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Short communication

# Molecular detection of *Rangelia vitalii* in domestic dogs from Uruguay

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ABSTRACT

The piroplasm *Rangelia vitalii* is the etiological agent of canine rangeliosis, a severe disease affecting domestic dogs in South America. Two domestic dogs from two different Departments (Salto and Treinta y Tres) of Uruguay presented with clinical signs such as apathy, anorexia, pale mucous membranes, jaundice, and hemorrhagic manifestations, suggestive of a canine vector-borne disease. Molecular analysis, based on PCR and DNA sequencing of portions of the 18S rRNA gene, revealed that both dogs were infected by *R. vitalii*. Two consensus sequences, one from Salto and one from Treinta y Tres, differed from each other by only 1 nucleotide (99.8% similarity) and were 99.8–100% identical to corresponding sequences of *R. vitalii* from Brazil and Argentina available in GenBank. Through phylogenetic analysis inferred by the 18S rRNA gene, the two Uruguayan sequences of *R. vitalii* were aligned with the corresponding sequences from 7 other *R. vitalii* sequences available in GenBank (5 from Brazil and, 2 from Argentina) under high bootstrap support. The two dogs of the present study were negative for *Ehrlichia canis* according to the *E. canis*-specific real-time PCR assay. Our findings not only confirm the occurrence of *R. vitalii* in Uruguay but also provide the southernmost record of this re-emerging agent. The only previous report of *R. vitalii* in Uruguay dated from 1976, a period when molecular analyses were not available. We provide the first molecular detection of *R. vitalii* in Uruguay. Currently, canine rangeliosis is confirmed to occur in Brazil, Argentina, and Uruguay.



## 1. Introduction

The piroplasm *Rangelia vitalii* is the etiological agent of canine rangeliosis, a severe disease affecting domestic dogs in South America. Although the first records of *R. vitalii* date from 1908 to 1914 (Carini, 1908; Pestana, 1910a,b; Carini and Maciel, 1914), this taxon was erroneously regarded as a synonym of *Babesia canis* by subsequent authors (Wenyon, 1926; Doflein and Reichenow, 1929; Moreira, 1938, 1939). *R. vitalii* was only recently confirmed through molecular analyses to be a valid, independent species (Soares et al., 2011). In domestic dogs, *R. vitalii* infects erythrocytes, leucocytes and endothelial cells (Pestana, 1910a; Carini and Maciel, 1914; Loretti and Barros, 2005; Da Silva et al., 2011; Soares et al., 2011), resulting in a variety of clinical signs such as anemia, thrombocytopenia, fever, apathy, anorexia, dehydration, splenomegaly, hepatomegaly,

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generalized lymphadenopathy, mucosal petechiation, bloody diarrhea, jaundice, and persistent bleeding from the nose, oral cavity and tips, margins and outer surface of the ear pinnae (Pestana, 1910b; Carini and Maciel, 1914; Braga, 1935; Fighera et al., 2010; França et al., 2010, 2013; Paim et al., 2012; Soares, 2014; Soares et al., 2014a).

A recent study demonstrated that the tick, *Amblyomma aureolatum*, is a competent vector of *R. vitalii* to dogs (Soares, 2014). The distribution of *A. aureolatum* includes Uruguay, southeastern and southern Brazil, and northeastern Argentina (Guglielmone et al., 2003). Not surprisingly, the occurrence of clinical cases of canine rangeliosis, confirmed in the laboratory by molecular analyses, has been restricted to sites within the distribution area of *A. aureolatum* in Brazil (Soares et al., 2011, 2014a; Lemos et al., 2012) and Argentina (Eiras et al., 2014). A recent study reported natural infection by *R. vitalii* among the wild canid *Cerdocyon thous* (crab-eating fox) in southeastern and southern Brazil (Soares et al., 2014b). Because *C. thous* is the most common wild host of *A. aureolatum* (Guglielmone et al., 2003; Labruna et al., 2005), it was suggested that this native canid could act as a natural reservoir of *R. vitalii* (Soares et al., 2014b).

*A. aureolatum* is one of the most common tick species occurring on domestic dogs in Uruguay (Venzal et al., 2003; Martins et al., 2014). Nonetheless, the presence of *R. vitalii* in Uruguay was previously reported in only the Department of Artigas, where the agent was tentatively identified by blood smear examination of a hunting dog that presented clinical signs compatible with canine vector-borne diseases (CVBD) (Sarasúa and Donati, 1976). Herein, we describe the occurrence of *R. vitalii* infecting domestic dogs from two Uruguayan Departments. For the first time, the presence of *R. vitalii* was confirmed in Uruguay by molecular analysis.

## 2. Materials and methods

Two domestic dogs, presenting clinical signs compatible with CVBD, were available for the present study. The following clinical signs were observed in the dogs: apathy, anorexia, pale mucous membranes, jaundice, and hemorrhagic manifestations (bleeding underneath the conjunctiva, also known as "hyposphagma", and persistent bleeding from the outer surface of the ear pinnae). One of the dogs was a mixed breed female, six months old, from Salto city, Salto Department and was attended in November 2012; the second dog was a pitbull male, five years old, from Treinta y Tres city, Treinta y Tres Department and was attended in May 2013. Although the two dogs were from urban areas, owners reported that their dogs visited surrounding forested areas approximately 12 days before disease onset. From each ill dog, blood samples were collected in EDTA-tubes and submitted to DNA extraction, which was completed using the AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biosciences, CA, USA). Clinical signs of the two dogs resolved after treatment with imidocarb dipropionate (5 mg/kg, subcutaneously, single dose in one dog; two doses at 10-day intervals in the second dog).

DNA samples were tested by PCR protocols targeting two overlapping fragments of the 18S rRNA gene of piroplasmid organisms of the genera *Babesia, Theileria*, and *Cytauxzoon*. The first PCR consisted of primers BAB33-57 (5′-GCC AGT AGT CAT ATG CTT GTC TTA A-3′) and BAB432-409 (5′-TTC CTT AGA TGT GGT AGC CGT TTC-3′), which amplify an approximately 370-bp long fragment (Spolidorio et al., 2009); the second PCR consisted of primers BAB143-167 (5′-CCG TGC TAA TTG TAG GGC TAA TAC A-3′) and BAB694-667 (5′-GCT TGA AAC ACT CTA RTT TTC TCA AAG-3′), which amplify an approximately 500-bp long fragment (Soares et al., 2011). Samples were also tested using an *E. canis*-specific TaqMan real-time PCR assay, as previously described (Doyle et al., 2005). Products generated by the first and second 18S rRNA PCR protocols were sequenced in an ABI automated sequencer (model ABI 3500 Genetic Analyzer; Applied Biosystems/Thermo Fisher Scientific, Foster City, CA) with the same primers (forward and reverse) used for each of the two PCR assays. The generated sequences were compared with each other and submitted to BLAST analyses (www.ncbi.nlm.nih.gov/blast) to infer the closest similarities available in GenBank.

Partial sequences (608-bp) of the 18S rRNA gene of piroplasmids derived from the two dogs were aligned with corresponding 18S rRNA sequences from 56 genotypes of the genera *Babesia*, *Rangelia*, *Theileria*, *Cytauxzoon* and *Hepatozoon* retrieved from GenBank, using Clustal/W v.1.8.1 (Thompson et al., 1994). A maximum likelihood phylogenetic tree using Tamura-Nei + G + I substitution model was generated using Mega 6.0.6 software (Tamura et al., 2013) with 100 bootstrap replicates. The substitution model was selected using Mega 6.0.6 software (Tamura et al., 2013) according to the lowest Bayesian Information Criterion (BIC) score. The sequence of *H. canis* was used as an outgroup.

## 3. Results

The two PCR protocols targeting two overlapping fragments of the piroplasmid 18S rRNA gene yielded amplicons of the expected size from the two dogs. For each dog, two DNA sequences, one of 370-bp (first PCR assay) and one of 500-bp (second PCR assay) were generated. These two sequences had overlapping fragments, as the 3′ end portion of the first PCR assay-sequence corresponded to the 5′ end portion of the second PCR assay-sequence. Therefore, they were aligned to form a consensus sequence of 608-bp. The two consensus sequences, one from Salto and one from Treinta y Tres, differed from each other by only 1 nucleotide (99.8% similarity), and when submitted to BLAST analyses, they were 99.8–100% identical to corresponding sequences of *R. vitalii* from Brazil and Argentina (GenBank accession nos. KF964146, JN880432, KF218606). The *R. vitalii* DNA sequences generated in the present study have been deposited in GenBank under the accession numbers KP202860 (*R. vitalii* strain Salto) and KP202861 (*R. vitalii* strain Treinta y Tres).

Through phylogenetic analysis inferred by the 18S rRNA gene, the two Uruguayan sequences of *R. vitalii* were aligned with the corresponding sequences from 7 other *R. vitalii* sequences available in GenBank (5 from Brazil and 2 from Argentina) under high bootstrap support (Fig. 1). The