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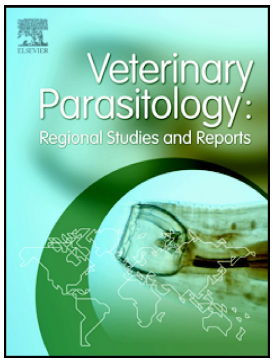

Use of molecular tools for the diagnosis of rangeliosis by Rangelia vitalii in Argentina: A case report

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Use of Molecular Tools for the Diagnosis of Rangeliosis by *Rangelia vitalii* in Argentina: a case report

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Abstract

Vector-borne pathogens are responsible for serious emerging diseases and *Rangelia vitalii*, the etiologic agent of canine rangeliosis, is one of the most pathogenic tick-borne pathogens for dogs in South America. This protozoan is transmitted by the *Amblyomma aureolatum* tick bite and the clinical features associated to the disease are fever, hemolytic anemia, jaundice, hepatosplenomegaly and bleeding from natural orifices, mainly from the ear egde. The reports of canine rangeliosis in Argentina are scarce. In the present study we report the detection of *Rangelia vitalii* in a naturally infected dog from Gualeguay, Entre Ríos, Argentina with history of tick infestation and clinical signs compatible with rangeliosis. An initial blood sample was positive to piroplasmids by blood smear examination and the molecular amplification of a fragment of the

18SrRNA gene. Sequencing of the fragment confirmed the pathogen identity. After treatment with imidocarb dipropionate, the clinical signs remitted and the blood smear tested negative.

Key words: Piroplasms*, Rangelia vitalii*, dogs, molecular diagnosis.

Introduction

Rangeliosis is an emerging tick borne disease that affects dogs from Brasil, (Loretti y Barros, 2004; Da silva et al., 2012; Franca et al 2010, 2014) Argentina, (Eiras et al., 2014; Sánchez et al., 2014) Uruguay (Soares et al., 2015, Rivero et al., 2017) and Paraguay (Inácio et al 2017*)*. It is caused by *Rangelia vitalii,* a tick-borne protozoan parasite from the Phylum Apicomplexa that is transmitted by the *Amblyomma aureolatum* tick-bite (Soares et al., 2018). In the life cycle of this parasite, domestic dogs and wild canines act as intermediary hosts. The clinical condition is characterized by fever, hemolytic anemia, jaundice, hepatosplenomegaly as well as spontaneous bleeding from natural orifices in the body, skin and particularly from the ear egde. Rangeliosis is also known as Nambi-uvú, which in Guaraní language means "bleeding ears". Unlike *Babesia vogeli and Babesia gibsoni*, *R. vitalii* merozoites can be localized free at plasma (merozoites type I and II), in red blood cells or monocytes/neutrophils (Loretti & Barros 2005, Soares et al., 2011; Sanchez et al., 2017). This feature facilitates the diagnosis of this parasite infection through blood smear examination. However, diagnosis confirmation could require the use of molecular methods that enable the characterization of the parasitic agent. In Argentina, Rangeliosis has been occasionally reported in Leando N. Alem, Misiones (Eiras et al., 2014) and in Concordia, Entre Ríos (Sanchez et al., 2014, 2017). The present work aimed to report the use of molecular methods for the diagnosis of Rangeliosis caused by *Rangelia vitalii* in a canine from Gualeguay, Entre Ríos,Argentina.

Case presentation

A 6-year-old male, Jack Russell Terrier mixed breed dog, native from a rural area of Gualeguay, Entre Ríos, Argentina, presented to the veterinary due to two-days history hyperthermia, icteric

mucous membrane, apathy, anorexia and a prolonged bleeding time from the ear edge. The dog had no travel history in recent months but had been infested with ticks. A Complete Blood Count (CBC) and a blood biochemical profile test revealed normochromic, normocytic anemia, thrombocytopenia, leukocytosis, elevated concentrations of urea, and activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST).In addition, a buffy coat cytology observation was performed and revealed free and intracellular basophilic elliptical structures in erythrocytes and leucocytes that were compatible with *R. vitalii* (Figure 1). In order to rule out the presence of the common tick-borne pathogen *Ehrlichia canis,* an immunochromatography test was performed which resulted negative. An empiric treatment against *R. vitalii* was started with imidocarb dipropionate (6 mg/kg IM) after atropinization (0.05mg/kg SC). Since the patient's clinical condition and the blood test values improved, a second imidocarb dipropionate dose was administered after 14 days of the first one. A control blood smear examination done after treatment resulted negative for *R.vitalii*

For further diagnostic confirmation, an aliquot of the blood sample was used for the molecular identification of possible pathogens. DNA was extracted from 200 µl of whole blood using a commercial kit (ADN PuriPrep-S; INBIO HIGHWAY) and PCR reactions were performed targeting a fragment of the *18SrRNA* (460 bp) common to the order Piroplasmida (Gubbels et al., 1999) and a nested PCR (200 bp) targeting the *p30* gene for *Ehrlichia canis* (Stich et al., 2002) . Both positive (*Babesia bovis* strain R1A, and *E. canis*) and negative (pure water) controls were used. An aliquot of 5 µl of the amplified product was analyzed by electrophoresis in 1% agarose gel stained with ethidium bromide. The *E. canis* PCR tested negative but given that the *18SrRNA* fragment common to the order Piroplasmida was amplified, the remaining amplification product was purified and both strands from the amplicon were sequenced and analyzed on an ABI 3500 genetic analyzer. The gene fragment targeted holds a hypervariable region that enables the genus and species identification. The resulting sequence (accession number MT036310) showed 100% identity with sequences from *R vitalii* deposited in GenBank (https://www.ncbi.nlm.nih.gov/). Moreover, a phylogenetic tree was built using the 410 bp sequence obtained and reference sequences from the

order Piroplasmida and the “Maximum Likelihood” algorithm with 1000 bootstrap iterations (Figure 2).

Discussion

Rangeliosis is an emerging infectious disease in our environment that affects dogs causing a severe hemolytic anemia and bleeding, due to endothelial dysfunction and a decrease in platelet count. The present study represents the southernmost report of a *R. vitalii* case in a dog from Argentina in South America.

Amblyomma aureolatum is a competent vector for *R. vitalii*, and transovarial and transstadial transmission has been proved in this vector (Soares et al., 2018). This tick species is distributed in natural areas of the northeast Argentina (Boero JJ, 1957; Ivancovich JC, 1973; Tarragona et al., 2012; Colombo et al., 2016; Nava et al., 2017), including Entre Ríos (Boero JJ, 1954; Guglielmone et al, 2002). Consequently, those dogs that are used as working animals or as hunters in rural areas without preventive acaricidal treatments, are at a higher risk of being bite by the vector (Carini and Maciel, 1914; Loretti and Barros, 2003; Soares et al, 2014, Rivero et al., 2017). Although to date, there are no cases of *R. vitalii* reported in densely populated urban zones, *A. aureolatum* has been described recently in a natural area of Buenos Aires city (Cicuttin et al., 2017).

Different drugs have been described for the treatment of Rangeliosis: imidocarb dipropionate, doxycycline or diminazeno aceturate (Loretti and Barros, 2004; Da Silva et al., 2011, Rivero et al., 2017). In the present case, the administration of two doses of imidocarb dipropionate with an interval of 14 days was enough for the remission of the clinical signs, and also a second blood smear performed after treatment resulted negative for this agent. This good response to the drug is in accordance with the treatment reported for “large forms of *Babesia*”, for which a combination of imidocarb dipropionate and atropine is used (Conrad et al., 1991; Baneth G, 2018).

Rhipicephalus sanguineus sensu lato (Debarbora et al., 2011; Nava et al., 2012; Nava et al., 2018; Borrás et al., 2019) is probably the most common tick in dogs in Argentina, and is a vector of many

pathogens, such as *Ehrlichia canis, Hepatozoon canis* and *Babesia vogeli* (Silva et al., 1998; Perez Tort et al., 2012; Eiras et al., 2008, 2013). These pathogens cause similar clinical signs and should be considered, either within the differential diagnosis or as co-infections.

Conclusion

There are different species of piroplasms that are circulating among the South American dogs. For this reason, the use of molecular tools allows professionals to get an accurate diagnosis, and at the same time are valuable for the study of epidemiological aspects of the parasite and its vectors. Rangeliosis should be suspected and included within the differential diagnoses in those areas where the *A. aureolatum* tick has been found, and where rural dogs and peri-urban dogs show clinical signs of anemia, fever, and bleeding. Similarly, it is a key point the use of acaricidal products regularly and systematically as a preventive measure to minimize the expose and risk of infection in dogs, as well as the early suspicion of this pathology by the veterinary community.

Declarations of competing interest:

The authors declare no conflict of interest.

Ethical statement:

This manuscript describes a clinical case. No animal experimentation occurred.

Acknowledgments

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References

Baneth G. 2018. Antiprotozoal treatment of canine babesiosis. Vet Parasitol. Apr 30;254:58-63. doi: 10.1016/j.vetpar.2018.03.001

Boero JJ. 1954. Los ixodideos de la República Argentina y sus huéspedes. Rev. Fac. Agr. Vet. 13: 505-514.

Boero JJ. 1957. Las garrapatas de la República Argentina (Acarina: Ixodoidea). Dpto. Edit. Univ. Buenos Aires. 113 pp

Borrás P, Sanchez J,Guillemi E,De la Fourniere S,Abadia M,Farber y Santini MS. 2019. Detección de bacterias de los géneros Ehrlichia, Anaplasma y Rickettsia en garrapatas Rhipicephalus Sanguineus s.l Pergamino, Argentina. Rev Argent Salud Pública; 10(41): 8-13.

Carini A y Maciel J. 1914. Sobre a molestia dos caes, chamada Nambi-Uvú, e o seu parasita (Rangelia vitalii). An Paul Med Cir 3:65-71.

Cicuttin G, Salvo María de, Nava S. 2017. Especies de garrapatas duras en un área urbana protegida de la Ciudad Autónoma de Buenos Aires. Rev Argent Salud Pública. Sep;8(32):7-12.

Colombo V; Pereira J; Nava S; Beldomenico P. 2016. Amblyomma aureolatum (Acari: Ixodidae): nuevos registros en las provincias de Buenos Aires y Santa Fe, Argentina. Revista Fave Ciencias Veterinarias. doi: www.dx.doi.org/10.14409/favecv.v15i1/2.5914

Conrad, P., Thomford, J., Yamane, I., Whiting, J., Bosma, L., Uno, T., Holshuh, H.J., Shelly, S., 1991. Hemolytic anemia caused by Babesia gibsoni infection in dogs. J. Am. Vet. Med. Assoc. 199, 601–605.

Da Silva A, França RT, Costa MM, Paim CB, Paim FC, Dornelles GL, Soares JF, Labruna MB, Mazzanti CM, Monteiro SG, Lopes STA. 2011. Experimental infection with Rangelia vitalii in dogs: Acute phase, parasitemia, biological cycle, clinical-pathological aspects and treatment. Exp Parasitol 128:347-352. doi: 10.1016/j.exppara.2011.04.010

Da Silva AS, Martins DB, Soares JF, Franca RT. 2012. Canine rangeliosis: the need for differential diagnosis. ParasitologyResearch. 112:1329-32. doi:10.1007/s00436-012-3160-y.

Eiras DF, Basabe J, Mesplet M, Schnittger L. First molecular characterization of Babesia vogeli in two naturally infected dogs of Buenos Aires, Argentina. Vet Parasitol. 2008 Nov 7;157(3-4):294-8. doi: 10.1016/j.vetpar.2008.07.037

Eiras DF, Craviotto MB, Vezzani D, Eyal O, Baneth G. 2013. First description of natural Ehrlichia canis and Anaplasma platys infections in dogs from Argentina. Comp Immunol Microbiol Infect Dis. Mar;36(2):169-73. doi: 10.1016/j.cimid.2012.11.008

Eiras DF, Craviotto MB, Baneth G, Moré G. 2014. First report of Rangelia vitalii infection (canine rangeliosis) in Argentina. Parasitology International. 63:729-34. doi: 10.1016/j.parint.2014.06.003

França RT, Da Silva AS, Loretti AP, Mazzanti CM, Lopes STA. 2014. Canine rangeliosis due to *Rangelia vitalii*: From first report in Brazil in1910 to current day - A review. Ticks Tick Borne Dis 5:466-474. doi: 10.1016/j.ttbdis.2014.04.005

Franca RT, Da Silva AS, Paim FC, Costa MM, Soares JF,Mazzanti CM, Lopes STA. 2010. Rangelia vitalii in dogs in southern Brazil. Comparative Clinical Pathology. 19:383-87. doi:10.1007/s00580-010-1041-2.

Gubbels JM, de Vos AP, van der Weide M, Viseras J, Schouls LM, de Vries E, Jongejan F. 1999. Simultaneous detection of bovine Theileria and Babesia species by reverse line blot hybridization. J Clin Microbiol. 37(6):1782-9.

Guglielmone AA, Mangold AJ, Boero C & Azcure R. 2002. Nuevo registro de Amblyomma aureolatum (Pallas, 1772) (= Amblyomma striatum Koch, 1844) en Entre Ríos, Argentina. Rev. FAVE-Ciencias Veterinarias. 1: 33-35.

Inácio EL, Pérez-Macchi S, Alabi A, Bittencourt P, Müller A. 2017.Prevalence and molecular characterization of piroplasmids in domestic dogs from Paraguay.Ticks Tick Borne Dis. 2019 Feb;10(2):321-327. doi: 10.1016/j.ttbdis.2018.11.009. Nov 17.

Ivancovich JC. 1973. Las garrapatas del Chaco y Formosa. Primera comunicación. Rev. Inv. Agropec. Ser. 4, 10: 9-24

Loretti AP y Barros SS. 2005. Hemorrhagic disease in dogs infected with an unclassified Intraendothelial Piroplasm in southern Brazil. Vet Parasitol 134:193-213.

Nava S, Mastropaolo M, Venzal JM, Mangold AJ, Guglielmone AA. 2012.Mitochondrial DNA Analysis of Rhipicephalus sanguineus sensu lato (Acari: Ixodidae) in the Southern Cone of South America. Vet Parasitol. 190(3-4):547-55. doi 10.1016/j.vetpar.2012.06.032

Nava S, Venzal J, Gonzalez Acuña D, Martins T, Guglielmone A. 2017. Ticks of the Southern Cone of America. 1st edition. Londres: Elsevier.

Nava S, Beati L, Venzal JM, Labruna MB, Szabó MPJ, Petney T, et al. 2018. Rhipicephalus sanguineus (Latreille, 1806): Neotype Designation, Morphological Re-Description of All Parasitic Stages and Molecular Characterization. Ticks Tick Borne Dis. 9(6):1573-1585. doi: 10.1016/j.ttbdis.2018.08.001

Pérez Tort, G.; Petetta, L. 2012. Estudio de 50 casos de hepatozoonosis en caninos naturalmente infectados en el Gran Buenos Aires, Argentina. Veterinaria Argentina. 24 (293): 1-10.

Rivero, R., Minoli, P., Parodi, P., Matto, C., Armúa-Fernández, MT., Gianneechini, E., Carvalho, L., & Venzal, JM. 2017. Descripción de un foco de rangeliosis canina en el litoral noroeste del Uruguay. Veterinaria (Montevideo), 53(208). https://doi.org/10.29155/VET.23.208.3

Sanchez RO, Moré G, Eiras DF. 2014. Piroplasmosis canina Rangelia vitalii (Protozoa, Piroplasmida) en la ciudad de Concordia, Entre Ríos. XX Reunión Científico Técnica de la AAVLD. Tucumán, Argentina.

Sánchez, R. O., More, G. A., & Eiras, D. F. 2017. Morfología de estructuras parasitarias de Rangelia vitalii en muestras de perros naturalmente infectados. Analecta Veterinaria, 37(2), 017.

Silva, MC.; Rodríguez, MS.; Rosa, A.; Preira, ME.; Márquez, AG. 1999.Hepatozoon canis: primer caso en Buenos Aires, Argentina. Revista de Medicina Veterinaria 80:489-492.

Soares JF, Costa FB, Girotto-Soares A, Silva ASD, França RT, Taniwaki SA, Dall'Agnol B, Reck J, Hagiwara MK, Labruna MB. 2018. Evaluation of the vector competence of six ixodid tick species for

Rangelia vitalii (Apicomplexa, Piroplasmorida), the agent of canine rangeliosis. Ticks and Tick-borne Diseases. 9(5): 1221-1234. https://doi.org/10.1016/j.ttbdis.2018.05.004

Stich RW, Rikihisa Y, Ewing SA, Needham GR, Grover DL, Jittapalapong S. 2002. Detection of Ehrlichia Canis in Canine Carrier Blood and in Individual Experimentally Infected Ticks With a p30-based PCR Assay. J Clin Microbiol. 40(2):540-6. doi: 10.1128/jcm.40.2.540-546.2002.

Tarragona EL, Eberhardt MAT, Zurvera D, Beldomenico PM & Mastropaolo M. 2012. Primer registro de Amblyomma aureolatum (Pallas, 1772) y Amblyomma Ovale Koch, 1844 (Acari: Ixodidae) en la provincia de Santa Fe, Argentina. FAVE Sección Ciencias Veterinarias. 11: 59- 63

Figures captions

Figure 1. (A) Free basophilic elliptical structures compatibles with *R. vitalii* merozoites type II. Blood smear. May Grunwald – Giemsa. 1000x. (B) Two merozoites within an erythrocyte. Blood smear. May Grunwald – Giemsa. 1000x. (C) Few merozoites within a monocyte. Blood smear. May Grunwald – Giemsa. 1000x

Figure 2. Phylogenetic tree using 410 bp sequences from the *18srRNA* gene of diverse Piroplasmida species and the Maximum Likelihood algorithm with 1.000 bootstrap iterations. A sequence from the *18srRNA* gene from *Hepatozoon canis* was used as an outgroup. The cluster formed by *R. vitalli* reference sequence and the sample sequence is marked with a box

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HIGHLIGHTS

- The southernmost case report of *Rangelia vitalii* in a dog from Argentina in South America
- Molecular detection and sequencing of *Rangelia vitalii*
- A good clinical response and blood tested values improvement after treatment with two doses of imidocarb dipropionate with an interval of 14 days.

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Declarations of competing interest:

The authors of "***Use of Molecular Tools for the Diagnosis of Rangeliosis by Rangelia vitalii in Argentina: a case report*****"** declare no conflict of interest.

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Ethical statement

This manuscript "***Use of Molecular Tools for the Diagnosis of Rangeliosis by Rangelia vitalii in Argentina: a case report"*** describes a clinical case. No animal experimentation occurred.

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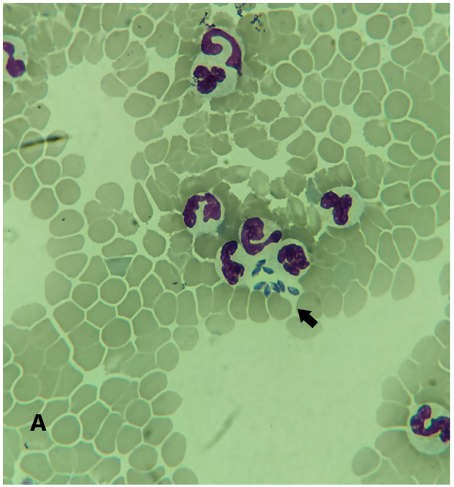
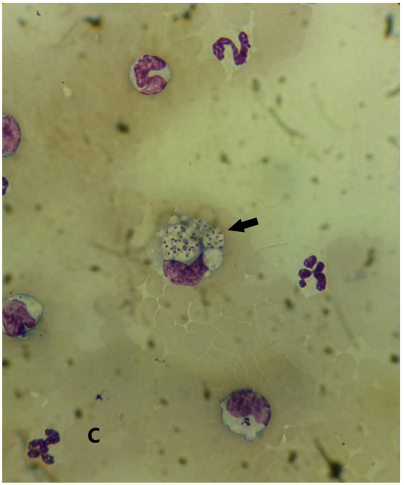




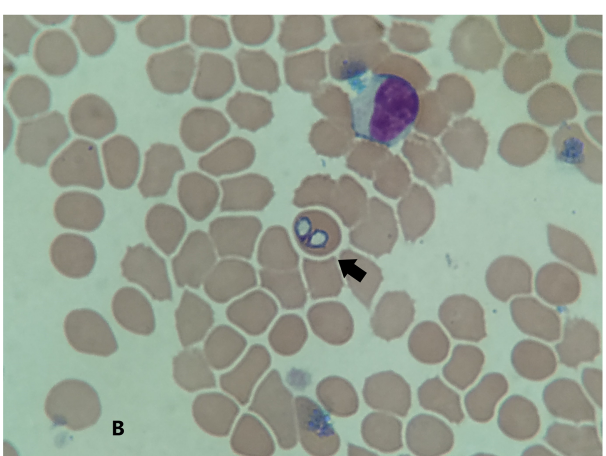
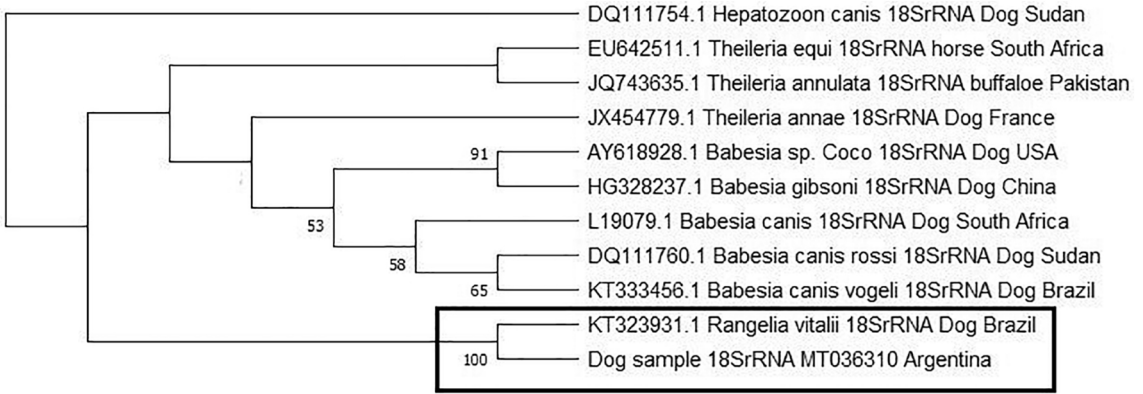


Figure 1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EU642511.1 Theileria equi 18SrRNA horse South Africa
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JX454779.1 Theileria annae 18SrRNA Dog France
91
AY618928.1 Babesia sp. Coco 18SrRNA Dog USA
HG328237.1 Babesia gibsoni 18SrRNA Dog China
53
L19079.1 Babesia canis 18SrRNA Dog South Africa
58
DQ111760.1 Babesia canis rossi 18SrRNA Dog Sudan
65
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KT323931.1 Rangelia vitalii 18SrRNA Dog Brazil
100
Dog sample 18SrRNA MT036310 Argentina

Figure 2