. O objetivo do presente estudo foi relatar um possível novo genótipo de *Ehrlichia* em um gato do Rio de Janeiro, Brasil. No total, 178 gatos foram selecionados aleatoriamente e amostras de sangue foram coletadas. O DNA foi extraído de amostras de sangue e utilizado em três reações de nested PCR baseadas na sequência do 16S rRNA para *E. canis*, *E. chaffeensis* e *Anaplasma* spp. Apenas um (0,56%) gato foi positivo na nested PCR para *E. chaffeensis*. O animal era um macho, castrado, sem raça definida, 16 anos, residente na cidade do Rio de Janeiro. Foram observadas pulgas no momento da amostragem, mas nenhum carrapato. O hemograma não apresentava alterações. O produto amplificado foi purificado e submetido a sequenciamento. A sequência parcial apresentou 100% de identidade com isolados de *Ehrlichia chaffeensis* pela análise BLAST. As informações relacionadas à distribuição geográfica, os achados patológicos e o potencial zoonótico da erliquiose felina expandiram-se mundialmente nos últimos anos. A identificação das espécies que afetam os animais domésticos, especialmente os gatos domésticos, é importante para o desenvolvimento de medidas de controle dessa doença, seja em animais, seja em humanos, devido à estreita interação entre eles. Um suposto novo genótipo de *Ehrlichia* circula na população de gatos do Rio de Janeiro. Mais estudos são necessários para caracterizar vetores, vias de transmissão e patogênese na infecção felina.

Palavras-chave: doenças transmitidas por vetores, felino, Anaplasmataceae, erliquiose, alterações clínicas, hematológicas

ACKNOWLEDGEMENTS

We thank cat owners for allowing the participation of their animals in the research.

REFERENCES

- ALMOSNY, N.R.P.; ALMEIDA, L.E.; MOREIRA, N.M.; MASSARD, C.L. Erliquiose clínica em gato (*Felis catus*). *Rev. Bras. Cienc. Vet.*, v.5, p.82-83, 1998.
- ALMOSNY, N.R.P.; MASSARD, C.L. Erliquiose felina: revisão. *Clín. Vet.*, v.4, p.30-32, 1999.
- ANDRÉ, M.R.; BACCARIM DENARDI, N.C.; SOUSA, K.C. *et al.* Arthropod-borne pathogens circulating in free-roaming domestic cats in a zoo environment in Brazil. *Ticks Tick Borne Dis.*, v.5, p.545-551, 2014.
- ANDRÉ, M.R.; CALCHI, A.C.; FURQUIM, M.E.C. *et al.* Molecular detection of Tick-Borne Agents in cats from Southeastern and Northern Brazil. *Pathogens*, v.11, p.106, 2022.
- ANDRÉ, M.R.; FILGUEIRA, K.D.; CALCHI, A.C. *et al.* Co-infection with arthropod-borne pathogens in domestic cats. *Rev. Bras. Parasitol. Vet.*, v.26, p.525-531, 2017.
- ANDRÉ, M.R.; HERRERA, H.M.; FERNANDES, S.J. *et al.* Tick-borne agents in domesticated and stray cats from the city of Campo Grande, state of Mato Grosso do Sul, midwestern Brazil. *Ticks Tick Borne Dis.*, v.6, p.779-786, 2015.
- BRAGA, I.A.; SANTOS, L.G.F.; MELO, A.L.T. *et al.* Hematological values associated to the serological and molecular diagnostic in cats suspected of *Ehrlichia canis* infection. *Rev. Bras. Parasitol. Vet.*, v.22, p.470-474, 2013.
- BRAGA, I.A.; TAQUES, I.I.G.G.; COSTA, J.S. *et al.* *Ehrlichia canis* DNA in domestic cats parasitized by *Rhipicephalus sanguineus* sensu lato (s.l.) ticks in Brazil - case report. *Braz. J. Vet. Res. Anim. Sci.*, v.54, p.412-415, 2017.

- BRAGA, Í.A.; TAQUES, I.I.G.G.; GRONTOSKI, E.C.; OLIVEIRA DIAS, I.S. *et al.* Exposure of domestic cats to distinct *Ehrlichia canis* TRP genotypes. *Vet. Sci.*, v.8, p.310, 2021.
- BRAGA, M.S.C.O.; ANDRÉ, M.R.; FRESCHI, C.R. *et al.* Molecular and serological detection of *Ehrlichia* spp. in cats on São Luís Island, Maranhão, Brazil. *Rev. Bras. Parasitol. Vet.*, v.21, p.37-41, 2012.
- CALCHI, A.C.; VULTAO, J.G.; ALVES, M.H. *et al.* *Ehrlichia* spp. and *Anaplasma* spp. in Xenarthra mammals from Brazil, with evidence of novel '*Candidatus Anaplasma* spp.'. *Sci. Rep.*, v.10, p.12615, 2020.
- CASTRO, M.B.; MACHADO, R.Z.; TOMAZ DE AQUINO, L.P.C. *et al.* Experimental acute canine monocytic ehrlichiosis: clinicopathological and immunopathological findings. *Vet. Parasitol.*, v.119, p.73-86, 2004.
- COSTA, P.S.G.; VALLE, L.M.C.; BRIGATTE, M.E.; GRECO, D.B. More about Human Monocytotropic Ehrlichiosis in Brazil: serological evidence of new nine cases. *Braz. J. Infect. Dis.*, v.10, p.7-10, 2006.
- DRYDEN, M.W.; PAYNE, P.A. Biology and control of ticks infesting dogs and cats in North America. *Vet. Ther.*, v.5, p.139-154, 2004.
- FONTALVO, M.C.; BRAGA, I.A., AGUIAR, D.M.; HORTA, M.C. Serological evidence of exposure to *Ehrlichia canis* in cats. *Ciênc. Anim. Bras.*, v.17, p.418-424, 2016.
- GUIMARÃES, A.; RAIMUNDO, J.M.; PEIXOTO, M.P. *et al.* Molecular detection, characterization of *Anaplasma* spp. in domestic cats from Rio de Janeiro state. *Acta Trop.*, v.191, p.239-242, 2019b.
- GUIMARÃES, A.; RAIMUNDO, J.M.; RODRIGUES, R.B. *et al.* *Ehrlichia* spp. infection in domestic cats from Rio de Janeiro State, southeast Brazil. *Rev. Bras. Parasitol. Vet.*, v.28, p.180-185, 2019a.
- HALL, T.A. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.*, v.41, p.95-98, 1999.
- JOHNSON, E.M.; EWING, S.A.; BARKER, R.W. *et al.* Experimental transmission of *Ehrlichia canis* (Rickettsiales: Ehrlichieae) by *Dermacentor variabilis* (Acari: Ixodidae). *Vet. Parasitol.*, v.74, p.277-288, 1998.
- KOCAN, A.A.; LEVESQUE, G.C.; WHITWORTH, L.C. *et al.* Naturally occurring *Ehrlichia chaffeensis* infection in coyotes from Oklahoma. *Emerg. Infect. Dis.*, v.6, p.477-480, 2000.
- KUMAR, S.; STECHER, G.; LI, M. *et al.* MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.*, v.35, p.1547-1549, 2018.
- LAPPIN, M.R., Update on flea and tick associated diseases of cats. *Vet. Parasitol.*, v.30, p.26-29, 2018.
- LITTLE, S.E. Ehrlichiosis and anaplasmosis in dogs and cats. *Vet. Clin. Small Anim.*, v.40, p.1121-1141, 2010.
- MACHADO, R.Z.; ANDRÉ, M.R.; WERTHER, K. *et al.* Migratory and carnivorous birds in Brazil: reservoirs for *Anaplasma* and *Ehrlichia* Species? *Vector Borne Zoonotic Dis.*, v.12, p.705-708, 2012.
- MACHADO, R.Z.; DUARTE, J.M.B.; DAGNONE, A.S.; SZABÓ, M.P.J. Detection of *Ehrlichia chaffeensis* in Brazilian marsh deer (*Blastocerus dichotomus*). *Vet. Parasitol.*, v.139, p.262-266, 2006.
- MALHEIROS, J.; COSTA, M.M.; AMARAL, R.B. *et al.* Identification of vector-borne pathogens in dogs and cats from Southern Brazil. *Ticks Tick Borne Dis.*, v.7, p.893-900, 2016.
- MASSUNG, R.F.; SLATER, K.; OWENS, J.H. *et al.* Nested PCR assay for detection of granulocytic ehrlichiae. *J. Clin. Microbiol.*, v.36, p.1090-1095, 1998.
- MURPHY, G.L.; EWING, S.A.; WHITWORTH, L.C. *et al.* A molecular and serologic survey of *Ehrlichia canis*, *E. chaffeensis* and *E. ewingii* in dogs and ticks from Oklahoma. *Vet. Parasitol.*, v.79, p.325-339, 1998.
- OLIVEIRA, L.S.; MOURÃO, L.C.; OLIVEIRA, K.A. *et al.* Molecular detection of *Ehrlichia canis* in cats in Brazil. *Clin. Microbiol. Infect.*, v.15, Supl.2, p.53-54, 2009.

- PEDRASSANI, D.; BIOLCHI, J.; GONÇALVES, L.R. *et al.* Molecular detection of vector-borne agents in cats in Southern Brazil. *Rev. Bras. Parasitol. Vet.*, v.28, p.632-643, 2019.
- PERSING, D.H. *PCR protocols for emerging infectious diseases*. A supplement to diagnostic molecular microbiology: principles and applications. Washington, D.C.: ASM Press, 1996. 180p.
- SACCHI, A.B.V.; DUARTE, J.M.B.; ANDRÉ, M.R.; MACHADO, R.Z. Prevalence and molecular characterization of Anaplasmatidae agents in free-ranging Brazilian marsh deer (*Blastocerus dichotomus*). *Comp. Immunol. Microbiol. Infect. Dis.*, v.35, p.325-334, 2012.
- SCHLOSS, P.D.; HANDELSMAN, J. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl. Environ. Microbiol.*, v.71, p.1501-1506, 2005.
- STUBBS, C.J.; HOLLAND, C.J.; REIF, J.S. *et al.* Feline ehrlichiosis. *Compend. Contin. Educ. Vet.*, Princeton, v.22, n.4, p.307-318, 2000.
- 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. *Nucl. Acids Res.*, v.22, p.4673-4680, 1994.
- WERTHER, K.; LUZZI, M.C.; GONÇALVES, L.R. *et al.* Arthropod-borne agents in wild Orinoco geese (*Neochen jubata*) in Brazil. *Comp. Immunol. Microbiol. Infect. Dis.*, v.55, p.30-41, 2017.
- YABSLEY, M.J. Natural history of *Ehrlichia chaffeensis*: vertebrate hosts and tick vectors from the United States and evidence for endemic transmission in other countries. *Vet. Parasitol.*, v.167, p.136-148, 2010.
- ZOBBA, R.; ANFOSSI, A.G.; VISCO, S. *et al.* Cell tropism and molecular epidemiology of *Anaplasma platys*-like strains in cats. *Ticks Tick Dis.*, v.6, p.272-280, 2015.