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FIRST REPORT OF *RANGELIA VITALII* IN A DOG FROM PARANÁ STATE, SOUTHERN BRAZIL

(Primeiro relato de infecção por Rangelia vitalii em um cão do Estado do Paraná, Sul do Brasil)

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RESUMO: A rangeliose, causada pelo piroplasma *Rangelia vitalii*, é uma doença conhecida por causar apatia, febre intermitente, sangramento de ponta de orelha e anemia. O presente trabalho relata a primeira detecção molecular de *R. vitalii* infectando um cão da região metropolitana de Curitiba, no Estado do Paraná. Um macho sem raça definida, de quatro anos de idade, apresentando apatia, perda de peso e sangramento de ponta de orelha foi encaminhado ao Hospital Veterinário da Universidade Federal do Paraná. O sequenciamento de uma amostra positiva para piroplasmas demonstrou uma semelhança de 99% com sequências parciais do gene 18S rDNA de *R. vitalii* depositadas no GenBank. Mais estudos são necessários para elucidar a patogenicidade, competência vetorial de carrapatos e epidemiologia desse protozoário no Estado do Paraná.

Palavras-chave: Rangeliose; piroplasmose; PCR.

ABSTRACT: Rangeliosis, caused by *Rangelia vitalii*, is known to causes apathy, intermittent fever, ear's margin bleeding and anemia. Herein, we report the first molecular detection of *R. vitalii* infecting a dog from the Curitiba metropolitan region, Paraná State, southern Brazil. A four-year-old male mixed-breed neighborhood dog, presenting apathy, weight loss, and ear's margin bleeding, was referred for clinical care at the Veterinary Teaching Hospital of the Universidade Federal do Paraná. Sequencing of the piroplasm positive sample showed ≥99% identity with *R. vitalii* 18S rDNA sequence deposited in GenBank. Further studies are needed to elucidate the pathogenicity, competence vector of ticks and epidemiology of this protozoa the State of Paraná.

Key Words: Rangeliosis; piroplasmosis; PCR.

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INTRODUCTION

Vector-borne diseases of humans and dogs are increasingly being recognized worldwide. Several emerging vector-borne pathogens have also been described and may represent a challenge in diagnosis, since clinical signs may be misinterpret (Dantas-Torres and Otranto, 2016). The piroplasm *Rangelia vitalii* is etiological agent of canine rangeliosis, and have been described infecting domestic and wild canids in South America (Eiras et al., 2014; Quadros et al., 2015; Soares et al., 2015, 2014), mainly in the South and Southeastern Brazilian states (Gottlieb et al., 2016; Lemos et al., 2017; Malheiros et al., 2016; Quadros et al., 2015; Silveira et al., 2016; Soares et al., 2014, 2011). Canine rangeliosis may cause a variety of clinical signs, such as apathy, intermittent fever, ear's margin bleeding and anemia (França et al., 2014), with an average lethality rate of 33,3% (Soares, 2014).

Amblyomma aureolatum has been implicated on the transmission of *R. vitalli* (Soares et al., 2011). Although, *Riphicephalus sanguineus* sensu lato ticks have been associated to *R. vitalii* infected dogs on both rural and suburban areas on Southern Brazil (França et al., 2014; Soares et al., 2011). A previous study has suggested that some *R. vitalii* strains are vector-specific in given geographic locations (Eiras et al., 2014), however this hypothesis should be further evaluated.

Canine piroplasmosis diagnosis is based on the epidemiology, clinical signs, and hematological and serum biochemical abnormalities (Eiras et al., 2014; Soares et al., 2011). Phylogenetic studies based on partial genes sequences (18S rRNA and 70 kDa heat shock protein) of *R. vitalii* have shown a close relationship between this protozoa and the *Babesia* sensu stricto clade (França et al., 2014; Soares et al., 2011). Thus, the definitive diagnosis is achieved by PCR-based assays (França et al., 2014). Herein, we report the first molecular detection of *R. vitalii* in a neighborhood dog from the Curitiba metropolitan region, Paraná State, southern Brazil.

CASE REPORT

A four-year-old male mixed-breed neighborhood dog, from the City of Campo Largo, Curitiba metropolitan region, was referred for clinical care at the Veterinary Teaching Hospital of the Universidade Federal do Paraná. Animal was presenting apathy, weight loss, and ear's margin bleeding, and despite ectoparasites have not been visualized during clinical examination, a canine vector-borne disease (CVBD) was suspected. Blood samples were collected by jugular venipuncture into EDTA tubes and stored at -4 °C until processing.

The packed cell volume (PCV) and total plasma protein (TPP) were measured by routine centrifugation and refractometry techniques, respectively as described elsewhere (Feldman et al., 2000); a PCV of 0.37-0.55 L/L and a TPP of 60-80 g/L were used as reference ranges (Feldman et al., 2000). The PCV concentration was 0.25 L/L and TPP concentration was 48 g/L, on the date of the first consultation.

The DNA was extracted from 200 uL blood using a commercial kit (Invitrogen, PureLink Genomic DNA Mini Kit), following the manufacturer's instructions. Negative control purifications using ultra-pure water were performed in parallel to monitor cross-contamination. A PCR for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed to ensure successful DNA extraction, as previously described

(Birkenheuer et al., 2003). Thereafter, the sample was screened by a conventional PCR using a primer set targeting piroplasms 18S rDNA gene as described elsewhere (Soares et al., 2011). The amplified PCR product (≈ 500bp) was directly purified (PureLink Quick Gel Extraction Kit, Invitrogen), evaluated by spectrophotometry for concentration and purity (NanoDrop® instrument, Thermo Scientific, Waltham, MA, EUA), and sequenced by Sanger method (ABI PRISM® 310 Genetic Analyser, Applied Biosystems, Foster City, CA, USA).

The assembled partial sequence of the 18S rDNA gene was compared with sequences deposited in GenBank database using the basic local alignment search tool (BLASTn) (Altschul et al., 1990). The piroplasm positive sample sequenced showed ≥99% identity with *R. vitalii* 18S rDNA sequence deposited in GenBank (accession nos. KU710789).

DISCUSSION

Herein, we described the first molecular detection of a natural infection by *R. vitalii* in a neighborhood dog from the Curitiba metropolitan region, Parana State, southern Brazil. Previous studies have shown a low prevalence of CVBD in neighborhoods dogs from Curitiba (Constantino et al., 2017). Conversely, *R. vitalli* have been detected infecting domestic and wild canids from the States of Rio Grande do Sul and Santa Catarina, southern Brazil (Gottlieb et al., 2016; Malheiros et al., 2016; Quadros et al., 2015). In the present study, besides ticks have not been visualized during sampling, *A. aureolatum* and *R. sanguineus* s.l. ticks, previously associated with *R. vitalii* transmission, were already found feeding on dogs from rural areas of Parana State (Arzua et al., 2005; Labruna et al., 2001). The neighborhood dog herein used to live close to rural areas of the City of Campo Largo. Rangeliosis is usually associated with dogs from rural areas or those that travel to those areas (França et al., 2014; Loretti and Barros, 2005), due to the possibility of infestation by *A. aureolatum* ticks (França et al., 2014) or proximity with wild canids, which are known hosts of *R. vitalii* (Loretti and Barros, 2005).

In the present study, dog was presenting weight loss, apathy, anemia, and ear's margin bleeding, suggesting rangeliosis (Eiras et al., 2014; França et al., 2014; Gottlieb et al., 2016). Hemorrhagic processes may leads to anemia and may be due to thrombocytopenia, which represent a drop in platelet count caused by decreased platelet production, aggregation or destruction (Paim et al., 2012). Anemia is a classic laboratory finding present in canine rangeliosis (Eiras et al., 2014; Fighera et al., 2010; França et al., 2014; Lemos et al., 2017; Silva et al., 2011). The hypoproteinemia observed herein may be explained by the anorexia, weight loss (Eiras et al., 2014; França et al., 2014; Lemos et al., 2017; Paim et al., 2012), and hypoalbuminemia (Fredo et al., 2017), commonly related with canine rangeliosis.

CONCLUSION

We report the first molecular evidence of *R. vitalii* infection in dogs from Paraná state. Further studies are needed to elucidate the pathogenicity, competence vector of ticks and epidemiology of this protozoa the State of Paraná.

REFERENCES

ALTSCHUL, S. F.; GISH, W.; MILLER, W.; MYERS, E. W.; LIPMAN, D. J. Basic local alignment search tool. **Journal of Molecular Biology**, v. 215, n. 3, p. 403–410, out. 1990. Available at:

<http://linkinghub.elsevier.com/retrieve/pii/S0022283605803602>. Access: dec. 7, 2017.

ARZUA, M.; ONOFRIO, V. C.; BARROS-BATTESTI, D. M. Catalogue of the tick collection (Acari, Ixodida) of the Museu de História Natural Capão da Imbuia, Curitiba, Paraná, Brazil. **Revista Brasileira de Zoologia**, v. 22, n. 3, p. 623–632, set. 2005. Available at: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0101-81752005000300015&lng=en&tlng=en>. Access: dec. 7, 2017.

BIRKENHEUER, A. J.; LEVY, M. G.; BREITSCHWERDT, E. B. Development and evaluation of a seminested PCR for detection and differentiation of Babesia gibsoni (Asian genotype) and B. canis DNA in canine blood samples. **Journal of clinical microbiology**, v. 41, n. 9, p. 4172–7, set. 2003. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/12958243>. Access: jun. 15, 2016.

CONSTANTINO, C.; DE PAULA, E. F. E.; BRANDÃO, A. P. D.; FERREIRA, F.; VIEIRA, R. F. da C.; BIONDO, A. W. Survey of spatial distribution of vector-borne disease in neighborhood dogs in southern Brazil. **Open veterinary journal**, v. 7, n. 1, p. 50–56, 2017. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/28331833>. Access: dec. 7, 2017.

DANTAS-TORRES, F.; OTRANTO, D. Best Practices for Preventing Vector-Borne Diseases in Dogs and Humans. **Trends in Parasitology**, v. 32, n. 1, p. 43–55, jan. 2016. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/26507152>. Access: dec. 7, 2017.

EIRAS, D. F.; CRAVIOTTO, M. B.; BANETH, G.; MORÉ, G. First report of Rangelia vitalii infection (canine rangeliosis) in Argentina. **Parasitology International**, v. 63, n. 5, p. 729–734, out. 2014. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/24970768>. Access: dec. 7, 2017.

FELDMAN, B. F.; ZINKL, J. G.; JAIN, N. C. **Schalm's veterinary hematology**. [s.l.] Lippincott Williams & Wilkins, 2000.

FIGHERA, R. A.; SOUZA, T. M.; KOMMERS, G. G.; IRIGOYEN, L. F.; BARROS, C. S. Patogênese e achados clínicos, hematológicos e anatomopatológicos da infecção por Rangelia vitalii em 35 cães (1985-2009). **Pesquisa Veterinária Brasileira**, v. 30, n. 11, p. 974–987, nov. 2010. Available at: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-736X2010001100012&lng=pt&tlng=pt>. Access: dec. 12, 2017.

FRANÇA, R. T.; DA SILVA, A. S.; LORETTI, A. P.; MAZZANTI, C. M.; LOPES, S. T. A. Canine rangeliosis due to Rangelia vitalii: From first report in Brazil in 1910 to current day – A review. **Ticks and Tick-borne Diseases**, v. 5, n. 5, p. 466–474, set. 2014. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/24950853>. Access: dec. 7, 2017.

FREDO, G.; LEITE-FILHO, R. V.; PIETZSCH, C. D. Á.; ANDRADE, C. P. de; DUDA, N. C. B.; NASCIMENTO, L. C. do; VALLE, S. de F.; SOARES, J. F.; SONNE, L.; FREDO, G.; LEITE-FILHO, R. V.; PIETZSCH, C. D. Á.; ANDRADE, C. P. de; DUDA, N. C. B.; NASCIMENTO, L. C. do; VALLE, S. de F.; SOARES, J. F.; SONNE, L. Rangeliosis: histopathological analysis, hematology and molecular detection of canine Rangelia vitalii in Rio Grande do Sul, Brazil. **Ciência Rural**, v. 47, n. 10, 17 ago. 2017. Available at: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-84782017001000501&lng=en&tlng=en>. Access: dec. 12, 2017.

GOTTLIEB, J.; ANDRÉ, M. R.; SOARES, J. F.; GONÇALVES, L. R.; OLIVEIRA, M. de; COSTA, M. M.; LABRUNA, M. B.; BORTOLINI, C. E.; MACHADO, R. Z.; VIEIRA, M. I. B. Rangelia vitalii, Babesia spp. and Ehrlichia spp. in dogs in Passo Fundo, state of Rio Grande do Sul, Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 25, n. 2, p. 172–178, jun. 2016. Available at: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1984-29612016000200172&lng=en&tlng=en>. Access: dec. 7, 2017.

LABRUNA, M. B.; SOUZA, S. L. P.; GUIMARÃES JR., J. S.; PACHECO, R. C.; PINTER, A.; GENNARI, S. M. Prevalência de carrapatos em cães de áreas rurais da região norte do Estado do Paraná. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 53, n. 5, p. 553–556, out. 2001. Available at: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-09352001000500007&lng=pt&tlng=pt>. Access: dec. 7, 2017.

LEMOS, T. D.; TOMA, H. K.; ASSAD, R. Q.; SILVA, A. V. da; CORRÊA, R. G. B.; ALMOSNY, N. R. P.; LEMOS, T. D.; TOMA, H. K.; ASSAD, R. Q.; SILVA, A. V. da; CORRÊA, R. G. B.; ALMOSNY, N. R. P. Clinical and hematological evaluation of Rangelia vitalii-naturally infected dogs in southeastern Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 26, n. 3, p. 307–313, 24 ago. 2017. Available at: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1984-29612017000300307&lng=en&tlng=en>. Access: dec. 7, 2017.

LORETTI, A. P.; BARROS, S. S. Hemorrhagic disease in dogs infected with an unclassified intraendothelial piroplasm in southern Brazil. **Veterinary Parasitology**, v. 134, n. 3–4, p. 193–213, 10 dez. 2005. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/16153781>. Access: dec. 7, 2017.

MALHEIROS, J.; COSTA, M. M.; DO AMARAL, R. B.; DE SOUSA, K. C. M.; ANDRÉ, M. R.; MACHADO, R. Z.; VIEIRA, M. I. B. Identification of vector-borne pathogens in dogs and cats from Southern Brazil. **Ticks and Tick-borne Diseases**, v. 7, n. 5, p. 893–900, jul. 2016. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/27266811>. Access: dec. 7, 2017.

NEVES, D. P. **Parasitologia humana**. 10. ed. São Paulo: Atheneu, 2000.

PAIM, C. B.; PAIM, F. C.; DA SILVA, A. S.; FRANÇA, R. T.; COSTA, M. M.; LEAL, C. A. M.; SOARES, J. F.; LABRUNA, M. B.; SCHETINGER, M. R. C.; MAZZANTI, A.; MAZZANTI, C. M.; MONTEIRO, S. G.; LOPES, S. T. A. Thrombocytopenia and platelet activity in dogs experimentally infected with Rangelia vitalii. **Veterinary Parasitology**, v. 185, n. 2–4, p. 131–137, 30 abr. 2012. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/22019200>. Access: dec. 12, 2017.

PASSOS, L. M. F.; GEIGER, S. M.; RIBEIRO, M. F. B.; PFISTER, K.; ZAHLER-RINDER, M. First molecular detection of Babesia vogeli in dogs from Brazil. **Veterinary Parasitology**, v. 127, n. 1, p. 81–85, 4 jan. 2005. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/15619377>. Access: dec. 12, 2017.

QUADROS, R. M.; SOARES, J. F.; XAVIER, J. S.; PILATI, C.; DA COSTA, J. L.; MIOTTO, B. A.; MILETTI, L. C.; LABRUNA, M. B. Natural Infection of the Wild Canid Lycalopex gymnocercus by the Protozoan Rangelia vitalii , the Agent of Canine Rangeliosis. **Journal of Wildlife Diseases**, v. 51, n. 3, p. 787–789, jul. 2015. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/25932667>. Access: dec. 7, 2017.

SILVA, A. S.; FRANÇA, R. T.; COSTA, M. M.; PAIM, C. B.; PAIM, F. C.; DORNELLES, G. L.; SOARES, J. F.; LABRUNA, M. B.; MAZZANTI, C. M.; MONTEIRO, S. G.; LOPES, S. T. A. Experimental infection with Rangelia vitalii in dogs: Acute phase, parasitemia, biological cycle, clinical-pathological aspects and treatment. **Experimental Parasitology**, v. 128, n. 4, p. 347–352, ago. 2011. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21570966>. Access: dec. 12, 2017.

SILVEIRA, J. A. G.; D'ELIA, M. L.; DE OLIVEIRA AVELAR, I.; DE ALMEIDA, L. R.; DOS SANTOS, H. A.; DE MAGALHÃES SOARES, D. F.; RIBEIRO, M. F. B.; DOS SANTOS LIMA, W.; ECCO, R. Rangelia vitalii in a free-ranging maned wolf (Chrysocyon brachyurus) and co-infections. **International journal for parasitology. Parasites and wildlife**, v. 5, n. 3, p. 280–285, dez. 2016. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/27761403>. Access: dec. 7, 2017.

SOARES, J. F. **História natural da rangeliose**. 2014. Universidade de São Paulo, 2014. Available at: <file:///C:/Users/Jessica Valente/Downloads/JOAO_FABIO_SOARES_Original.pdf>. Access: dez. 12, 2017.

SOARES, J. F.; CARVALHO, L.; MAYA, L.; DUTRA, F.; VENZAL, J. M.; LABRUNA, M. B. Molecular detection of Rangelia vitalii in domestic dogs from Uruguay. **Veterinary Parasitology**, v. 210, n. 1–2, p. 98–101, 30 maio 2015. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/25843009>. Access: dec. 7, 2017.

SOARES, J. F.; DALL'AGNOL, B.; COSTA, F. B.; KRAWCZAK, F. S.; COMERLATO, A. T.; ROSSATO, B. C. D.; LINCK, C. M.; SIGAHI, E. K. O.; TEIXEIRA, R. H. F.; SONNE, L.; HAGIWARA, M. K.; GREGORI, F.; VIEIRA, M. I. B.; MARTINS, J. R.; RECK, J.; LABRUNA, M. B. Natural infection of the wild canid, Cerdocyon thous, with the piroplasmid Rangelia vitalii in Brazil. **Veterinary Parasitology**, v. 202, n. 3–4, p. 156–163, 28 maio 2014. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/24685025>. Access: dec. 7, 2017.

SOARES, J. F.; GIROTTO, A.; BRANDÃO, P. E.; DA SILVA, A. S.; FRANÇA, R. T.; LOPES, S. T. A.; LABRUNA, M. B. Detection and molecular characterization of a canine piroplasm from Brazil. **Veterinary Parasitology**, v. 180, n. 3–4, p. 203–208, 25 ago. 2011. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21489694>. Access: dec. 7, 2017.