SENSITIVITY AND SPECIFICITY OF DIAGNOSTIC METHODS FOR CANINE VISCERAL LEISHMANIASIS: A COMPARATIVE ANALYSIS OF IMMUNOCHROMATOGRAPHIC RAPID TEST (TR DPP), ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA), AND POLYMERASE CHAIN REACTION (PCR) USING BLOOD SAMPLES

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1. BACKGROUND

Canine visceral leishmaniasis (CVL) is a zoonosis considered a neglected tropical disease, caused by parasites of the *Leishmania* genus. It is an illness with clinical signs that vary according to the response of each host organism, and it can be asymptomatic or symptomatic. The main clinical signs reported are onychogryphosis, dermatitis, ulcerative lesions in mucosal regions, and ocular alterations such as uveitis, blepharitis, and hyphema. Laboratory alterations are nonspecific, and diagnosis can sometimes be complex.[1-4]

The main reservoir of the disease in urban environments is the dog. In wildlife environments, the protozoan has been isolated in marsupials, wild canids, and rodents. Due to factors such as the adaptability of vectors to urban environments and climate change, as well as the increasing proximity of dogs to humans, the number of CVL cases has expanded significantly.[3-5]

Brazil is among the countries with the highest number of registered CVL cases, alongside Bangladesh, Sudan, Ethiopia, India, and Nepal. The species responsible for the visceral form of the disease in Latin America is *Leishmania infantum*. The known vectors capable of transmitting the disease in the aforementioned region are *Lutzomyia longipalpis* and *L. cruzi*, from the *Psychodidae* family.[6]

When taking a blood meal from the reservoirs, female phlebotomine sandflies can become infected with amastigote forms. These forms then transform into the promastigote form in the arthropod's gastrointestinal tract. The promastigote is the flagellated infective form, which is transmitted to the vertebrate host through the vector's bite. Upon infecting the host, promastigotes parasitize phagocytes and revert to the amastigote form, spreading through lymphatic and hematogenous routes.[5,6]

The officially recommended diagnosis in Brazil consists of screening through the immunochromatographic rapid test (TR DPP®) and confirming the infection via the enzyme-linked immunosorbent assay (ELISA).[7] However, recent studies indicate that the sensitivity and specificity of these tests may vary. A study involving 975 dogs compared results of DPP, ELISA, and real-time PCR (qPCR), finding that nearly one in five (174/887; 19.6%) of the dogs negative for DPP tested positive for LVC by qPCR.[8] Comparative research indicated that in canine populations with a seroprevalence below 30% for LVC, DNA detection by PCR reached 80%.[9] Tests using DPP and in-house ELISA showed cross-reaction rates with *Babesia canis* of 44% and 22%, respectively.[10]

Brazilian researchers conducted a systematic review followed by meta-analysis in 2013 to assess the accuracy of serological tests used for diagnosing canine visceral leishmaniasis in the Americas. Fifteen studies using crude antigen (ELISA), eleven studies on indirect immunofluorescence (IFAT), and three studies on the dual-platform immunochromatographic test (DPP) were meta-analyzed. The authors concluded that ELISA with crude antigens and DPP have moderate accuracy for diagnosing CVL. They pointed to the need for studies focused on validating and improving diagnostic tests for the disease.[11] To date, there has been no publication of a systematic review followed by a meta-analysis to evaluate the immunochromatographic test and ELISA as diagnostic methods for CVL globally.

In this context, the search for a definitive diagnosis of CVL is of utmost importance to obtain more accurate results. This will lead to a more reliable record of this zoonosis. Consequently, official bodies, health professionals, and citizens will be able to implement more effective control and combat strategies for the disease. Additionally, with accurate and early diagnosis, the patient can undergo more targeted interventions.

Thus, the objective of this study is, through the use of evidence-based medicine methodologies, specifically systematic review and meta-analysis, to evaluate

whether the sensitivity and specificity of the TR DPP® and ELISA are higher than those of PCR and its variations in diagnosing CVL using blood samples.

2. OBJECTIVES

2.1 General Objective

To conduct a systematic review and meta-analysis to evaluate whether the sensitivity and specificity of the TR DPP® and ELISA are higher than those of PCR and its variations in diagnosing CVL using blood samples.

3. METHODS

The data will be obtained through a systematic review, following the recommendations of Cochrane Collaboration. After extraction, they will be submitted to meta-analysis. The PICO methodology will be used to structure the research question. P represents the population to be studied, in this case: dogs diagnosed with Visceral Leishmaniasis. I refers to the index test, where the immunochromatographic test and enzyme-linked immunosorbent assay (ELISA) will be evaluated. C corresponds to the comparator tests, which are the Polymerase Chain Reaction (PCR) and its variations. The outcomes, represented by O, consist of the sensitivity and specificity of the aforementioned tests.

The databases PUBMED/NCBI, SCIENCE DIRECT, SCOPUS, SCIELO, Web Of Science, and the Virtual Health Library will be used to search for scientific evidence. Grey literature will not be considered. The search strategy will involve a combination of subject descriptors and Boolean operators: sorology, leishmania, enzyme-linked immunosorbent assay, dogs (dogs OR canis familiaris), polymerase chain reaction.

The questions to be answered are:

● Is the sensitivity and specificity of the TR DPP® and ELISA higher than that of PCR and its variations, in the diagnosis of VL, based on blood samples?

3.1 Eligibility Criteria Definition

The following criteria will be considered for inclusion: i) primary studies involving dogs with LV, of any age, breed, and sex; ii) studies using serological tests

(TR DPP® and ELISA) from blood samples for CVL diagnosis; iii) studies with confirmed infection by PCR or its variations, using blood tissue samples, lymphoid tissue, skin tissue, or conjunctival swabs; iv) studies written in English, Spanish, or Portuguese; v) studies published in the last twenty-two years. Studies not meeting any of these criteria will be excluded from the review.

3.2 Documentation of the Systematic Review Methodology

The articles identified will be pre-selected based on titles and abstracts, which will be reviewed by two independent researchers. Subsequently, the shortlisted articles will undergo full-text review to assess their eligibility for inclusion in the review. In both stages, selection will be carried out by two independent reviewers. Articles not meeting the criteria will be excluded, and those deemed eligible by both reviewers will be included in the study. In case of disagreement, a third reviewer will be consulted. The primary reason for exclusion will be recorded and will be included in the selection flow diagram.

For selection, a standardized clinical data sheet will be used, designed according to the recommendations of the Cochrane Collaboration, with pre-defined inclusion and exclusion criteria. Furthermore, the sheet will contain data that will be used sequentially to summarize the scientific evidence and assess the feasibility of performing the meta-analysis. Key information will include species, sex, number of patients included in the study, reference standard used, types of samples used in both the reference standard and index test, and the methodology employed in both tests, including target genes and primers.

3.3 Data Extraction

Data will be extracted using a form developed according to the recommendations of the Cochrane Collaboration[12], aiming to assess the heterogeneity of the studies and the variables that may influence the sensitivity and specificity of each technique. The collected evidence will be tabulated and summarized to form the basis for the meta-analytic analyses.

The scientific evidence extracted using the standardized form will be organized into Microsoft Office Excel® spreadsheets.

The quality of the methods used in the primary studies will undergo a critical evaluation through the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) protocol.[12] The results will be presented in both tabular and graphical formats.

3.4 Meta-analytic Summarization and Statistical Analysis

The data obtained from the systematic literature review will be tabulated in spreadsheets and subjected to meta-analysis. The results will be graphically assessed through a sensitivity analysis, plotting each risk difference (RD) against its corresponding standard error (funnel plot). The absence of publication bias will be considered when there is a homogeneous distribution of RDs on both sides of the funnel plot. Furthermore, the asymmetry of the funnel plot will be formally tested using Egger's regression.[13]

Studies whose risk difference lies outside the normal pyramid of the funnel plot will be considered potential outliers, and their influence on the overall result will be tested by excluding them individually from the analysis. Any significant change in the overall result will lead to the removal of the study from the analysis; otherwise, the study will be retained. The software Review Manager 5.4.1 and Jamovi 2.3.19.0 will be used for all statistical analysis and graphical synthesis of the results.

4. TIMELINE OF THE REVIEW PROCESS

STAGE	STARTED	COMPLETED
Literature review and team selection	**YES**	**YES**
Pilot project and adjsutments	**YES**	**YES**
Eletronic database search	**YES**	**YES**
Data selection	**NO**	**NO**
Data extraction	**NO**	**NO**
Quality appraisal	**NO**	**NO**
Synthesis	**NO**	**NO**
Meta-analysis	**NO**	**NO**

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