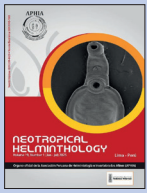
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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

CLINICAL/PARACLINICAL FINDINGS IN DOGS WITH *LEISHMANIA INFANTUM* (ROSS, 1903) IN TRANSMISSION AREAS, URUGUAY

HALLAZGOS CLÍNICOS/PARACLÍNICOS EN PERROS CON *LEISHMANIA INFANTUM* (ROSS, 1903) EN ÁREAS DE TRANSMISIÓN, URUGUAY

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ABSTRACT

Canine visceral leishmaniasis (CVL), caused by *Leishmania infantum* (Ross, 1903), is an emerging disease in Uruguay, posing significant public and animal health challenges. This study aimed to evaluate the relationship between clinical signs and hematological, hepatic, and renal alterations in dogs seropositive for *L. infantum* in Uruguay. Canids were classified according to the absence/presence of clinical signs as asymptomatic (no apparent signs of disease), oligosymptomatic (less than three clinical signs compatible with CVL), polysymptomatic (three to six clinical signs), and hypersymptomatic (with more than six clinical signs). Laboratory analysis revealed significant alterations in hematological, hepatic, and renal parameters as the number of clinical signs increased. Polysymptomatic and hypersymptomatic dogs exhibited significant decreases in erythrocytes, hemoglobin, hematocrit, mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), alongside increased segmented neutrophils and decreased lymphocytes. Hepatic function tests showed reductions in total and direct bilirubin, albumin, and the albumin/globulin ratio, while renal parameters such as urea and creatinine increased significantly with clinical severity. The multidimensional analysis highlighted distinct clusters of clinical signs and laboratory parameters that reflect the systemic effects of the disease. These findings highlight the progressive nature of CVL, characterized by worsening of erythropoiesis, hepatic dysfunction, and renal impairment. The study emphasizes the importance of early diagnosis and monitoring of clinical and laboratory parameters to manage this emerging disease

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effectively. The emergence of CVL in Uruguay calls for increased surveillance and control measures to mitigate its impact on both canine and human populations.

Keywords: Canines – visceral leishmaniasis – clinical status –hematological/biochemical profiles – global behavior – Uruguay

RESUMEN

La leishmaniasis visceral canina (LVC), causada por *Leishmania infantum* (Ross 1903), es una enfermedad emergente en Uruguay que plantea importantes desafíos para la salud pública y animal. Este estudio tuvo como objetivo evaluar la relación entre los signos clínicos y las alteraciones hematológicas, hepáticas y renales en perros seropositivos a *L. infantum* en Uruguay. Los cánidos se clasificaron según la ausencia/presencia de signos clínicos como asintomáticos (sin signos aparentes de enfermedad), oligosintomáticos (menos de tres signos clínicos compatibles con LVC), polisintomáticos (tres a seis signos clínicos) e hipersintomáticos (con más de seis signos clínicos). El análisis de laboratorio reveló alteraciones significativas en los parámetros hematológicos, hepáticos y renales a medida que aumentaba el número de signos clínicos. Los perros polisintomáticos e hipersintomáticos exhibieron disminuciones significativas en eritrocitos, hemoglobina, hematocrito, hemoglobina corpuscular media (HCM) y concentración de hemoglobina corpuscular media (HCCM), junto con un aumento de neutrófilos segmentados y una disminución de linfocitos. Las pruebas de función hepática mostraron reducciones en la bilirrubina total y directa, la albúmina y la relación albúmina/globulinas, mientras que los parámetros renales como la urea y la creatinina aumentaron significativamente con la gravedad clínica. El análisis multidimensional destacó grupos distintos de signos clínicos y parámetros de laboratorio que reflejan los efectos sistémicos de la enfermedad. Estos hallazgos resaltan la naturaleza progresiva de la LVC, caracterizada por el empeoramiento de la eritropoyesis, la disfunción hepática y el deterioro renal. El estudio enfatiza la importancia del diagnóstico temprano y el monitoreo de los parámetros clínicos y de laboratorio para manejar esta enfermedad emergente de manera efectiva. La aparición de LVC en Uruguay requiere una mayor vigilancia y medidas de control para mitigar su impacto tanto en las poblaciones caninas como humanas.

Palabras clave: Caninos – leishmaniosis visceral – estado clínico – perfiles hematológicos/bioquímicos – comportamiento global – Uruguay

INTRODUCTION

Canine visceral leishmaniasis is a parasitic zoonosis primarily transmitted by vectors. Although vectorial transmission remains the main route of infection, other forms of transmission have been identified, such as blood transfusions, and maternal-fetal transmission, albeit with a lesser epidemiological impact. The presence of green areas, gardens, and abandoned lots in cities, along with the growing pet population, is contributing to the spread of this traditionally rural disease into urban areas (Organización Panamericana de la Salud, 2023). In the Americas, the epidemiology is linked to the etiological agent *Leishmania infantum* (Ross, 1903), the vector *Lutzomyia longipalpis* (Lutz & Neiva, 1912) — preferred due to its wide distribution and adaptation to peridomestic environments — and the dog (*Canis familiaris familiaris*, Linnaeus, 1758), which represents the quintessential domestic-urban reservoir (Quinnell & Courtenay, 2009; Romero & Boelaert, 2010; Organización Panamericana de la Salud, 2023). Symptomatic or asymptomatic infected dogs can be infectious to sandflies and thus play a key role in maintaining transmission (OPS – OMS, 2019).

Canine visceral leishmaniasis is considered a systemic disease, generally chronic in its progression, and its clinical manifestations are notable for their variety and extent, depending, among other factors, on the predominant immune response of the infected animal (Ciaramella & Corona, 2003; Solano-Gallego *et al.*, 2011; Scayola *et al.*, 2024). Cutaneous lesions are reported as the most frequent and include scaly dermatitis, onychogryphosis, nasal and footpad hyperkeratosis, alopecic areas, and ulcerations. Additionally, general signs such as lymphadenomegaly, weight loss, mucosal pallor, ocular

lesions, bleeding disorders, locomotor abnormalities, renal involvement, and others are prominent (Solano-Gallego *et al*., 2011; Solano-Gallego, 2013; Scayola *et al*., 2019).

The progression of the infection involves serological, parasitological, hematological and biochemical profile alterations. In this regard, Reis *et al*. (2006) report a direct correlation between these changes and the clinical stage of the disease. They demonstrated that the severity of clinical signs was related to the parasitic density in different tissues, such as skin, bone marrow, and spleen, as well as to higher antibody titers in symptomatic dogs, resulting in a lower albumin/globulin ratio compared to asymptomatic and uninfected controls. For their part, Giunchetti *et al*. (2008) observed more intense hepatic inflammatory reactions in symptomatic dogs, associated with a higher frequency of parasitism compared to asymptomatic dogs. They also reported an association between hepatic histological changes and the progression of biochemical alterations according to the clinical forms of the infection. Although hematological and biochemical parameters are not pathognomonic for CVL, they are useful in assessing clinical status and can serve as prognostic indicators. Laboratory findings include dysproteinemia, anemia, thrombocytopenia, and azotemia (Reis *et al*., 2006; da Costa-Val *et al*., 2007; Scayola *et al*., 2024).

Considering the epidemiological relevance of canine involvement in the transmission of visceral leishmaniasis and as a holistic approach to evaluating the infection, the objective was proposed to relate and visualize the global behavior of clinical signs and hematological and biochemical profiles in *L. infantum*-seropositive canids in areas with transmission in Uruguay.

MATERIALS AND METHODS

Study area description and involved animal population

The study took place in areas where the disease is actively spreading, meaning these are regions where both dogs and humans have been reported to contract the infection locally. These areas are located in the capital city of the Salto department, Uruguay, in the western littoral region on the eastern bank of the Uruguay River (31° 23’ 18.0” S, 57° 57’ 38.0” W). The climate is temperate subtropical, with average annual temperatures and rainfall of approximately 18-19 °C and 1400 mm, respectively (Red Académica Uruguaya, 2024).

A cross-sectional study was conducted on an estimated subpopulation (Yofre *et al*., 2012) that included 43 dogs (*C. familiaris familiaris*) with a mean age of 4.9 years, diagnosed with naturally acquired visceral leishmaniasis. Diagnosis was made by the presence of anti-*Leishmania* antibodies using the immunochromatographic technique with the recombinant antigen rK39 (Kalazar Detect Canine Rapid Test, InBios).

The dogs were classified based on the absence/presence of clinical signs as asymptomatic (no apparent signs of disease) or symptomatic (with clinical signs compatible with CVL). The symptomatic group was further divided into oligosymptomatic (fewer than three clinical signs), polysymptomatic (three to six clinical signs), and hypersymptomatic (more than six clinical signs) subgroups (modified from Pozio *et al*., 1981). The frequency of clinical signs was recorded and these were grouped into the main clinical presentations: visceral (anorexia, vomiting, sneezing, diarrhea, lameness, fever), circulatory (mucosal pallor, conjunctivitis, lymphadenomegaly), and cutaneous (alopecia, onychogryphosis, nasal and footpad hyperkeratosis, pruritus, petechiae). The clinical groups were established based on the results of the multidimensional scaling.

Analysis of hematological and biochemical profiles

Two blood samples (5 mL total) were collected from each animal via the cephalic vein using sterile 21G butterfly needles (Experimental Protocol No. 2/15, approved by the Ethics Committee on Animal Use, CEUA, and the Council of Cenur Litoral Norte, Universidad de la República, on 24/06/2015), with prior consent from the owners or caretakers. One sample was collected in a tube containing ethylenediaminetetraacetic acid (EDTA) anticoagulant for hematological analysis, which included the study of the red blood cell series, white blood cell series, and platelet count. Within the red blood cell series, the number of erythrocytes, hemoglobin, hematocrit percentage, and hematimetric indices—mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)—were determined. For the white blood cell series, total and differential leukocyte counts were performed, including relative and absolute counts of neutrophils, lymphocytes, monocytes, and eosinophils.

The other sample was collected in a dry tube containing separation gel, without anticoagulant, for biochemical evaluation of liver and kidney function. The hepatic

biochemical profile included measurements of direct, indirect, and total bilirubin; cholesterol; total proteins, albumin, globulins, and the albumin/globulin ratio; and the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT). The renal biochemical profile included serum urea and creatinine levels.

Analyses were performed at a private clinical laboratory using the Horiba ABX Pentra 60 (Japan) analyzer for red blood cell, white blood cell, and platelet counts, hemoglobin, hematocrit, and hematimetric indices. Differential leukocyte counts were performed by microscopic examination of blood smears stained with May Grünwald-Giemsa. Automated analysis of hepatic and renal biochemical parameters was conducted using the AMS Alliance SAT 450 (Italy) analyzer.

Statistical analysis

The relationship between hematological and biochemical parameters and the number of clinical signs was evaluated using Student's t-test, grouping dogs into asymptomatic and oligosymptomatic versus polysymptomatic and hypersymptomatic categories. Additionally, the relationship between these parameters and the number of clinical signs, both overall and categorized into visceral, circulatory, and cutaneous presentations, was assessed using Pearson correlation (Sokal & Rohlf, 2002). A Pearson correlation coefficient (r) of 0.35, with 41 degrees of freedom, was considered the threshold for significance. A 95% confidence level was used, and statistical analyses were performed using Microsoft Excel 2016, Minitab 11, and R Package ggplot2 and scales.

For a global visualization of how clinical signs relate to each other and to hematological and biochemical parameters, multidimensional scaling was performed. Distance matrices were constructed using squared correlation coefficients (r^2) of clinical signs relative to parameters, and vice versa, for all 43 dogs, using the Perceptual Mapping (PERMAP) 11.6 software.

Ethic aspects: The experimental protocol was appproved by the Ethics Committee in the Use of Animals of the University of the Republic (CEUA/CENUR Litoral Norte, protocol N°2/15).

RESULTS

Description of clinical manifestations

Based on the classification considering the number of clinical signs in the dogs, 14% were asymptomatic, 21% oligosymptomatic, 30% polysymptomatic, and 35% hypersymptomatic. The main clinical signs recorded included skin lesions in 78.38% of cases, weight loss in 72.97%, mucosal pallor in 56.76%, lymphadenomegaly in 51.35%, conjunctivitis and polyuria/polydipsia in 45.95%, lethargy in 43.50%, petechiae in 32.50%, and anorexia in 28.50% of symptomatic dogs.

Relationship between hematological, hepatic, and renal parameters in Leishmania-seropositive dogs with the number of clinical signs and clinical presentations

When comparing asymptomatic and oligosymptomatic animals with polysymptomatic and hypersymptomatic ones (Table 1), variations in hematological, hepatic, and renal parameters were observed as the number of clinical signs increased. In the red blood cell series, there was a significant decrease in erythrocytes, hemoglobin, hematocrit, MCH, and MCHC as the number of clinical signs increased.

In the white blood cell series, polysymptomatic and hypersymptomatic dogs showed a significant increase in the relative values of segmented neutrophils and a relative and absolute decrease in lymphocytes (Table 1).

In liver function tests, total and direct bilirubin, albumin, and the albumin/globulins ratio decreased significantly in dogs with more than three clinical signs. However, no significant variation was observed in total proteins or globulins levels, despite being elevated in most cases; nor in the activities of the enzymes ALT, AST, ALP and GGT in relation to the number of clinical signs (Table 1).

Regarding the renal profile, both urea and creatinine increased significantly with the greater the number of clinical signs in the dogs (Table 1).

Table 1. Relationship between hematological, hepatic, and renal parameters in asymptomatic and oligosymptomatic dogs compared to polysymptomatic and hypersymptomatic dogs. Significance level.

Parameters	p-value	Parameters	p-value
Red blood cells	6.54131e-06 (↓)	Total bilirubin	0.00615069 (↓)
Hemoglobin	1.55078e-06 (↓)	Direct bilirubin	7.1157e-05 (↓)
Hematocrit	2.26542e-06 (↓)	Indirect bilirubin	0.06627826
MCV	0.09968413	AST	0.15712271
MCH	0.0176781 (↓)	ALT	0.48862227
MCHC	0.00296652 (↓)	GGT	0.14019129
Platelet count	0.37199956	ALP	0.2093285
White blood cells	0.90943021	Total proteins	0.27730504
Segmented neutrophils (%)	0.02027718 (↑)	Albumin	0.0311152 (↓)
Eosinophils (%)	0.87756432	Globulins	0.3914846
Lymphocytes (%)	0.00129336 (↓)	Albumin/globulins ratio	0.000624886 (↓)
Monocytes (%)	0.96856919	Urea	0.01786212 (↑)
Segmented neutrophils	0.23938512	Creatinine	0.04866968 (↑)
Eosinophils	0.65318959		
Lymphocytes	0.00350947 (↓)		
Monocytes	0.23014687		

p = significance value: (↑) = significant increase, (↓) = significant decrease.

Table 2 shows the relationship between hematological, hepatic, and renal parameters according to clinical presentation (visceral, cutaneous, and circulatory) and the total number of clinical signs. A significant decrease in red blood cells, hemoglobin, hematocrit, and MCHC was observed as the number of clinical signs increased in the overall affected dogs, as well as in those with cutaneous and circulatory presentations. In the latter, a decrease in MCH and a near-significant decrease in MCV were also noted.

Table 2. Relationship between hematological, hepatic, and renal parameters according to clinical presentation and the total number of clinical signs in *Leishmania*-positive dogs

Parameter	Visceral	Cutaneous	Circulatory	Overall
Red blood cells	-0.251	-0.45 (↓)	-0.63 (↓)	-0.5 (↓)
Hemoglobin	-0.264	-0.5 (↓)	-0.67 (↓)	-0.54 (↓)
Hematocrit	-0.261	-0.49 (↓)	-0.67 (↓)	-0.54 (↓)
MCV	-0.069	-0.23	-0.35	-0.28
MCH	-0.093	-0.32	-0.41 (↓)	-0.34
MCHC	-0.16	-0.43 (↓)	-0.4 (↓)	-0.37 (↓)
Platelet count	0.198	0.232	0.32	0.261
White blood cells	0.074	0.161	0.23	0.153
Segmented neutrophils (%)	0.064	0.264	0.38 (↑)	0.276

(Continúa Table 2)

(Continúa Table 2)

Parameter	Visceral	Cutaneous	Circulatory	Overall
Eosinophils (%)	0,257	0,108	-0,07	0,146
Lymphocytes (%)	-0.246	-0.42 (↓)	-0.45 (↓)	-0.45 (↓)
Monocytes (%)	0,039	0,065	0	0,027
Segmented neutrophils	0,025	0,045	0,14	0,088
Eosinophils	0,139	-0,05	-0,11	0,028
Lymphocytes	-0,152	-0,38 (↓)	-0,34	-0,35
Monocytes	0,017	-0,1	-0,14	-0,1
Total bilirubin	-0,19	-0,24	-0,41 (↓)	-0,27
Direct bilirubin	-0,329	-0,37 (↓)	-0,51 (↓)	-0,42 (↓)
Indirect bilirubin	-0,097	-0,14	-0,32	-0,16
AST	-0,059	0,172	0,34	0,14
ALT	-0,168	-0,01	0,12	-0,04
GGT	0,16	0,253	0,1	0,222
ALP	-0,06	0,025	0,1	0,028
Total proteins	0,132	0,263	0,2	0,225
Albumin	-0,184	-0,31	-0,44 (↓)	-0,36 (↓)
Globulins	-0,104	0,124	0,1	0,029
Albumin/globulins ratio	-0,153	-0,48 (↓)	-0,49 (↓)	-0,44 (↓)
Urea	0,312	0,299	0,36 (↑)	0,352
Creatinine	0,338	0,247	0,27	0,328

Values (↑) = significant increase, values (↓) = significant decrease.

In the white blood cell series, an increase in segmented neutrophils was observed in dogs with circulatory presentations, while a decrease in lymphocytes was noted in the overall group and in those with cutaneous and circulatory involvement. This trend was also observed in absolute lymphocyte counts for the cutaneous presentation and was nearly significant in the overall group (Table 2).

Regarding liver function, a decrease in total bilirubin was detected in dogs with circulatory presentations, and a decrease in direct bilirubin was observed in those with cutaneous and circulatory presentations, as well as in the overall group. The albumin/globulins ratio also decreased significantly in the overall group and in dogs with cutaneous and circulatory presentations. However, albumin levels decreased only in the overall group and in dogs with circulatory presentations. Serum urea was significantly increased in dogs with circulatory involvement and was near the significance threshold in the overall group. As in the previous analysis, no significant increases in total proteins or globulins were observed across different clinical presentations or with an increasing number of clinical signs in the overall group (Table 2).

Global behavior of clinical signs and hematological, hepatic and renal profiles in Leishmania-seropositive dogs

This multidimensional scaling analysis allowed for the visualization of how clinical signs and laboratory parameters behaved collectively in a 2D graph, showing proximity relationships and the emergence of pseudo-axes that provided physiological meaning to the data distribution. Based on these premises, the results are interpreted in two ways.

1- *Relationships between clinical signs and laboratory parameters in Leishmania-seropositive dogs*

The clinical signs observed in *Leishmania*-seropositive dogs were grouped into distinct presentations based on

their pathophysiological significance, as illustrated in Fig. 1:

- Circulatory presentation: Enlarged lymph nodes, conjunctivitis, and mucosal pallor. These signs are related to systemic immune responses and hematological alterations.
- Cutaneous presentation: Alopecia, onychogryphosis, nasal and footpad hyperkeratosis, pruritus, and petechiae. These findings highlight dermal involvement and localized immune responses.
- Visceral presentation: Fever, anorexia, vomiting, sneezing, lameness, and diarrhea. These signs reflect the systemic dissemination of the parasite.
- Renal involvement: Polyuria/polydipsia, indicative of renal dysfunction likely caused by immune-mediated glomerulonephritis and tubular damage.
- Independent signs: Lethargy and weight loss, which did not integrate into any specific group, are general manifestations of chronic systemic disease.

These clinical patterns demonstrate the multisystemic nature of leishmaniasis, driven by immune dysregulation, chronic inflammation, and parasite dissemination.

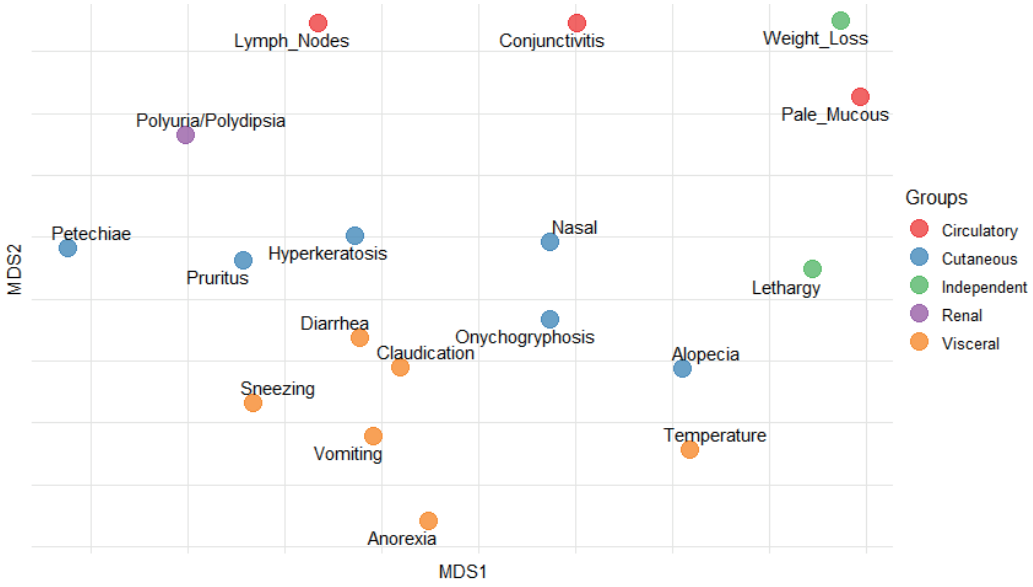


Figure 1. Relationship between clinical signs in *Leishmania*-seropositive dogs and hematological, hepatic, and renal parameters

2- Relationships between laboratory parameters and clinical signs in Leishmania-seropositive dogs

Fig. 2 illustrates the clustering of hematological, hepatic, and renal parameters into four distinct sectors, highlighting their associations with the clinical signs:

- Liver/Red Blood Cells Group: This group includes red blood cells, hemoglobin, hematocrit, hematimetric indices (MCV, MCH, MCHC), albumin, the albumin/globulins ratio, and platelets. It reflects the liver's role in erythropoiesis and protein synthesis.
- Immune System/Liver Group: Located at the center, this group includes immune system cells such as white blood cells, neutrophils, eosinophils, monocytes, and lymphocytes, along with hepatic enzymes (AST, ALT, GGT, and ALP). Cholesterol lies between this group and the liver/red blood cells group, indicating its relationship with both. These findings underscore the liver's critical role in maintaining immune and metabolic homeostasis.
- Liver/Hemoglobin Group: This group comprises total bilirubin, indirect bilirubin, direct bilirubin, and globulins, reflecting the liver's role in bilirubin metabolism and globulin production.
- Renal Group: Urea and creatinine were clustered separately, forming the renal group, which underscores the kidney's role in waste product elimination and its vulnerability to immune-mediated damage in leishmaniasis.
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The visualization highlights the distinct clustering of laboratory parameters and their associations with clinical signs, providing a comprehensive understanding of the pathophysiological relationships in *Leishmania*-seropositive dogs.

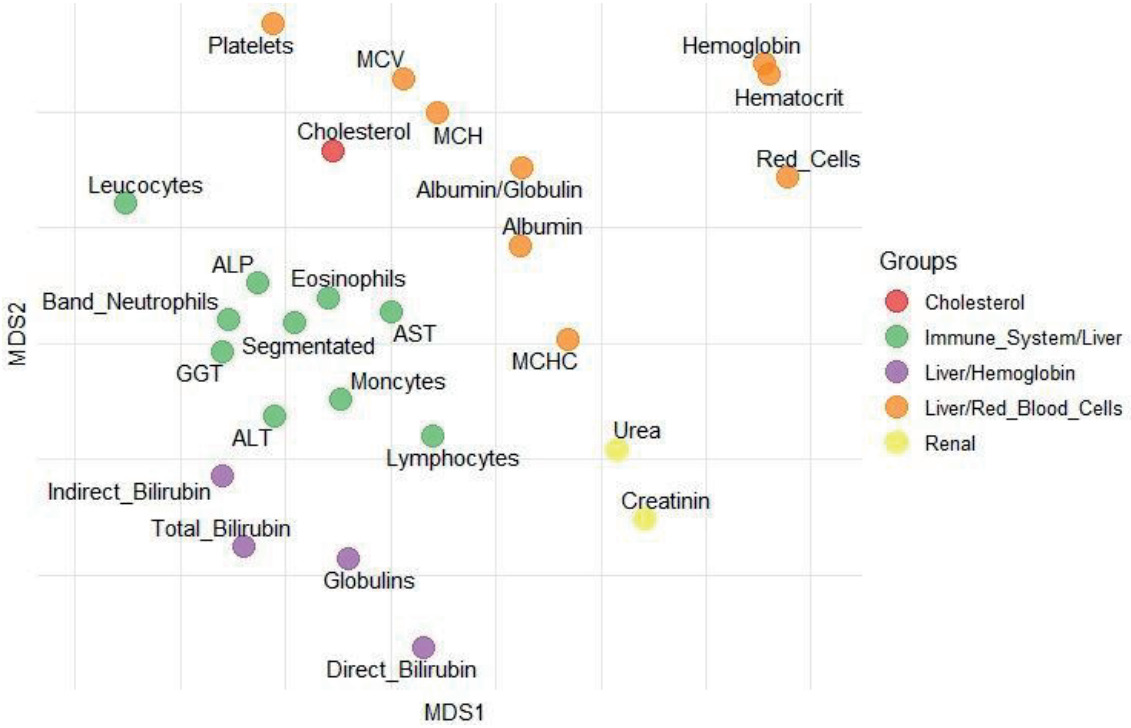


Figure 2. Relationship between hematological, hepatic and renal parameters with respect to clinical signs in *Leishmania*-seropositive dogs

DISCUSSION

Regarding the clinical manifestations of dogs seropositive for *L. infantum*, it was found that 14% showed no signs compatible with the disease. However, these asymptomatic animals exhibited decreased platelet counts and increased total proteins and globulins. The primary clinical manifestations recorded align with reports from various studies (Ciaramella & Corona, 2003; Dias *et al.*, 2008; Perego *et al.*, 2014; Sales *et al.*, 2017).

Hematological parameters

In the analysis of hematological parameters relative to the number of clinical signs present, a significant decrease in erythrocyte count, hemoglobin, and hematocrit was observed in dogs with three or more clinical signs. This relationship has been described by Reis *et al.* (2006), Nicolato *et al.* (2013), and Dodovski *et al.* (2020). Conversely, Freitas *et al.* (2012) noted reductions in erythrocytes, hematocrit, and hemoglobin in animals with clinical manifestations but found no significant differences compared to asymptomatic dogs and negative controls. It is suggested that the clinical severity of the disease is significantly associated with the erythrogram, and a factor limiting the erythropoietic response may be linked to higher parasite loads in the bone marrow as well as elevated uremia levels (Reis *et al.*, 2006; Ribeiro *et al.*, 2013; Waugh *et al.*, 2024). In contrast, da Costa-Val *et al.* (2007) found no correlation between hematocrit values and the intensity of clinical signs in dogs. They also identified anemia as responsible for classic clinical manifestations of canine leishmaniasis, such as lethargy, weakness, and weight loss, which, alongside other protozoan actions and the host's immune response, define case presentations. Additionally, anemia leads phlebotomines to feed for longer periods or ingest more blood from animals, both factors contributing to higher infection rates. The decrease in MCH concentration would indicate impaired iron homeostasis (Meléndez-Lazo *et al.*, 2018).

Platelet counts showed no significant alterations as the number of clinical signs increased. This is because thrombocytopenia was recorded in 83.33% of asymptomatic dogs. In this regard, Foglia-Manzillo *et al.*

(2013) reported thrombocytopenia and anemia as the most common early alterations, while da Costa-Val *et al.* (2007) found no correlation between clinical status and platelet count. However, Sales *et al.* (2017) described a trend toward decreased platelet counts in advanced stages of infection and Muniz dos Santos *et al.* (2023) concluded that the evaluation of thrombocytopenia is important in the follow-up of CVL.

Regarding white blood cell parameters, no significant relationship was found between total leukocyte count and the number of clinical signs. This, along with normal leukocyte counts in most *Leishmania*-positive dogs, indicates the disease's minimal influence on this parameter (Ribeiro *et al.*, 2013). Conversely, Reis *et al.* (2006) documented a significant decrease in absolute leukocyte counts in symptomatic animals, while Sales *et al.* (2017) reported an increase. Notably, although eosinophils showed no changes with increasing clinical signs, eosinopenia was observed in all animals, reaching 83% in asymptomatic cases. Absolute neutrophil concentration remained unchanged as clinical signs increased, consistent with Reis *et al.* (2006), but Sales *et al.* (2017) detected neutrophilia in poly- and hypersymptomatic dogs. Significant lymphopenia in poly- and hypersymptomatic dogs compared to asymptomatic and oligosymptomatic ones was also observed by Reis *et al.* (2006), da Costa-Val *et al.* (2007), and Sales *et al.* (2017), attributed to the disease's immunosuppressive effect and compensatory lymphocyte migration to lymphoid organs increased in advanced stages, serving as a marker of poor prognosis (Geisweid *et al.*, 2012; Nicolato *et al.*, 2013; Muniz dos Santos *et al.*, 2023).

Hepatic and renal parameters

Evaluation of liver function via enzymatic activity showed no differences with increased clinical signs. In this regard, Kiral *et al.* (2004) and Freitas *et al.* (2012) observed no significant elevations of transaminases compared to negative controls and Baxarias *et al.* (2023) found increases in liver enzymes in 13.2% of cases.

The significant decrease in total and direct bilirubin with three or more clinical signs may relate to reduced erythrocyte production (Shcherbinina, 2007). Despite increased total proteins and globulins in 76.74% and 93.02% of seropositive animals, respectively, no differences were found relative to the number of clinical signs, likely because these parameters were already elevated in 66.67% of asymptomatic dogs. Similarly, Argôlo- Montargil *et al.* (2018) and Baxarias *et al.* (2023) observed hyperproteinemia in asymptomatic dogs. In contrast, Freitas *et al.* (2012) recorded significantly higher total protein and globulin levels in the symptomatic group compared to asymptomatic and negative control grups. Hyperproteinemia and hyperglobulinemia are considered among the most common alterations in CVL (Kiral *et al.*, 2004; Sales *et al.*, 2017; Camoletto *et al.*, 2020), possibly associated with elevated anti-*Leishmania* antibody levels (Câmara *et al.*, 2017).

The significant decrease in albuminemia and albumin/globulins ratio with a greater number of clinical signs aligns with findings by Reis *et al.* (2006), Giunchetti *et al.* (2008), and Sales *et al.* (2017). These findings were reported by Amusategui *et al.* (2003) in advanced stage patients and by Foglia-Manzillo *et al.* (2013) in dogs after 12 months of infection. However, Ribeiro *et al.* (2013) found no correlation between the number of clinical signs and proteinogram parameters.

Elevated urea and creatinine levels in infected canines (Alves *et al.*, 2013; Câmara *et al.*, 2017) were significantly higher in poly- and hypersymptomatic dogs and are considered indicators of poor prognosis. The significant increase in urea would reflects early renal damage, as creatinine changes occur when most nephrons become dysfunctional (Abbehusen *et al.*, 2017; Paludo *et al.*, 2013).

Clinical evolution and disease progression

The presentation of clinical signs and laboratory parameters in asymptomatic and oligosymptomatic infected dogs compared to poly- and hypersymptomatic ones may represent disease progression. In this sense, Foglia-Manzillo *et al.* (2013) proposed that infection gradually progresses from a seemingly normal, temporary clinical state to an active intermediate stage with few clinical manifestations, evolving to the classic, severe, terminal form with more clinical signs and altered blood, hepatic, and renal parameters. Meanwhile, Donato *et al.* (2024) demonstrated the relevance of blood cell index measurements in asymptomatic and symptomatic dogs.

Clinical staging revealed distinct patterns. The circulatory presentations included signs visible through mucous membranes and lymphadenomegaly, involving peripheral and lymphatic circulation, respectively. A right-to-left axis was observed, progressing from superficial mucosal pallor to deeper conjunctival inflammation and ending with lymphatic system alterations. The renal presentations were found to be separate, acting individually but closely with the circulatory presentations and the proximity to the lymphadenomegaly may be related to the excessive

circulation of antibodies through the lymphatic system that transit to the kidney participating in the renal alteration (Verde *et al.*, 2016).

The cutaneous presentations showed clinical signs distributed along an axis from external to deeper skin layers (Harvey & Mckeever, 2001). This axis begins with superficial alopecia, onychogryphosis, and nasal/plantar hyperkeratosis, progressing to deeper vascular damage evident in petechiae.

Visceral manifestations studied here were limited to those assessed clinically, excluding signs requiring complex analyses. Many visceral signs are regulated by the central nervous system (e.g., hypothalamus and brainstem), such as diarrhea, sneezing, vomiting, anorexia, and fever. These also result from interrelated factors, such as chronic inflammatory effects on appetite control centers and high TNF-α, IL-1, and IL-6 levels inducing cachexia (Radostits *et al.*, 2007; Costa *et al.*, 2023).

Relationships between hematological, hepatic, and renal parameters in seropositive dogs and clinical signs revealed hepatic involvement in systemic alterations except renal function. An inverse relationship between red blood cell components and bilirubin was observed, separating erythrocyte formation (liver/red blood cell group: Fig. 2 upper end) and destruction (liver/hemoglobin group: Fig. 2 lower end), as bilirubin is a hemoglobin catabolite. Albumin, responsible for bilirubin transport (Hayes, 2004), was located between these groups. Proximity of erythrocytes, hemoglobin, and hematocrit was logical, as decreases in these parameters manifest as anemia. Nearby hematimetric indices reflected anemia type. In the liver/red blood cells group, the location of platelets was also highlighted, since thrombocytopenia can cause bleeding, altering erythrocyte counts, with both parameters at opposite ends. In the center of Fig. 2, the relationship between the liver and its connection to the immune system is represented (Aguilar, 2010; Bevilacqua & Canziani, 2014; Waugh *et al.*, 2024).

The study on CVL caused by *L. infantum* provides crucial insights into the systemic effects of the disease, combining findings from Uruguay with a multidimensional analysis of clinical and laboratory data. Laboratory analyses showed progressive alterations in hematological, hepatic, and renal parameters as clinical severity increased. Polysymptomatic and hypersymptomatic dogs showed affected erythropoiesis, characterized by decreases in red blood cells, hemoglobin, hematocrit, MCH, and MCHC, as well as increases in segmented neutrophils and decreases in lymphocytes. Liver function tests indicated reductions in total and direct bilirubin, albumin, and the albumin/globulins ratio, while renal parameters such as urea and creatinine increased significantly with clinical severity. The multidimensional analysis highlighted distinct clusters of clinical signs and laboratory parameters that reflect the systemic effects of the disease. Clinical signs were grouped into circulatory, cutaneous, visceral, and renal presentations, emphasizing the multisystemic nature of CVL. The liver emerged as a key organ involved in erythropoiesis, immune regulation, protein synthesis, and bilirubin metabolism. Renal involvement, characterized by elevated urea and creatinine, highlighted the role of immune-mediated glomerulonephritis and tubular damage in disease progression. The progressive nature of CVL, from an asymptomatic state to a severe form with worsening clinical and laboratory manifestations, emphasizes the importance of early diagnosis and close monitoring of hematological, hepatic, and renal parameters. This analysis also highlights the utility of integrating clinical and laboratory data to comprehensively characterize disease progression, providing a framework for improved diagnostic and therapeutic strategies.

In conclusion, CVL represents an emerging health threat in Uruguay and other endemic regions, characterized by worsening of erythropoesis, hepatic dysfunction, and renal impairment as clinical severity increases. The findings emphasize the importance of implementing control measures, increasing surveillance, and advancing clinical management strategies to mitigate the disease's impact on canine and human populations.

Author contributions: CRediT (Contributor Roles Taxonomy)

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