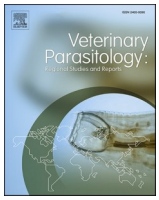
Veterinary Parasitology: Regional Studies and Reports 64 (2025) 101333



Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr



Original Article



Rangelia vitalii in naturally infected dogs in southern Brazil: clinical classification of the disease into acute and subacute phases

Felipe Carniel [a], Gustavo Willian Pandolfo [b], Paulo E. Ferian [a], Julieta Volpato [a], Maysa Garlet Nunes Xavier [a], Mariângela Lovatel [a], Marília Luciani [a], Eloiza Zoboli [a], João Fabio Soares [c], Renata Assis Casagrande [b,*]

[a] *Hospital de Clínicas Veterinárias Prof. Lauro Ribas Zimmer (HCV), Centro de Ciências Agroveterinárias (CAV), Universidade do Estado de Santa Catarina (UDESC), Av. Luiz de Camões, 2090, CEP 88520-000, Lages, SC, Brazil*

[b] *Laboratório de Patologia Animal (LAPA). Centro de Ciências Agroveterinárias (CAV), Universidade do Estado de Santa Catarina (UDESC), Av. Luiz de Camões, 2090, CEP 88520-000 Lages, SC, Brazil*

[c] *Laboratório de Protozoologia e Rickettsioses Vetoriais (ProtozooVet), Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves, 9090, CEP 91540-000 Porto Alegre, RS, Brazil*

ARTICLE INFO

Keywords:
Hematology
Parasitic disease
Protozoan
Pathology
Rangeliosis

ABSTRACT

Canine rangeliosis, caused by *Rangelia vitalii*, is an extravascular hemolytic disease. This study aimed to establish a clinical classification of the disease into acute and subacute phases and to describe the clinical, hematological, and pathological findings in naturally infected dogs. Eighteen dogs were included and submitted a complete blood count, serum biochemistry, ear margin blood smear, abdominal ultrasonography, polymerase chain reaction for *R. vitalii* and *Babesia* spp., and three dogs were necropsied. All animals were positive for *R. vitalii* and negative for *Babesia* spp. The most consistent findings in the acute phase included thrombocytopenia, macrocytosis, Howell-Jolly bodies, coarse hypoechoic splenomegaly, hyperthermia, and tachycardia. In the subacute phase, findings included moderate thrombocytopenia, macrothrombocytes, anisocytosis, and polychromasia, anemia, coarse hypoechoic splenomegaly, hypoproteinemia and hypoalbuminemia, increased urinary gamma-glutamyl transferase levels, lethargy, and anorexia. Thrombocytopenia and splenomegaly were the most frequent alterations, present in all cases. In blood smears, the protozoan was in its free form in 61.1 % of cases. The pathological findings were characteristic of extravascular hemolytic disease, with the most severe tissue parasitism was observed in the intestine, heart, pancreas, and bladder. This study to describe the clinical features of rangeliosis, emphasizing a clinical classification of the disease into to acute and subacute phases.

1. Introduction

Rangelia vitalii is the etiological agent of canine rangeliosis, an extravascular and immune-mediated hemolytic disorder (Fighera et al., 2010). Until the mid-20th century, several researchers considered *R. vitalii* to be synonymous with *Babesia canis* (Levine, 1973; Peirce, 2000). However, morphological, molecular, and phylogenetic analyses have demonstrated that *R. vitalii* is a distinct species of piroplasm, confirming its taxonomic validity (Soares et al., 2011). The tick *Amblyomma aureolatum* is recognized as the natural vector of this protozoan (Soares et al., 2018). Wild canids have been identified as natural reservoirs of *R. vitalii*, maintaining the agent in enzootic cycles in Atlantic Forest regions (Fredo et al., 2015; Soares et al., 2014; Lorenzo et al., 2021a, 2021b).

Canine rangeliosis is a severe piroplasmosis that has been described in various regions, including Brazil (Fighera et al., 2010; Soares et al., 2011; Rodrigues et al., 2022), Uruguay (Soares et al., 2015), and Argentina (Eiras et al., 2014; Borrás et al., 2019). In Santa Catarina, the first report of the disease in dogs was made by Rodrigues et al. (2022), assessing the occurrence of *R. vitalii* in dogs that exhibited clinical signs of hemoparasitosis. Additionally, cases of rangeliosis have been documented in wild canids (Soares et al., 2014; Fredo et al., 2015; Quadros et al., 2015; Silveira et al., 2016; Souza et al., 2019; Lorenzo et al., 2021a, 2021b). In a case reported in Santa Catarina, a pampas fox was diagnosed with rangeliosis (Quadros et al., 2015; Silva et al., 2018).

The disease was first clinically described in 1908 an was categorized

* Corresponding author.
E-mail address: renata.casagrande@udesc.br (R.A. Casagrande).

https://doi.org/10.1016/j.vprsr.2025.101333
Received 13 February 2025; Received in revised form 14 August 2025; Accepted 18 August 2025
Available online 19 August 2025
2405-9390/© 2025 Elsevier B.V. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

into acute (icteric), subacute (hemorrhagic), and chronic (benign) forms (Pestana, 1910; Carini and Maciel, 1914). While acute and subacute forms have been reported in more recent studies, the chronic form has not been observed in naturally infected domestic dogs (França et al., 2010; Soares et al., 2011; Martins et al., 2016). This study highlights the need to reconsider the classification and nomenclature of the disease, as most cases described here demonstrated an overlap of clinical forms (concurrent icteric and hemorrhagic forms), a phenomenon also observed by Krauspenhar et al. (2003).

The presumptive diagnosis of the disease is based on patient history, epidemiology, clinical signs, hematologic and serum biochemistry findings. Regenerative anemia and thrombocytopenia are common laboratory findings (Loretti and Barros, 2005; França et al., 2013; Fredo et al., 2017). Biochemical findings are limited, with most data derived from experimental studies (Silva et al., 2011). To date, there are no detailed studies on the primary ultrasonographic and urinalysis findings in this disease. The definitive diagnosis is based on blood smears, polymerase chain reaction (PCR), or *post-mortem* histopathological examination (Fighera et al., 2010; França et al., 2010; Soares et al., 2011, 2018).

The objectives of this study were to establish a clinical classification of the disease into acute and subacute phases, and to comprehensively describe the clinical signs, hematological alterations, treatment, and *post-mortem* pathological findings associated with *Rangelia vitalii* infection in naturally infected dogs from southern Brazil.

2. Materials and methods

This study was conducted at the Veterinary Hospital of the University of Santa Catarina State (UDESC), in Lages, Santa Catarina, southern Brazil, between August 2017 and May 2019. Eighteen dogs presenting a clinical history and physical examination findings compatible with rangeliosis were submitted a complete blood count, serum biochemistry, ear prick blood smear, abdominal ultrasonography, real-time PCR for *R. vitalii* and conventinal PCR for *Babesia* spp. Dogs with hypoproteinemia and hypoalbuminemia also submitted urine collection by cystocentesis for physical and chemical analysis, as well as urinary gamma-glutamyl transferase (GGT) measurement. This study included 18 dogs diagnosed with rangeliosis, which tested negative for babesiosis and did not present clinical signs or laboratory abnormalities suggestive of other concomitant comorbidities.

Epidemiological data such as age, sex, breed, geographic origin, and presence of ectoparasites were obtained from the animals' clinical records and physical examinations at the time of admission. Blood samples were collected from all dogs by jugular venipuncture and were immediately submitted to the clinical pathology laboratory for complete blood count and biochemical analyses. Packed cell volume was determined by microhematocrit. Total erythrocyte and leukocyte counts, and hemoglobin concentration were performed by an automated cell counter (SDH-3 Vet, Labtest Diagnóstica, Lagoa Santa, Brazil). Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated as described by Feldman et al. (2000).

Abdominal ultrasonography was performed in B-mode using a SonoScape S8ExpV® apparatus and multifrequency linear and microconvex transducers (5 to 10 MHz and 2 to 5 MHz, respectively). Ear-prick blood smears were stained with a rapid stain and examined under microscopy for search hemoparasites, platelet estimation, and differential leukocyte count. Biochemical analyses were performed using commercial kits specific on an automated analyzer (Labmax Plenno, Labtest Diagnóstica, Lagoa Santa, Brazil). Thoracic radiography was performed only in dogs presenting respiratory signs.

The biochemical tests performed were urea (Enzymatic UV), creatinine (1010. Colorimetric – Alkaline picrate - Jaffé), alanine aminotransferase (ALT) (Kinetic UV-IFCC), alkaline phosphatase (ALP) (Colorimetric - Modified Bowers and McComb), total serum protein (TSP) (Colorimetric - Biuret), albumin (Colorimetric - Bromocresol green), globulins (subtraction of albumin value from TSP) and gamma-glutamyl transferase (GGT) (Modified Szasz).

Urinalysis included chemical, physical, and sediment examinations. Reagent strips (Labor Import, DUS10) were used for chemical analysis. Physical parameters (volume, colour, appearance, odor, and urine specific gravity) were assessed as described by Navarro (1996). Sediment analysis was performed under light microscopy (1000×). Urinary GGT was measured by a commercial kit for automated analysis (Labmax Plenno, Labtest Diagnóstica, Lagoa Santa, Brazil) by modified Szasz technique.

For PCR, 500 μL aliquots of blood were submitted to DNA extraction by phenol-chloroform method according to Sangioni et al. (2005). The assay for *R. vitalii* diagnosis was TaqMan assay, which includes the 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′), and a probe at position 772–790 [5′- 6-FAM (CCT TAT CAA ATC ATT CTT C) MGB NFQ −3′]. This primer pair corresponds to amplification of a 180-bp fragment of the hsp70 gene of *R. vitalii* (Soares et al., 2018). The molecular diagnosis of *Babesia* spp. was performed by PCR through a reference laboratory. All patients were treated simultaneously with imidocarb dipropionate 6 mg/kg subcutaneously, as a single dose, repeated in 14 days, and dexamethasone 1 mg administered slowly intravenously, once a day (SID), for 3 days.

Three cases ($n = 3$) that resulted in death were sent to the Animal Pathology Laboratory (LAPA/UDESC) for necropsy. Samples of brain, tonsils, lungs, heart, liver, spleen, pancreas, lymph nodes, kidneys, bladder, adrenals, testes, epididymis, prostate, thyroid, parathyroid, stomach, and intestine were collected and fixed in 10 % buffered formalin. The samples were, routinely processed being paraffin embedded, after, sectioned at 3 μm using a standard microtome, stained with hematoxylin and eosin (H&E), and evaluated by light microscopy.

After completing the sampling and data analysis, the animals were classified into two clinical groups: acute phase ($n = 7$) and subacute phase ($n = 11$). This classification was developed as part of the present study, based on a combination of parameters including the duration of clinical evolution, physical examination findings, clinical signs, and laboratory results (Braga, 1935).

Table 1 presents the proposed classification criteria for the acute and subacute phases of rangeliosis. To classify an animal into one of the phases, it was required to meet at least three criteria, with at least one being among the main criteria defined by the authors.

3. Results

3.1. Epidemiology

Of the 18 cases included in this study, 10 were males (10/18 [55.6 %]) and eight were females (8/18 [44.4 %]). Age ranged from 8 months to 12 years (mean 5.4 years). Fifteen animals were mixed breed (15/18 [83.33 %]), one Poodle (1/18 [5.55 %]), one Labrador (1/18 [5.55 %]), and one Pitbull (1/18 [5.55 %]). All dogs resided in rural or peri-urban areas surrounded by forests and had significant flea infestations. Ticks were observed in only 38.9 % of the dogs at the time of diagnosis, and these were identified as *A. aureolatum*, according to dichotomous keys (Barros-Battesti et al., 2006).

3.2. Clinical and radiographic findings

The clinical signs observed in the seven patients classified as acute phase and the eleven patients in the subacute phase at the time of diagnosis are presented in Table 2. Only one dog in the subacute phase (1/11 [9.1 %]) presented with dyspnea, which was classified as expiratory and mild. Cardiopulmonary auscultation revealed crackles in the caudal lung fields. Thoracic radiography revealed diffuse bronchointerstitial opacity tending towards alveolar, in the caudodorsal lobes and the presence of a lobar sign in the accessory lobe. Thoracic

Table 1
Criteria employed for the classification of rangeliosis into acute and subacute phases in 18 naturally infected dogs.

Phase	Main criteria	Secondary criteria
Acute	1. Moderate to severe thrombocytopenia (between 1000 and 50,000/μL); 2. Mild or undetectable anemia; 3. Frequent presence of structures compatible with *R. vitalii* within erythrocytes, leukocytes, or free in the bloodstream; 4. Mild lymphoreticular hyperplasia (splenomegaly, hepatomegaly, and/or lymphadenopathy); 5. Hyperthermia; 6. Petechiae, ecchymoses, and/or hemorrhagic diathesis.	1. Acute, mild, nonspecific, or even imperceptible clinical signs. The main include: apathy, tachycardia, and vomiting.; 2. Variable leukogram: total leukocyte count within reference intervals; leukopenia due to neutropenia, eosinopenia, and monocytopenia.
Subacute	1. Moderate to severe anemia (packed cell volume between 5 and 20 %); 2. Moderate thrombocytopenia (between 70,000 - 150,000/μL); 3. Reticulocytosis >1 %; 4. Moderate to marked lymphoreticular hyperplasia (splenomegaly, hepatomegaly, and/or lymphadenopathy); 5. Free or intraleukocytic forms of *R. vitalii* are more likely to be found; Intraerythrocytic forms are less common.	1. Severe clinical signs (evolution of approximately two weeks). The main include: apathy, anorexia, progressive weight loss, pale or icteric mucous membranes, vomiting, diarrhea, ear margin bleeding, melena, petechiae, and ecchymoses; 2. Biochemical panel with hypoproteinemia, hypoalbuminemia, decreased albumin:globulin (A:G), and/or increased urea; 3. Variable leukogram: leukocytosis with neutrophilia (frequently a regenerative left shift); total leukocyte count within reference intervals; leukopenia due to neutropenia; eosinopenia.

Table 2
Clinical signs presented by dogs naturally infected with *Rangelia vitalii* classified in acute ($n = 7$) and subacute ($n = 11$) phases.

Clinical signs	Acute phase*	Subacute phase*
Apathy	0	100
Anorexia	0	90,9
Progressive weight loss	0	81,8
Pale mucous membranes	14,3	81,8
Icteric mucous membranes	0	18,2
Vomiting	28,6	81,8
Hypothermia	0	54,5
Hyperthermia	100	0
Tachycardia	71,5	54,5
Submandibular and popliteal lymphadenomegaly	57,2	54,5
Ear margin bleeding	0	54,5
Dehydration	0	45,4
Diarrhea	0	36,4
Petechiae and ecchymoses	57,2	27,3
Melena	0	18,2
Expiratory dyspnea	0	9,1
Epistaxis	0	9,1
Edema pelvic limbs	0	9,1

* Values are represented as a percentage.

radiography was repeated 24 h after the initiation of treatment, and the lung fields appeared within normal radiographic patterns.

3.3. Hematological and serum biochemistry findings

Hematology results for all 18 cases, divided into acute and subacute phases, are presented in Table 3. In the acute phase, additional hematological abnormalities were observed, including Howell-Jolly bodies (7/7 [100 %]), macroplatelets (7/7 [100 %]), anisocytosis and polychromasia (5/7 [71.4 %]), activated monocytes (3/7 [42.9 %]), spherocytes (3/7 [42.9 %]), activated lymphocytes (1/7 [14.3 %]), and Mott cells (1/7 [14.3 %]). These findings were not included in Table 3, as it focuses on the main hematological parameters presented as median values and interquartile ranges. Only one dog in this group presented with anemia, classified as normocytic normochromic, associated with leukopenia and thrombocytopenia, thus characterizing pancytopenia.

In the subacute phase, findings included: macroplatelets (11/11 [100 %]), anisocytosis and polychromasia (9/11 [81.8 %]), reactive lymphocytes (4/11 [36.4 %]), spherocytes (3/11 [27.3 %]), toxic neutrophils (2/11 [18.2 %]), Döhle bodies (1/11 [9.1 %]), and hypersegmented neutrophils (1/11 [9.1 %]). The animals in the subacute phase presented bicytopenia due to anemia and thrombocytopenia (11/11 [100 %]), with two of these presenting pancytopenia (2/11 [18.2 %]). One of the most debilitated dogs had a hematocrit of 8 % and showed marked regenerative anemia. Anemias were classified as: normocytic normochromic (5/11 [45.5 %]), macrocytic hypochromic (3/11 [27.3 %]), and macrocytic normochromic (3/11 [27.3 %]). Serum

Table 3
Median values (25 % percentile; 75 % percentile) of hematological parameters, platelet count, and leukogram of 18 dogs naturally infected with *Rangelia vitalii* in the acute (n = 7) and subacute (n = 11) phases.

Hematologicas findings	Acute phase	Subacute phase	Reference intervals*
Erythrogram			
Erythrocytes (x10^6/uL)	5,6 (4,44; 6,03)	2,22 (1,53; 2,59)	5,5-8,5
Hemoglobin (g/dL)	13,1 (10,35; 13,2)	5,55 (4,02; 6,25)	12–18
Hematocrit (%)	37 (30,5; 39)	17 (12,00; 19,5)	37–55
Mean corpuscular volume (fL)	66,1 (64,8; 69,65)	76,85 (72,02; 80,92)	60–77
Mean corpuscular hemoglobin concentration (%)	32 (31,85; 33,95)	32,2 (31,2; 34,12)	32–36
Reticulocytes (%)	0,05 (0; 0,16)	1,71 (1,24; 4,21)	To 1 %
Erythrocytes nucleated (/100 leukocytes)	5 (3; 7,5)	5,5 (2,75; 10,5)	–
Platelet Count			
Platelets (x10^{3}u/L)	67 (51,5; 73,5)	115,5 (91,5; 127,75)	200–500
Leukogram (x10^{3}u/L)			
Total leukocytes	6.218 (5.859; 6.341)	18.904 (9.575; 22.665)	6.000–17.000
Segmented neutrophils	5.236 (3.915; 5.260)	15.123 (6.507; 19.836)	3.000–11.500
Bands neutrophils	49 (24,5; 86,5)	335 (200; 870)	0–300
Metamyelocytes neutrophils	0	0	0
Myelocytes neutrophils	0	0	0
Lymphocytes	1.228 (1.018; 1.495)	1.871 (1.584; 2772)	1.000–4.800
Eosinophils	0 (0; 49)	92 (0; 304)	100–1.250
Basophils	0	0	Rare
Monocytes	0 (0; 150)	311 (50; 513)	150–1.350

* Reference intervals: Jain, N.C. (1993).

Table 4
Median values (25 % percentile; 75 % percentile) of biochemical parameters of 18 dogs naturally infected with *Rangelia vitalii* in the acute (n = 7) and subacute (n = 11) phases.

Biochemical findings	Acute phase	Subacute phase	Reference intervals*
Urea	33 (30–40)	111 (80,5–136)	21–59,9
Creatinine	0,93 (0,81-0,96)	0,82 (0,69-1,33)	0,5-1,5
Alanine aminotransferase	34 (29–42)	31 (24,25-55,5)	21–102
Alkaline phosphatase	97 (88–118)	109,5 (79–167,5)	20–156
Gamma-glutamyltransferase	2,5 (2,5-2,5)	2,5 (2,5-3,62)	1,2-6,4
Total serum protein	6,12 (5,98-6,21)	5,2 (4,91-5,59)	5,4-7,1
Albumin	2,93 (2,68-3,06)	1,82 (1,36-1,93)	2,6-3,3
Globulins	3,37 (3,01-3,52)	3,58 (3,25–3,93)	2,7-4,4
A:G ratio (albumin: globulin)	0,87 (0,76-1,03)	0,5 (0,4-0,55)	0,59-1,11

* Reference intervals: Kaneko et al. (2008).

biochemistry results for all 18 patients at the time of diagnosis are presented in Table 4.

In ear-tip blood smears, 11/18 cases (61.1 %) showed at least one of the parasitic forms of *R. vitalii*: intraerythrocytic merozoites, intraerythrocytic trophozoites, free merozoites in the bloodstream, or schizonts in monocytes (Figs. 1 and 2). The parasitic forms and their occurrence in each phase of the disease are described, as presented in Table 5. In seven cases (7/18 [38.9 %]), the diagnosis of rangeliosis was confirmed by the presence of schizonts within leukocytes. In another seven animals (7/18 [38.9 %]), structures compatible with *R. vitalii* were not found in blood smears; however, clinical, epidemiological, hematological, biochemical, and ultrasonographic data were sufficient to initiate treatment while PCR was processed. In six dogs (6/18 [33.3 %]), the diagnosis of piroplasmosis was confirmed by hematological findings associated with the presence of intraerythrocytic or free structures in the bloodstream.

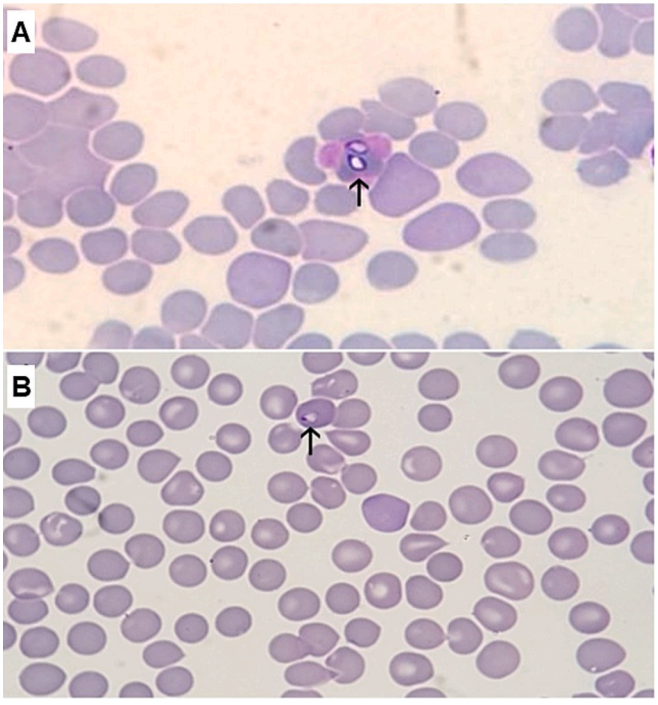


Fig. 1. Intraerythrocytic stages of *Rangelia vitalii* in canine blood smears. A) Intraerythrocytic merozoites (arrow). B) Intraerythrocytic trophozoites (arrow).

3.4. Ultrasound findings

The most common ultrasonographic alterations in the acute phase (*n* = 7) were hypoechoic and coarse splenomegaly (7/7 [100 %]), hypoechoic hepatomegaly (4/7 [57.1 %]), jejunal and medial iliac lymphadenopathy (4/7 [57.1 %]), and gallbladder wall thickening (2/7 [28.6 %]). In the subacute phase (*n* = 11), the main findings were: hypoechoic and coarse splenomegaly (11/11 [100 %]), hypoechoic and coarse hepatomegaly (8/11 [72.7 %]), jejunal and medial iliac lymphadenopathy (8/11 [72.7 %]), gallbladder wall thickening (6/11 [54.5 %]), mild anechoic peritoneal effusion (5/11 [45.5 %]), mesenteric reactivity (4/11 [36.4 %]), pancreatic hypoechogenicity (4/11 [36.4 %]), renal hyperechogenicity (4/11 [36.4 %]), hypoechoic hepatopathy (3/11 [27.3 %]), gastric muscular layer hyperechogenicity (3/11 [27.3 %]), and small intestinal loop thickening (1/11 [9.1 %]).

3.5. Urinalysis findings

In the subcacute phase, the dogs presented hypoproteinemia and hypoalbuminemia and were submitted to urinalysis and urinary GGT dosage. Among the observed alterations were: increased urinary GGT [11/11 [100 %]], proteinuria (9/11 [81.82 %]), and glycosuria (6/11 [54.5 %]). Urinary GGT values in patients ranged from 130 to 298 UI/L. Glycosuria and proteinuria were mild.

3.6. PCR results

The dogs were positive for *R. vitalii* (18/18 [100 %]) and negative for *Babesia* spp. (0/18 [0.0 %]) by PCR.

3.7. Treatment

The dogs received treatment with imidocarb (6 mg/kg, subcutaneously) and dexamethasone (1 mg/kg, intravenously). Only two dogs (2/18 [11.1 %]) presented sialorrhea and vomiting as side effects of imidocarb administration and were treated with atropine sulfate (0.02 mg/kg, subcutaneously). Supportive therapies varied according to each patient's clinical condition and included: fluid therapy (15/18 [83.3 %]), vitamin and mineral supplements (12/18 [66.7 %]), antacids (8/18 [44.4 %]), antiemetics (8/18 [44.4 %]), and, in severe cases, blood transfusion (3/18 [16.7 %]). The dogs were monitored with complete blood counts 24 and 48 h after treatment. After the first 24 h of treatment, 83.3 % of patients presented a significant leukocytosis with neutrophilia. Three dogs were unsuccessful in treatment had intense parasitemia and arrived to begin treatment in very advanced stage of the disease.

Dogs that presented with thrombocytopenia with values ranging from 100,000 to less than 200,000/uL,had platelet counts normalized within 24 h. However, in dogs with more severe thrombocytopenia (<100,000u/L), the recovery of physiological levels required up to 96 h. Animals in the acute phase and presenting intraerythrocytic structures experienced a hematocrit drop of up to 15 % after the administration of imidocarb and dexamethasone, and no patient presented structures compatible with *R. vitalii* in blood smears performed 24 and 48 h after the initiation of treatment.

3.8. Pathological findings

The dogs (*n* = 3) that died in this study were classified as being in the subacute phase of the disease at the time of death. They presented a combination of lesions consistent with an extravascular hemolytic disorder. External mucous membranes, subcutaneous tissue, and serosa showed yellow (2/3 [66.67 %]) to markedly pale (1/3 [33.33 %]), and also splenomegaly was marked (3/3 [100 %]) (Fig. 3), and in one

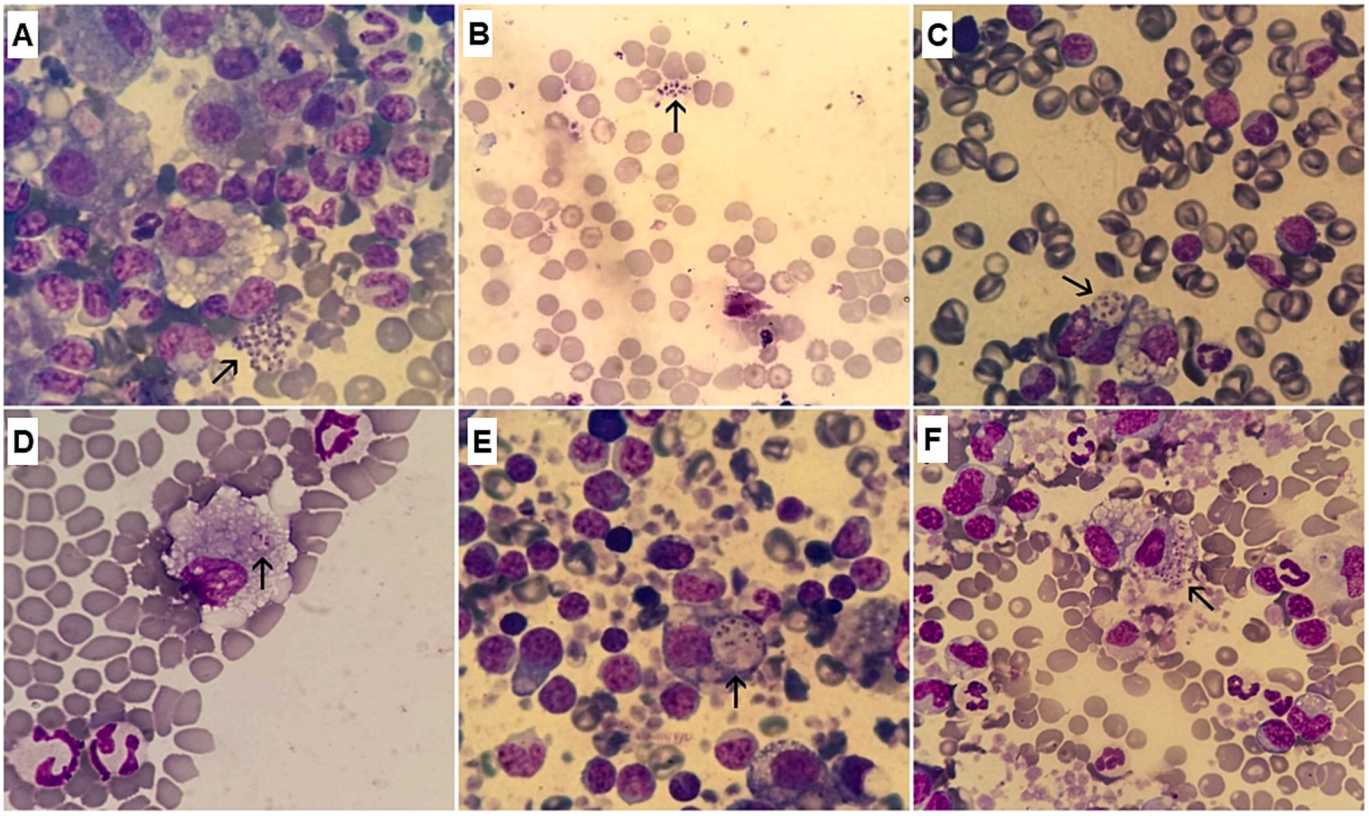


Fig. 2. *Rangelia vitalii* stages in canine blood smears. A, B) Free in the bloodstream (arrow). C—F) Schizonts within monocytes (arrow).

Table 5
Parasitic forms of *Rangelia vitalii*, divided into acute (n = 7) and subacute (n = 11) phases in 18 naturally infected dogs with *R. vitalii*.

Parasitic forms	Acute phase	Subacute phase
Intraerythrocytic (trophozoite and merozoite)	4/7 [40 %])	1/11 [9,1 %])
Free	–	1/11 [9,1 %])
Schizont in leukocytes	2/7 [20 %])	5/11 [45,5 %])
Mixed: intraerythrocytic and intraleukocyte simultaneously	2/7 [20 %])	–

section, the spleen showed red pulp hyperplasia (2/3 [66.67 %]). Superficial and deep lymph nodes showed moderate enlargement (3/3 [100 %]).

The liver exhibited a accentuated lobular pattern (3/3 [100 %]); diffuse marked hepatomegaly (2/3 [66.67 %]); diffuse orange (2/3 [66.67 %]) (Fig. 3) and red-orange (1/3 [33.33 %]); with multiple lacerations showing hemorrhage, fibrinous exudate deposition, and clots adherent to the edges (1/3 [33.33 %]) and gallbladder full of bile with a grainy appearance (2/3 [66.67 %]).

The lungs were heavy and remained distended when removed from the thorax, presenting a moist, shiny, and pale surface (3/3 [100 %]) moderate (2/3 [66.67 %]) to mild (1/3 [33.33 %]) amount of fluid. Multiple petechiae and ecchymoses were also observed distributed throughout all lobes (2/3 [66.67 %]) (Fig. 3). In the severe case, the trachea was filled with a large amount of yellow-turbid frothy material, extending from the middle third to the bronchial branching. In the heart, multifocal pale areas were observed in the myocardium (1/3 [33.33 %]) and multiple petechiae near the coronaries (1/3 [33.33 %]).

The kidneys presented a yellow (2/3 [66.67 %]) to pale (1/3 [33.33 %]). In dogs where the kidneys were yellow, jaundice was marked. In the region of the urinary bladder trigone, there was a focal area of hemorrhage (1/3 [33.33 %]). In the intestines, there was a large amount of darkened fecal content with blood remnants (2/3 [66.67 %]). Petechiae were observed along the entire mucosa of the small intestine (1/3 [33.33 %]). In the cranial skeleton and central nervous system, multiple petechiae were found in the dura mater (1/3 [33.33 %]) and submeningeal ecchymoses in both cerebral hemispheres (1/3 [33.33 %]).

Histopathologically, numerous round protozoa, approximately 2.0 µm in diameter, with light blue cytoplasm and violet nucleus, forming parasitophorous vacuoles compatible with *R. vitalii* were observed in the vascular endothelium of various organs (Fig. 4). The intensity of parasitic structures morphologically compatible with *R. vitalii* observed in histopathology in the different tissues are described in Table 6, according to the same criteria proposed by Fredo et al. (2017).

In liver, a mild multifocal lymphoplasmacytic infiltrate was observed in all cases, as well as a macrophage infiltrate in one (1/3 [33.33 %]), in addition to moderate to severe multifocal centrilobular coagulative necrosis (2/3 [66.67 %]), areas of hemorrhage (1/3 [33.33 %]), marked diffuse (1/3 [33.33 %]) to moderate multifocal cholestasis (1/3 [33.33 %]) and a moderate amount of myeloid and erythroid precursors in the sinusoids (1/3 [33.33 %]). The kidneys presented a mild multifocal lymphoplasmacytic infiltrate in all cases, hyaline casts were also observed in the tubular lumen in a multifocal and discrete manner (1/3 [33.33 %]) and mild thickening of Bowman's capsule with structures compatible with *R. vitalii* in the glomerular mesangium (1/3 [33.33 %]).

In the spleen, extramedullary hematopoiesis (3/3 [100 %]), red pulp hyperplasia (2/3 [66.67]), erythrophagocytosis (2/3 [66.67 %]) and mild multifocal hemosiderosis (1/3 [33.33 %]) were observed. The pancreas presented a mild to moderate multifocal lymphoplasmacytic infiltrate in all three cases (3/3 [100 %]). The lymph nodes showed moderate diffuse congestion (2/3 [66.67 %]), erythrophagocytosis (1/3 [33.33 %]), as well as neutrophilic infiltrate and foci of hemorrhage (1/3 [33.33 %]). In the bone marrow, myeloid and erythroid hyperplasia

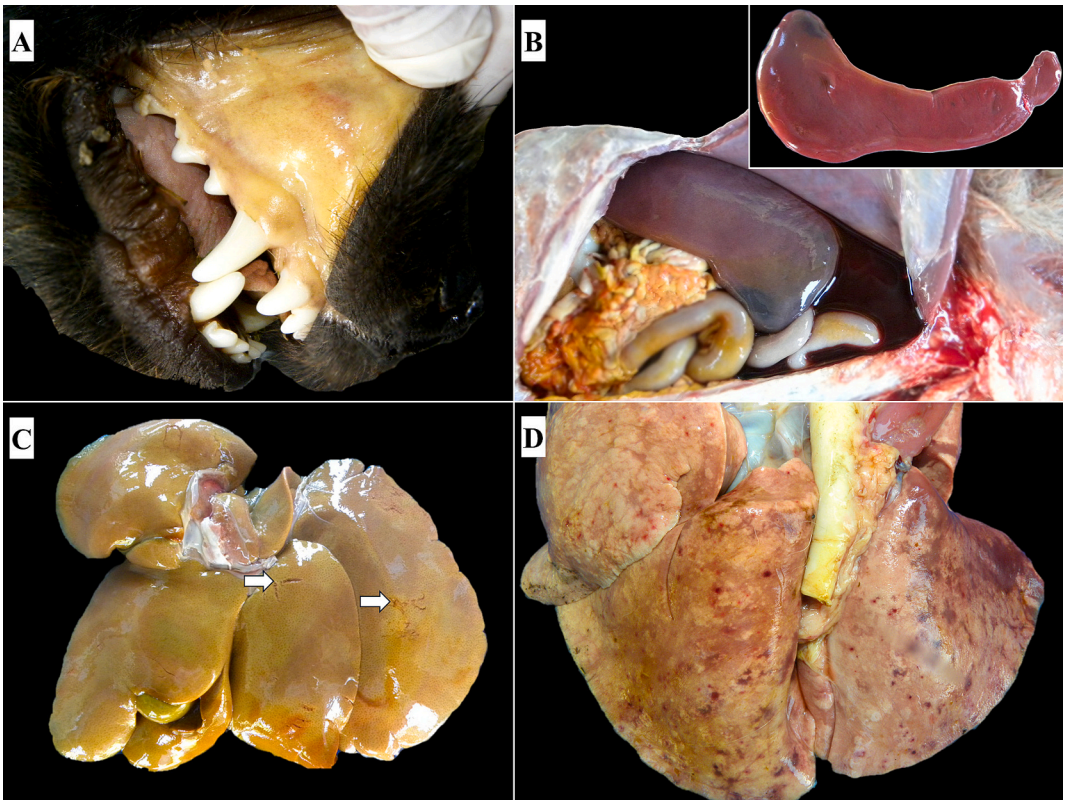


Fig. 3. Gross features in dogs naturally infected with *Rangelia vitalii* A) Diffusely yellow oral mucosa. B) Spleen markedly enlarged in the abdominal cavity with a moderate amount of free blood. Insert: evidencing splenomegaly. C) Liver with a marked lobular pattern, moderate diffuse hepatomegaly, diffuse orange and multiple parenchymal lacerations (arrow). D) Lungs non-collapsed, with a moist, shiny, pale surface, and multiple petechiae and ecchymoses distributed throughout all lobes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was observed (1/3 [33.33 %]).

In the lungs, moderate to mild edema (3/3 [100 %]) were observed and moderate multifocal thrombosis and hemorrhage were observed (1/3 [33.33 %]) and a mild amount of megakaryocytes within capillaries (1/3 [33.33 %]), in addition to a predominantly neutrophilic and macrophage infiltrate in one case. In the heart, a mild to moderate multifocal lymphoplasmacytic infiltrate was observed in all cases (3/3 [100 %]) and hemorrhage near the coronaries (1/3 [33.33 %]). In the meninges, a mild multifocal lymphoplasmacytic infiltrate (3/3 [100 %]), mild multifocal hemorrhage (1/3 [33.33 %]), and evidence of a large amount of myeloid and erythroid precursors in the vascular lumen were observed in one case (1/3 [33.33 %]).

4. Discussion

The natural vector of *R. vitalii* is the tick *A. aureolatum* (Soares et al., 2018), which inhabits temperate regions such as the Atlantic forests of southeastern Brazil (Guglielmone et al., 2003; Labruna et al., 2005). The fact that most animals did not present ticks at the time of diagnosis can be explained that the adult form of *A. aureolatum* has an engorgement period of approximately 12 days (Pinter et al., 2004), while the period for rangeliosis first clinical manifestations is longer (Soares et al., 2018).

Since its initial descriptions, rangeliosis has been considered a disease that primarily affects young dogs and, less frequently, adult to elderly dogs (Pestana, 1910; Carini, 1948). Similar findings were observed in Santa Catarina, where the few reported cases involved young dogs (Rodrigues et al., 2022). This study demonstrated that the average age of the affected dogs was 5.4 years, with more than 40 % of cases involving dogs over 8 years old. Similar results have been reported by Fighera et al. (2010) and Fredo et al. (2017), suggesting that dogs of any age can develop the disease. Furthermore, no sexual predisposition has been reported in the literature (Fighera et al., 2010; Fredo et al., 2017; Rodrigues et al., 2022), as was also observed in the profiles of the patients in this study.

The clinical division of rangeliosis into its three phases (Braga, 1935) is not applicable to the cases observed in more recent studies (Soares et al., 2014). The chronic phase has not been observed clinically in naturally infected domestic canines, as the piroplasm *R. vitalii* is considered a highly pathogenic protozoan for dogs (Silva et al., 2011; Soares et al., 2014; Lorenzo et al., 2021a, 2021b). It is also highly likely that all the animals described in this study, which presented the subacute phase of the disease, would have die if specific treatment and supportive measures had not been taken in time, reinforcing the premise that the chronic phase of the disease does not occur in domestic dogs, but only in wild canids, which act as reservoirs for the protozoan (Soares et al., 2014; Souza et al., 2019; Lorenzo et al., 2021a, 2021b).

Many details regarding the clinical course of the disease remain uncertain. This is primarily because the first descriptions of the disease were made many years ago, when confirmatory diagnostic methods were scarce, and the disease was often confused with other conditions, such as toxoplasmosis and babesiosis (Pestana, 1910; Carini, 1948). The first molecular confirmation of *R. vitalii* was performed by PCR (Soares et al., 2011); however, the standardization of the technique for specific detection of the protozoan was only achieved several years later (Soares et al., 2014; Soares et al., 2018).

In the acute phase, which is now well-documented, authors report initially mild and nonspecific clinical signs, such as apathy, hyperthermia, tachycardia, petechiae, and ecchymosis (Silva et al., 2011; Martins et al., 2016). Frequently, intraerythrocytic structures of *R. vitalii* are observed in blood smears during this phase of the disease, where there is a higher replication of the protozoan within erythrocytes, resulting in a peak of parasitemia and more pronounced thrombocytopenia (Silva

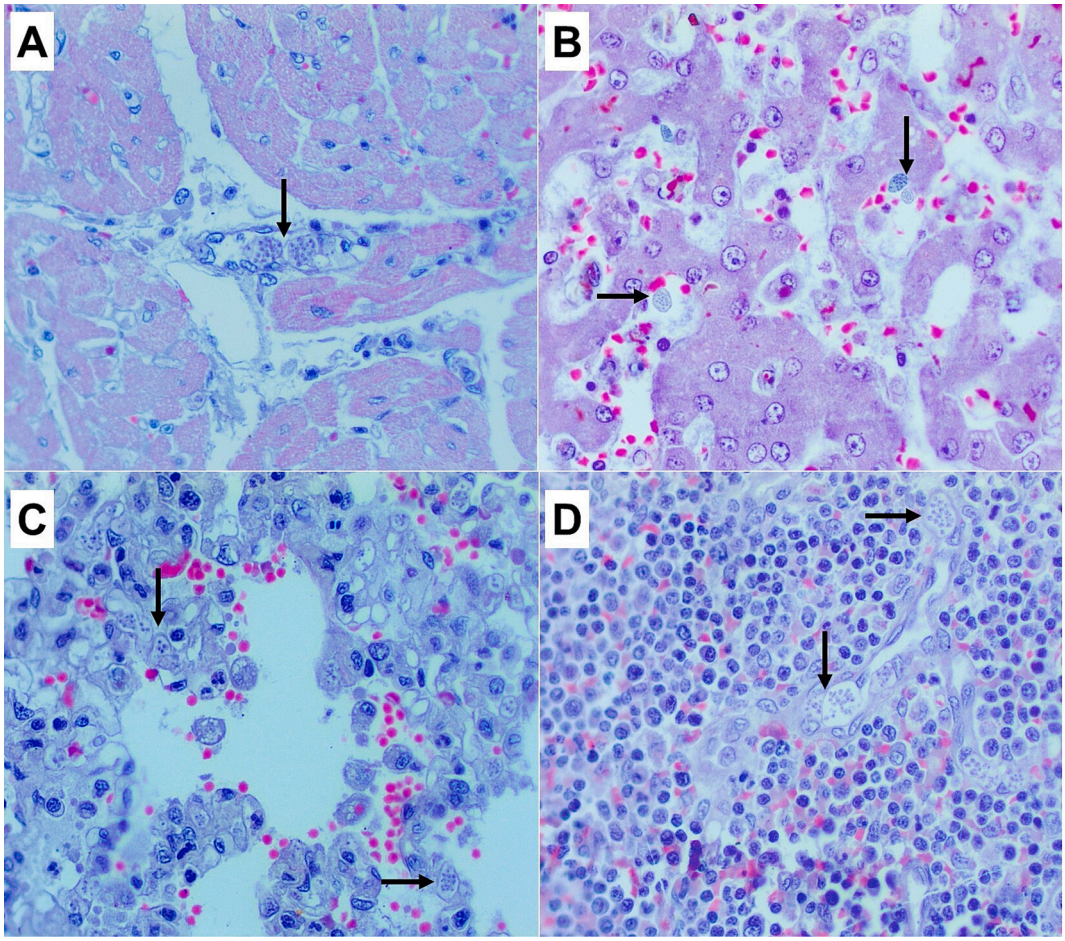


Fig. 4. Histopathological features in dogs naturally infected with *Rangelia vitalii*. Numerous parasitic structures morphologically compatible with *R. vitalii* (rounded, approximately 2.0 μm in diameter, light blue cytoplasm, violet nucleus) forming parasitophorous vacuoles in the vascular endothelium of the (a) Heart, (b) Liver, (c) Lung, and (d) Lymph node. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 6
Characterization of parasitism intensity in different organs observed in the histopathological analysis of three dogs naturally infected with *Rangelia vitalii*.

Organ	Case 1	Case 2	Case 3
Spleen	–	–	+
Liver	–	–	+
Intestine	+++	++	+
Lungs	–	–	+
Hearth	+++	++	+
Lymph nodes	+	+	–
Pancreas	+++	+	–
Kidneys	+	+	+
Bladder	+++	++	+
Bone marrow	–	–	–
Choroid plexus	+++	–	–
Thyroid and parathyroid	+++	–	–
Testicle, epididymis and prostate	+++	–	+

References: (–) negative. (+) mild. (++) moderate. (+++) strong

et al., 2011).

The subacute phase was characterized by the classic signs of rangeliosis (including regenerative anemia, persistent thrombocytopenia, and mild to moderate leukocytosis), which appears to be preceded by an acute phase with the characteristics described above. However, the subacute phase is characterized by a more intense and progressive clinical presentation, with the main finding being anemia, accompanied by a lower quantity of circulating protozoa, similar to what has been documented in the literature (Pestana, 1910; Carini and Maciel, 1914; Lemos et al., 2017). The dogs in this phase had a reticulocyte index >1 %, indicating some level of bone marrow regeneration, except for the most debilitated dog, which had a hematocrit of 8 % and strongly regenerative anemia. This may have occurred due to prolonged hemolysis, leading to a decrease erythrocyte lifespan, iron sequestration by the mononuclear phagocyte system, and a decrease in regenerative capacity, possibly due to bone marrow exhaustion (Schalm et al., 1975).

Normocytic normochromic anemias may be related to immune competence, the acute phase of infection, or immune-mediated depression of bone marrow activity (Cowgill et al., 2003; Thrall, 2010). A study by França et al. (2013) found that, on the first day post-infection, dogs presented normocytic normochromic anemias, and approximately twenty days later, they began to show macrocytic hypochromic anemias. In the study by Rodrigues et al. (2022), conducted in the same region as the present study, both dogs presented normocytic normochromic anemia, which was also the most observed in our research.

Macrocytic hypochromic anemias are commonly observed in rangeliosis, as hemolytic and immune-mediated hemolytic anemias often present signs of regeneration on hematological examinations. These signs include anisocytosis, polychromasia, reticulocytosis, nucleated red blood cells, and Howell-Jolly bodies (Thrall, 2010). In some cases, the presence of spherocytes, erythrophagocytosis, and agglutination suggests that the pathogenesis of this hemolysis involves an immune-mediated component (Fighera et al., 2010; Thrall, 2010).

The leukopenia with neutropenia and eosinopenia observed in some cases may occur during acute inflammation or in inflammatory states,

and has been previously described in rangeliosis (França et al., 2010; Fredo et al., 2017). The leukocytosis observed in the subacute phase of the disease is directly related to the inflammatory process. The increased neutropoiesis results in the release of toxic neutrophils into the bloodstream and stimulation of other leukocyte (Schultze, 2010; Thrall, 2010), which was observed in this study with the presence of activated lymphocytes, Döhle bodies, and toxic neutrophils.

Thrombocytopenia was the most consistent hematological finding in this study, present in all cases and always accompanied by macrothrombocytes, indicating a bone marrow response to the decreased number of circulating platelets (Paim et al., 2012). In the acute phase of the disease, thrombocytopenia was considered severe, being below 70,000/μL in almost all cases. When the platelet count is below 100,000/μL, the risk of naturally bleeding increases, which is a common finding in rangeliosis (Vanderschueren et al., 2000; Fighera et al., 2010).

Thrombocytopenia in this phase is associated with splenomegaly due to the inflammatory and infectious process induced by the disease, the vasculitis caused by the replication of the protozoan in the vascular endothelium, and the immune-mediated component leading to platelet destruction. Splenomegaly increases platelet sequestration, exacerbating the intensity of thrombocytopenia (Paim et al., 2012). In the subacute phase, thrombocytopenia is less severe, with average values around 120,000/μL. The presentation of vasculitis and capillary fragility persists, though less intense than in the acute phase, but is still sufficient to maintain the risk of naturally bleeding (Paim et al., 2012; Paim et al., 2013).

The presentation forms of *R. vitalii* in blood smears include intraerythrocytic structures (trophozoites and merozoites), intraleukocytic structures, and free merozoites in the bloodstream. Only the intraleukocytic form is considered a pathognomonic finding of the protozoan (Soares et al., 2014). In five cases described in this article, intraerythrocytic structures compatible with *R. vitalii* were found, which are more commonly observed in the acute phase of the disease, where there is a fever peak and parasitemia (Silva et al., 2011).

Schizonts in monocytes and free merozoites were also found in both phases of the disease, corroborating studies on the extraerythrocytic cycle of the protozoan. This cycle demonstrates the protozoan's ability to parasitize neutrophils, monocytes, and vascular endothelial cells, and it can even be found free in the bloodstream.

Regarding biochemical findings, the increase in urea may be related due to anorexia, degradation of endogenous proteins (Kaneko et al., 2008) or the onset of acute kidney injury (Grauer, 2005). Hypoproteinemia and hypoalbuminemia may be due to hemorrhage and anorexia.

The dogs in the subacute phase presented increased urinary GGT. Accompanying this increase, glycosuria, proteinuria, and increased urea were also observed. Those are indicative of acute kidney injury. Urinary GGT may be elevated even before functional changes occur in the kidneys, demonstrating its early detection in cases of acute tubular injury (Grauer, 2005). Therefore, we can affirm that the protozoan *R. vitalii* seems to have the potential to cause acute kidney injury.

The treatment of rangeliosis is based on protozoacidal drugs and complementary and supportive treatments. Imidocarb can cause some side effects in patients, including salivation, vomiting, diarrhea, lacrimation, and weakness. These effects can be minimized with atropine sulfate at the appropriate dose (0.02 mg/kg). (Loretti and Barros, 2004; França et al., 2010; Greene, 2015). In this study, only two dogs presented salivation and vomiting after imidocarb administration.

The abrupt decrease in hematocrit values after treatment was observed only in acute phase patients, where parasitemia peaks and anemia is not usually established. Thus, imidocarb dipropionate leads to the lysis of all infected erythrocytes, consequently resulting in increased sequestration and removal of these cells by the spleen, leading to a decrease in packed cell volume (Tilson, 2003).

Ultrasonographic findings in this study were predominantly nonspecific and affected multiple organs, which is consistent with a systemic infection targeting capillary endothelial cells (França et al., 2014; Soares et al., 2023). The most frequent alteration was hypoechoic splenomegaly, observed in all patients. This finding may reflect extramedullary hematopoiesis, infection, or vascular compromise (Pennick and D'Anjou, 2015), especially when interpreted alongside laboratory data indicative of hemolytic disease. In the three dogs that died, histopathological examination confirmed the ultrasonographic alterations, and structures compatible with *R. vitalii* were observed in the affected organs.

Hypoechoic hepatomegaly or hepatopathy was also visualized in all dogs. It is known that ultrasonography has low accuracy for differentiating diffuse hepatic alterations, and there are multiple differential diagnoses for the described finding. Among them, congestion and acute hepatitis are the main ones, which may be associated with cases of leptospirosis, for example (Pennick and D'Anjou, 2015). The liver parenchyma was described as coarse in eight patients, which may be related to the areas of coagulative necrosis and hemorrhage found in the histopathology, lesions commonly observed as heterogeneous areas of variable echogenicity by ultrasonography (Pennick and D'Anjou, 2015).

We observed hepatic lesions such as hepatomegaly with accentuated lobular pattern, red-orange discoloration, hepatocyte atrophy, centrilobular necrosis, cholestasis, erythrophagocytosis, and extramedullary hematopoiesis, as previously described in the literature (Fighera et al., 2010; Fredo et al., 2017). Additionally, a mononuclear inflammatory infiltrate was present in two dogs, confirming the inflammatory component already reported by Fighera et al. (2010).

Gallbladder wall thickening can be caused by inflammation or edema, commonly observed in association with cholangiohepatitis or hypoalbuminemia, respectively (Pennick and D'Anjou, 2015). Eight patients exhibited this alteration, with six presenting hypoalbuminemia and two showing multifocal or diffuse cholestasis on histopathology. However, none of these patients had evidence of bile duct dilatation or wall thickening on ultrasonography. Some authors mention the difficulty in delineating bile ducts, even when dilated (Pennick and D'Anjou, 2015). Additionally, the gallbladder was full and contained a granular content, findings similar to those described by Fighera et al. (2010), due to extravascular hemolysis and hyperbilirubinemia.

Lymphadenomegaly is a common finding in rangeliosis, occurring mainly due to lymphoreticular hyperplasia and erythrophagocytosis (Silva et al., 2011; França et al., 2014). In the necropsied dogs, all superficial and deep lymph nodes were enlarged; however, no lymphoreticular hyperplasia was observed upon histological evaluation in any of the three animals, which contrasts with the findings reported by Fighera et al. (2010).

Peritoneal effusion was detected in five patients, being discrete and anechoic, suggesting a fluid with low cellularity, such as pure transudates (Pennick and D'Anjou, 2015). All these dogs presented with severe hypoalbuminemia, which could be the effusion secondary to a reduction in oncotic pressure. Mesenteric reactivity was visualized in four patients, who didn't present peritoneal effusion in association. The hyperechogenicity of the mesentery, as a single finding, is suggestive of inflammation, such as peritonitis or vasculitis (Pennick and D'Anjou, 2015).

On gastric ultrasonography, the increased echogenicity of the muscular layer, without diffuse wall thickening, was described in 3 of the 11 dogs in the subacute phase, being an uncommon finding but could possibly associated with gastritis (Pennick and D'Anjou, 2015). The pancreatic hypoechogenicity visualized in four patients suggests acute pancreatitis, confirmed histologically by lymphoplasmacytic infiltrate. In the study by Fighera et al. (2010), foci of hemorrhage were observed in the pancreas of three patients with rangeliosis, which could be considered a differential diagnosis observed imaging findings.

The mixed pulmonary pattern, composed of bronchointerstitial opacity with tendencies an alveolar pattern, in the case of rangeliosis, has as its main diagnosis pulmonary edema, which a main cause of death along with severe anemia in dogs with rangeliosis and frequently

presents an inflammatory pattern (Fighera et al., 2010; Thrall, 2018), being observed in the three dogs submitted to necropsy. The inflammatory infiltrate was composed of neutrophils and macrophages, which differs from what is typically described in the literature, where the infiltrate is mononuclear (Fighera et al., 2010; Fredo et al., 2017).

Thoracic radiography in a dog in the subacute phase with expiratory dyspnea revealed a bronchointerstitial pulmonary pattern with alveolar tendencies. After 24 h of treatment initiation, thoracic radiography revealed lung fields within normal limits, highlighting the importance of thoracic radiography in dogs with suspected rangeliosis and expiratory dyspnea, including inflammatory pulmonary edema as a differential diagnosis.

Hemorrhagic foci reported in this study in the intestine, lung, heart, and bladder were also found in a previous study (Fighera et al., 2010). In two cases, multiple petechiae in the dura mater and submeningeal ecchymoses in the cerebral hemispheres were observed, findings that are poorly described in the literature.

Parasitophorous vacuoles of *R. vitalii* were found by histopathology in the vascular endothelium of organs of the endocrine, cardiorespiratory, urogenital, gastrointestinal and neurological systems of forty-nine necropsied dogs in Rio Grande do Sul (Fredo et al., 2017). These findings are similar to those found in this study and demonstrated attention to the systemic distribution of the protozoan.

The intensity of parasitism was considered greater histologically in the intestine, heart, pancreas, and bladder. The testes, epididymis, and prostate were also organs with a high predilection for the parasite. Histology revealed that most dogs presented with mononuclear inflammatory infiltrate. In the meninges, pancreas, heart, kidneys, and liver of the three necropsied dogs, the infiltrate was predominantly lymphoplasmacytic. One dog presented with neutrophilic infiltration in the lungs and lymph nodes, and in the lungs, there was also macrophage infiltration. The predominantly lymphoplasmacytic mononuclear infiltrates have been frequently described previously in other studies (Fighera et al., 2010; Fredo et al., 2017; Lorenzo et al., 2021a, 2021b).

Numerous histological alterations observed by Fighera et al. (2010) in the lymphoreticular and hematopoietic systems were also found in this study. These included erythrophagocytosis and extramedullary hematopoiesis in the spleen, elevated numbers of erythroid and myeloid precursors in the liver, erythrophagocytosis in lymph nodes, and erythroid and myeloid hyperplasia in the bone marrow. Histopathological findings in the lungs revealed edema, thrombosis, multifocal hemorrhage, a discrete number of megakaryocytes within capillaries, and neutrophilic and macrophagic infiltrates. Mononuclear infiltrates, primarily composed of macrophages, were also described in previous studies (Fighera et al., 2010; Fredo et al., 2017). These inflammatory infiltrates are similar to those described in cases of interstitial or alveolar edema secondary to vasculitis in canine ehrlichiosis and appear to be the most plausible explanation for pulmonary edema in rangeliosis (Paim et al., 2013).

Lastly, an important differential diagnosis for rangeliosis is aflatoxicosis, as its clinical signs and macroscopic lesions may be similar. These signs include icteric mucous membranes, hematemesis, hematochezia, or melena, as well as bleeding in the skin, eyes, ears, and mouth (Wouters et al., 2013). At necropsy, icterus, hemorrhages in multiple organs, and a yellow, enlarged liver with an evident lobular pattern are observed. In some cases, there may be chronic ascites with a yellow, reduced liver, irregular surface, and firm consistency. However, the histological features differ significantly from the cases of rangeliosis reported in this article, including fatty degeneration of hepatocytes, biliary duct hyperplasia, cholestasis, and, in chronic cases, hepatic fibrosis (Wouters et al., 2013). Additionally, historical information such as exposure to contaminated feed, recent dietary changes, or outbreaks in animals consuming the same batch of feed is important for distinguishing aflatoxicosis from rangeliosis.

5. Conclusion

In this work we propose classification for rangeliosis divided into acute and subacute phases. In the acute phase, the main findings included severe thrombocytopenia, macrocytosis, Howell-Jolly bodies, coarse hypoechoic splenomegaly, hyperthermia, and tachycardia. In the subacute phase, findings included thrombocytopenia, macrothrombocytes, anisocytosis, polychromasia, anemia, thrombocytopenia, coarse hypoechoic splenomegaly, hypoproteinemia and hypoalbuminemia, increased urinary GGT levels, lethargy, and anorexia. Urinary GGT and urinalysis proved to be early markers of acute tubular injury in rangeliosis. Ear margin blood smear and histopathology were effective for diagnosis. Ultrasonography proved to be an important tool during the diagnosis of rangeliosis even in mild cases, with splenomegaly being observed in both phases. Treatment based on dexamethasone and imidocarb was effective in 15/18 dogs. Therefore, establishing a classification of rangeliosis not only enhances the understanding of the different clinical presentations but also facilitates accurate diagnosis, management, and treatment of this condition.

CRediT authorship contribution statement

Felipe Carniel: Writing – original draft, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Gustavo Willian Pandolfo:** Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Paulo E. Ferian:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Julieta Volpato:** Methodology, Investigation, Formal analysis, Conceptualization. **Maysa Garlet Nunes Xavier:** Methodology, Investigation, Data curation, Conceptualization. **Mariângela Lovatel:** Methodology, Investigation, Data curation, Conceptualization. **Marília Luciani:** Methodology, Investigation, Data curation, Conceptualization. **Eloiza Zoboli:** Resources, Methodology, Investigation, Data curation, Conceptualization. **João Fabio Soares:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Renata Assis Casagrande:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Ethical statement

This study was conducted in compliance with all applicable international, national, and institutional guidelines for the care and use of animals. The animals included in this study were attended as part of routine clinical care, and only standard diagnostic procedures were performed. Therefore, ethical approval was not required. Additionally, three dogs underwent post-mortem examination after succumbing naturally to Rangelia infection, and no experimental interventions were conducted. All procedures followed ethical principles and veterinary medical standards.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

The coauthor JFS is funded by CNPq (Grant # 308112/2025–3) and RAC is funded by CNPq (Grant # 313408/2021–1).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Barros-Battesti, D.M., Arzua, M., Bechara, G., 2006. Carrapatos de importância médico-veterinária da região neotropical: um guia ilustrado para identificação de espécies. Butantan, São Paulo.

Borrás, P., Salvador, F., Rinaldi, V., Armitano, R., Farber, M., Sanchez, R., Mori, L., Guillemi, E., 2019. Use of molecular tools for the diagnosis of rangeliosis by *Rangelia vitalii* in Argentina: a case report. Vet. Parasitol. Reg. Stud. Rep. 21, 100426.

Braga, A., 1935. Contribuição ao estudo diferencial das piroplasmoses dos cães. Bol. Vet. Exército 3, 1–16.

Carini, A., 1948. Sobre o ciclo de desenvolvimento exo-eritrocitário de um piroplasma do cão. Arq. Inst. Biol. 285, 49–52.

Carini, A., Maciel, J., 1914. Sobre a moléstia dos cães, chamada nambi-uvú, e o seu parasita (*Rangellia vitalli*). An. Paul. Med. Cirurg. 3, 65–71.

Cowgill, E.S., Neel, J.A., Grindem, C.B., 2003. Clinical application of reticulocyte counts in dogs and cats. Vet. Clin. North Am. Small Anim. Pract. 33, 1223–1244.

Eiras, D.F., Craviotto, M.B., Baneth, G., Moré, G., 2014. First report of *Rangelia vitalii* infection (canine rangeliosis) in Argentina. Parasitology 63, 729–734.

Feldman, B.F., Zinkl, J.G., Jain, N.C., Jensen, A.L., 2000. Validation of veterinary diagnostic tests in hematology laboratories. In: Feldman, B.F., Zinkl, J.G., Jain, N.C. (Eds.), Schalm's Veterinary Hematology, 5th ed. Lippincott Williams & Wilkins, Philadelphia, pp. 20–28.

Fighera, 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). Pesqui. Vet. Bras. 30, 974–987.

França, R.T., Silva, A.S., Paim, F.C., Costa, M.M., Soares, J.F., Mazzanti, C.M., 2010. *Rangelia vitalii* in dogs in southern Brazil. Comp. Clin. Pathol. 19, 383–387.

França, R.T., Silva, A.S., Costa, M.M., Paim, F.C., Soares, J.F., Labruna, M.B., Mazzanti, C.M., Lopes, S.T.A., 2013. Hematologic and bone marrow changes in dogs experimentally infected with *Rangelia vitalii*. Vet. Clin. Pathol. 42, 31–39.

França, R.T., Silva, A.S., Loretti, A.P., Mazzanti, C.M., Lopes, S.T.A., 2014. Canine rangeliosis due to *Rangelia vitalii*: from first report in Brazil in 1910 to current day – a review. Ticks Tick Borne Dis. 5, 466–474.

Fredo, G., Bianchi, M.V., De Andrade, C.P., Souza, S.O., Leite-Filho, R.V., Bandinelli, M. B., Sonne, L., 2015. Natural infection of wild canids (*Cerdocyon thous* and *Lycalopex gymnocercus*) with the intraendothelial piroplasm *Rangelia vitalii* in southern Brazil. J. Wildl. Dis. 51, 880–884.

Fredo, G., Leite-Filho, R.V., Pietzsch, C.Á., Andrade, C.P., Duda, N.C.B., Nascimento, L. C., Valle, S.F., Soares, J.F., Sonne, L., 2017. Rangeliosis: histophological analysis, hematology and molecular detection of canine *Rangelia vitalii* in Rio Grande do Sul, Brazil. Cienc. Rural 47, 1–9.

Grauer, G.F., 2005. Early detection of renal damage and disease in dogs and cats. Vet. Clin. North Am. Small Anim. Pract. 35, 581–596.

Greene, C.E., 2015. Infectious Disease of the Dog and Cat, 4th ed. Saunders, Georgia.

Guglielmone, A.A., Estrada-Peña, A., Mangold, A.J., Barros-Battesti, D.M., Labruna, M.B., Martins, J.R., Venzal, J.M., Arzua, M., Keirans, J.E., 2003. *Amblyomma aureolatum* (Pallas, 1772) and *Amblyomma ovale* (Koch, 1844): hosts, distribution and 16S rDNA sequences. Vet. Parasitol. 113, 273–288.

Jain, N.C., 1993. Comparative hematology of common domestic animals. In: Jain, N.C. (Ed.), Essentials of Veterinary Hematology. Lea and Febiger, Philadelphia.

Kaneko, J.J., Harvey, J.W., Bruss, M.L., 2008. Clinical Biochemistry of Domestic Animals. Academic Press, California.

Krauspenhar, C., Fighera, R.A., Barros, C.S.L., Graça, D.L., 2003. Protozoa-related immune-mediated hemolytic anemia – case report. Biennal Conf. Soc. Trop. Vet. Med. 7, 39.

Labruna, M.B., Jorge, R.S., Sana, D.A., Jácomo, A.T., Kashivakura, C.K., 2005. Ticks (Acari: Ixodida) on wild carnivores in Brazil. Exp. Appl. Acarol. 36, 149–163.

Lemos, T.D., Toma, H.K., Assad, R.Q., Silva, A.V., Corrêa, R.G.B., Almosny, N.R.P., 2017. Clinical and hematological evaluation of *Rangelia vitalii*-naturally infected dogs in southeastern Brazil. Braz. J. Vet. Parasitol. 26, 307–313.

Levine, N.D., 1973. Protozoan Parasites of Domestic Animals and of Man. Burgess Publishing, Minneapolis.

Lorenzo, C., Bianchi, M.V., Ehlers, L.P., Vielmo, A., Pereira, P.R., Almeida, B.A., Andrade, C.P., Girotto-Soares, A., Pavarini, S.P., Driemeier, D., Soares, J.F., Sonne, L., 2021a. *Rangelia vitalii* molecular and histological quantification in tissues comparing crab-eating foxes (*Cerdocyon thous*) and domestic dogs. Ticks Tick Borne Dis. 12 (5), 101731.

Lorenzo, C., Boabaid, F.M., Oliveira, L.G.S., Bianchi, M.V., Félix, M.L., Armúa-Fernández, M.T., Soares, J.F., Venzal, J.M., Sonne, L., 2021b. *Rangelia vitalii* in free-living crab-eating foxes (*Cerdocyon thous*) in Uruguay. Ticks Tick Borne Dis. 12, 101765.

Loretti, A.P., Barros, S.S., 2004. Parasitismo por *Rangelia vitalli* em cães ("Nambiuvú", "Peste de sangue") – Uma revisão crítica sobre o assunto. Arq. Inst. Biol. 71, 101–131.

Loretti, 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, D.B., Silva, A.S., Oliveira, E., Labruna, M.B., Soares, J.F., 2016. Canine rangeliosis: a rare case of hyperparasitemia in the acute phase. Acta Sci. Vet. 44, 1–4.

Navarro, C.E.K., 1996. Manual de urinálise veterinária. São Paulo Varela.

Paim, C.B., Paim, F.C., Silva, A.S., França, R.T., Costa, M.M., Leal, C.A.M., Soares, J.F., Labruna, M.B., Schetinger, M.R.C., Mazzanti, A., Mazzanti, C.M., Monteiro, S.G., Lopes, S.T.A., 2012. Thrombocytopenia and platelet activity in dogs experimentally infected with *Rangelia vitalii*. Vet. Parasitol. 185, 131–137.

Paim, F.C., Silva, A.S., Paim, C.B., França, R.T., Costa, M.M., Duarte, M.M.M.F., Sangoi, M.B., Moresco, R.N., Monteiro, S.G., Lopes, S.T.A., 2013. Increased cytokine and nitric oxide levels in serum of dogs experimentally infected with *Rangelia vitalii*, Korean. J. Parasitol. 51, 133–137.

Peirce, M.A., 2000. Order Piroplasmorida. In: Lee, J.J., Leedale, G.F., Bradbury, P. (Eds.), The Illustrated Guide to the Protozoa. Society of Protozoologists, Lawrence, pp. 347–353.

Pennick, D., D'Anjou, M., 2015. Atlas of small animal ultrasonography. Wiley Blackwell, Iowa.

Pestana, B.R., 1910. O nambiuvú. Rev Med São Paulo 22, 423–426.

Pinter, A., Dias, R.A., Gennari, S.M., Labruna, M.B., 2004. Study of the seasonal dynamics, life cycle, and host specificity of *Amblyomma aureolatum* (Acari: Ixodidae). J. Med. Entomol. 41, 324–332.

Quadros, R.M., Soares, J.F., Xavier, J.S., Pilati, C., Costa, J.L., Miotto, B.A., Labruna, M. B., 2015. Natural infection of the wild canid *Lycalopex gymnocercus* by the protozoan *Rangelia vitalii*, the agent of canine rangeliosis. J. Wildl. Dis. 51, 787–789.

Rodrigues, R.G., Soares, J.F., Soares, A.G., Ferian, P.E., Milbratz, G.H., Chryssadifis, A.L., Moura, A.B., 2022. First report of canine rangeliosis in domestic dogs from different regions of Santa Catarina state, Brazil. Vet. Parasitol. Reg. Stud. Rep. 36, 1–4.

Sangioni, L.A., Horta, M.C., Vianna, M.C., Gennari, S.M., Soares, R.M., Galvão, M.A., Schumaker, T.T., Ferreira, F., Vidotto, O., Labruna, M.B., 2005. Rickettsial infection in animals and Brazilian spotted fever endemicity. Emerg. Infect. Dis. 11, 265–270.

Schalm, O.W., Jain, N.C., Carrol, E.J., 1975. Veterinary Hematology. Lea & Febinger, Philadelphia.

Schultze, A.E., 2010. Interpretation of canine leukocyte responses. In: Weiss, D.J., Wardrop, K.J. (Eds.), Schalm's Veterinary Hematology. Blackwell Publishing, Iowa.

Silva, A.S., França, R.T., Costa, M.M., Paim, C.B., Paim, F.C., Dornelles, G.L., Lopes, S.T., 2011. Experimental infection with *Rangelia vitalii* in dogs: acute phase, parasitemia, biological cycle, clinical-pathological aspects and treatment. Exp. Parasitol. 128, 347–352.

Silva, M.R.L., Mattoso, C.R.S., Costa, A., Saito, M.S., Tchaicka, L., O'Dwyer, L.H., 2018. *Rangelia vitalii* and *Hepatozoon canis* coinfection in pampas fox *Lycalopex gymnocercus* from Santa Catarina state, Brazil. Braz. J. Vet. Parasitol. 27, 377–383.

Silveira, J.A.G., D'Elia, M.L., Avelar, I.O., Almeida, L.R., Santos, H.A., Soares, D.F.M., Ribeiro, M.F.B., Lima, W.S., Ecco, R., 2016. *Rangelia vitalii* in a free-ranging maned wolf (*Chrysocyon brachyurus*) and co-infections. Int. J. Parasitol. Parasites Wildl. 5, 280–285.

Soares, J.F., Girotto, A., Brandão, P.E., Silva, A.S., França, R.T., Lopes, S.T.A., Labruna, M., 2011. Detection and molecular characterization of a canine piroplasm from Brazil. Vet. Parasitol. 180, 203–208.

Soares, J.F., Dall'Agnol, B., Costa, F.B., Krawczak, F.S., Comerlato, A.T., Rossato, B.C.D., Linck, C.M., Sigahi, E.K.O., Teixeira, R.H.F., Sonne, L., Hagiwara, M.K., Gregori, F., Vieira, M.I.B., Martins, J.R., Reck, J., Labruna, M.B., 2014. Natural infection of the wild canid, *Cerdocyon thous*, with the piroplasmid *Rangelia vitalii* in Brazil. Vet. Parasitol. 202, 156–163.

Soares, J.F., Carvalho, L., Maya, L., Dutra, F., Venzal, J.M., Labruna, M.B., 2015. Molecular detection of *Rangelia vitalii* in domestic dogs from Uruguay. Vet. Parasitol. 210, 98–101.

Soares, J.F., Costa, F.B., Girotto-Soares, A., Da Silva, A.S., França, R.T., Taniwaki, S.A., Dall'Agnol, B., Reck, J., Hagiwara, M.K., Labruna, M.B., 2018. Evaluation of the vector competence of six ixodid tick species for *Rangelia vitalii* (Apicomplexa, Piroplasmorida), the agent of canine rangeliosis. Ticks Tick-borne Dis. 9, 1221–1234.

Piroplasmoses. In: Soares, J.F., Jericó, M.M., Neto, J.P. Andrade, Kogika, M.M. (Eds.), 2023. Tratado de Medicina Interna de Cães e Gatos, 2ª ed. Guanabara Koogan, Rio de Janeiro, pp. 819–838.

Souza, V.K., Dall'Agnol, B., Souza, U.A., Webster, A., Peters, F.B., Favarini, M.O., Reck, J., 2019. Detection of *Rangelia vitalii* (Piroplasmida: Babesiidae) in asymptomatic free-ranging wild canids from the Pampa biome, Brazil. Parasitol. Res. 118, 1337–1342.

Thrall, M.A., 2010. Hematologia e Bioquímica Clínica Veterinária, São Paulo, Roca.

Thrall, D.E., 2018. Canine and feline lung. In: Textbook of Veterinary Diagnostic Radiology, St Louis. Elsevier.

Tilson, D.M., 2003. Spleen. In: Slatter, D.H. (Ed.), Textbook of Small Animal Surgery, 3rd ed. Saunders, Philadelphia.

Vanderschueren, S., Weerdt, A., Malbrain, M., Vankersschaever, D., Frans, E., Wilmer, A., Bobbaers, H., 2000. Thrombocytopenia and prognosis in intensive care. Crit. Care Med. 28, 1871–1876.

Wouters, A.T.B., Casagrande, R.A., Wouters, F., Watanabe, T.T.N., Boabaid, F.M., Cruz, C.E.F., Driemeier, D., 2013. An outbreak of aflatoxin poisoning in dogs associated with aflatoxin B1–contaminated maize products. J. Vet. Diagn. Invest. 25, 282–287.