Arq. Bras. Med. Vet. Zootec., v.78, n.4, e13798, 2026

http://dx.doi.org/10.1590/1678-4162-13798
Case Report- Veterinary Medicine

Natural infection by *Rangelia vitalii* pestana, 1910, in dog from the state of Minas Gerais, Brazil – a case report

[I*nfecção natural por* Rangelia vitalii pestana, *1910 em cão do estado de Minas Gerais, Brasil – relato de caso*]

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ABSTRACT

Rangeliosis is a severe, emerging hemoparasitic disease affecting dogs in South America, caused by the protozoan *Rangelia vitalii* and transmitted by *Amblyomma aureolatum*, an ixodid tick found in the Atlantic Forest biome. Although cases are commonly reported in southern Brazil and in southeastern states such as São Paulo and Rio de Janeiro, reports from the state of Minas Gerais remain scarce. This study describes a natural infection by *R. vitalii* in a dog from the metropolitan region of Belo Horizonte, Minas Gerais, Brazil. Peripheral blood smears revealed intra- and extra-erythrocytic piroplasms. Molecular testing detected DNA from the order Piroplasmida (18S rRNA and *hsp70* genes). Genetic sequencing of the amplified piroplasmid fragments revealed 99.83% similarity with *R. vitalii*. This is the first study to report the ante-mortem molecular diagnosis of *R. vitalii* in a dog, along with clinical treatment and recovery, in the state of Minas Gerais, Brazil.

Keywords: hemopathogens, hemoparasites, epidemiology, canids

RESUMO

A rangeliose é uma doença hemoparasitária grave e emergente, que afeta cães na América do Sul, causada pelo protozoário Rangelia vitalii *e transmitida pelo carrapato ixodídeo* Amblyomma aureolatum*, encontrado no bioma da Mata Atlântica. Casos são comumente relatados na região Sul do Brasil e em estados do Sudeste, como São Paulo e Rio de Janeiro, enquanto em Minas Gerais os relatos ainda são escassos. Este estudo descreve uma infecção natural por* R. vitalii *em um cão da região metropolitana de Belo Horizonte, Minas Gerais, Brasil. Esfregaços de sangue periférico revelaram piroplasmas intra e extraeritrocitários. Testes moleculares detectaram DNA da ordem Piroplasmida (genes 18S rRNA e hsp70). O sequenciamento genético dos fragmentos amplificados revelou 99,83% de similaridade com* R. vitalii*. Este é o primeiro estudo a relatar o diagnóstico molecular* ante mortem *de* R. vitalii *em um cão, juntamente com o tratamento clínico e a recuperação, no estado de Minas Gerais, Brasil.*

Palavras-chave: hemopatógenos, hemoparasitas, epidemiologia, canídeos

INTRODUCTION

Rangeliosis is a hemoparasitic disease caused by the protozoan *Rangelia vitalii*, a piroplasm capable of parasitizing erythrocytes, neutrophils, monocytes, and endothelial cells of both wild and domestic canids (Fighera *et al*., 2010; Carniel *et al*., 2025). The ixodid tick *Amblyomma aureolatum*, the vector of *R. vitalii*, is found in South America—in countries such as Brazil, Argentina, Uruguay, Paraguay, French Guiana, and Suriname—with its occurrence predominantly recorded in higher-altitude regions of the Atlantic Forest biome (Soares, 2018; Borrás *et al*., 2020). For this reason, rangeliosis has only been reported on this continent, mainly in the cooler regions of southern Brazil (Oliveira *et al*., 2023).

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Submitted: January 15, 2026. Accepted: April 2, 2026.

In southeastern Brazil, cases have also been reported, primarily in the states of São Paulo (Silva *et al*., 2019b) and Rio de Janeiro (Palmer *et al*., 2024). Although the prevalence and geographic distribution of the disease are well established, sporadic cases have been documented in other regions of the country (Silveira *et al*., 2016). This suggests that, like other vector-borne pathogens, *R. vitalii* may be considered a re-emerging disease, expanding into non-endemic areas and remaining underdiagnosed (Soares *et al*., 2018).

In this context, the objective of this study is to describe the molecular diagnosis of rangeliosis in a naturally infected female dog from the state of Minas Gerais, Brazil, a non-endemic region.

ETHICAL ASPECTS

This study was not submitted to the Animal Use Ethics Committee (CEUA), as it consists of a clinical case report without experimental procedures. The animal described herein underwent only the diagnostic and therapeutic interventions clinically indicated for its condition, with no additional procedures or modifications to clinical management performed for the purposes of this study.

CASUISTRY

A six-year-old, 11.5-kg female French Bulldog presented with a history of inappetence, vomiting, diarrhea, and hematuria. The owner reported that, seven days prior to the consultation, the dog had accessed a forested area in the municipality of Nova Lima, Minas Gerais, Brazil. There was no history of ectoparasite infestation or travel. On physical examination, the dog was lethargic, with an adequate body condition score, mildly icteric mucous membranes, splenomegaly, hypothermia, and dehydration.

Hematological analysis revealed normocytic normochromic anemia and thrombocytopenia. Peripheral blood smears showed intraerythrocytic structures suggestive of Babesia spp., with an estimated parasitemia rate of 0.48% (Fig. 1). Serum biochemistry revealed hypoglobulinemia and increased urea and AST levels.

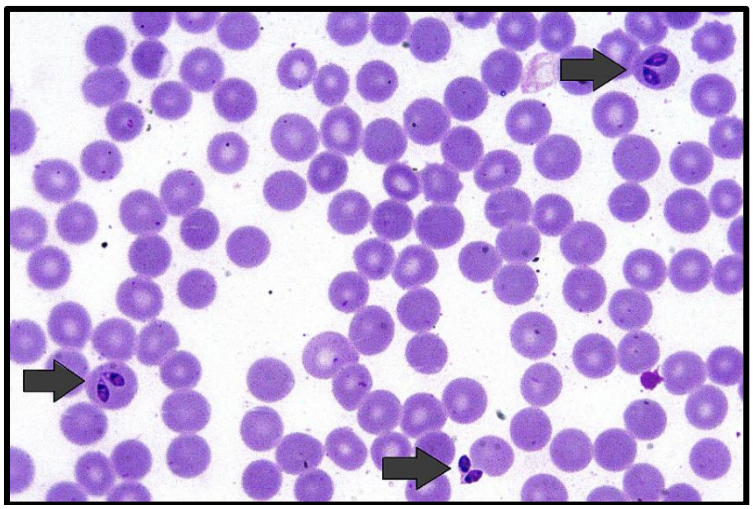

Figure 1. Blood smears from a female dog naturally infected with *Rangelia vitalii*. Intra- and extraerythrocytic piroplasm merozoites morphologically suggestive of *Babesia* spp. (red arrows). Hematoxylin and eosin stain, 100× objective.

Blood samples were submitted to the Protozoology Laboratory for molecular diagnosis of hemopathogens. DNA was extracted from 300 µL of whole blood using the Wizard® DNA extraction kit (Promega, USA). To verify the quality of the extraction, DNA integrity, and the presence of possible reaction inhibitors, the samples were tested for amplification of the gene encoding glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) (Birkenheuer *et al*., 2003). Assays were performed for the detection of agents belonging to the order

Piroplasmida/*Hepatozoon* spp., *Ehrlichia* spp., *Anaplasma* spp., *Leishmania* spp., and hemotropic *Mycoplasma*.

All primers, nucleotide sequences, target genes, fragment sizes, and cycling conditions used in this study are detailed in the works cited in Table 1. Ultrapure sterile water (Life Technologies®, USA) was used as a negative control, and DNA from samples of naturally infected hosts, previously confirmed by PCR and genetic sequencing (Castillo *et al*., 2024), was used as a positive control.

Table 1. Primer sequences used to identify the hemopathogens investigated in the present study

Agents		Primer	Sequences (5'-3')	Target	Fragment (bp)	Reference
Piroplasmida/ *Hepatozoon* spp. screening	1st reaction	RIB-19	CGGGATCCAACCTGGTTGATCCTGC	18S rRNA	1700	Zahler *et al.*, 2000
		RIB-20	CCGAATTCCTTGTTACGACTTCTC			
	2nd reaction	BAB-Rum F	ACCTCACCAGGTCCAGACAG	18S rRNA	430	Silveira *et al.*, 2011
		BAB-Rum R	GTACAAAGGGCAGGGACGTA			
Piroplasmida screening		HSP70F2	GGATCAACAAYGGMAAGAAC	*hsp70*	720	Soares *et al.* 2011
		HSP70R2	GBAGGTTGTTGTCCTTVGTCAT			
Babesia vogeli characterization		BCV-18S-F	GTGAACCTTATCACTTAAAGG	*ITS*	602	Kaur *et al.*, 2020
		BCV-18S-R	CAACTCCTCCACGCAATCG			
Ehrlichia spp. screening	1st reaction	N516SCH 1 F	ACGGACAATTGCTTATAGCCTT	16S rRNA	1195	Kawahara *et al*. 2009
		N516SCH 1 R	ACAACTTTTATGGATTAGCTAAAT			
	2nd reaction	N516SCH 2 F	GGGCACGTAGGTGGACTAG	16S rRNA	443	Kawahara *et al*. 2009
		N516SCH 2 R	CCTGTTAGGAGGGATACGAC			
Anaplasma spp. screening	1st reaction	GE3a	CACATGCAAGTCGAACGGATTATTC	16S rRNA	932	Massung *et al.*, 1998
		GE10R	TTCCGTTAAGAAGGATCTAATCTCC			
	2nd reaction	GE9F	AACGGATTATTCTTTATAGCTTGCT	16S rRNA	546	Massung *et al*. 1998
		GE2	GGCAGTATTAAAAGCAGCTCCAGG			
Hemotropic *Mycoplasma* screening/characterization		HBT F	ATACGGCCCATATCCCTACG	16S rRNA	600	Criado-Fornelio *et al.*, 2003
		HBT R	TCGCTCCACCACTTGTTCA			
Leishmania spp. screening/characterization		LITSR	CTGGATCATTTT CCGATG	*ITS1*	300-350	El Tai *et al.* 2000
		L5.8S	TGATACCACTTA TCGCACTT			
gapdh		GAPDH F	CCTTCATTGACCTCAACTACAT	*gapdh*	400	Birkenheuer *et al.*, 2003
		GAPDH R	CCAAAGTTGTCATGGATGACC			

The sample tested positive for the *gapdh* gene, and subsequent molecular assays detected the presence of DNA from the order Piroplasmida (18S rRNA and *hsp70*) and hemotropic *Mycoplasma*. To confirm the identity of the piroplasm, the amplified product (*hsp70* gene) was purified using the QIAquick PCR Purification Kit (Qiagen Biotechnology, Brazil) and sequenced (Sanger *et al*., 1977) on an ABI 3130 Genetic Analyzer (Applied Biosystems, USA). Sequencing was performed at the Oswaldo Cruz Foundation (Fiocruz) using the BigDye® Direct Cycle Sequencing Kit v3.1 (Applied Biosystems) with the same primers employed in the PCR and the POP-7™ polymer.

The obtained sequence (GenBank submission number: PQ261164) exhibited 99.83% identity and an E-value of 0 with *R. vitalii* detected in domestic dogs (*Canis familiaris*; JF279603) and

wild canids (*Cerdocyon thous*: PP357051; *Chrysocyon brachyurus*: KU507417) sampled in Brazil.

A phylogenetic tree was constructed using MEGA (Molecular Evolutionary Genetics Analysis) version 11. Sequence alignment was performed with the ClustalW algorithm, incorporating reference sequences retrieved from GenBank. The evolutionary model was selected based on the lowest Bayesian Information Criterion (BIC) and corrected Akaike Information Criterion (AICc) values. The Maximum Likelihood (ML) method, with 1,000 bootstrap replicates, was employed (Fig. 2).

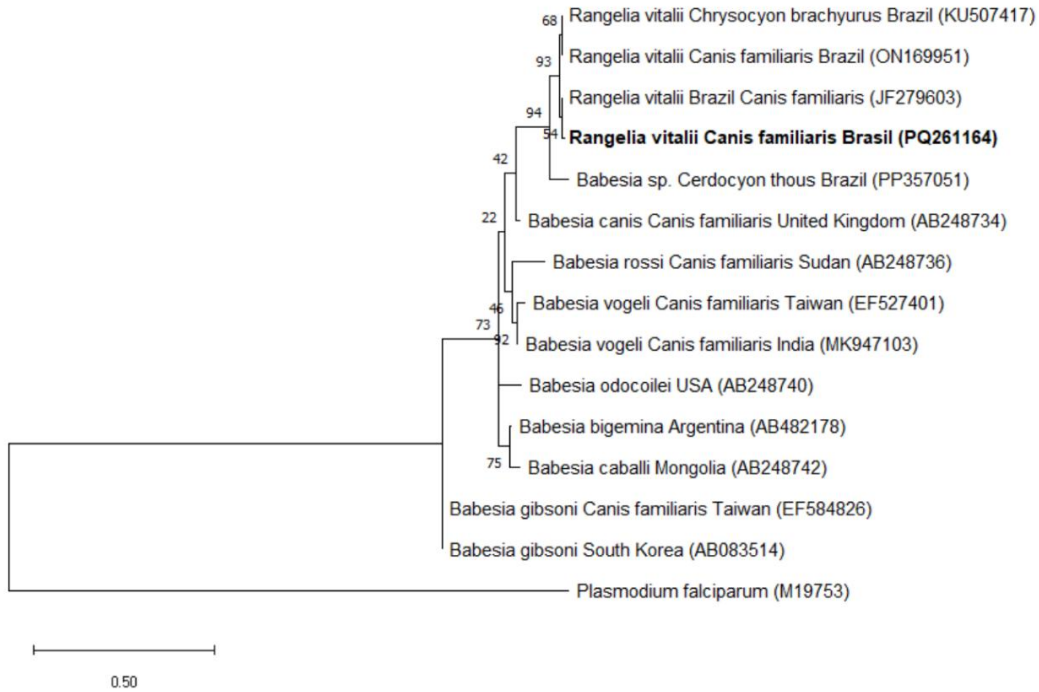

Figure 2. Phylogenetic tree based on a 614-bp alignment of the *hsp70* gene of Piroplasmida, encompassing 21 nucleotide sequences, constructed using the Maximum Likelihood (ML) method and the K2+G+I evolutionary model. Bootstrap values from 1,000 replicates are indicated at the nodes; values below 50 are not shown. The scale bar represents evolutionary distance. The sequence detected in the present study is highlighted in bold, with the GenBank accession number in parentheses. *Plasmodium falciparum* was used as the outgroup.

Supportive therapy was instituted in combination with the administration of imidocarb dipropionate (6mg/kg, subcutaneously), with a repeat dose after 15 days. Complete resolution of clinical signs and hematological abnormalities was observed 49 days after hospital admission.

DISCUSSION

In Brazil, *R. vitalii* infection in dogs has been reported in the states of Rio Grande do Sul (Lorenzo *et al*., 2021), Paraná (Silva *et al*., 2019a), Santa Catarina (Carniel *et al*., 2025), São Paulo (Batista *et al*., 2023), and Rio de Janeiro (Palmer *et al*., 2024). Reports from the state of Minas Gerais are scarce. Moreira *et al*. (2013) described a post-mortem diagnosis of rangeliosis in a dog from the municipality of Caeté; however, the animal's history was limited. In another report, Silveira *et al*. (2016) presented post-mortem findings of the disease in a free-ranging maned wolf (*Chrysocyon brachyurus*) rescued from a peri-urban area in the municipality of Rio Acima. These data, together with the case reported here, highlight the potential underdiagnosis of this hemopathogen in Minas Gerais, where the presence of the vector *A. aureolatum* is recognized (Rodrigues *et al*., 2002). Notably, the municipality of Nova Lima, where the patient resided, is only 17 km from

Rio Acima and 45 km from Caeté, all located within the metropolitan region of Belo Horizonte. This area represents a transition zone between the Cerrado and Atlantic Forest biomes, with an average altitude of 722 m and peaks reaching 1,583 m (Werneck *et al*., 2010). Importantly, in the present case, there was no history of travel to endemic areas, suggesting a possible autochthonous infection.

Rangeliosis is frequently reported in mixed-breed dogs from peri-urban or rural environments. In the present case, the patient, a French Bulldog, lived in an apartment but had a history of access to areas with native vegetation, which may represent a risk factor for the disease (Silva *et al*., 2019a; Palmer *et al*., 2024). Soares *et al*. (2018) demonstrated that exposure to forested areas and potential contact with wild animals can contribute to *R. vitalii* infection in dogs. In this context, it is important to emphasize that unplanned urban expansion may facilitate the emergence of wildlife-associated diseases, such as rangeliosis, by increasing contact between humans, domestic animals, wildlife, and vectors.

Although the owner denied tick infestation, certain nonspecific clinical signs presented by the patient reinforced the suspicion of infection by ixodid-transmitted hemopathogens, allowing the establishment of a diagnostic plan (Carniel *et al*., 2025). Coinfection with the hemoparasites *R. vitalii* and *Mycoplasma* spp., as observed in the present case, may exacerbate the clinical condition, intensifying anemia, thrombocytopenia, and systemic signs.

Compared to other hemopathogens affecting dogs, *R. vitalii* is recognized for causing severe disease (Soares *et al*., 2014). The severity of the clinical presentation and the opportunity to discuss the case with parasitologists in an academic setting were crucial for including rangeliosis as a differential diagnosis. This highlights the need to disseminate information about the disease even in areas considered non-endemic, facilitating appropriate treatment of affected animals and supporting animal health surveillance actions.

Blood smears are valuable tools for confirming the presence of piroplasm. In the case of *R. vitalii*, intracellular forms can be observed five days post-exposure, with a progressive increase in parasitemia until the eleventh day (Paim *et al*., 2012). In the present study, the hemoprotozoan was microscopically identified as intra- and extraerythrocytic piriform parasitic forms. However, according to Carniel *et al*. (2025), *Rangelia vitalii* is observed in blood smears of only approximately 61,1% of naturally infected dogs.

Blood smear evaluations may be insufficient for the diagnosis of rangeliosis. This is because *R. vitalii* is genetically related to hemoprotozoans of the genus *Babesia*, with which it shares similar morphology under light microscopy (Soares *et al*., 2011). Distinction between *R. vitalii* and *Babesia* species with merozoites >2.5 μm is only possible microscopically when the protozoan is identified in non-erythrocytic cells, such as leukocytes or endothelial cells, which was not observed in the present case.

In the present study, the diagnosis of rangeliosis was only possible through molecular analysis followed by nucleotide sequencing. Phylogenetic analysis placed the obtained sequence within a well-supported clade (bootstrap = 94) alongside other *R. vitalii* strains previously identified in *C. familiaris*, *C. brachyurus*, and *C. thous* from Brazil. This highly supported grouping suggests a close evolutionary relationship among the isolates, regardless of the domestic or wild origin of the hosts. Such a phylogenetic pattern reinforces the hypothesis of potential ecological interactions between domestic dogs and wild canids, facilitating vector sharing and, consequently, bidirectional pathogen flow between distinct populations.

CONCLUSION

Rangeliosis should be considered in the differential diagnosis of dogs presenting with anemia, jaundice, and fever in the state of Minas Gerais, Brazil, where the disease is not yet recognized as endemic despite favorable conditions for the tick vector. This report describes the first confirmed antemortem, autochthonous diagnosis of rangeliosis in a dog from this state, supporting the hypothesis of either geographic expansion of the parasite or underdiagnosis of the infection in the region.

FUNDING

This study was financed by scholarships from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), financial support for the field, lab work and Research productivity grant from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (315466/2021-9; CNPq/SECTICS/ CAPES/FAPs no 46/2024 and 308527/2025-9), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) (APQ-02531–24).

CONTRIBUTORS

ALM Ribeiro: Conceptualization, Investigation, Methodology, Data curation, Writing – original draft. PHC Rodrigues: Investigation, Methodology, Data curation, Writing – original draft. L Fachini: Investigation, Methodology. JPS Alves: Investigation, Methodology. AC Nepomuceno: Methodology, Formal analysis. JAG da Silveira: Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Supervision, Writing – review & editing. LED de Oliveira: Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Supervision, Writing – review & editing.

DATA AVAILABILITY STATEMENT

The research data are available upon request.

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Editor-chefe: Marcelo Resende de Souza
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