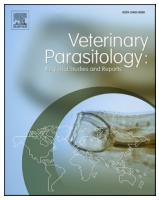
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Original Article



First report of canine rangeliosis in domestic dogs from different regions of Santa Catarina State, Brazil

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A R T I C L E I N F O

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A B S T R A C T

Rangeliosis is the disease caused by *Rangelia vitalii*, a parasite reported in dogs from southeastern and southern Brazil, Uruguay, Paraguay, and Argentina. This protozoan is transmitted by the ixodid *Amblyomma aureolatum*, and infects erythrocytes, leukocytes, and vascular endothelial cells of the host. The common clinical signs, such as prostration, fever, anemia, thrombocytopenia, anorexia, weight loss, and dehydration, are also found in other infections, like canine babesiosis and ehrlichiosis. The similar clinical presentation with other diseases, as well as the indistinguishable morphology with intraerythrocytic *Babesia canis*, complicates the disease diagnostic. In the present study, blood samples from dogs presenting clinical signs compatible with hemoparasitosis were investigated for rangeliosis. The dogs were treated at veterinary clinics in the cities of Blumenau and Lages, in the State of Santa Catarina, Brazil. Blood samples from 17 dogs were analyzed by PCR. The samples were screened by a conventional piroplasma-PCR and the positives confirmed by a specific *R. vitalii*-qPCR. Two animals (2/17; 11.8%) were positive for *R. vitalii*, one from Blumenau and the other from Lages. Both animals presented unspecific signs of hemoparasitosis, such as apathy, anemia, and anorexia. The results indicate the necessity of molecular assays for the proper identification of the hemoparasite, and to investigate the real prevalence of rangeliosis in the State of Santa Catarina.

1. Introduction

Hemoparasitosis are among the most important and common infectious diseases in veterinary practice. In dogs, the most frequent hemoparasites found in Brazil are bacteria of the genus *Ehrlichia* and protozoa of the genus *Babesia*, both transmitted by the tick *Rhipicephalus sanguineus* sensu lato (Almosny, 2002; Vieira et al., 2011).

Dogs with ehrlichiosis or babesiosis present clinical signs of apathy, anemia, fever, and thrombocytopenia, which may vary in intensity. Animals infected by protozoan hemoparasites may also present jaundice, because of intra- and extravascular hemolysis. Due to the similarity between the clinical signs caused by these agents, the differential diagnosis based only on clinical examination is almost impossible (Almosny, 2002; Irwin, 2009; Fighera et al., 2010; Harrus and Waner, 2011).

Rangelia vitalii is a reemerging canine hemoparasite, which has been reported in domestic dogs from rural and peri-rural areas in the southeast and south regions of Brazil, but also in Argentina, Uruguay, and Paraguay (Pestana, 1910; Loretti and Barros, 2005; Soares et al., 2011; Correa et al., 2012; Eiras et al., 2014; Soares et al., 2015; Borrás et al., 2019; Inácio et al., 2019). Additionally, this protozoan was identified in wild canids from southern Brazil and Uruguay (Soares et al., 2014; Fredo et al., 2015; Quadros et al., 2015; Silveira et al., 2016; Lorenzo et al., 2021).

The most common clinical signs of canine rangeliosis are apathy, anorexia, anemia, jaundice, and spontaneous bleeding of extremities, such as ears, snout, and paws. The Guaraní communities call it *nambyuvú*, which means “bleeding ears”. The infected dogs present splenomegaly, and hemolytic anemia with signs of erythroid regeneration may be detected in the hemogram (Fighera et al., 2010; Silva et al., 2011; Fredo et al., 2015). After the appearance of regenerative anemia, the agent can be found in lymph nodes, spleen, bone marrow and heart

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(Fighera et al., 2010).

R. vitalii was initially confounded with other protozoa, as it may infect different cell types, like erythrocytes, monocytes, neutrophils, and capillary endothelial cells (Fighera et al., 2010; Soares et al., 2018). The intra-erythrocytic stage is morphologically indistinguishable from *Babesia vogeli*, with the typical pear-shaped or rounded cells, but despite of being phylogenetically close to *Babesia* genus, it is indeed a different species (Soares et al., 2011). The intra-leucocytic and intra-endothelial stages have been misidentified as *Leishmania* sp. and *Toxoplasma gondii*, respectively, and hence molecular analyses are deemed necessary for its correct identification (Fighera et al., 2010; Soares et al., 2011).

Recently, it was demonstrated that *R. vitalli* can be transmitted to dogs by the tick *Amblyomma aureolatum*, but not by *Rhipicephalus sanguineus* sensu lato, *Amblyomma ovale*, *Amblyomma tigrinum*, and *Amblyomma sculptum*, suggesting that *A. aureolatum* is the natural vector of this protozoan. This is the first protozoan transmitted vertically in *Amblyomma* ticks, as *R. vitalli* is passed by transstadial and transovarial routes, indicating a very long co-evolution between these parasites (Soares et al., 2018).

A. aureolatum adult ticks are mainly found in wild carnivores, specially from Canidae family (Guglielmone et al., 2003). In the state of Santa Catarina, Brazil, *A. aureolatum* was found parasitizing dogs in rural areas of the Serrano Plateau and Itajaí Valley mesoregions, putting these canines at risk of being infected by *R. vitalii* (Bellato et al., 2003; Lavina et al., 2014; Soares et al., 2014).

There is a lack of information about canine rangeliosis in the state of Santa Catarina and, to this date, there is no confirmation of the occurrence of such disease in domestic dogs in the municipalities of Blumenau (Itajaí Valley mesoregion) and Lages (Serrano Plateau mesoregion). Considering the severity of this disease, the presence of the tick-vector *A. aureolatum* in these regions, and the little information about canine hemoparasites in Santa Catarina, the objective of this work was to verify the occurrence of *R. vitalii* in dogs that presented clinical signs of hemoparasitosis, treated at veterinary clinics in Blumenau and Lages (Santa Catarina, Brazil).

2. Material and methods

This investigation was approved by the Ethics Committee on the Use of Animals (CEUA), of the Centro de Ciências Agroveterinárias, Santa Catarina State University under protocol number 2474060516.

2.1. Sample and data collection

Blood samples from dogs presenting clinical signs of hemoparasitosis were obtained during clinical attendance in Veterinary Clinics from the municipalities of Blumenau and Lages, in the mesoregions of Itajaí Valley and Serrano Plateau, respectively, Santa Catarina state, Brazil. Samples were collected from 2017 to 2018.

The information about gender, age, breed, address, type of food, number of canines in the property, whether the animal is free or enclosed in a certain area of the property, whether it moves between urban and rural areas, as well as the observation or history of parasitism by ticks were obtained during the anamnesis.

A blood sample was collected by venipuncture of the cephalic vein, in a tube containing EDTA. The hemogram, biochemical and blood smear analysis were carried out just after collection, and an aliquot was kept frozen at −20 °C until the time of molecular testing.

2.2. Molecular investigation protocols

The DNA extraction from the blood was performed individually using the Purelink Genomic DNA Mini Kit® (Invitrogen®), following the manufacturer's protocol.

The samples were screened by a conventional PCR (BAB2), using the generic primers BAB-143-167 (5′-CCGTGCTAATTGTAGGGCTAATACA-3′) and BAB-694-667 (5′-GCTTGAAACACTCTARTTTTCTCAAAG-3′), which amplify a fragment of approximately 551 bp of the 18S rRNA gene from piroplasmids (Soares et al., 2011).

Positive samples were then tested in a specific protocol for detection of *R. vitalii* using a qPCR protocol designed to amplify a 180 bp fragment of the *R. vitalii* HSP70 gene (Soares et al., 2018). The Taqman Real-Time PCR protocol was carried out with forward and reverse primers Rv751–770 (5′- GCGTATCCCGAAGATTCAAA-3′) and Rv930–911 (5′-AGT GAA AGC GGT GCA ACA TC-3′), respectively, and the probe 772–790 [5′-6- FAM (CCTTATCAAATCATTCTTC) MGB NFQ −3′].

Additionally, the samples were investigated for a possible infection by *E. canis*, using a specific qPCR protocol, with the forward and reverse primers dsb-321 (5′–TTGCAAAATGATGTCTGAAGATATGAAACA–3′) and dsb-671 (5′–CTGCTCAACCAAGAAATGTATCCCCTA–3′), respectively, and the probe *E. canis* - specific (5′–FAM AGCTAGTGCTGCTTGGGCAACTTTGAGTGAA–BHQ–1–3′) (Doyle et al., 2005).

3. Results and discussion

The origin of the animals (n = 17) presenting clinical signs of hemoparasitosis attended in Veterinary Clinics from Santa Catarina, Brazil, is shown in Table 1.

The clinical signs detected in the attended dogs suspected of hemoparasitosis are presented in Table 2. The most frequent clinical sign was anemia, detected by pale mucous membranes and low red blood counts (below 5.5 × 10^6/μl; data not shown) verified in 47.1% (8/17) of the animals.

All the tested samples were negative for *E. canis*. Three dogs (3/17; 17.6%) were positive in the generic PCR for piroplasmids. From these animals, two (2/17; 11.8%) were positive in the specific qPCR for *R. vitalii*, one dog from Blumenau and one from Lages. All the data collected from the studied animals are compiled and presented in Table 3.

The *R. vitalii*-positive dog from Blumenau was a three-years old mixed breed male, living in the rural area of the municipality. During the clinical examination, the dog presented severe prostration, anemia, and jaundice. The owner reported that the animal was anorexic, had free access to forest, rural and urban areas, and has been parasitized by ticks; however, no specimens were found during the clinical examination. Blood count revealed normocytic normochromic anemia, thrombocytopenia, and leukocytosis due to neutrophilia. The blood serum was intensely jaundiced, and the serum biochemistry showed an increase in alkaline phosphatase. No hemoparasites were found in the blood smear. The animal received fluid therapy (500 mL of Ringer's solution, 5 mL/kg/h IV) with atropine sulfate (0.04 mg/kg IV), 15 min before the application of a single dose of imidocarb dipropionate (5 mg/kg IM). The treatment with doxycycline hydrochloride (5 mg/kg BID PO) was also implemented, but the dog died three days after the start of treatment.

The second *R. vitalii*-positive dog was a one-year-old mixed breed female from the urban area of Lages. The animal presented signs of anemia, jaundice, loose stools and prostration. Splenomegaly was detected in ultrasound examination. The owner reported anorexia, and the presence of ticks in the animal's ear one week before the examination, but no specimens were found during the physical inspection. Also,

Table 1
Geographical origin of samples from canines suspected of hemoparasitosis treated at Veterinary Clinics in Santa Catarina, Brazil.

Municipality	Region		Total
	Rural	Urban	
Blumenau	1 (16,6%)	5 (83,4%)	6
Lages	0	11 (100%)	11
Total	1 (5,9%)	16 (94,1%)	17

Table 2
Clinical signs and parasitism observed in dogs attended at Veterinary Clinics in Santa Catarina, Brazil, with signs of hemoparasitosis.

Clinical signs or parasitism	n (%)
Anemia + epistaxis	1 (5,9%)
Anemia + ear bleeding	1 (5,9%)
Anemia	4 (23,5%)
Anemia + apathy + anorexia	1 (5,9%)
Anemia + jaundice	1 (5,9%)
Anorexia + jaundice	1 (5,9%)
Apathy + anorexia	1 (5,9%)
Apathy	2 (11,7%)
Epistaxis	1 (5,9%)
Jaundice	1 (5,9%)
Ticks' infestation	1 (5,9%)
Ear bleeding	2 (11,7%)
Total	17

it was a free-roaming dog, with reported access to rural and forest areas. Two blood counts and blood smear analysis were carried out with this dog, with the exams being repeated two days after the initial attendance. Normocytic normochromic anemia, macrothrombocytopenia, and neutropenic leukopenia were observed in the first hemogram, as well as the presence of activated macrophages, with erythrophagocytosis and leukophagocytosis. In the second blood count, hypochromic macrocytic anemia and intensified thrombocytopenia were detected. No hemoparasites were detected in the blood smears. The serum was moderately jaundiced, but all other biochemical markers were normal. The treatment with doxycycline hydrochloride (5 mg/kg BID PO) for 28 days, and dipyrone (25 mg/kg TID SC) for seven days was instituted. The animal was discharged and, according to the owners, had a good recovery.

Rangelia vitalii parasitism in wild and domestic canids has been reported in southern Brazil and other South American countries (Eiras et al., 2014; Soares et al., 2014; Fredo et al., 2015; Quadros et al., 2015; Soares et al., 2015). This is the first confirmation of canine rangeliosis in the Itajaí Valley and the Serrano Plateau mesoregions, Santa Catarina, Brazil. In the present study, it was possible to verify compatible clinical signs in domestic dogs attended in Veterinary Clinics from Blumenau and Lages, with the verification by qPCR, a very sensitive and specific test (Soares et al., 2018).

Rangeliosis is a disease that most frequently affects young dogs, up to five-years old, as reported initially by Pestana (1910) and corroborated by other authors (França et al., 2010; Correa et al., 2012). The positive animals from Lages and Blumenau also fit into this age group, however, older dogs can also be infected, as a 12-year-old mixed bred bitch that tested positive in Argentina (Eiras et al., 2014). Jaundice, anemia, and anorexia are frequent clinical signs detected in dogs infected by *R. vitalii* (Fighera et al., 2010), as well as ear bleeding and melena (Fighera et al., 2010). In the present study, both infected dogs presented jaundice, anemia, and anorexia, however, they did not show ear bleeding or melena.

A. aureolatum is the main vector of *R. vitalii* (Soares et al., 2018), and hence its presence in the environment is directly related with the occurrence of the protozoan. This species is found primarily in forests, within sylvatic environment (Guglielmone et al., 2003; Loretti and Barros, 2005; Soares et al., 2018). In the geographical regions of origin of the positive animals investigated in the present study, this ixodid has already been observed parasitizing domestic dogs (Bellato et al., 2003; Lavina et al., 2014) and wild canids (Quadros et al., 2015). The positive dogs from this study did not present ticks during the physical examination, but both had history of parasitism, as reported by their owners. Additionally, their owners also confirmed that the dogs had access to forests, as they were free-roaming-dogs and therefore they might have been parasitized by *A. aureolatum*. History of parasitism and contact with forests are risk factors for *R. vitalii* infection (Loretti and Barros, 2005).

4. Conclusion

Animals affected by rangeliosis may only present general signs of hemoparasitosis, like anorexia, anemia, and jaundice, and not the *Rangelia*-related spontaneous bleedings. It may lead to inaccurate diagnosis of this disease, a false negative, if investigated solely by clinical examination and hemogram/biochemical analysis. The present work demonstrates the importance of molecular investigation for the diagnosis of *R. vitalli* infection, and the routine use of this technique should be encouraged. This is the first report of canine rangeliosis in domestic dogs from Santa Catarina, Brazil, and due to the scarcity of epidemiological data on canine rangeliosis in this state, further studies of this nature should be carried out in order to evaluate the real frequency of such important parasitosis in this and other regions, with the objective to inform Veterinary professionals about the disease, for appropriate treatment.

Ethical statement

This investigation was approved by the Ethics Committee on the Use of Animals (CEUA), of the Agroveterinary Sciences Centre, Santa Catarina State University under protocol number 2474060516.

Conflict of interest

The aforementioned authors declare that there is no conflict of

Table 3
Individual data from the dogs suspected of hemoparasitosis, attended at Veterinary Clinics in Santa Catarina, Brazil.

Animal	Gender	Age (years)	Breed	Observed signs and parasitism	Piroplasmid PCR result	Municipality	Area of origin
1	Female	2	Saint Bernard	Anemia, epistaxis	Negative	Lages	Urban
2	Male	4	Mixed breed	Anemia, bleeding	Negative	Lages	Urban
3	Female	10	Mixed breed	Anemia	Negative	Blumenau	Urban
4	Female	2	Yorkshire	Anemia	Negative	Lages	Urban
5	Male	17	Pinscher	Anemia	Negative	Lages	Urban
6	Male	5	English Pointer	Anemia	Negative	Lages	Urban
7	Male	1	Maltese	Anemia, apathy, anorexia	Negative	Lages	Urban
8	Female	1	Mixed breed	Anemia, jaundice	Positive for *Rangelia vitalli*	Lages	Urban
9	Male	3	Mixed breed	Anorexia, jaundice	Positive for *Rangelia vitalli*	Blumenau	Rural
10	Male	10	Shih-Tzu	Apathy, anorexia	Negative	Blumenau	Urban
11	Female	10	Yorkshire	Apathy	Positive for piroplasmid	Blumenau	Urban
12	Female	12	Mixed breed	Apathy	Negative	Blumenau	Urban
13	Female	15	Mixed breed	Epistaxis	Negative	Lages	Urban
14	Female	4	Mixed breed	Jaundice	Negative	Lages	Urban
15	Male	2	German Shepherd	Ticks' infestation	Negative	Blumenau	Urban
16	Male	2	Mixed breed	Ear bleeding	Negative	Lages	Urban
17	Male	3	Pit Bull Terrier	Ear bleeding	Negative	Lages	Urban

interest in the production of this manuscript.

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