



Natural infection of the wild canid, *Cerdocyon thous*, with the piroplasmid *Rangelia vitalii* in Brazil

João F. Soares^{a,1}, Bruno Dall'Agnol^{b,1}, Francisco B. Costa^a, Felipe S. Krawczak^a, Alexandra T. Comerlato^c, Bruna C.D. Rossato^d, Camila M. Linck^d, Eduardo K.O. Sigahi^e, Rodrigo H.F. Teixeira^c, Luciana Sonne^f, Mitika K. Hagiwara^a, Fabio Gregori^a, Maria Isabel B. Vieira^d, João R. Martins^b, José Reck^b, Marcelo B. Labruna^{a,*}

^a Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Av. Prof. Orlando Marques de Paiva 87, São Paulo, SP, Brazil

^b Instituto de Pesquisas Veterinárias Desidério Finamor, Fundação Estadual de Pesquisa Agropecuária, Eldorado do Sul, RS, Brazil

^c Parque Zoológico Municipal "Quinzinho de Barros", Soarocaba, SP, Brazil

^d Faculdade de Agronomia e Medicina Veterinária, Universidade de Passo Fundo, Passo Fundo, RS, Brazil

^e Departamento de Vigilância em Saúde, Secretaria de Saúde, Prefeitura Municipal de Mogi das Cruzes, SP, Brazil

^f Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:

Received 20 January 2014

Received in revised form 18 February 2014

Accepted 26 February 2014

Keywords:

Rangelia vitalii

Piroplasm

Cerdocyon thous

Domestic dog

Babesia

Brazil

ABSTRACT

Canine rangelioidosis, caused by the piroplasmid protozoan *Rangelia vitalii*, is currently recognized as a reemerging disease that affects domestic dogs in Brazil. In the present study, piroplasmid infection was searched in wild canids (20 *Cerdocyon thous* and 4 *Lycalopex gymnocercus*) in Brazil. Molecular analysis, based on PCR and DNA sequencing of a portion of the 18S rRNA gene, revealed that 30% (6/20) *C. thous* were infected by *R. vitalii*. Blood and bone marrow samples from one of the *R. vitalii*-infected *C. thous* were inoculated into a domestic dog, which developed clinical rangelioidosis that was confirmed by molecular tests. However, the *C. thous* donor showed no clinical, hematological or biochemical alterations, even though its *R. vitalii* infection status was confirmed for at least 80 days. These observations suggest that *R. vitalii* is not as highly pathogenic for *C. thous* as it is for domestic dogs. Phylogenetic analysis inferred by the 18S rRNA gene placed *R. vitalii* embedded in the clade '*Babesia sensu stricto*', consisting of a number of species that represent truly the genus *Babesia*. It is proposed that the species *R. vitalii* should be transferred to the genus *Babesia*. The present study expands our knowledge on the natural history of *R. vitalii*, suggesting that it might have a natural cycle involving the wild canid *C. thous*. Further studies are needed to confirm that *C. thous* is a natural reservoir of *R. vitalii* in Brazil.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Piroplasmids are tick-borne protozoan parasites that infect blood cells of numerous wild and domestic

vertebrates worldwide. The piroplasmid *Rangelia vitalii* is the etiologic agent of rangelioidosis, a canine disease that was described in the beginning of the previous century in the state of São Paulo, southeastern Brazil (Pestana, 1910; Carini and Maciel, 1914). Because a number of subsequent authors (Wenyon, 1926; Doflein and Reichenow, 1929; Moreira, 1938, 1939; Levine, 1973; Peirce, 2000) considered *R. vitalii* a synonym of *Babesia vogeli* (reported as *Babesia canis*), rangelioidosis was widely neglected during the

* Corresponding author. Tel.: +55 11 3091 1394; fax: +55 11 3091 7928.

E-mail address: labruna@usp.br (M.B. Labruna).

¹ These authors contributed equally to this work.

second half of the 20th century. During the last decade, a number publications from southern Brazil highlighted *R. vitalii* as a reemerging agent of a severe canine piroplasmosis, especially among rural dogs (Krauspenhar et al., 2003; Loretto and Barros, 2005; Figuera, 2007; Figuera et al., 2008, 2010; França et al., 2010). In 2011, the validity of the taxon *R. vitalii* was proposed by molecular methods based on phylogenetic analyses inferred by partial sequences of the 18S rRNA and *hsp70* genes (Soares et al., 2011). The infection by *R. vitalii* has been confirmed solely in domestic dogs from southern and southeastern Brazil (Soares et al., 2011, 2013a,b; Lemos et al., 2012).

The Brazilian fauna of wild canids is composed by six native species (Cheida et al., 2011). Literature records on piroplasmids infecting these native canids are very scarce, and have been based solely on morphological identification of intraerythrocytic piroplasmid forms in blood smears from *Chrysocyon brachyurus* (Serra-Freire et al., 1995; Cansi et al., 2012), *Lycalopex vetulus* (Martins et al., 2006), *Lycalopex gymnocercus* (Ruas et al., 2003), and *Cerdocyon thous* (Paraense and Vianna, 1948; Massard et al., 1981). The crab-eating fox, *C. thous*, is widely distributed in South America, from Uruguay and northern Argentina to the lowlands of Bolivia and Venezuela, also occurring in Colombia, Guyana and Suriname. In Brazil, it is found in all major biomes except for the Amazon (Cheida et al., 2011). The Pampas fox, *L. gymnocercus*, has a more restricted distribution, occurring in the southern cone of South America, except for Chile (Cheida et al., 2011). In the present study, piroplasmid infection was searched for in *C. thous* and *L. gymnocercus* from Brazil. We report the first molecular detection of a piroplasmid agent in South American free-ranging wild canids, as well as the effects of the inoculation of this agent in a domestic dog.

2. Materials and Methods

2.1. Wild canids

In May 2012, a free-ranging, young, adult female (approximately 5 kg) of *C. thous* (animal #1) was rescued in Carazinho Municipality, state of Rio Grande do Sul, southern Brazil, and taken to the nearby Veterinary Teaching Hospital of the University of Passo Fundo due to a traumatic amputating injury in the hind limb. Clinical evaluation was performed, and blood samples collected 0, 69, and 80 days after admission were used for molecular detection for piroplasmids (described below), whereas blood samples collected in both dry and EDTA tubes at the 21st day were used for biochemical and hematological evaluations, respectively. At the 80th day, the animal died of unknown etiology; bone marrow aspirates and spleen samples were collected.

From July 2012 to August 2013, tissue samples (blood or internal organs) were collected from 23 wild canids from different areas of the states of Rio Grande do Sul and São Paulo (southeastern Brazil) (Table 1). Animal #2 was rescued due to trauma caused by fighting with domestic dogs; animals #3–13 and 21–23 were road-killed, whereas animals #14–20, and 24, were sampled in captivity at zoos. From road-killed animals, the collected material varied

accordingly to the availability of tissues found in the carcasses. From living animals sampled at the zoos, only blood in EDTA was collected.

2.2. Inoculation of domestic dog

Three ml of bone marrow aspiration and 10 ml of blood was collected from *C. thous* #1 on the 80th day after its admission to the hospital, and sent to the University of São Paulo under refrigeration. Twenty hours later, the samples were intravenously inoculated into an 8-month domestic dog, derived from our Beagle experimental kennel, where dogs have never experienced tick infestations, are regularly vaccinated and dewormed, and are regularly tested to certify that they are free of tick-borne diseases. The domestic dog was clinically evaluated and had its rectal temperature measured daily, as well as blood samples taken in EDTA-tubes 3 times per week for blood cell and platelet counts, and PCR targeting piroplasmids during a period of 32 days after inoculation. An additional blood collection at 69 days post-inoculation (dpi) was used for PCR analysis. During this period, the dog was kept in a room with no environmental contamination with ticks.

2.3. Molecular and phylogenetic analyses

Animal blood or tissue samples were subjected to DNA extraction using the DNeasy Blood & Tissue Kit (Qiagen®, Hilden, Germany), according to the manufacturer's instructions. All DNA samples from wild canids were initially tested by one of the following two PCR assays, one targeting a ~700-bp fragment of the Carnivore mitochondrial DNA (mtDNA) control region containing the first hypervariable segment (HVS-I), using primers MTLPRO2 and CCR-DR1, as previously described (Tchaicka et al., 2007); or a PCR assay targeting a 359-bp fragment of the vertebrate mitochondrial cytochrome b gene (*cyt b*), as previously described (Steuber et al., 2005). For detection of piroplasmids, all samples were tested by a PCR protocol using primers BAB143-167 and BAB694-667, targeting a ~500-bp fragment of the piroplasmid 18S rRNA gene, as previously described (Soares et al., 2011). PCR products were electrophoresed through a 1.5% agarose gel, stained with ethidium bromide, and examined by UV transillumination. Amplicons of the expected size were purified with ExoSap (USB, Cleveland, OH) and sequenced in an automatic sequencer (Applied Biosystems/PerkinElmer, model ABI Prism 310 Genetic, Foster City, CA) according to the manufacturer's protocol. Generated sequences were submitted to BLAST analysis (Altschul et al., 1990) to determine the closest similarities in GenBank.

Partial sequences (549-nt) of the 18S rRNA gene of piroplasmids derived from the wild canids were aligned with corresponding 18S rRNA sequences from 53 genotypes of the genera *Babesia*, *Theileria*, *Cytauxzoon* and *Hepatozoon* retrieved from Genbank, using Clustal/W v.1.8.1 (Thompson et al., 1994). A maximum likelihood phylogenetic tree using GTR+G+I substitution model was generated using Mega 5.2.2 software (Tamura et al., 2011) with 100 bootstrap replicates. The substitution model was select using Mega 5.2.2 software (Tamura et al., 2011) according

Table 1Wild canids (*Cerdocyon thous* and *Lycalopex gymnocercus*) sampled in the states of Rio Grande do Sul (RS) and São Paulo (SP), and tested by PCR for the presence of *Rangelia vitalii* DNA.

Animal number	Species	Origin				Animal condition	Collected samples tested by PCR	PCR result	
			Municipality	Coordinates					Elevation (m)
				South	West				
1	<i>C. thous</i>	Carazinho, RS	28°17'	52°47'	603	Rescued due to trauma	Blood Bone marrow Spleen Liver	+ + + +	
2	<i>C. thous</i>	Viamão, RS	30°03'	51°00'	60	Rescued due to trauma			
3	<i>C. thous</i>	Cachoeira do Sul, RS	30°02'	52°53'	26	Road-killed	Carotid Muscle	+ —	
4	<i>C. thous</i>	Cachoeira do Sul, RS	30°02'	52°53'	26	Road-killed	Lung Carotid	+ +	
5	<i>C. thous</i>	Cachoeira do Sul, RS	30°02'	52°53'38"	26	Road-killed	Lung Liver	+ —	
6	<i>C. thous</i>	Itaqui, RS	29°06'46"	56°17'30"	66	Road-killed	Muscle	—	
7	<i>C. thous</i>	Restinga Seca, RS	29°48'	53°22'	49	Road-killed	Lung	—	
8	<i>C. thous</i>	São Borja, RS	28°38'23"	55°50'00"	69	Road-killed	Muscle	—	
9	<i>C. thous</i>	São Luiz Gonzaga, RS	28°24'	54°57'	251	Road-killed	Muscle	—	
10	<i>C. thous</i>	Mogi das Cruzes, SP	23°31'	46°11'	780	Road-killed	Lymph node Carotid Spinal marrow	+ + —	
11	<i>C. thous</i>	Botucatu, SP	22°53'	48°26'	804	Road-killed	Spleen	—	
12	<i>C. thous</i>	Sorocaba, SP	23°30'	47°27'	600	Road-killed	Spleen	—	
13	<i>C. thous</i>	Sorocaba, SP	23°30'	47°27'	600	Road-killed	Spleen	—	
14	<i>C. thous</i>	Sorocaba, SP	23°30'19"	47°26'15"	580	Zoo	Blood	—	
15	<i>C. thous</i>	Sorocaba, SP	23°30'19"	47°26'15"	580	Zoo	Blood	—	
16	<i>C. thous</i>	Sorocaba, SP	23°30'19"	47°26'15"	580	Zoo	Blood	—	
17	<i>C. thous</i>	Sorocaba, SP	23°30'19"	47°26'15"	580	Zoo	Blood	—	
18	<i>C. thous</i>	Sorocaba, SP	23°30'19"	47°26'15"	580	Zoo	Blood	—	
19	<i>C. thous</i>	Sorocaba, SP	23°30'19"	47°26'15"	580	Zoo	Blood	—	
20	<i>C. thous</i>	Sorocaba, SP	23°30'19"	47°26'15"	580	Zoo	Blood	—	
21	<i>L. gymnocercus</i>	Cachoeira do Sul, RS	30°02'	52°53'	26	Road-killed	Liver	—	
22	<i>L. gymnocercus</i>	Cachoeira do Sul, RS	30°02'	52°53'	26	Road-killed	Heart Liver	— —	
23	<i>L. gymnocercus</i>	Uruguaiana, RS	29°35'13"	56°51'36"	65	Road-killed	Blood Liver Heart	— — —	
24	<i>L. gymnocercus</i>	Cachoeira do Sul, RS	30°02'	52°53'	26	Zoo	Blood	—	

+: positive; —: negative; RS: Rio Grande do Sul; SP: São Paulo.

to the lowest Bayesian Information Criterion (BIC) score. The sequence of *Hepatozoon canis* was used as outgroup.

2.4. Hematological and biochemical evaluation

EDTA-blood samples were processed within 24 h after collection. Blood serum samples were separated by centrifugation and kept at -20°C until use. Packed cell volume, hemoglobin, erythrocytes, leukocytes and platelets were determined in an automatic hematology analyzer (bc2800 – Mindray, France). Differential leukocyte count was performed on blood smears stained with Rosenfeld staining (Rosenfeld, 1947). Blood smears were prepared within 10 min after blood collection. Biochemical analyses were determined in an automatic analyzer (Labmax240, Japan) using the commercial kits Randox® (serum urea, total protein, and albumin); Labtest® (serum creatinine) and Byosystems® (ALT, FA, and bilirubin). The parameters reported by Gomes (2006) and Feldman et al. (2000a,b) were taken as references values for *C. thous* and domestic dogs, respectively.

2.5. Ethics statements

This work was authorized by the ICMbio Cetas (state of Rio Grande do Sul), and was approved by the Ethical Committee of Animal Use of the Faculty of Veterinary Medicine of the University of São Paulo (protocol 2248/2011).

3. Results

3.1. Wild canids

The *C. thous* #1 (Table 1) was found infested by *Amblyomma aureolatum* adult ticks, but had no clinical signs of tick-borne diseases; fever, anemia, lymphadenopathy, skin hemorrhagic lesions, or jaundice were not observed. In addition, hematological and biochemical analyses performed on day 21 showed no abnormalities, apart from a slight increase in total protein (Table 2). Blood samples collected on 0 and 69 days after admission, and bone marrow and spleen samples collected on day 80 were PCR-positive for piroplasmids.

Regarding the remaining wild canids (animals #2 to #24), 5 out of 19 *C. thous* were PCR-positive for piroplasmids, whereas the 4 *L. gymnocercus* were negative. Overall, 30% (6/20) *C. thous* were PCR-positive. Considering only the free-ranging *C. thous*, 46.2% (6/13) were PCR-positive, since all captive animals were PCR-negative. Amplicons generated in all piroplasma-PCR assays were DNA sequenced, and showed to be 99–100% identical to corresponding 18S rRNA sequences of *R. vitalii* available in GenBank (HQ150006, JN880430, JN880431, JN880432, JN880433, KF218605, KF218606). DNA samples from all 24 wild canids yielded amplicons of the expected size by the mtDNA control region or the *cyt b* PCR assay. Taxonomic identification of 13 free-ranging canids (including the five ones that were *R. vitalii*-PCR positive) were confirmed by sequencing their mtDNA control region or *cyt b* partial sequence, which were 98–100% identical to corresponding sequences of *C. thous*

Table 2

Results of the hematological and biochemical analyses performed on blood samples of *Cerdocyon thous* #1 at the 21st day after admission to the hospital.

Parameters	<i>C. thous</i> #1	Reference values (Gomes, 2006)
Erythrocytes ($\times 10^6/\mu\text{L}$)	4.8	4.31–6.77
Hemoglobin (g/dL)	14.6	12.96–16.88
Packed cell volume %	44	38–49
VCM (fL)	91.7	68–95
CHCM (%)	33.2	31–38
Total Leukocytes ($\times 10^3/\mu\text{L}$)	10	8.1–13.9
Segmented (/ μL)	6700	5758–10,387
Eosinophils (/ μL)	1300	189–1336
Lymphocytes (/ μL)	1900	1062–2357
Monocytes (/ μL)	100	0–354
Platelets ($\times 10^3/\mu\text{L}$)	165	n.a.
Total Protein (g/dL)	7.6	5.47–7.09
Albumine	34.1	n.a.
ALT	17	n.a.
Creatinine	0.8	0.37–1.11
FA	51	n.a.
Urea	41	22.46–71.84

n.a.: not available.

(DQ309764, EF107009, EF107010, EF107022, EF107030) or *L. gymnocercus* (EF107034, EF107037) in GenBank.

DNA sequences generated in the present study have been deposited in GenBank under the accession numbers KF964146–KF964151 for 18S rRNA partial sequences of *R. vitalii*, KF964152–KF964154 for mtDNA *cyt b*, and KF964155–KF964164 for mtDNA control region partial sequences of wild canids.

3.2. Domestic dog

The domestic dog that was inoculated with *C. thous* #1-blood and bone marrow showed a number of clinical abnormalities, i.e., anorexia, apathy, decreased body condition, vomiting, mild dehydration, mildly pale mucous membranes, mild fever, and bloody diarrhea, which started on the 11th dpi and lasted until 17–20 dpi. The most marked hematological alteration was thrombocytopenia, which started on the 6th dpi and lasted the whole observation period (Fig. 1). Other hematological alterations observed during this period were decreased values for packed cell volume, hemoglobin, neutrophils, and total leukocytes, as shown in Fig. 1. Blood samples were tested by the Piroplasma-PCR on 0, 2, 4, 32, and 69 dpi; except for days 0 and 2, all samples were PCR-positive. PCR products were DNA sequenced and showed to be 100% identical to *R. vitalii* (HQ150006, JN880433). Blood smears revealed intraerythrocytic forms compatible with *R. vitalii* from 6 to 20 dpi (Fig. 2). Within this period, blood smears also revealed anisocytosis, polychromasia, erythroblasts, larger platelets, and reactive lymphocytes. Supportive therapy, initiated at 12 dpi, was continued until 20 dpi, when the animal improved its clinical condition.

3.3. Phylogenetic analysis

The phylogenetic tree (Fig. 3) depicts that all six sequences of *R. vitalii* generated in the present study

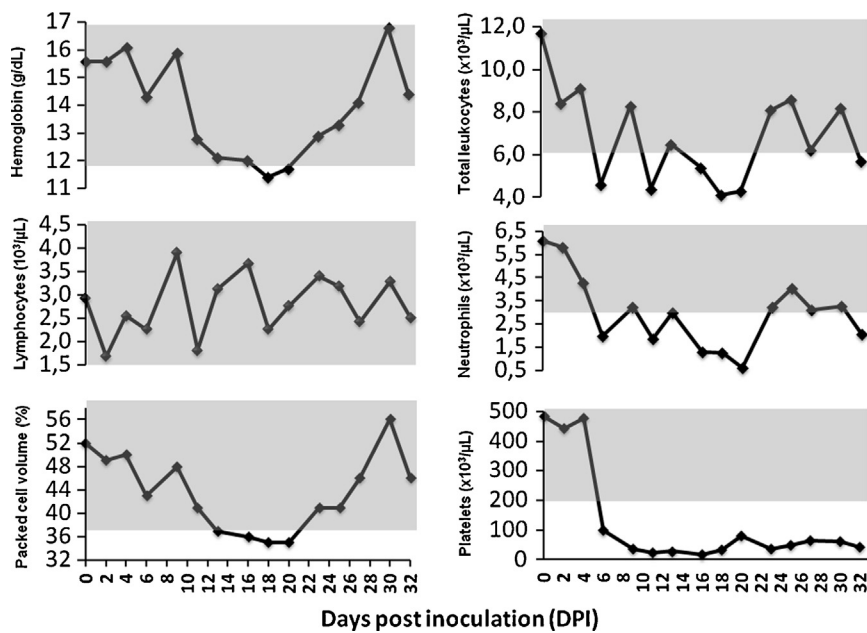


Fig. 1. Hematologic values for a dog that was inoculated with blood and bone marrow from a *Rangelia vitalii*-infected *Cerdocyon thous*, and observed for 32 days post inoculation. Shaded areas represent laboratory reference range for domestic dogs according to Feldman et al. (2000a,b).

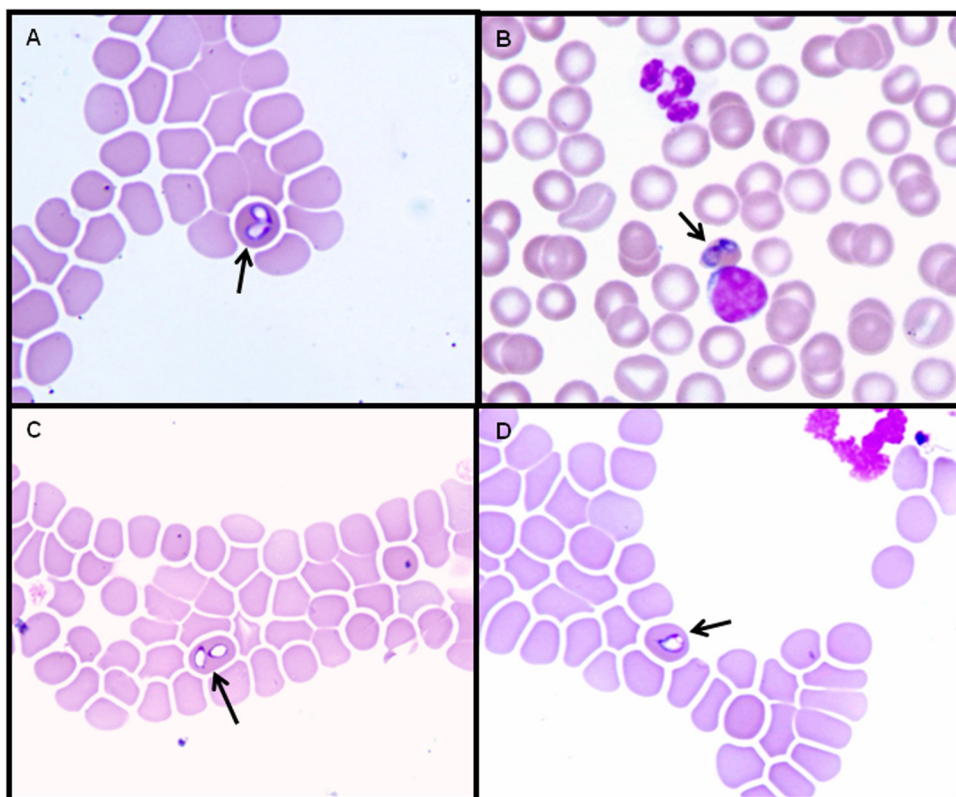
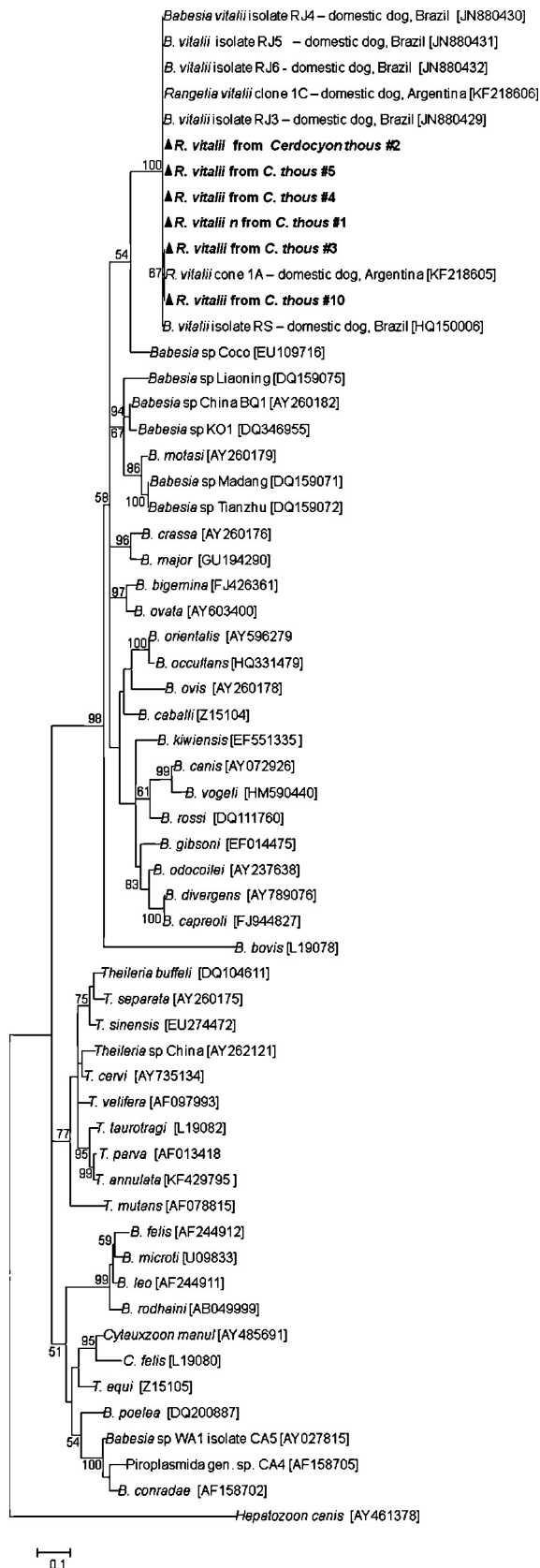


Fig. 2. Intraerythrocytic forms (arrows) compatible with *Rangelia vitalii* found in Rosenfeld-stained thin blood smears collected from a domestic dog at the 11th day after being inoculated with *R. vitalii*-infected tissues from a *Cerdocyon thous*.



formed a cluster with all previous 18S rRNA sequences of *R. vitalii* amplified from domestic dogs in Argentina and Brazil, under 100% bootstrap support. This clade clustered within a much larger clade composed exclusively by sequences related to the '*Babesia sensu stricto*' species, under high bootstrap support (98%).

4. Discussion

Herein, we provide the first molecular detection of a piroplasmid agent in free-ranging canids native to South America. Currently, the only piroplasmid agents that have been reported infecting domestic dogs in South America are *B. vogeli*, *Babesia gibsoni*, and *R. vitalii* (Passos et al., 2005; Trapp et al., 2006; Soares et al., 2011). Among these three agents, *R. vitalii* is the only one that has not been reported outside South America; therefore, it is likely to be native to this continent. Because the domestic dog was introduced to South America by humans in a relatively recent period (Leonard et al., 2002), one would expect that *R. vitalii* would have coevolved with a native canid in South America. Our findings of *R. vitalii* infecting free-ranging *C. thous* from two geographically separated states of Brazil (Rio Grande do Sul and São Paulo) could corroborate a hypothesis that *R. vitalii* is naturally associated with *C. thous* in South America. This statement is reinforced by the following epidemiological observations: (i) it has been demonstrated in an ongoing study that the tick *A. aureolatum* is so far the only competent vector of *R. vitalii* in Brazil (Soares et al., 2012); (ii) *A. aureolatum* is the most common tick species reported to infest *C. thous* in southeastern and southern Brazil (Guglielmone et al., 2003; Labruna et al., 2005), which include the currently known areas of occurrence of canine rangelioidosis (Soares et al., 2013a,b); (iii) in the present study, *R. vitalii* was detected in 46.2% (6/13) of free-ranging *C. thous*, and at the same time, none of the 7 captive *C. thous* were infected; these contrasting findings would be explained by the fact that infestations by *A. aureolatum* would be unlikely in captive *C. thous*, just as has been observed among urban dogs that do not have access to Atlantic rainforest areas within the distribution area of *A. aureolatum* (Ribeiro et al., 1997; Moraes-Filho et al., 2009).

In the present study, the *R. vitalii* isolate obtained from *C. thous* #1 was demonstrated to be highly pathogenic for a domestic dog, which developed severe rangelioidosis that required supportive therapy. This observation corroborates previous studies that have demonstrated *R. vitalii* to cause severe disease in domestic dogs under natural conditions (Krauspenhar et al., 2003; Loretto and Barros, 2005; Figuera, 2007; Figuera et al., 2008, 2010; França et al., 2010; Soares et al., 2013a,b). Among the six *R. vitalii*-infected *C. thous* of the present study, clinical evaluation could be performed

Fig. 3. Maximum likelihood phylogenetic tree of 18S rRNA partial sequences (549-nt) of *Rangelia vitalii* and other piroplasms. Numbers on the nodes indicate bootstrap values from 100 replicates. Only bootstrap values >50 are shown. Numbers in brackets are GenBank accession numbers. The *R. vitalii* sequences generated in the present study are in bold, indicated by a black arrow head.

on just one animal (*C. thous* #1), since the other ones were road-killed. Despite keeping the infection for at least 80 days, no clinical, hematological or biochemical alteration was notably observed in *C. thous* #1, even though it was potentially under capture stress and had a hind limb injury. These observations suggest that *R. vitalii* is not as highly pathogenic for *C. thous* as it is for domestic dogs, although it is a premature concept, since we have made clinical observations on a single *R. vitalii*-infected *C. thous*. Interestingly, it has been reported that the African agent *Babesia rossi* is highly pathogenic for domestic dogs, and at the same time, it is not pathogenic for spleen-intact black-backed jackals (*Canis mesomelas*) and African wild dogs (*Lycaon pictus*); these two wild canids are included as natural hosts for the *Haemaphysalis* ticks that transmit *B. rossi* in the sub-Saharan Africa (Neitz and Steyn, 1947; Van Heerden, 1980; Penzhorn, 2011).

Our phylogenetic analysis inferred by the 18S rRNA gene placed *R. vitalii* embedded in the clade '*Babesia sensu stricto*', consisting of a number of species that represent truly the genus *Babesia* (Schnittger et al., 2012). Under such circumstance, the species *R. vitalii* should be transferred to the genus *Babesia*, as recently suggested in another study (Soares et al., 2011). It has been proposed that the '*Babesia sensu stricto*' species have two main phenotypic characters: transovarial transmission in ticks, and absence of schizonts (Schnittger et al., 2012). Interestingly, *R. vitalii* was originally assigned to this monotypic genus because it was observed doing schizogony within endothelial cells (Carini and Maciel, 1914), which has been demonstrated by immunohistochemistry (Loretto and Barros, 2005). Placing *R. vitalii* in the genus *Babesia* would create an exception for the classical phenotypic classification of piroplasmids, although the occurrence of schizogony has not been deeply investigated in a number of '*Babesia sensu stricto*' species. Indeed, recent molecular phylogenetic analyses have confirmed many of the phenotype-based classic taxonomic classifications of piroplasmids; however, genus-level reallocations of a few species have also been proposed (Lack et al., 2012; Schnittger et al., 2012).

In spite of being neglected for more than five decades, canine rangelioidosis is now recognized as a reemerging disease in Brazil. The present study expands our knowledge on the natural history of *R. vitalii*, suggesting that it might have a natural cycle involving the wild canid *C. thous*. Further studies are needed to confirm the hypothesis that *C. thous* is a natural reservoir of *R. vitalii* in Brazil.

Acknowledgments

We are grateful to Maria Helena S. Pelissari, Maria Luísa Franchini, and Samanta Miyashiro for their technical help in hematological and biochemical analyses, to Guilherme Klafke for valuable suggestions, and to Ugo Souza and Anelise Webster for technical support. This work was supported by the Brazilian Funding Agencies CAPES, CNPq, FAPERGS (Edital PROBIC/PROBITI 2012/2013, Edital 17/2012 DTI, and Edital 01/2013 PqG) and FAPESP.

References

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410.
- Cansi, E.R., Bonorino, R., Mustafa, V.S., Guedes, K.M.R., 2012. Multiple parasitism in wild maned Wolf (*Chrysocyon brachyurus*, Mammalia: Canidae) in Central Brazil. *Comp. Clin. Pathol.* 21, 489–493.
- Carini, A., Maciel, J., 1914. Sobre a moléstia dos cães, chamada Nambi-Uvú, e o seu parasita (*Rangelia vitalii*). *An. Paul. Med. Cir.* 3, 65–71.
- Cheida, C.C., Nakano-Oliveira, E., Fusco-Costa, R., Rocha-Mendes, F., Quadros, J., 2011. Ordem carnívora. In: Reis, N.R., Peracchi, A.L., Pedro, W.A., Lima, I.P. (Eds.), *Mamíferos do Brasil*, 2nd ed, pp. 250–254.
- Doflein, F., Reichenow, E., 1929. *Lehrbuch der Protozoenkunde*. Jena: fünfte Auflage, 1027–1028.
- Feldman, B.F., Zinkl, J.G., Jain, N.C., 2000a. *Schalm's Veterinary Hematology*, vol. 302., 5th ed. Lippincott Williams & Wilkins, Philadelphia, pp. 110–116.
- Figuera, R.A., 2007. Rangeliose. *Acta Sci. Vet.* 35, 261–263.
- Figuera, R.A., Souza, T.M., Silva, M.C., Brum, J.S., Graça, D.L., Kommers, G.D., Irigoyen, L.F., Barros, C.S.L., 2008. Causas de morte e razões para eutanásia de cães da Mesorregião do Centro Ocidental Rio-Grandense (1964–2004). *Pesq. Vet. Bras.* 28, 223–230.
- Figuera, R.A., Souza, T.M., Kommers, G.G., Irigoyen, L.F., Barros, C.S.L., 2010. Patogênese e achados clínicos, hematológicos e anatomopatológicos da infecção por *Rangelia vitalii* em 35 cães (1985–2009). *Pesq. Vet. Bras.* 30, 974–987.
- França, R.T., Silva, A.S., Paim, F.C., Costa, M.M., Soares, J.F., Mazzanti, C.M., Lopes, S.T.A., 2010. *Rangelia vitalii* in dogs in southern Brazil. *Comp. Clin. Pathol.* 19, 383–387.
- Gomes, M.S., 2006. Carnívora-canidae. In: Cubas, Z.S., Silva, J.C.R., Catão-Dias, J.L. (Eds.), *Tratado de animais selvagens-Medicina Veterinária*. Ed Roca, São Paulo, p. p497.
- Feldman, B.F., Zinkl, J.G., Jain, N.C., 2000b. *Schalm's Veterinary Hematology*, 5th ed. Lippincott Williams & Wilkins, Philadelphia, pp. 110–116.
- Guglielmone, A.A., Estrada-Peña, A., Mangold, A.J., Barros-Batesti, D.M., Labruna, M.B., Martins, J.R., Venzal, J.M., Arzua, M., Keirans, J.E., 2003. *Amblyomma aureolatum* (Pallas, 1772) and *Amblyomma ovale* Kock 1844, hosts, distribution and 16S rDNA sequences. *Vet. Parasitol.* 113, 273–288.
- Krauspenhar, C., Figuera, R.A., Graça, D.L., 2003. Anemia hemolítica em cães associada a protozoários. *Medvet – Rev. Cient. Med. Vet. Pequenos Anim. Anim. Estim.* 1, 273–281.
- Labruna, M.B., Jorge, R.S., Sana, D.A., Jácomo, A.T.A., Kashivakura, C.K., Furtado, M.M., Ferro, C., Perez, A.S., Silveira, L., Santos Jr., T.S., Marques, S.R., Morato, R.G., Nava, A., Adania, C.H., Teixeira, R.H., Gomes, A.A., Conforti, V.A., Azevedo, F.C., Prada, C.S., Silva, J.C., Batista, A.F., Marvulo, M.F., Morato, R.L., Alho, C.J., Pinter, A., Ferreira, P.M., Ferreira, F., Barros-Batesti, D.M., 2005. Ticks (Acari: Ixodida) on wild carnivores in Brazil. *Exp. Appl. Acarol.* 36, 149–163.
- Lack, J.B., Reichard, M.V., Van Den Bussche, R.A., 2012. Phylogeny and evolution of the Piroplasmida as inferred from 18S rRNA sequences. *Int. J. Parasitol.* 42, 353–363.
- Lemos, T.D., Cerqueira, A.M.F., Toma, H.K., Silva, A.V., Corrêa, R.G.B., Paludo, G.R., Massard, C.L., Almosny, N.R.P., 2012. Detection and molecular characterization of piroplasm species from naturally infected dogs in southeast Brazil. *Rev. Bras. Parasitol. Vet.* 21, 137–142.
- Leonard, J.A., Wayne, R.K., Wheeler, J., Valadez, R., Guillén, S., Vilà, C., 2002. Ancient DNA evidence for old world origin of new world dogs. *Science* 298, 1613–1616.
- Levine, N.D., 1973. *Protozoan Parasites of Domestic Animals and of Man*, 2nd ed. Burgess Publishing, Minneapolis, pp. 406 p.
- Loretto, A.P., Barros, S.S., 2005. Hemorrhagic disease in dogs infected with an unclassified intraendothelial piroplasm in southern Brazil. *Vet. Parasitol.* 134, 193–213.
- Martins, T.F., Curotto, S., Paz e Silva, F.M., Teixeira, C.R., Takahira, R.K., Lopes, R.S., 2006. *Ancylostoma* sp. e *Babesia* sp. associado ao parasitismo por *Rhipicephalus sanguineus* (Acari: Ixodidae) em Raposinha – do – campo (*Pseudalopex vetulus*) (Carnívora: Canidae) no Centro de Recuperações de Animais Silvestres da FMVZ – Unesp – Botucatu – SP. In: XV Congresso da Sociedade Paulista de Zoológicos, 2006, São Pedro – SP. Anais do XV Congresso da Sociedade Paulista de Zoológicos, Available from: <http://www.spzoo.org.br/anais2006/1.pdf>
- Massard, C.A., Massard, C.L., Lopes, C.W.G., Serra-Freire, N.M., 1981. Babesiose canina e sua transmissão experimental. *Rev. Bras. Med. Vet.* 4, 28–35.
- Moraes-Filho, J., Pinter, A., Pacheco, R.C., Gutmann, T.B., Barbosa, S.O., Gonzáles, M.A.R.M., Muraro, M.A., Cecílio, S.R.M., Labruna, M.B., 2009. New epidemiological data on Brazilian spotted fever in an endemic

- area of the state of São Paulo, Brazil. *Vector-Borne Zoonotic Dis.* 9, 73–78.
- Moreira, J., 1938. Sobre a natureza do nambiuvú dos cães. *Arq. Inst. Biol. de S. Paulo* 9, 315–319.
- Moreira, J., 1939. O Nambiuvú. *O Biológico* 6, 113–116.
- Neitz, W.O., Steyn, H.P., 1947. The transmission of *Babesia canis* (Piana and Galli-Valerio, 1895) to the black-backed jackal [*Thos mesomelas mesomelas* (Schreber)] with a discussion of the classification of the piroplasmids of the Canidae. *J. S. Afr. Vet. Med. Assoc.* 18, 1–12.
- Paraense, W.L., Vianna, Y.L., 1948. Algumas observações sobre a babesiose dos cães no Rio de Janeiro. *Mem. Inst. Oswaldo Cruz* 46, 595–603.
- Passos, L.M., Geiger, S.M., Ribeiro, M.F.B., Pfister, K., Zahlerinder, M., 2005. First molecular detection of *Babesia vogeli* in dogs from Brazil. *Vet. Parasitol.* 127, 81–85.
- Trapp, S.M., Messick, J.B., Vidotto, O., Jojima, F.S., Morais, H.S.A., 2006. *Babesia gibsoni* genotype Asia in dogs from Brazil. *Vet. Parasitol.* 141, 177–180.
- Peirce, M.A., 2000. Order piroplasmorida. In: Lee, J.J., Leedale, G.F., Bradbury, P. (Eds.), *The Illustrated Guide to the Protozoa*, vol. I, 2nd ed. Society of Protozoologists, Lawrence, pp. 347–353.
- Penzhorn, B.L., 2011. Why is Southern African canine babesiosis so virulent? An evolutionary perspective. *Parasit. Vect.* 51, 1–6.
- Pestana, B.R., 1910. O nambiuvú. *Rev. Méd. S. Paulo* 22, 423–426.
- Ribeiro, V.L.S., Weber, M.A., Fetzter, L.O., Vargas, C.R.B., 1997. Espécies e prevalência das infestações por carrapatos em cães de rua da cidade de Porto Alegre, RS, Brasil. *Ciênc. Rural* 27, 285–289.
- Rosenfeld, G., 1947. Corante pancrômico para hematologia e citologia clínica, Nova combinação dos componentes do May-Grunwald e do Giemsa num só corante de emprego rápido. *Mem. Inst. Butantan* 20, 329–335.
- Ruas, J.L., Farias, N.A.R., Soares, M.P., Brum, J.G.W., 2003. *Babesia* sp. em graxaim do campo (*Lycalopex gymnocercus*) no Sul do Brasil. *Arq. Inst. Biol.* 70, 113–114.
- Schnittger, L., Rodriguez, A.E., Florin-Christensen, M., Morrison, D.A., 2012. *Babesia*: a world emerging. *Infect. Genet. Evol.* 12, 1788–1809.
- Serra-Freire, N.M., Teixeira, R.H.F., Amorim, M., Gazeta, G.S., Nunes, A.L.V., Yada, H.S., Teixeira, C., 1995. Babesiose associada ao parasitismo por carrapatos em lobo guará. In: XIX Congresso da Sociedade de Zoológicos do Brasil: Foz do Iguaçu, Arquivos da Sociedade de Zoológicos do Brasil, 9 p.
- Soares, J.F., Giroto, A., Brandão, P.E., Da Silva, A.S., França, R.T., Lopes, S.T.A., Labruna, M.B., 2011. Detection and molecular characterization of a canine piroplasm from Brazil. *Vet. Parasitol.* 180, 203–208.
- Soares, J.F., Costa, F.B., Soares, H.S., Da Silva, A.S., França, R.T., Miyashiro, S., Lopes, S.T.A., Monteiro, S.G., Hagiwara, M.K., Labruna, M.B., 2012. Caracterização morfológica, molecular e estudos dos ixodídeos vetores de *Rangelia vitalii*. In: XVII Congresso Brasileiro de Parasitologia Veterinária Set/2012 São Luiz do Maranhão, Brasil, Anais XVII Congresso Brasileiro de Parasitologia Veterinária, p. 221, Available from: <http://www.cbpv.com.br/congressos/Anais.XVII.CBPV.FINAL.pdf>
- Soares, J.F., Giroto, A., Dalmolin, M.L., França, R.T., Hlavac, N.R.C., Moroz, L.R., Alves, C.B.R., Salomão, E.L., Pelissari, M.H.S., Franchini, M.L., Miyashiro, S., Lopes, S.T.A., Lacerda, L.A., Hagiwara, M.K., Labruna, M.B., 2013a. Detecção molecular de *Rangelia vitalii* nos Estados de São Paulo, Santa Catarina e Rio Grande do Sul. In: IV Simpósio Brasileiro de Acarologia: Maio/2013 Bento Gonçalves-RS, Brasil 2013, Anais do IV Simpósio Brasileiro de Acarologia, CD-ROM.
- Soares, J.F., Corrêa, S., Pelissari, M.H.S., Franchini, M.L., Miyashiro, S., Hagiwara, M.K., Labruna, M.B., 2013b. Detecção molecular de *Rangelia vitalii* no Estado de Minas Gerais. In: III Simpósio Estadual de Doenças Transmitidas por Carrapatos: Outubro/2013 Campinas-RS, Brasil 2013. Boletim Epidemiológico Paulista, vol. 10, Anais, p. 25, Available at: <http://www.saude.sp.gov.br/resources/ccd/homepage/bepa/edicoes-2013/edicao.118.-outubro.especial.carrapatos.parte.2.2.pdf>
- Steuber, S., Abdel-Rady, A., Clausen, P., 2005. PCR-RFLP analysis: a promising technique for host species identification of blood meals from tsetse flies (Diptera: Glossinidae). *Parasitol. Res.* 97, 247–254.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony method. *Mol. Biol. Evol.* 28, 2731–2739.
- Tchaicka, L., Eizirik, E., Oliveira, T.G., Candido Jr., J.F., Freitas, T.R.O., 2007. Phylogeography and population history of the crab-eating fox (*Cerdocyon thous*). *Mol. Ecol.* 16, 819–838.
- Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22, 4673–4680.
- Van Heerden, J., 1980. The transmission of *Babesia canis* to the wild dog (*Lycaon pictus*) (Temminck) and black-backed jackal *Canis mesomelas* (Schreber). *J. S. Afr. Vet. Assoc.* 18, 119–120.
- Wenyon, C.M., 1926. Protozoology: A Manual for Medical Men, Veterinarians and Zoologists, vol. II., 1st ed. Baillière Tindall and Cox, London, pp. 991–1022.