PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 45:e07578, 2025
DOI: 10.1590/1678-5150-PVB-7578

Original Article
Small Animal Diseases

Outbreak of *Trypanosoma evansi* in dogs utilized in wild boar (*Sus scrofa*) population control[1]

Ananda S. Ries[2], Isac J. Roman[2], Renata D. Mazaro[3,4], Ana Paula G. Mortari[2], Gilneia Rosa[2], Alana P. Herbichi[2], Douglas M. Lorenzetti[3], Guilherme R. Cassanego[3,5], Gabriela Hartmann[3], Juliana F. Cargnelutti[6], Rafael A. Fighera[3], Fernanda S.F. Vogel[2]*

ABSTRACT.- Ries AS, Roman IJ, Mazaro RD, Mortari APG, Rosa G, Herbichi AP, Lorenzetti DM, Cassanego GR, Hartmann G, Cargnelutti JF, Fighera RA, Vogel FSF. **Outbreak of *Trypanosoma evansi* in dogs utilized in wild boar (*Sus scrofa*) population control.** *Pesquisa Veterinária Brasileira 45:e07578, 2025.* Laboratório de Doenças Parasitárias, Departamento de Medicina Veterinária Preventiva, Universidade Federal de Santa Maria, Camobi, Santa Maria, RS, Brazil. E-mail: fernanda.vogel@ufsm.br

Trypanosoma evansi can affect a broad range of domestic and wild animals, such as feral pigs, horses, cattle, goats, dogs, and other carnivores. The growth in wild boar (*Sus scrofa*) populations is considered a significant risk factor, given their status as known reservoirs. This article reviews the epidemiological, clinicopathological, and molecular dimensions, including the identification and genetic characterization, of a *T. evansi* outbreak in dogs. Thirty-four dogs, utilized for wild boar management, underwent physical examinations, complete blood counts, hemoparasite detection via direct microscopic examination of blood smears, DNA detection using polymerase chain reaction, and serological testing for antibodies against *Trypanosoma* spp., alongside *post mortem* analyses of two deceased animals. Clinical signs observed included changes in the color of ocular, oral, and genital mucous membranes, lymphadenomegaly, subcutaneous edema, and anemia. *Trypanosoma* spp. DNA was detected in blood and tissue samples, while blood smears (8/34) revealed the presence of organisms morphologically consistent with the trypomastigote forms of *Trypanosoma* spp. Serological testing failed to detect antibodies. Our results suggest that the dogs were likely infected with *T. evansi* through contact with the blood and/or tissues of infected wild boars. Given the spread of wild boars and their role as reservoirs of *T. evansi*, we emphasize the need to incorporate this protozoan species into routine diagnostic procedures for dogs and the urgency of preventive measures to reduce contact between dogs and wildlife, along with wild boar control initiatives.

INDEX TERMS: Oral transmission, dogs, *Trypanosoma evansi*, PCR, anatomopathological findings, *Sus scrofa.*

RESUMO.- [Surto de *Trypanosoma evansi* em cães utilizados no controle populacional de javalis (*Sus scrofa*).] *Trypanosoma evansi* pode afetar uma ampla variedade de animais domésticos e selvagens, como javalis, cavalos, bovinos, caprinos, cães e outros carnívoros. O crescimento das populações de javalis (*Sus scrofa*) é considerado um fator de risco significativo, visto que são conhecidos como reservatórios. Este artigo revisa as dimensões epidemiológicas,

[1] Received on April 17, 2025.
Accepted for publication on June 30, 2025.

[2] Graduate Program in Veterinary Medicine, Laboratório de Doenças Parasitárias (LADOPAR), Departamento de Medicina Veterinária Preventiva, Universidade Federal de Santa Maria (UFSM), Camobi, Santa Maria, RS, Brazil. * Corresponding author: fernanda.vogel@ufsm.br

[3] Laboratório de Patologia Veterinária (LPV), Departmento de Patologia, Centro de Ciências da Saúde (CCS), Universidade Federal de Santa Maria (UFSM), Camobi, Santa Maria, RS, Brazil.

[4] Departamento de Agricultura, Biodiversidade e Florestas, Universidade Federal de Santa Catarina (UFSC), Curitibanos, SC, Brazil.

[5] Graduate Program in Wildlife Animals, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu, SP, Brazil.

[6] Laboratório de Bacteriologia (LABAC), Departamento de Medicina Veterinária Preventiva, Universidade Federal de Santa Maria (UFSM), Camobi, Santa Maria, RS, Brazil.

clinicopatológicas e moleculares, incluindo a identificação e caracterização genética, de um surto de *T. evansi* em cães. Trinta e quatro cães, utilizados no manejo de javalis, foram submetidos a exames físicos, hemogramas completos, detecção de hemoparasitas por exame microscópico direto de esfregaços sanguíneos, detecção de DNA utilizando a reação em cadeia da polimerase (PCR) e testes sorológicos para anticorpos contra *Trypanosoma* spp., além de análises *post mortem* de dois animais que vieram a óbito. Nossos resultados sugerem que a infecção dos cães por *T. evansi* provavelmente ocorreu devido ao contato com sangue e/ou tecidos de javalis infectados. Considerando a disseminação dos javalis e sua importância como reservatórios de *T. evansi*, ressaltamos a necessidade de incorporar essa espécie do protozoário na rotina diagnóstica de cães e a urgência de medidas preventivas para reduzir o contato entre cães e animais selvagens, junto com iniciativas de controle dos javalis.

TERMOS DE INDEXAÇÃO: Transmissão oral, caninos, cães, *Trypanosoma evansi*, PCR, achados anatomopatológicos, *Sus scrofa.*

INTRODUCTION

Trypanosoma evansi is a protozoan found in tropical and subtropical areas, likely introduced to the Americas during colonization through the introduction of chronically infected animals (Desquesnes et al. 2013). It causes a disease commonly known as "surra" (Greif et al. 2018). In Africa, its transmission cycle is closely associated with the vector of the genus Glossina spp., known as tsetse flies (Desquesnes et al. 2013). Although this vector genus is not present in the Americas, other flies such as tabanids and Stomoxys can act as mechanical vectors (Desquesnes et al. 2013). In addition to mechanical transmission, dogs can become infected through the ingestion of raw meat and blood from infected hosts (Desquesnes et al. 2013). *T. evansi* can affect a wide range of domestic and wild animals, including feral pigs, horses, cattle, goats, dogs, and other carnivores (Desquesnes et al. 2013). Dogs, similar to horses, can exhibit the disease in two phases: an acute phase that can lead to death if not treated promptly, and a chronic phase in which the dog becomes a carrier for an indefinite period (Brun et al. 1998). In Brazil, occurrences are often reported in dogs living in endemic areas, such as the Pantanal (Franciscato et al. 2007). An outbreak of T. evansi in dogs was described in 2005 in Rio Grande do Sul (southern Brazil) (Colpo et al. 2005), with another case in the central region of the state in 2007 (Franciscato et al. 2007); in both instances, the dogs had not traveled to endemic areas (Colpo et al. 2005, Franciscato et al. 2007). In 2018, the first case of a dog diagnosed with *T. evansi* was reported in the western border of Rio Grande do Sul near Uruguay, specifically in the city of Rivera (Greif et al. 2018).

Reports of outbreaks or clinical cases of T. evansi in horses are common in South America (Aregawi et al. 2019). While Brazil is not yet considered endemic, neighboring countries such as Venezuela and Argentina are classified as such, and the movement of animals between Brazil and Argentina is regular, with cases correlating with this epidemiology (Conrado et al. 2005). The increase in wild boar (Sus scrofa) populations is considered a significant risk factor, as they are deemed reservoirs (Herrera et al. 2008). Given this context, this article discusses the epidemiological, clinicopathological, and molecular aspects, as well as the identification and genetic characterization of an outbreak of trypanosomiasis caused by T. evansi in dogs from rural areas used for wild boar management in the western border of Rio Grande do Sul (southern Brazil).

MATERIALS AND METHODS

Ethical approval. No approval from research ethics committees was required to accomplish the goals of this study because experimental work was conducted with samples received from a veterinary hospital.

The study included 34 dogs from three rural properties in central Rio Grande do Sul, showing an association of clinical signs suggestive of trypanosomiasis, such as reduced body condition score, submandibular lymphadenomegaly, oral mucosal pallor, and subcutaneous edema, among others. These dogs were utilized for wild boar management on farms in the western border of the state (Fig. 1). Each animal underwent a physical exam and blood collection for a complete blood count, hemoparasite screening via blood smears, DNA detection using polymerase chain reaction (PCR), and serological tests for *Trypanosoma* spp. antibodies.

Clinical analysis and sample collection. The physical examination involved superficial lymph node palpation, body temperature measurement, cardiac and pulmonary auscultation, mucosal color evaluation, and an overall health condition assessment. Additionally, the body condition score was determined for each dog according to the World Small Animal Veterinary Association using a 9-point scale (Freeman et al. 2011), where scores of 1-3 indicate underweight, 4-5 ideal weight, and 6-9 overweight.

Two dogs that passed away underwent necropsy, with various organs and tissues fixed in 10% buffered formalin for histopathology, stained with hematoxylin-eosin (HE). Epidemiological data were collected from the owners during clinical evaluations. For DNA analysis, tissue samples underwent DNA extraction and PCR analysis. Blood samples were taken for antibody detection, blood smear preparation, and DNA extraction for subsequent PCR analysis.

Direct examination and serological test. Staining for hemoparasite research was done using Panotic Rapid® (Laborclin, Brazil). Indirect immunofluorescence reaction with slides sensitized with promastigote forms of *Trypanosoma vivax* was used for

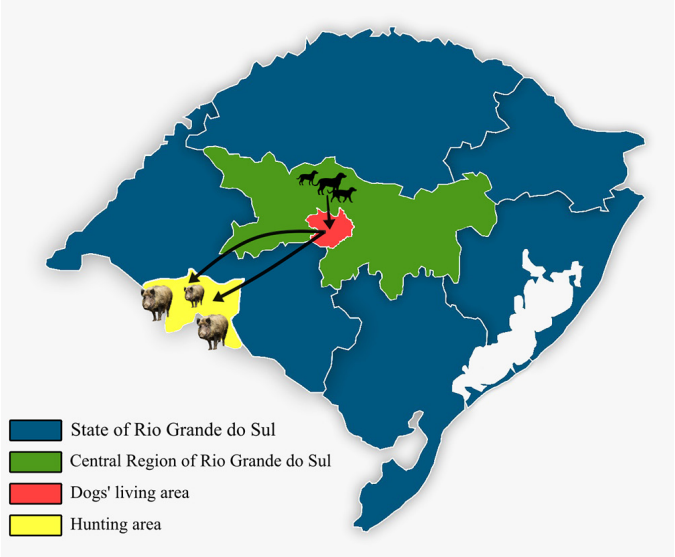

Fig. 1. Illustration of the hunting area location (Campanha Region) and the dogs' habitat area (central region of Santa Maria).

antibody detection. A commercial fluorescein-labeled anti-dog IgG (rabbit anti-dog IgG FITC®, F7884, Sigma-Aldrich, USA) served as the secondary antibody. Slides were examined under a fluorescent microscope at 400x magnification, considering samples reactive if showing total fluorescence with a titer of ≥ 1:40, and non-reactive if no fluorescence was observed, using included positive and negative controls for reference.

Molecular analysis. Blood samples underwent DNA extraction using the Wizard® Genomic DNA Purification Kit (Promega, USA) with modifications to the manufacturer's protocol. During the lysis of red and white blood cells, the cell lysis solution was added, followed by incubation at 65 °C for 30 minutes. The samples were vortexed every 10 minutes and then centrifuged at 16,000 × g for 10 minutes. After discarding the supernatant, the pellet was vortexed again, and a nuclei lysis solution was added for another 30-minute incubation at 65 °C with intermittent vortexing. Following this, a protein precipitation solution was introduced, and the mixture was centrifuged at 16,000 × g for 10 minutes. The DNA pellet was then rehydrated with 50 µl of rehydration solution. Fragments of heart, esophagus, spleen, lymph node, and colon from each deceased animal were processed into two separate pools for DNA extraction using the PureLink® Genomic DNA Kit (Invitrogen, USA), adhering to the mammalian tissue lysis preparation protocol.

The DNA samples were stored at -20 °C until PCR analysis. The PCR assays utilized 25 µL reaction volumes containing 2 µL of total DNA (100-200 ng), 1.25 mM $MgCl_2$, 2.5 mM Taq buffer 10x, 1 U Taq DNA polymerase (0.2 µL) (Thermo Fisher Scientific, USA), 20 µM deoxynucleotides, 1 µM of each primer, and ultrapure water. A positive control used DNA from *Trypanosoma evansi*, with ultrapure water serving as a negative control. The primers used were Kin1, 5'-GCG TTC AAA GAT TGG GCA AT-3' (reverse) and Kin2, 5'-CGC CCG AAA GTT CAC C-3' (forward), targeting conserved regions of rDNA 18S and 5.8S to amplify ITS1 for *Trypanosoma* subgenera, species, or types, as indicated by the sizes of the amplified products: *T. vivax* (305 bp), *T. simiae* (435 bp), *T. theileri* (455 bp), *T. equiperdum*, *T. evansi* and *T. brucei* (540 bp), *T. congolense* Kenya Coast (680 bp), *T. congolense* savannah (750 bp), and *T. congolense* forest (780 bp), as described in (Desquesnes et al. 2001).

The PCR protocol included an initial denaturation at 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at 53 °C for 1 minute, and extension at 72 °C for 1 minute, ending with a final extension at 72 °C for 5 minutes. Electrophoresis of PCR products was conducted on a 1.5% agarose gel, stained with Diamond™ Nucleic Acid Dye (Promega, USA), and visualized under ultraviolet light. Positive samples were prepped for nucleotide sequencing; 20 µL of each PCR product was purified using the PureLink® Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen, USA), and sequenced in duplicate. Sequence analysis involved the Staden Package for consensus sequences and a nucleotide BLAST against the NCBI database. Additional PCR reactions targeted a 205 bp fragment of the RoTat 1.2 variable surface glycoprotein gene specific to *T. evansi*, as described by (Claes et al. 2004): 5'-GCGGGGTGTTTAAAGCAATA-3' and 5'-ATTAGTGCTGCGTGTGTTCG-3'. Dogs were treated with diminazene aceturate according to the manufacturer's guidelines and monitored for remission and recurrence of infection.

RESULTS

A total of 34 dogs were evaluated; two died a few days after diagnosis and underwent necropsy. This report describes their epidemiological, clinical, hematological, and pathological findings.

Epidemiological findings

Out of the 34 dogs in the study, 21 (61.8%) were male, and 13 (38.2%) were female. All dogs were intact. Their ages ranged from nine to 60 months, with a mean age of 23.3 months and a median of 22.5 months, primarily young adults. A majority (27/34, 79.4%) were between 12 and 36 months old. Most dogs (29/34, 85.3%) were purebred, with a smaller proportion (5/34, 14.7%) being mixed breed. The breeds included Dogo Argentino (11/29, 32.4%), English Pointer (7/29, 20.6%), Galgo da Campanha (6/29, 17.6% – not yet recognized by the Brazilian Confederation of Cynophilia), Pampas Deerhound (4/29, 11.8%), and Pit Bull (1/29, 2.9%) (Table 1).

Clinical findings

During the physical examination, the body condition score was rated as 4 in 22 dogs (64.7%) and 3 in the remaining 12 dogs (35.3%) (Fig. 2-7). We noted changes in the color of the ocular, oral, and genital mucous membranes in six of the 34 dogs assessed (17.7%). These membranes appeared mildly (4/6) or moderately (2/6) pale (Fig. 3). When palpating the superficial lymph nodes, we found that in most cases (29/34, 85.3%), at least one lymph center exhibited increased volume (lymphadenomegaly). The pre-scapular (22/29, 64.7%) and submandibular (21/29, 61.8%) lymph nodes were the most commonly affected (Fig. 4). Among the dogs with pale mucous membranes, two also presented slight icterus of the penile mucosa (Fig. 5). The popliteal (10/29, 29.4%) and superficial inguinal (9/29, 26.5%) lymph nodes were less frequently involved. Bilateral nodal involvement was more common (16/22, 72.7%), with unilateral involvement seen in only six cases (6/22, 27.3%), always in the submandibular lymph nodes. Subcutaneous edema was observed in 10 of the 34 dogs (29.4%), primarily mild (8/10) and involving either exclusively or concurrently the prepuce (Fig. 6), the scrotum (hydrocele, Fig. 7), the ventral abdominal region, and/or the pelvic limbs. Only two dogs (5.9%) were presented with a mild fever at the time of consultation, measuring 39.9 °C and 40.5 °C, respectively.

Hematological findings

In the complete blood count, 12 dogs (35.3%) showed anemia of various severities, with hematocrits ranging from 16% to 36%. Erythrocyte counts and hemoglobin levels varied from 2.3 to 5.4 million/mM^3 and 5.3 to 11.3 g/dL, respectively. Anemia was consistently normocytic normochromic (12/12), with mean corpuscular volume between 65.2 and 70.6 femtoliters and mean corpuscular hemoglobin concentration between 32.8% and 33.3%. Red cell distribution width values ranged from 12 to 15. Leukocyte counts were altered in most dogs (25/34, 73.5%), with leukocytosis ranging from 17,500 to 32,500 leukocytes/mM^3. Leukocytosis was consistently attributed to a mild to moderate increase in neutrophils (19/34, 55.8%), lymphocytes (2/34, 5.9%), or both (4/34, 11.8%). Thrombocytopenia was noted in a minority (5/34, 14.7%) of dogs, with platelet counts ranging from 138,000 to 190,000 platelets/mm^3.

In eight cases (8/34, 23.5%), blood smears revealed the presence of organisms. These were free-floating among erythrocytes, elongating up to 30 µm, and featured abundant cytoplasm, a clearly defined undulating membrane, a free flagellum, a central nucleus, and a small, nearly invisible

Table 1. Epidemiological and clinical findings of the dog population under study

Case	Breed	Gender	Age (months)	BCC	Visible mucosas	Lymph nodes				T °C	Observations
						SM	PE	IS	PP		
Dog 1	Galgo da Campanha[a]	Female	9	4	Normocorad	-[b]	+[c]	-	-	39.1	-
Dog 2	Pit Bull	Female	15	4	Normocorad	++[d]	-	-	++	35.9	-
Dog 3	MB	Female	9	4	Normocorad	+	(E)[e] +	+	+	38.9	-
Dog 4	Galgo da Campanha	Male	21	4	Normocorad	++	++	-	+	39.3	-
Dog 5	Pampas Deerhound	Female	13	3	Normocorad	++	+	+	+	39.2	-
Dog 6	Galgo da Campanha	Male	36	4	Normocorad	+	+	+	+	38.3	-
Dog 7	Pampas Deerhound	Male	13	3	Moderate pallor with slight jaundice of the penile mucosa	-	(E) +	-	-	39.9	Mild subcutaneous edema of the prepuce
Dog 8	Dogo Argentino	Female	33	4	Normocorad	+	-	-	-	39.1	-
Dog 9	Dogo Argentino	Male	36	4	Normocorad	-	-	+	-	38.1	-
Dog 10	Dogo Argentino	Male	9	4	Normocorad	-	++	-	-	38.5	-
Dog 11	English pointer	Female	14	4	Normocorad	-	+	+	+	37.9	-
Dog 12	Dogo Argentino	Female	9	4	Normocorad	-	-	-	-	38.0	-
Dog 13	Galgo da Campanha	Male	17	4	Mild pallor	-	+	-	+	38.9	-
Dog 14	Dogo Argentino	Male	36	4	Normocorad	-	-	-	-	38.6	-
Dog 15	Galgo da Campanha	Male	18	3	Normocorad	-	-	-	-	37.7	-
Dog 16	MB	Male	12	4	Mild pallor	+	(D)[f] ++	++	-	38.5	Moderate subcutaneous edema in the ventral abdominal region and prepuce
Dog 17	English pointer	Female	36	4	Normocorad	+	(D) +	+	-	38.3	-
Dog 18	Pampas Deerhound	Male	48	4	Normocorad	+	++	-	-	38.6	Mild subcutaneous edema of the prepuce
Dog 19	English pointer	Male	12	3	Normocorad	+	(E) +	-	-	38.5	-
Dog 20	Dogo Argentino	Female	24	4	Normocorad	-	++	-	+	38.4	-
Dog 21	Galgo da Campanha	Male	18	3	Normocorad	+	-	-	-	38.3	Mild subcutaneous edema of the prepuce
Dog 22	MB	Female	24	3	Normocorad	+	-	-	-	38.1	-
Dog 23	English pointer	Male	24	3	Normocorad	++	+	-	-	38.3	Mild subcutaneous edema of the prepuce
Dog 24	English pointer	Male	36	3	Normocorad	++	+	-	-	39.3	Mild subcutaneous edema of the prepuce
Dog 25	Dogo Argentino	Male	36	4	Mild pallor	+	+	+	-	38.6	Mild subcutaneous edema of the prepuce and scrotum (hydrocele)
Dog 26	MB	Female	9	4	Normocorad	-	-	-	-	38.4	-
Dog 27	English pointer	Female	36	3	Normocorad	+	+	-	-	38.4	-
Dog 28	Dogo Argentino	Male	12	4	Normocorad	+	+	-	-	38.0	-
Dog 29	Dogo Argentino	Male	30	3	Moderate pallor with slight jaundice of the penile mucosa	-	+	-	+	40.5	Mild subcutaneous edema in the pelvic limbs
Dog 30	Pampas Deerhound	Male	60	3	Normocorad	+	+	-	-	38.0	-
Dog 31	MB	Female	24	4	Normocorad	+	-	-	-	37.9	-
Dog 32	English pointer	Male	24	4	Normocorad	-	-	-	-	38.0	Mild subcutaneous edema of the prepuce
Dog 33	Dogo Argentino	Male	24	3	Normocorad	-	-	-	-	38.8	-
Dog 34	Dogo Argentino	Male	18	4	Moderate pallor	+	+	+	+	39.1	Subcutaneous edema in the ventral abdominal region, prepuce, and scrotum (hydrocele)

BCC = Body condition score, SM = submandibular lymph nodes, PE = prescapular lymph nodes, IIS = superficial inguinal lymph nodes, PP = popliteal lymph nodes, MB = mixed breed; [a] Breed not yet recognized by the Brazilian Kennel Club (BKC), [b] (-) no changes, [c] (+) mild increase, [d] (++) moderate increase, [e] (E) = only the left lymph node, [f] (D) = only the right lymph node.

kinetoplast – these are the hallmark morphological characteristics of *Trypanosoma* spp. Trypomastigotes. These organisms appeared in significant numbers, varying from 1-3 per high-power field (hpf [40x]) in half of the cases (4/34, 11.8%), while in the remaining cases, they were less numerous, ranging from 0-1 per hpf. No other signs of hemoparasites within erythrocytes, leukocytes, or platelets were identified in the blood smears examined.

Anatomopathological findings

The two dogs that died were identified as Dogs 29 and 34 in Table 1. Upon examination, both displayed moderately pale ocular, oral, and genital mucous membranes. Notably, there was mild jaundice in the mucous membranes, especially within the penile mucosa. In Dog 34, similar findings were observed in the scrotal region's subcutaneous tissue, known as hydrocele. The ventral abdominal subcutaneous tissue appeared moist, shiny, and gelatinous, indicating edema (Fig. 8). The spleen was uniformly and moderately enlarged (diffuse splenomegaly) (Fig. 9), soft to the touch, and uniformly red upon sectioning. Moderate cardiomegaly was observed (Fig. 10), coupled with bilateral ventricular dilation and mild hypertrophy of the left ventricular myocardium in Dog 29. The submandibular, pre-scapular, axillary, cranial sternal, superficial inguinal, and popliteal lymph nodes were uniformly and moderately enlarged (lymphadenopathy) (Fig. 11). When cut, these nodes were soft, moist, and shiny, exhibiting a whitish or brownish appearance with some preservation of the cortico-medullary distinction. Whitish, multifocal, or coalescing nodules indicative of follicular lymphoid hyperplasia obscured parts of the nodal parenchyma (florid nodal pattern). In Dog 29, the tonsils were similarly enlarged (tonsillomegaly), losing their folded structure to follicular hyperplasia, as seen in numerous white, multifocal to coalescing spots.

Histologically, the lymph nodes exhibited an increase in both the number and volume of lymphoid follicles and germinal centers. In nodes where macroscopic examination showed partial loss of cortico-medullary distinction due to coalescing nodules, lymphoid follicles were also found in the paracortex and medulla. The medullary cords were notably thickened with plasma cells, their precursors (plasmablasts), and macrophages containing brown-golden granular pigment indicative of hemosiderin. Some lymph nodes showed intense hemosiderosis, corresponding to a brownish cut surface upon necropsy. The tonsils had comparable follicular lymphoid hyperplasia. The spleen's white pulp displayed marked hyperplasia with concentric thickening of the periarteriolar lymphatic sheaths, accompanied by plasmacytosis of the red pulp, where a significant accumulation of plasma cells and plasmoblasts made identifying Billroth's cords and splenic sinuses challenging.

Some plasma cells contained large cytoplasmic granules of hyaline material (Russell bodies), giving them a morule-like appearance (Mott cells). The kidneys showed a mononuclear inflammatory infiltrate primarily consisting of plasma cells and a moderate thickening of the glomerular basement membrane due to hyaline material deposition and increased tuft cellularity (membranoproliferative glomerulonephritis). Liver examination revealed hypertrophy of Kupffer cells, mild hemosiderosis, presence of erythroid precursors (rubricytes and metarubricytes) in sinusoids, and discrete centrilobular degeneration and necrosis.

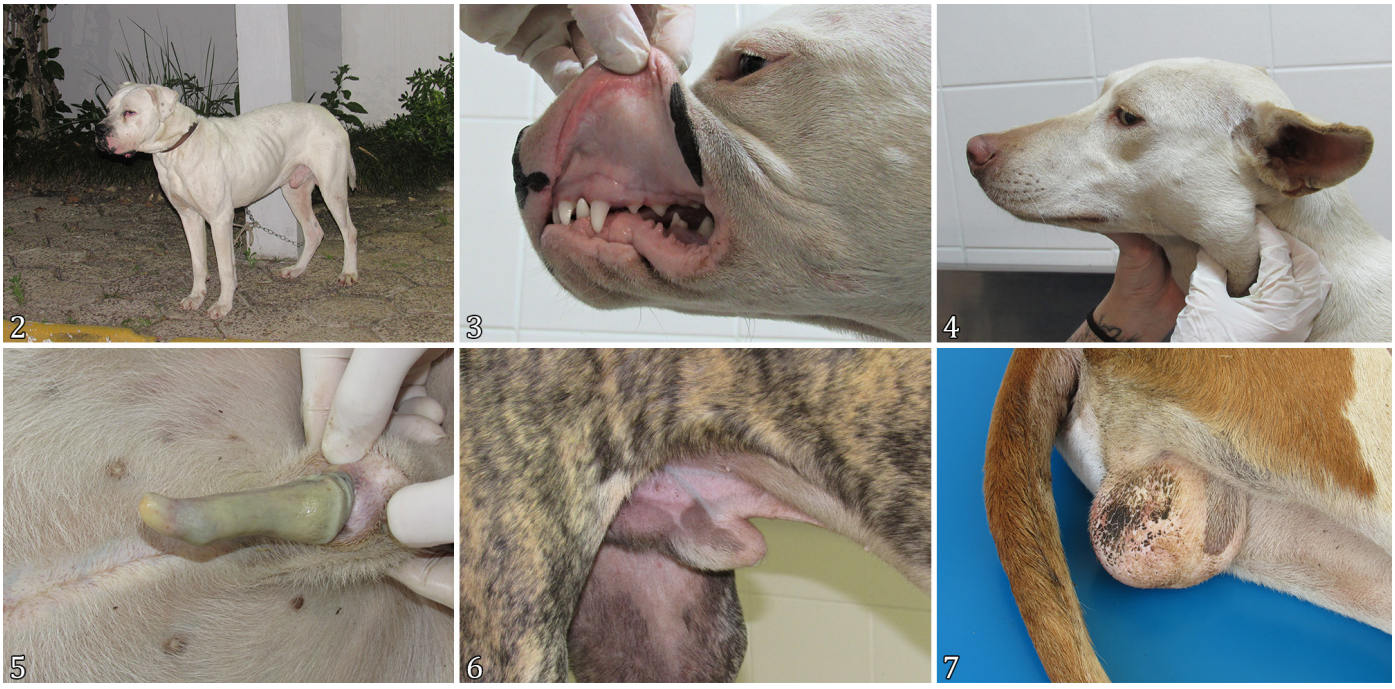

Fig. 2-7. Changes observed during clinical evaluation of dogs affected by *Trypanosoma evansi* in the present study. (**2**) Poor nutritional status (body condition score 3) in a dog with *T. evansi* trypanosomiasis. (**3**) Pallor of the oral mucosa in a dog with *T. evansi* trypanosomiasis. (**4**) Submandibular lymphadenomegaly in a dog with trypanosomiasis. (**5**) Mild jaundice of the penile mucosa in a dog with *T. evansi* trypanosomiasis. (**6**) Preputial edema in a dog with *T. evansi* trypanosomiasis. (**7**) Hydrocele in a dog with trypanosomiasis caused by *T. evansi*.

Serological diagnosis

Anti-*Trypanosoma* spp. antibodies (IgG) were not detected in any of the 34 samples analyzed.

Molecular diagnosis

DNA of *Trypanosoma spp.* was detected in six blood samples. Tissue samples from the two necropsied animals also tested positive for *Trypanosoma spp.* These positive samples underwent sequencing, achieving consensus sequences. BLAST analysis revealed identities of ≥ 98% with *Trypanosoma evansi* and *Trypanosoma brucei*. In PCR tests specific for *T. evansi* and *Trypanosoma cruzi*, blood samples tested positive for *T. evansi*, while tissue samples tested negative for *T. cruzi*.

Treatment response

Of the 32 dogs treated with a single 3.5 mg/kg dose of diminazene aceturate via deep intramuscular injection, 18 exhibited clinical improvement, weight gain, and normalization of mucous membrane coloration. The remaining 14 dogs, showing no initial improvement, received a second treatment with the same dosage and medication. Of these, four died, although the remaining 10 showed a favorable treatment response and clinical improvement.

DISCUSSION

Based on the results presented, it is evident that the infection of dogs by *Trypanosoma evansi* likely occurred through contact with the blood and/or tissues of infected wi*ld boars*. These animals are considered reservoirs for *T. evansi*, and since their infection is typically subclinical, they are cryptic reservoirs. The detection of *T. evansi* DNA in wild boars in southern Brazil has been confirmed (Freitas et al. 2024), indicating that the proximity of dogs and humans to this species could pose a significant health risk since wild pigs are reservoirs of *Trypanosoma spp.* The presence of infected wild boars can facilitate the transmission of the agent both vectorially and through close contact with blood and tissues during wild boar management practices (Herrera et al. 2008, 2007). Dogs involved in wild boar management likely became

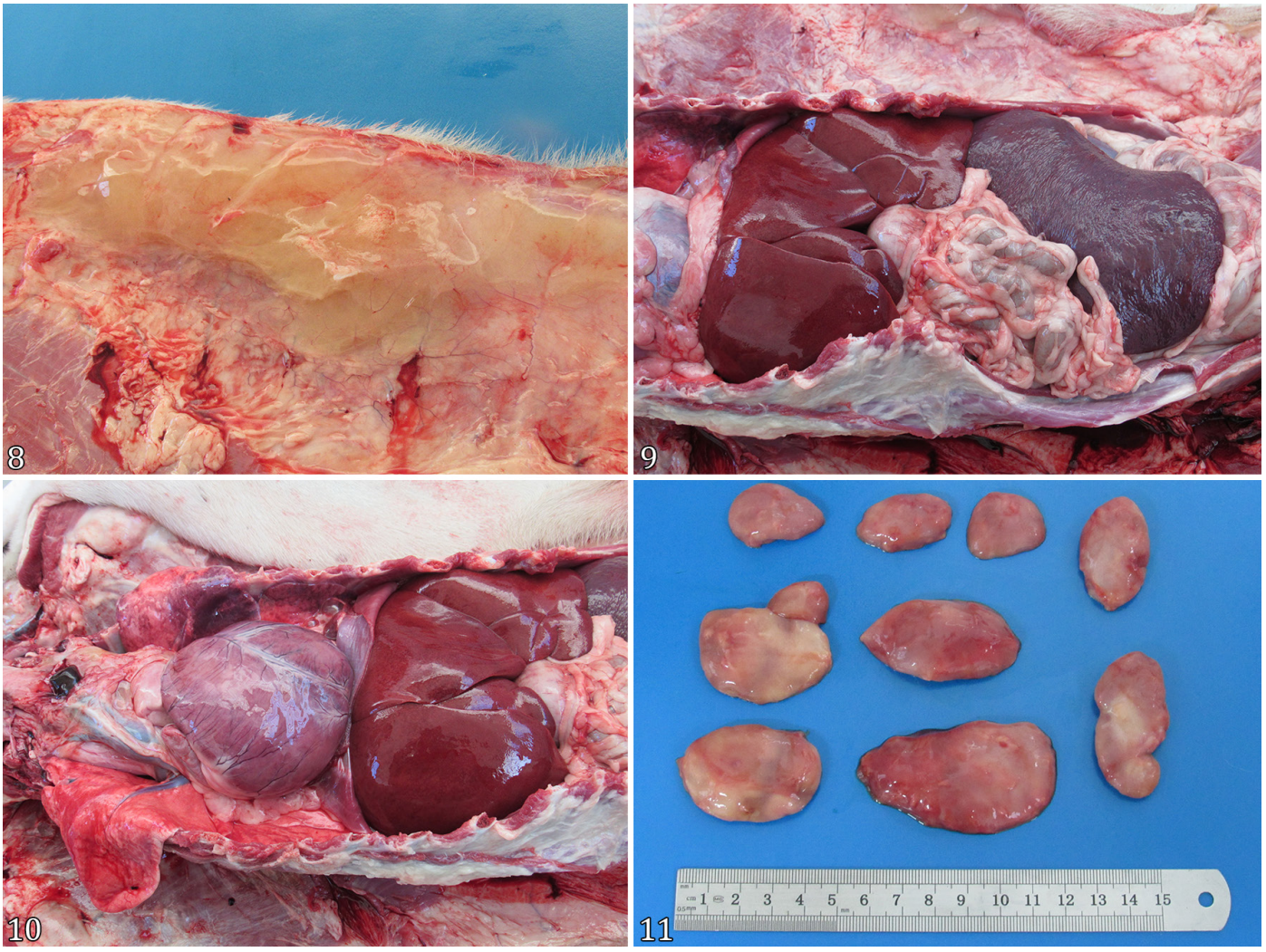

Fig. 8-11. Anatomopathological changes observed during necropsy of dogs affected by *Trypanosoma evansi* in the present study. (**8**) Subcutaneous edema in the ventral abdomen of a dog with trypanosomiasis caused by *T. evansi.* (**9**) Diffuse splenomegaly in a dog with trypanosomiasis caused by *T. evansi.* (**10**) Cardiomegaly in a dog with trypanosomiasis caused by *T. evansi.* (**11**) Generalized lymphadenomegaly in a dog with trypanosomiasis caused by *T. evansi*.

infected with *T. evansi* orally, through the ingestion of blood and/or tissues. This mode of transmission has been verified by several authors (Desquesnes et al. 2013, Panigrahi et al. 2015, Van Bree et al. 2018, Morelli et al. 2019, Sangeetha et al. 2022). It is an important route of transmission for carnivores.

Dogs are susceptible to *T. evansi* infection, which can lead to hyperthermia, anemia, mucosal pallor, progressive weight loss, weakness, and palpable lymph nodes (Aquino et al. 1999). These signs, along with macroscopic findings such as hepatomegaly and splenomegaly, were observed in dogs *from this study, supporting literature data* (Jaimes-Dueñez et al. 2017). The necropsied dogs also showed pale mucous membranes, jaundice, and lymphadenomegaly, indicating a disseminated infection. The anemia observed is associated with the damage caused by the parasite's flagellar movement and the resultant increase in proteolytic enzymes and metabolites, leading to hemolytic events (Jaimes-Dueñez et al. 2017). The observed anemia is linked to the damage caused by the flagellar movement of the parasite, along with the resulting increase in proteolytic enzymes and metabolites, which leads to hemolytic events (Jaimes-Dueñez et al. 2017). Anemia causes a loss of appetite and subsequent weight loss in animals, as well as predisposing them to jaundice. The systemic immune response in the parasitized host correlates with the observed lymphadenopathy. It may result in damage to the liver and spleen due to parasitic migration, organ removal, or extramedullary hematopoiesis (Jaimes-Dueñez et al. 2017, Simões et al. 2018).

Geographically close to the region where the animals originated, there was a report of trypanosomiasis affecting a dog (Greif et al. 2018), marking the first description of this agent in Uruguay. In addition, Freitas et al. (2024) described the detection of *Trypanosoma* spp. in tissues of wild boars in a geographical area similar to that reported in Uruguay, as well as in the dogs observed in our study. Although vector-borne transmission is a principal mode of transmission, with potential vectors such as tabanids (*Tabanus triangulum*, *Tabanus fuscus*, etc.) and *Stomoxys* species (*Stomoxys calcitrans* and other *Stomoxys* spp.) present in the studied region, transmission in these cases likely occurred orally. This hypothesis is supported by the observation that among the total number of dogs from the three properties involved, only those that had direct contact with the blood and tissues of wild boars were affected. Notably, these dogs were part of a group used for controlling the wild boar population. Within this group were scent-tracking dogs that did not engage in the hunt and, thus, did not fall ill. Therefore, the group of scent-tracking dogs that were exposed to the same environment but remained asymptomatic supports the likely oral transmission source. Access to the tissues of the animals killed before the appearance of clinical signs was not possible; such information could have further substantiated the probable transmission route (Sangeetha et al. 2022).

The treatment employed for the animals was diminazene aceturate, selected for its efficacy against *T. evansi* due to its higher therapeutic index and lower incidence of resistance (Howes et al. 2011). Research indicates that daily intramuscular administration of diminazene aceturate at 3.5 mg/kg for five consecutive days enhances penetration through the blood-brain barrier, thereby facilitating the parasite's elimination from the brain (Howes et al. 2011). Further studies suggest that two combined doses of diminazene aceturate and phenazone, administered preferably with a 24-hour interval to prevent recurrence, have been effective in rendering peripheral blood smears parasite-free on the third day post-treatment (Behera et al. 2017). In our study, an initial dose of diminazene aceturate was administered to 32 animals; among these, 18 demonstrated clinical improvement consistent with the literature, which reports improvement in consciousness and vital parameters by the third day post-treatment, while hematological and biochemical parameters returned to near-normal levels within 14 days (Behera et al. 2017). Nonetheless, 14 animals did not show improvement after a single treatment and required a second application. Starting treatment with only one dose, contrary to what is recommended in veterinary medicine literature, might be linked to deteriorating clinical conditions. This could offer a possible explanation for the death of four out of the 14 animals that needed a second treatment.

Regarding diagnosis, the direct method exhibited higher sensitivity for detection compared to serological and molecular methods. The serological method's lower sensitivity is likely due to collections performed during the outbreak, where antibody titers were below the detection threshold of the test used. Issues related to sample extraction and PCR reaction sensitivity may explain the molecular method's low number of confirmed samples. The absence of anti-*Trypanosoma* spp. antibodies in dogs that tested positive on blood smears and/or for DNA detection by PCR, or exhibited clinical signs indicative of *T. evansi* infection, aligns with other studies that have also reported a higher number of animals positive for DNA/blood smears compared to those identified through antibody detection (Porfirio et al. 2018).

The study of wild animal species such as capybaras (*Hydrochoerus hydrochaeris*), coatis (*Nasua nasua*), and wild boars is crucial. These species, previously reported as significant reservoirs for the disease, should be considered a risk factor for both maintaining and transmitting the parasite (Silva et al. 1995). The study by (Freitas et al. 2024) underscores the involvement of wild boars in trypanosomiasis' maintenance and transmission to domestic animals, including dogs, thereby underlining the importance of incorporating *Trypanosoma* spp. in dogs' routine clinical assessments. The documentation of *T. evansi* cases in equines within the region (Conrado et al. 2005), the detection of this protozoan's DNA in wild boar tissues (Freitas et al. 2024), and the findings of the present study underscore the agent's circulation and the critical need to include this protozoan in dogs' routine clinical assessments. Furthermore, it emphasizes the necessity for meticulous management of dogs involved in wild boar control operations.

CONCLUSION

Through anatomopathological, cytological, and molecular findings, we observed the ability of *Trypanosoma evansi* to infect dogs exposed to biological materials from wild boars. Based on our findings, we emphasize the importance of including *T. evansi* in routine dog diagnoses. We also highlight the need for preventive measures to limit contact between dogs and wild animals, as well as manage wild boar populations, which seem to have been the source of transmission in this outbreak. Considering that wild boars are viewed as reservoirs for *T. evansi*, they pose a risk to a wide variety of species, not just dogs.

Acknowledgments.- Ananda S. Ries and Fernanda S.F. Vogel are recipients of fellowships from the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq). This study was partially funded by the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES) – Finance code 001. The authors would also like to thank "Atlas AssessoriaLinguística" for language editing.

Conflicts of interest statement.- The authors have no financial or proprietary interests in any material discussed in this article.

Credit author statement.- Conceptualization: Ananda S. Ries, Isac J. Roman, Renata D. Mazar, Ana Paula G. Mortari, Gilneia da Rosa, Alana P. Herbichi, Douglas M. Lorenzetti, Guilherme R. Cassanego, Gabriela Hartmann, Juliana F. Cargnelutti, Rafael A. Fighera, Fernanda S.F. Vogel. Data curation: Ananda S. Ries, Isac J. Roman. Formal analysis: Ananda S. Ries, Isac J. Roman, Rafael A. Fighera. Methodology: Ananda S. Ries, Isac J. Roman. Supervision: Rafael A. Fighera, Fernanda S.F. Vogel. Writing – original draft: Ananda S. Ries, Isac J. Roman. Writing – review and editing: Ananda S. Ries, Isac J. Roman, Rafael A. Fighera, Fernanda S.F. Vogel. All authors contributed to the study's conception and design.

Data availability statement.- The authors declare that all data used are available in this article.

REFERENCES

Aquino LPCT, Machado RZ, Alessi AC, Marques LC, Castro MB, Malheiros EB. Clinical, parasitological and immunological aspects of experimental infection with Trypanosoma evansi in dogs. Mem Inst Oswaldo Cruz 1999; https://doi.org/10.1590/S0074-02761999000200025

Aregawi WG, Agga GE, Abdi RD, Büscher P. Systematic review and meta-analysis on the global distribution, host range, and prevalence of *Trypanosoma evansi*. Parasit Vectors 2019; https://doi.org/10.1186/S13071-019-3311-4

Behera SK, Sarma K, Behera P, Ali MA. Therapeutic management of trypanosomosis with ophthalmic involvement in a dog. J Parasit Dis 2017; https://doi.org/10.1007/S12639-017-0953-Z

Brun R, Hecker H, Lun Z-R. *Trypanosoma evansi* and *T. equiperdum*: distribution, biology, treatment and phylogenetic relationship (a review). Vet Parasitol 1998; https://doi.org/10.1016/S0304-4017(98)00146-0

Claes F, Radwanska M, Urakawa T, Majiwa PAO, Goddeeris B, Büscher P. Variable surface glycoprotein RoTat 1.2 PCR as a specific diagnostic tool for the detection of *Trypanosoma evansi* infections. Kinetoplastid Biol Dis 2004; https://doi.org/10.1186/1475-9292-3-3

Colpo CB, Monteiro SG, Stainki DR, Colpo ETB, Henriques GB. Infecção natural por *Trypanosoma evansi* em cães. Ciência Rural 2005; https://doi.org/10.1590/S0103-84782005000300038

Conrado AC, Lopes STA, Oliveira LSS, Monteiro SG, Vargas DLB, Bueno A. Infecção natural por *Trypanosoma evansi* em cavalos na região Central do Estado do Rio Grande do Sul. Ciência Rural 2005; https://doi.org/10.1590/S0103-84782005000400029

Desquesnes M, Dargantes A, Lai D-H, Lun Z-R, Holzmuller P, Jittapalapong S. *Trypanosoma evansi* and surra: a review and perspectives on transmission, epidemiology and control, impact, and zoonotic aspects. Biomed Res Int 2013; https://doi.org/10.1155/2013/321237

Desquesnes M, McLaughlin G, Zoungrana A, Dávila AMR. Detection and identification of *Trypanosoma* of African livestock through a single PCR based on internal transcribed spacer 1 of rDNA. Int J Parasitol 2001; https://doi.org/10.1016/S0020-7519(01)00161-8

Franciscato C, Lopes STA, Teixeira MMG, Monteiro SG, Wolkmer P, Garmatz BC, Paim CB. Cão naturalmente infectado por *Trypanosoma evansi* em Santa Maria, RS, Brasil. Ciência Rural 2007; https://doi.org/10.1590/S0103-84782007000100049

Freitas BR, Rosa G, Roman IJ, Gressler LT, Cargnelutti JF, Vogel FSF, Cunha RC. Molecular detection of DNA from *Trypanosoma* spp. and *Leishmania* spp. in wild boar (*Sus scrofa*) tissues. Vet Parasitol Reg Stud Rep 2024; https://doi.org/10.1016/J.VPRSR.2023.100970

Greif G, Faral-Tello P, Vianna CS, Hernandez A, Basmadjian Y, Robello C. The first case report of trypanosomiasis caused by *Trypanosoma evansi* in Uruguay. Vet Parasitol Reg Stud Rep 2018; https://doi.org/10.1016/J.VPRSR.2017.11.002

Herrera HM, Abreu UGP, Keuroghlian A, Freitas TP, Jansen AM. The role played by sympatric collared peccary (*Tayassu tajacu*), white-lipped peccary (*Tayassu pecari*), and feral pig (*Sus scrofa*) as maintenance hosts for *Trypanosoma evansi* and *Trypanosoma cruzi* in a sylvatic area of Brazil. Parasitol Res 2008; https://doi.org/10.1007/S00436-008-1021-5

Herrera HM, Rademaker V, Abreu UGP, D'Andrea PS, Jansen AM. Variables that modulate the spatial distribution of *Trypanosoma cruzi* and *Trypanosoma evansi* in the Brazilian Pantanal. Acta Trop 2007; https://doi.org/10.1016/J.ACTATROPICA.2007.03.001

Howes F, Da Silva AS, Athayde CL, Costa MM, Corrêa MMB, Tavares KCS, Miletti LC, Lopes STA, Amaral AS, Schmidt C. A new therapeutic protocol for dogs infected with *Trypanosoma evansi.* Acta Scient Vet 2011;39(3):988.

Jaimes-Dueñez J, Triana-Chávez O, Mejía-Jaramillo AM. Parasitological and molecular surveys reveal high rates of infection with vector-borne pathogens and clinical anemia signs associated with infection in cattle from two important livestock areas in Colombia. Ticks Tick Borne Dis 2017; https://doi.org/10.1016/J.TTBDIS.2016.12.002

Morelli G, Bastianello S, Catellani P, Ricci R. Raw meat-based diets for dogs: Survey of owners' motivations, attitudes and practices. BMC Vet Res 2019; https://doi.org/10.1186/s12917-019-1824-x

Panigrahi PN, Mahendran K, Jena SC, Behera P, Mahajan S, Arjun K, Dey S. *Trypanosoma evansi* infection in a German shepherd dog — Apparent successful treatment using serial low dose of diminazene aceturate. Vet Parasitol Reg Stud Rep 2015; https://doi.org/10.1016/J.VPRSR.2016.03.003

Porfirio GEO, Santos FM, Macedo GC, Barreto WTG, Campos JBV, Meyers AC, André MR, Perles L, Oliveira CE, Xavier SCC, Andrade GB, Jansen AM, Herrera HM. Maintenance of *Trypanosoma cruzi*, *T. evansi* and *Leishmania* spp. by domestic dogs and wild mammals in a rural settlement in Brazil-Bolivian border. Int J Parasitol Parasites Wildl 2018; https://doi.org/10.1016/J.IJPPAW.2018.10.004

Sangeetha SG, Nehra AK, Raguvaran R, Banerjee PS, Ram H. Oral transmission of *Trypanosoma evansi* infection in a German Shepherd dog—a rare case report. Comp Clin Pathol 2022; https://doi.org/10.1007/s00580-022-03324-6

Silva RAMS, Arosemena NAE, Herrera HM, Sahib CA, Ferreira MSJ. Outbreak of trypanosomosis due to *Trypanosoma evansi* in horses of Pantanal Matogrossense, Brazil. Vet Parasitol 1995; https://doi.org/10.1016/0304-4017(94)00757-4

Simões MV, Romano MMD, Schmidt A, Martins KSM, Mrin-Neto JA. Chagas disease cardiomyopathy. Int J Cardiovasc Sci 2018; https://doi.org/10.5935/2359-4802.20180011

Van Bree FPJ, Bokken GCAM, Mineur R, Franssen F, Opsteegh M, van der Giessen JWB, Lipman LJA, Overgaauw PAM. Zoonotic bacteria and parasites found in raw meat-based diets for cats and dogs. Vet Rec 2018; https://doi.org/10.1136/VR.104535