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Rangelia vitalii molecular and histological quantification in tissues comparing crab-eating foxes (Cerdocyon thous) and domestic dogs

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ABSTRACT

Rangeliosis is a condition transmitted by the tick *Amblyomma aureolatum* and caused by the protozoan parasite *Rangelia vitalii* in canids. In domestic dogs, the disease causes a severe hemolytic disease, while in wild canids the piroplasm is often detected without any clinical abnormality. This study aimed to detect and quantify the number of copies of the *R. vitalii Hsp70* gene (indirect parasite burden) in several organs of domestic and South American wild canids (*Cerdocyon thous* and *Lycalopex gymnocercus*) to elucidate distinct clinical presentations of rangeliosis in these species. A total of seven domestic dogs that died due to rangeliosis and 38 wild foxes were initially included, with all dogs presenting histological and molecular features of rangeliosis, while eight *C. thous* were positive at the molecular analysis for *R. vitalii*. Fragments of 22 organs collected from domestic ($n = 7$) and wild foxes ($n = 8$) were employed for histological and molecular quantification using real-time polymerase chain reaction aiming at the *Hsp*70 gene. Histologically, parasitophorous vacuoles were constantly detected in the dogs, while these were detected only in two *C. thous*. Parasitic burden was significantly higher in the digestive, cardiorespiratory, endocrine, genitourinary, and skeletal-muscle systems of domestic dogs when compared to wild foxes. In the hematopoietic system of wild canids, some organs, such as the lymph nodes and tonsils, presented significantly lower amounts of *R. vitalii*, while other organs (spleen, bone marrow, and blood) had results similar to those of domestic dogs. Additionally, the central nervous system of both domestic and wild canids presented a similar quantity of *R. vitalii*. The etiological agent is possibly maintained through an asexual reproductive process (merogony) in both domestic and wild species. Nonetheless, a limited or short-duration schizogony phase occurs in *C. thous*, which would designate this species as a possible reservoir host for the agent. Dogs, in contrast, would most likely act as accidental hosts, presenting a severe and more pathogenic schizogony phase, resulting in characteristic clinical and pathological rangeliosis.

1. Introduction

Rangelia vitalii is a protozoan parasite, which belongs to the Piroplasmorida order and is phylogenetically related to the Babesiidae family, whose members cause the hemolytic disease rangeliosis in dogs (Soares et al., 2011). The condition affects mainly domestic dogs (*Canis lupus familiaris*) living in rural or peri-urban areas (Loretti and Barros, 2005; Fredo et al., 2017), and is strictly linked to the vector *Amblyomma aureolatum* distribution among these areas (Soares et al., 2018), considering that this tick is restricted to neotropical regions (Ribeiro et al., 1997; Evans et al., 2000).

The life cycle of *R. vitalii* has been partially elucidated, sharing its

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features with a genetic group of *Babesia* sensu stricto, such as transovarial transmission in the tick vector (Soares et al., 2018), as well as merogony within erythrocytes of vertebrate hosts (Votýpka et al., 2017; Jalovecka et al., 2018, 2019). However, the life cycle differs in terms of some features, such as schizogony reproduction (Pestana, 1910; Carini and Maciel, 1914; Loretti and Barros, 2005) since frequently the agent is observed microscopically within parasitophorous vacuoles in the cytoplasm of endothelial cells of several tissues (Pestana, 1910; Carini and Maciel, 1914; Loretti and Barros, 2005; Fighera et al., 2010; Fredo et al., 2017).

In addition to domestic dogs, wild canids such as the crab-eating fox (*Cerdocyon thous*), pampas fox (*Lycalopex gymnocercus*), and maned-wolf (*Chrysocyon brachyurus*) are also susceptible hosts to *R. vitalii* infection (Soares et al., 2014; Fredo et al., 2015; Quadros et al., 2015; Silveira et al., 2016; Silva et al., 2018; Copat et al., 2019). Additionally, *C. thous* is considered the most common wild host of *A. aureolatum* (Labruna et al., 2005), and previous studies suggested that the fox could act as an asymptomatic carrier or reservoir of *R. vitalii* (Soares et al., 2014; Souza et al., 2019). Considering these features, the present work aimed to detect and quantify parasite burden of *R. vitalii* in several organs of domestic and wild canids (*C. thous*) through histological and molecular analysis to clarify some aspects related to a distinct clinical-pathological presentation of rangeliosis between wild foxes and domestic dogs.

2. Materials and methods

2.1. Sample selection

This study was conducted between December 2018 and July 2020 through sample collection of tissues of domestic dogs and wild canids received for necropsy at the Veterinary Pathology Laboratory from the Universidade Federal do Rio Grande do Sul, located in Porto Alegre city, Rio Grande do Sul state, Brazil. The domestic dogs were referred from veterinary hospitals or private clinics from the metropolitan area of Porto Alegre city, with a presumptive clinical diagnosis of rangeliosis before postmortem examination was conducted. The criteria for the inclusion of dogs in this investigation included gross and microscopic changes typical of rangeliosis, while domestic dogs that did not present any of these changes were excluded from further analyses. Conversely, the wild foxes were either referred similarly from private clinics, zoological facilities, and wildlife rehabilitation centers or an active search was conducted at highways of the Rio Grande do Sul state aiming for acquiring wild canids killed by a motor vehicle collision. This procedure was authorized by the Instituto Chico Mendes of Biodiversity Conservation (ICMBio) and registered at SISBIOs under the numbers 67,053-1 2 and 73,807-1. All wild foxes were analyzed independently of the presence of gross lesions and/or suspicion of rangeliosis. The autolysis state of these animals (*post mortem* changes) was classified according to McAloose et al. (2018). Information contained within the necropsy reports, such as species, breed, sex, age, previous treatment, and ectoparasites presence, was compiled and summarized.

2.2. Parasitophorous vacuoles quantification and average parasite burden

Fragments of organs of multiple systems were collected for the histopathological examination, which included the cardiorespiratory system (heart and lungs), central nervous system (cerebrum, cerebellum, and spinal cord), hematopoietic system (bone marrow, spleen, lymph node, and tonsils), digestive tract (salivary gland, stomach, liver, pancreas, small and large intestines), endocrine system (thyroid/parathyroid and adrenal glands), genitourinary system (kidneys, urinary bladder, testicle/ovary, and prostate/uterus), and locomotor system (skeletal muscle [posterior thigh]). Tissues were fixed in 10% neutral buffered formalin, routinely processed for histology, and stained for Hematoxylin and Eosin. From these organs, we performed a histological quantification of the parasitophorous vacuoles (PV) of *R. vitalii* located within endothelial cells by counting the total number of parasitic structures within 10 high-power fields (HPF; 400 ×), and, then, we estimated an average parasite burden by splitting the total count of parasitic structures in 10 HPF by 10, which was further classified as mild (+; 1–5 PV), moderate (++; 6–10 PV), and severe (+++; >10 PV), as described previously for domestic dogs (Fredo et al., 2017). Additionally, other concomitant microscopic lesions were also evaluated to associate the agent with the cause of death of these canids. Moreover, parasitic forms were investigated in the cytoplasm of erythrocytes through histological sections.

2.3. DNA extraction

Approximately 0.05 gm of the same tissues previously listed at the histological process and whole blood samples from both domestic dogs and wild canids were frozen for later DNA extraction. This was conducted using the commercial kit QIAamp DNA Mini Kit (QIAGEN®, Chatsworth, CA, EUA) according to the manufacturer's instructions, except with regards to the amount of tissue.

2.4. Quantitative real-time PCR (qPCR)

Molecular detection of *R. vitalii* was obtained through real-time PCR employing the following primers Rv751-770-forward (5′- GCG TAT CCC GAA GAT TCA AA-3 ′) and Rv930-911-reverse (5′- AGT GAA AGC GGT GCA ACA TC-3 ′), additional to a TaqMan probe at the position 772-790 (5′- 6-FAM CCT TAT CAA ATC ATT CTT C MGB NFQ −3′). This set of primers amplified a fragment of 180 base pairs (bp) from the *Hsp70* gene of *R. vitalii* (Soares et al., 2018), which has a single copy in piroplasms (Jie et al., 1996; GenBank-gene: BBOV_III007800, BBOV_IV003490, BBBOND_0306850, BBBOND_0100100, BEWA_018760), with the following adjustments: 10x of PCR buffer, 2 mM of each deoxyribonucleotide triphosphate, 1.5 mM of $MgCl_2$, 1 U of Platinum Taq Polymerase (5000 U/mL) (Invitrogen®, Carlsbad, CA), 30 pmol of each initiator and probe, Rox, 2.0 μL of model DNA, and molecular grade water to complete a final volume of 25 μL. For each reaction, a standard curve was inserted for the absolute quantification. The qPCR was performed on a StepOne™ Real-Time PCR System (Applied Biosystems, CA, EUA).

2.5. Standard curve development for absolute quantification (qPCR)

A commercially developed artificial plasmid (Invitrogen®, Carlsbad, CA) with 2647 bp containing a fragment of 306 bp of the *Hsp70* gene of *R. vitalii* was used. The initial number of plasmid copies received was 1.75×10^{12} in 5000 ng (equivalent to the number of copies of the *Hsp70* gene of *R. vitalii*), and from this, serial dilutions were performed on the basis of 3×10^{11} at three copies of the plasmid. Time and temperature conditions (denaturation, annealing, and extension) for real-time PCR were set as previously described (Soares et al., 2018). Considering nine standard curves, the efficiency of the qPCR was demonstrated to be 98.19% (mean slope= −3.366), a 0.973 coefficient of correlation (R^2), and a 40.747 y-intercept, based on the formula proposed by Bustin et al., 2009.

2.6. Coinfections in wild foxes

Samples of the spleen and lymph node of the foxes that presented positive results at the PCR for the *Hsp70* gene of *R. vitalii* were also sent to the Virology Laboratory at UFRGS, and the virome detected was described in another study (Weber et al., 2020). Furthermore, at the histological exam other parasites coinfecting these canids were also evaluated.

2.7. Statistical analysis

To deal with zero values, before logarithmic transformation (log10)

the copy number values received an offset equal to half of the smallest positive value recorded (Welham et al., 2014). The transformed copy number and PV counting were tested for normality using the Shapiro-Wilk test. According to non-parametric distribution and sample numbers, inter-species comparisons were conducted using the Mann-Whitney U test. Data was shown using median, range, and number of observations. The significance level for the null hypothesis rejection is less than 5% ($p < 0.05$). The statistical analysis employed IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, 2013) and GraphPad Prism for Windows, version 6.01 (La Jolla, California, 2012).

3. Results

3.1. Domestic dogs

From December 2018 to July 2020, 891 dogs underwent necropsy, of which seven (0.8%) were affected by rangeliosis, which was confirmed through histological and molecular features. Neither of these animals were previously treated with antiprotozoal drugs.

Six dogs were mixed breed, while one was a Yorkshire Terrier. Males were slightly overrepresented (57%; 4/7), while females accounted for three cases (43%). Age was reported in five cases (71%), ranging between 2 and 13 years, and with a median age of 6 years.

Histologically, PV containing schizonts of *R. vitalii* (Fig. 1) within the cytoplasm of endothelial cells were observed and quantified in several organs, as depicted in Table 1. No parasitic form was observed within the cytoplasm of erythrocytes.

3.2. Wild foxes

Samples of 38 wild foxes were collected, of which 31 were crab-eating foxes (*C. thous*) and seven were pampas foxes (L. *gymnocercus*). All foxes were free-ranging animals, and upon necropsy, traumatic lesions related to vehicle collision were consistently observed, with an exception of case #1 (data previously published by Copat et al., 2019). This *C. thous* was referred to a zoo after capture, with clinical signs and hematological findings suggestive of canine rangeliosis. Therefore, it was treated 24 h before death with imidocarb hydrochloride and dexamethasone. Additionally, *C. thous* #4 had microscopical lesions suggestive of canine distemper infection.

A total of eight crab-eating foxes (out of 31; 26%) were found to be positive for *R. vitalii* using PCR, while none of the pampas foxes were positive at this examination. *Amblyomma aureolatum* was detected in 11% (4/38) of the wild canids and 37% (3/8) of the positive *C. thous*.

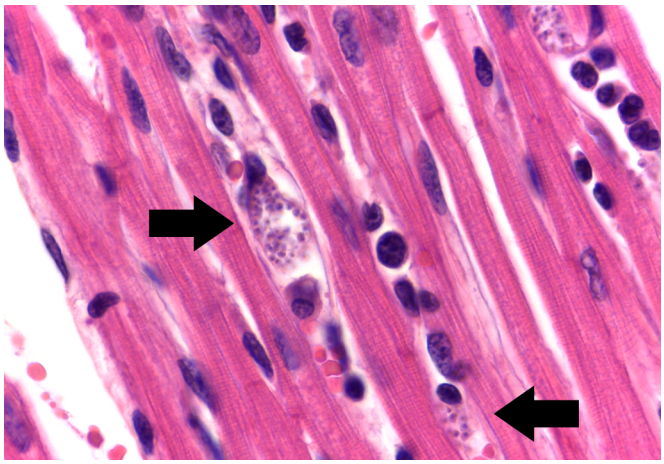


Fig. 1. Domestic dog with Rangeliosis. Heart. Endothelial cells have numerous intracytoplasmic parasitophorous vacuoles of *Rangelia vitalii* (arrow). Hematoxylin and eosin (400x).

More data regarding the foxes, like sex, autolysis degree, histological analysis, PCR results, and presence or absence of ectoparasites, additional to possible coinfections, is available in Table 2. With regards to the positive cases, PV was observed histologically only in two of these cases (2/8; Table 3).

3.3. Comparison between domestic dogs and wild foxes (C. thous)

The quantity of *R. vitalii Hsp70* gene (indirect parasite burden) was significantly lower in *C. thous* in the digestive tract, cardiorespiratory, endocrine, genitourinary, and skeletal muscle systems when compared to domestic dogs (Fig. 2). Nonetheless, a peculiar result was observed for the hematopoietic system, with distinct quantifications according to different organs: the quantity of that gene in the lymph nodes and tonsils was significantly lower in *C. thous* when compared to dogs, while in the spleen, bone marrow, and whole blood it was similar between both species. Additionally, the central nervous system had similar reduced quantification results in both species, which were also corroborated through the absolute results of parasite burden from *R. vitalii* presented in Table 4.

When compared to domestic dogs, the wild foxes (*C. thous*) also presented, histologically, a minor amount of PV in the lungs, heart, thyroid, parathyroid, adrenal gland, kidneys, prostate/uterus, lymph node, spleen, stomach, small and large intestines, pancreas, bone marrow, and urinary bladder, while no difference was detected in the central nervous system (Fig. 3). Testicles/ovaries, salivary gland, tonsils, and skeletal muscle were not analyzed due to the small number of samples, which precluded further statistical analyses.

4. Discussion

During the analyzed period, we detected a frequency of 0.8% of rangeliosis in domestic dogs, which is slightly lower than that observed in previous investigations conducted by other researchers within the same state (6,8–7.5%; Gottlieb et al., 2016; Malheiros et al., 2016) and in Rio de Janeiro (5,8%; Lemos et al., 2012). However, these studies were conducted with blood samples of dogs referred for clinical evaluation, while our study investigated numerous samples of organs of dogs presented for necropsy examination. Another investigation, conducted on dogs submitted to necropsy in another pathology laboratory in the Rio Grande do Sul state, pointed out that rangeliosis was the cause of death or reason for euthanasia in 0.4% of dogs (Fighera et al., 2008), similar to the current study. Additionally, in other South American countries, such as Paraguay, the prevalence of detection of *R. vitalii* was equally low and estimated at 0.26% (Inácio et al., 2019). A lower prevalence of *R. vitalii* in domestic dogs observed in retrospective studies employing necropsy database, when compared to the hematological findings of alive dogs, may be related to early diagnosis and treatment, which may reduce the mortality of dogs due to this condition.

Rangelia vitalii detection in *C. thous* submitted to necropsy (26%) was quite similar to that obtained in another study that used blood samples of free-living *C. thous* (25%; Souza et al., 2019). However, no positive cases for L. *gymnocercus* were detected in our investigation, which is probably related to a lower occurrence of *R. vitalii* in this specimen, as described previously (Souza et al., 2019). The frequencies of detection of *R. vitalii* in both studies, conducted with distinct methodologies, were relatively similar, as the wild canids of the present work most often died because of traumatic events related to a vehicle collision, with no influence of *R. vitalii* in these deaths, similar to a previous investigation of detection of the piroplasm from blood samples collected of free-living *C. thous* (Souza et al., 2019)

Amblyomma aureolatum, the only tick proven to act as a vector for the transmission of *R. vitalii* (Soares et al., 2018), was detected in positive *C. thous*, similar to the results of another investigation (Souza et al., 2019). This ectoparasite requires environments with high humidity and mild temperatures to survive (Guglielmone et al., 2003; Pinter et al.,

Table 1
Histological quantification of *Rangelia vitalii* in several organs of naturally infected domestic dogs.

System	Organ	Dog number						
		1	2	3	4	5	6	7
Cardiorespiratory	Heart	+++	+++	+++	+++	+++	+++	+++
	Lungs	+++	+++	+++	+++	+++	+++	+++
Central nervous	Cerebrum	+	–	+	++	+	–	+
	Cerebellum	+	–	++	+	+	–	+
	Spinal cord	NC	NC	+	NC	+	NC	–
Hematopoietic	Bone marrow	+	+	+	+	+	+	–
	Spleen	+	+	++	+++	+++	–	+++
	Lymph node	+++	+++	+++	+++	+++	+++	+++
	Tonsil	NC	NC	NC	NC	NC	NC	NC
Digestive	Salivary gland	NC	NC	NC	NC	NC	++	NC
	Stomach	+++	+++	+++	+++	+++	+++	+++
	Liver	+	+++	+++	+++	+++	++	+++
	Pancreas	+++	+++	+++	+++	+++	+++	+++
	Small intestine	+++	+++	+++	+++	+++	+++	+++
	Large intestine	+++	+++	+++	+++	+++	+++	+++
Endocrine	Thyroid and parathyroid	NC	+++	+++	+++	+++	NC	+++
	Adrenal	+++	+++	+++	+++	+++	++	+++
Genitourinary	Kidney	+++	+++	+++	+++	+++	+++	+++
	Urinary bladder	+++	+++	+++	+++	+++	+++	+++
	Testicle/ovary	++	+	NC	NC	NC	NC	NC
	Prostate/uterus	+++	+++	NC	NC	NC	NC	+++
Other	Skeletal muscle	NC	NC	NC	+++	NC	–	NC

(+) mild; (++) moderate; (+++) severe; (-) absent/not detected; (NC): sample not collected.

Table 2
Individual identification, sex, autolysis degree, microscopical, presence/absence of ectoparasites, and possible coinfections in eight crab-eating foxes (*Cerdocyon thous*) with positive results at the polymerase chain reaction for *Rangelia vitalii*.

Individual identification	Sex	Autolysis degree*	*R. vitalii* parasitophorous vacuoles at histology	Ectoparasites	Coinfections
1	M	3	+	*Amblyomma aureolatum*	ND
2	F	3	+	Absent	*Bordetella bronchiseptica* and *Proteus* sp., *Angiostrongylus* sp.
3	M	4	–	Absent	*Angiostrongylus* sp.
4	F	2	–	Absent	Canine distemper virus
5	F	3	–	Absent	ND
6	M	2	–	*A. aureolatum*	ND
7	M	2	–	Absent	ND
8	M	2	–	*A. aureolatum*	ND

+: positive; -: negative. F: female; M: male; ND: not detected; *: (1) alive. (2) mild autolysis. (3) moderate autolysis. (4) severe autolysis. (5) mummified. (MCALOOSE et al., 2018).

2004), which are frequently observed in the Brazilian biomes at the Atlantic Forest and Southern Plains (Pampa) (Alvares et al., 2013). Additionally, these areas are frequently shared geographically with the crab-eating fox (*C. thous*) (Cheida et al., 2011). It is likely that both species coevolved in these areas, which enabled *C. thous* to act as the main host of the adult phase of *A. aureolatum* (Labruna et al., 2005) and, therefore, it probably led to constant contact with the piroplasm carried by the tick.

Microscopically, the schizogony stage of *R. vitalii,* located within endothelial cells of domestic dogs, was a consistent feature, mainly in the heart, small and large intestines, lungs, lymph nodes, kidneys, pancreas, urinary bladder, and stomach, as evidenced in another similar investigation (Fredo et al., 2017). *Rangelia vitalii* was related previously to *Babesia* spp. sensu stricto, using molecular phylogenetic analysis (Soares et al., 2011), but *Babesia* spp. usually do not undergo schizogony in vertebrate hosts during their life cycle (Schreeg et al., 2016; Jalovecka et al., 2018, 2019). Although we detected schizogony stages of *R. vitalii* in two *C. thous*, histological observation of these parasites was not possible in most of the cases, which were regarded as natural hosts. Some factors, such as coinfections, environmental damage pressure, climatic variations, or immunodepression, may be associated with increase susceptibility to piroplasm infection in wild canids (Alvarado-Rybak et al., 2016), which may be reflected in the clinical presentation of these animals.

Rangelia vitalii has been previously assigned as the cause of death in a *C. thous,* as severe schizogony was observed histologically, in addition to other gross and histological typical changes of rangeliosis (Copat et al., 2019). Nonetheless, the qPCR results of this case (*C. thous* #1) were similar to those of the remaining positive cases; therefore, we did not find an apparent difference between these canids. Nevertheless, this crab-eating fox had been previously treated, which possibly interfered with the results; therefore, the real parasite burden in this canid remains unknown.

Domestic dogs presented a higher *R. vitalii* parasite burden at the digestive, cardiorespiratory, endocrine, genitourinary, and skeletal muscle systems when compared to *C. thous*. This finding may be explained by a severe and long-lasting schizogony reproductive phase in the accidental host, which would lead to the death of the animal. Similarly, an experimental study showed that domestic cats (*Felis catus*) acted as likely accidental hosts of *Cytauxzoon felis*, with clinical signs and schizogony stages evidenced in all tissues upon necropsy (Blouin et al., 1987). Conversely, the wild host (lynx; *Lynx rufus*) presented a

Table 3
Histological quantification of *Rangelia vitalii* in several organs of naturally infected crab-eating foxes (*Cerdocyon thous*).

System	Organ	*C. thous*							
		1	2	3	4	5	6	7	8
Cardiorespiratory	Heart	++	+++	–	–	–	–	–	–
	Lungs	++	++	–	–	–	–	–	–
Central nervous	Cerebrum	–	+	–	–	–	–	–	–
	Cerebellum	+	NC	–	–	–	–	–	–
	Spinal cord	NC	–	–	–	–	–	–	–
Hematopoietic	Bone marrow	–	–	–	–	–	–	–	–
	Spleen	–	+	–	–	–	–	–	–
	Lymph node	–	NC	–	–	–	–	–	–
	Tonsil	NC	NC	–	–	–	–	–	–
Digestive	Salivary gland	–	NC	–	–	–	–	–	–
	Stomach	NC	NC	–	–	–	–	–	–
	Liver	+	–	–	–	–	–	–	–
	Pancreas	++	+++	–	–	–	–	–	–
	Small intestine	++	++	–	–	–	–	–	–
	Large intestine	NC	++	–	–	–	–	–	–
Endocrine	Thyroid and parathyroid	NC	NC	–	–	–	–	–	–
	Adrenal gland	–	NC	–	–	–	–	–	–
Genitourinary	Kidneys	++	++	–	–	–	–	–	–
	Urinary bladder	+	NC	–	–	–	–	–	–
	Testicle/ovary	–	NC	–	–	–	–	–	–
	Prostate/uterus	NC	NC	–	–	–	–	–	–
Other	Skeletal muscle	NC	–	–	–	–	–	–	–

(+) mild; (++) moderate; (+++) severe; (-) absence/not detected; (NC): samples not collected.

short schizogony stage after exposure, wherein PV was not detected in the tissues histologically, after 30 days post-exposure, despite significant and detectable parasitemia (Blouin et al., 1987). Therefore, those authors suggested that the schizogony reproductive phase is brief in the wild felid, while the merogony phase in the blood appears to be longer (Blouin et al., 1987), similar to the observations made in the present study, wherein the schizogony phase was poorly detected histologically in *C. thous*. This is further supported by the extended period in which merogony phase forms of *R. vitalii* may be detected in *C. thous* (Soares et al., 2014), similar to *C. felis* infection in lynx (Blouin et al., 1987). Nonetheless, *R. vitalii* infection has been previously associated with the death of *C. thous* (Copat et al., 2019), and *C. felis* was also associated with the death of L. *rufus* (Nietfeld, and Pollock, 2002), which suggests that these agents can occasionally induce a more pathogenic and prolonged schizogony phase in these species.

Another previous molecular investigation revealed that free-ranging *C. thous* may act as carriers of *R. vitalii* that do not show any clinical or hematological abnormality related to this infection (Souza et al., 2019). Thus, wild canids probably present a mild schizogony phase, which may be undetectable or brief, explaining the absence of clinical signs in these animals. Similar findings have been reported for *Babesia rossi* infection, which presents higher pathogenicity in domestic dogs, while not being pathogenic for black-backed jackals (*Canis mesomelas*) (Neitz and Steyn, 1947) and African wild dogs (*Lycaon pictus*) (Van Heerden, 1980; Penzhorn, 2011). The findings of these authors suggested that *B. rossi* coevolved with both hosts in Sub-Saharan Africa, while most of the domestic dogs were introduced recently from Europe, Asia, and North and South America (Penzhorn, 2011). Similarly, the hypothesis of coevolution could be applied to *R. vitalii* and native canids of South America (Soares et al., 2014; Mitchell, 2017), considering that existing lineages of domestic dogs in this location were introduced after the European colonization of North and South America (Leonard et al., 2002; Mitchell, 2017).

The quantitative results obtained using whole blood, bone marrow, and spleen samples showed that *C. thous* did not present any statistically significant difference compared to domestic dogs in these tissues. Therefore, *C. thous* and dogs with rangeliosis defined as the cause of death, exhibiting clinical signs and characteristic gross/microscopic lesions, presented a similar *R. vitalii* parasite burden in these tissues. Merogony seems to occur similarly in both dogs and wild canids, which may reflect similar quantification values in hematopoietic tissues, such as the bloodstream, spleen, and bone marrow (Homer et al., 2000; Jalovecka et al., 2018). This has been previously demonstrated in both domestic and wild hosts through the detection of merozoites of *R. vitalii* within erythrocytes (Soares et al., 2011; Copat et al., 2019; Silva et al., 2018). In contrast, the quantification obtained for *R. vitalii Hsp70* gene at the central nervous system did not differ between both species, which could be explained by the fact that large piroplasms, such as *R. vitalii* (2–3.5 μm) (Pestana, 1910; Carini and Maciel, 1914; Soares et al., 2011), are not easily associated with infection at this site, similar to the observations made in dogs infected by *Babesia canis* (Daste et al., 2013). For instance, in Europe, neurological infections by *B. canis* are recorded at low levels ranging from 1 to 3% of the cases (Màthé et al., 2006; Matijatko et al., 2009, 2010; Daste et al., 2013). Additionally, in cattle infected by *Babesia bigemina* or *Babesia bovis*, only the latter, a small *Babesia*, can cause neurological infections (Bock et al., 2014)

Rangelia vitalii is maintained in both domestic and wild foxes (*C. thous*) through a merogony phase within erythrocytes. However, as observed in domestic cats infected by *C. felis*, the schizogony phase is more pathogenic. Furthermore, this correlation is a plausible hypothesis for *R. vitalii* infection in domestic dogs and could explain the severity of the condition in these canids. This has been shown in the current investigation in the form of a higher number of copies of *R. vitalii* identified using PCR, as well as through the frequent detection of PV (schizogony phase) upon histopathological investigation. Additionally, this parasitic form is considered highly pathogenic owing to the marked endothelial damage, which, in addition to the erythrocyte phase, causes severe anemia and thrombocytopenia in domestic dogs, leading to death. We therefore suggest that the domestic dog may act as an accidental host in the life cycle of *R. vitalii*, considering that both *A. aureolatum* and *C. thous* are species endemic to South American countries (Guglielmone et al., 2003; Cheida et al., 2011), while domestic

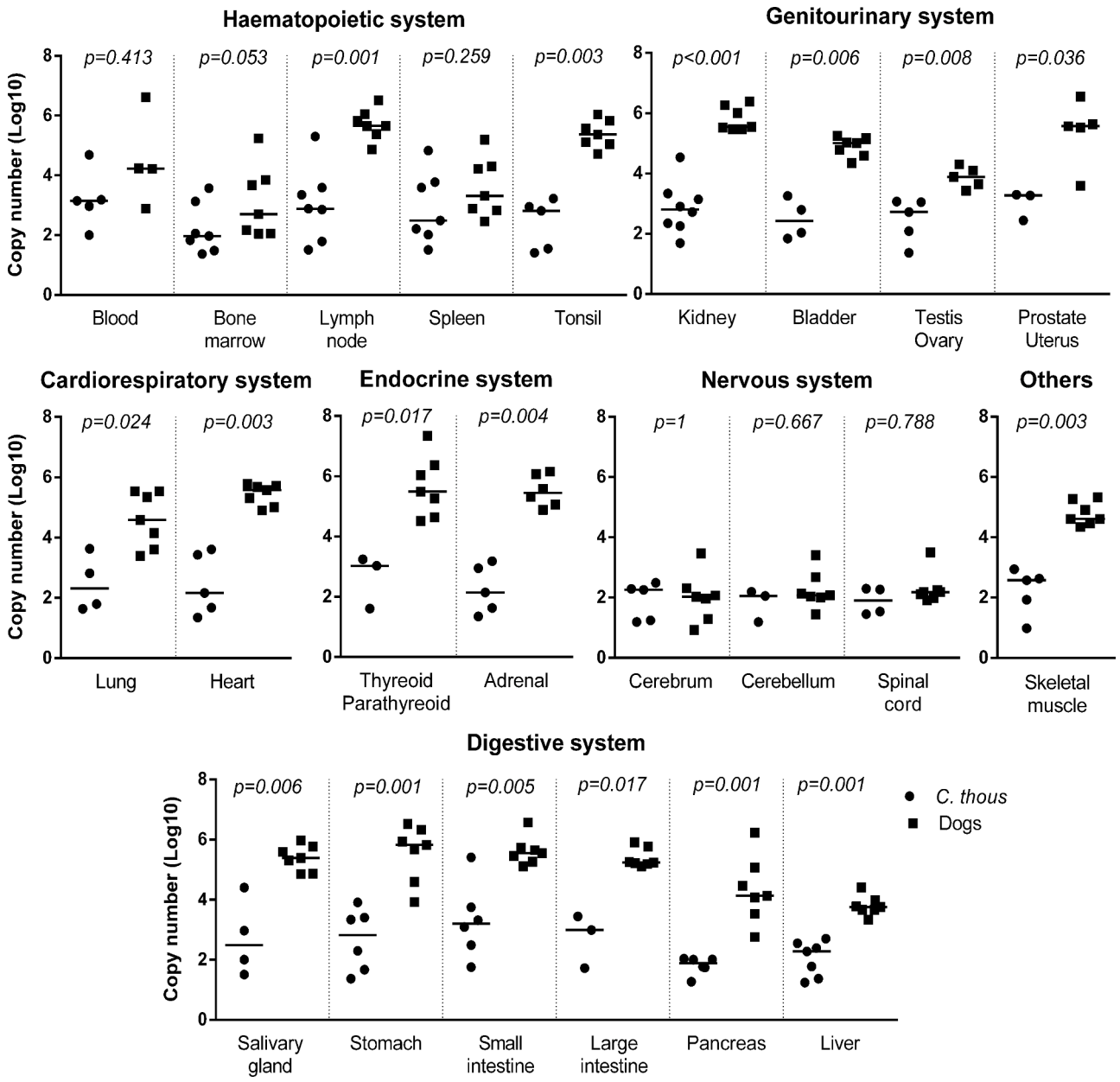


Fig. 2. Inter-species comparison of copy number (*Rangelia vitalii hsp*70 gene fragment) in tissues of *Cerdocyon thous* and domestic dogs using Mann-Whitney U test. The $p < 0.05$ is statistically significant.

Table 4
Absolute results of copy numbers of the R. vitalii *Hsp*70 gene /DNA uL according to each system.

System	Median (Range) Domestic dogs	*Cerdocyon thous*
Digestive	85,320 (20–1838,640)	51 (0–128,555)
Cardiorespiratory	109,688 (195–865,088)	72 (0–2132)
Endocrine	155,744 (16,493–10,864,007)	68 (0–866)
Genitourinary	75,448 (0–1756,501)	262 (0–17,391)
Hematopoietic	25,959 (0–2088,366)	154 (0–99,315)
Nervous	59 (3–1573)	10 (0–152)
Other	20,460 (10,941–104,023)	23 (0–214)

dogs are likely exotic (Leonard et al., 2002; Mitchell, 2017). The current investigation builds upon the findings of previous studies conducted in *C. thous* naturally infected by *R. vitalii* (Soares et al., 2014; Fredo et al., 2015; Souza et al., 2019), which indicated this species as the natural host of the hemoparasite. Possibly, the wild fox coevolved with *A. aureolatum*, the only proven vector of *R. vitalii*, which is widely distributed (endemic) in the area inhabited by *C. thous* (Soares et al., 2018). Thus, *C. thous* probably develop a limited or brief schizogony phase during *R. vitalii* infection, which would make the wild fox a reservoir for the piroplasmid.

CRediT authorship contribution statement

Cíntia De Lorenzo: Conceptualization, Investigation, Methodology, Writing - original draft. **Matheus Viezzer Bianchi:** Investigation, Writing - review & editing. **Luiza Presser Ehlers:** Investigation, Writing - review & editing. **Andreia Vielmo:** Investigation, Writing - review & editing. **Paula Reis Pereira:** Investigation, Writing - review & editing. **Bruno Albuquerque de Almeida:** Methodology, Writing - review & editing. **Caroline Pinto de Andrade:** Investigation, Writing - review & editing. **Aline Girotto-Soares:** Investigation, Writing - review & editing.

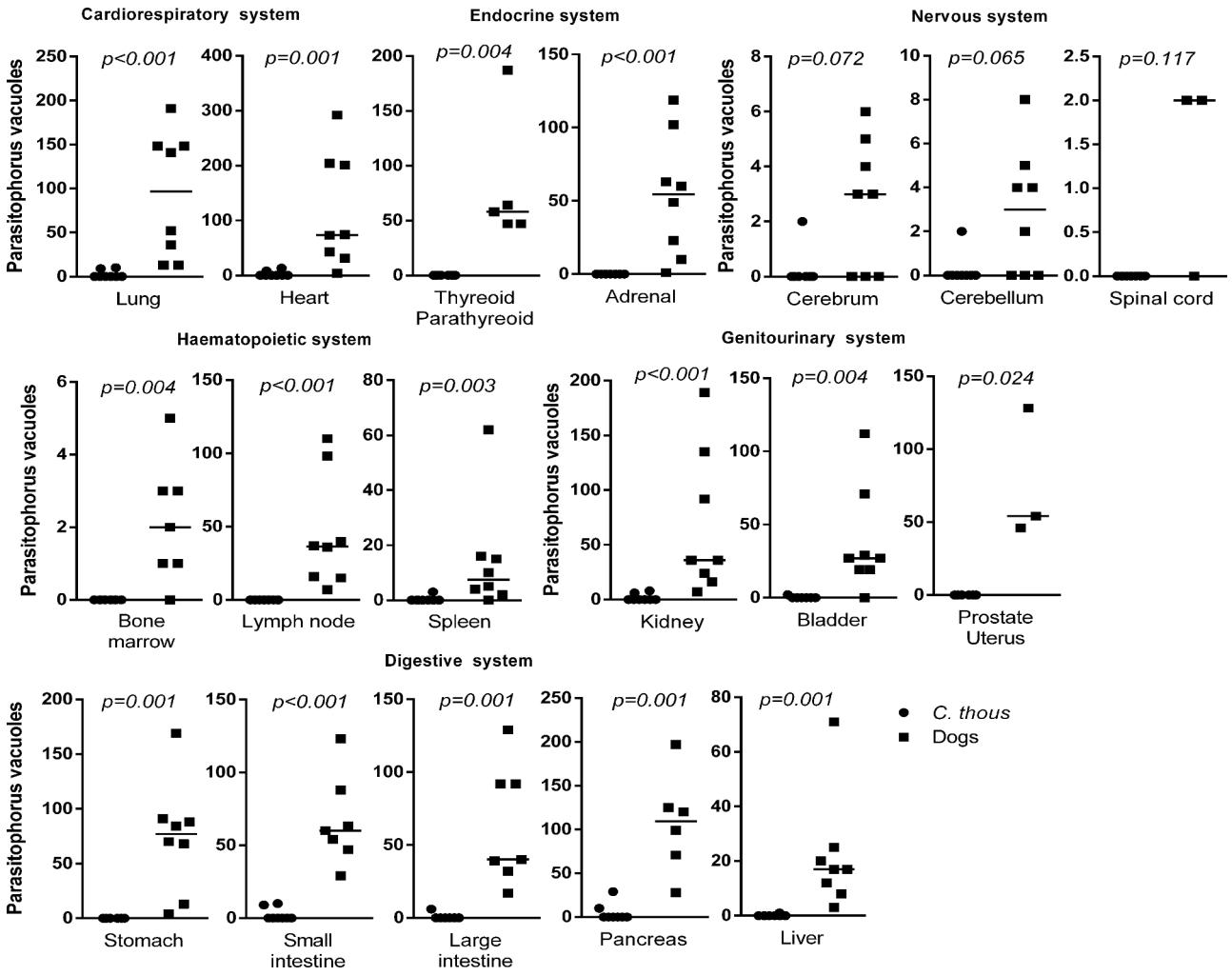


Fig. 3. Inter-species comparison of parasitophorous vacuoles in tissues of *Cerdocyon thous* and domestic dogs using Mann-Whitney U test. The $p < 0.05$ is statistically significant.

Saulo Petinatti Pavarini: Investigation, Writing - review & editing. **David Driemeier:** Investigation, Writing - review & editing. **João Fabio Soares:** Conceptualization, Investigation, Methodology, Writing - review & editing. **Luciana Sonne:** Conceptualization, Investigation, Project administration, Writing - review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest regarding this publication.

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