CASE REPORT

# Molecular detection of *Leishmania (Leishmania) infantum* in vitreous humor of a dog with visceral leishmaniasis

## Detecção molecular de *Leishmania (Leishmania) infantum* no humor vítreo de um cão com leishmaniose visceral

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## Abstract

The vitreous humor is a biological matrix that has been poorly explored in Canine Visceral Leishmaniasis (CVL). Its study represent an alternative approach for investigating *Leishmania infantum*. This report describes the molecular detection and isolation of *L. infantum* in the vitreous humor of a dog from an endemic area in northwestern São Paulo State, Brazil. An adult male dog presented with progressive weight loss, lymphadenomegaly, and signs of bilateral chronic uveitis associated with vision loss. Infection was confirmed by Dual Path Platform (DPP®) and ELISA serological tests. Lymph node and bone marrow samples were collected before euthanasia; spleen, liver, cornea, lens, and vitreous humor samples were collected afterwards. Samples of spleen, liver, lymph node, and ocular tissues were analyzed in parasitological culture. Additionally, real-time PCR (qPCR) was performed on bone marrow and ocular samples, using the canine GAPDH gene as an endogenous quality control. The cornea and lens yielded negative results in both methodologies. qPCR confirmed the presence of *L. infantum* DNA in the bone marrow and vitreous humor. The isolation and molecular detection of *L. infantum* in the vitreous humor demonstrate intraocular involvement in CVL and reinforce the parasite's presence in different tissue compartments. These findings broaden our understanding of the tissue tropism of *L. infantum* and contribute to knowledge about the parasite's dissemination in the ocular tissues of naturally infected dogs.

**Keywords:** *Leishmania (L.) infantum*, canine visceral leishmaniasis, qPCR, vitreous humor, ocular involvement.

## Resumo

O humor vítreo é uma matriz biológica ainda pouco explorada na leishmaniose visceral canina (LVC). Seu estudo representa uma abordagem alternativa para a investigação de *Leishmania infantum*. Este relato descreve a detecção molecular e o isolamento de *L. infantum* no humor vítreo de um cão proveniente de uma área endêmica no noroeste do Estado de São Paulo, Brasil. Um cão macho adulto apresentava perda de peso progressiva, linfadenomegalia e sinais de uveíte crônica bilateral associados à perda de visão. A infecção foi confirmada pelos testes sorológicos Dual Path Platform (DPP®) e ELISA. Amostras de linfonodo e medula óssea foram coletadas antes da eutanásia; após o procedimento, foram obtidas amostras de baço, fígado, córnea, cristalino e humor vítreo. Amostras de baço, fígado, linfonodo e tecidos oculares foram submetidas à cultura parasitológica. Adicionalmente, foi realizada PCR em tempo real (qPCR) em amostras de medula óssea e tecidos oculares, utilizando o gene canino GAPDH como controle endógeno de qualidade. A córnea e o cristalino apresentaram resultados negativos em ambas as metodologias. A qPCR confirmou a presença de DNA de *L. infantum* na medula óssea e no humor vítreo. O isolamento e a detecção molecular de *L. infantum* no humor vítreo demonstram o envolvimento intraocular na LVC e reforçam a presença do parasito em diferentes compartimentos teciduais. Esses achados ampliam a compreensão sobre o tropismo tecidual de *L. infantum* e contribuem para o conhecimento acerca da disseminação do parasito nos tecidos oculares de cães naturalmente infectados.

**Palavras-chave:** *Leishmania (L.) infantum*, leishmaniose visceral canina, qPCR, humor vítreo, envolvimento ocular.

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## Introduction

Visceral leishmaniasis (VL) is a parasitic zoonosis caused by protozoa of the *Leishmania donovani* complex, with *Leishmania* (*Leishmania*) *infantum* being the main species responsible for urban transmission in Brazil and other countries in the Americas. Domestic dogs are recognized as the primary urban reservoirs of infection and play a central epidemiological role in maintaining transmission of the parasite to phlebotomine sandflies (Dantas-Torres, 2009; Baneth et al., 2008).

Canine visceral leishmaniasis (CVL) is characterized as a chronic, multisystemic infection with heterogeneous clinical manifestations, including dermatological changes, weight loss, lymphadenomegaly, hepatosplenomegaly, and ocular involvement. Ophthalmic changes are frequently reported in naturally infected dogs and may affect the conjunctiva, cornea, uveal tract, retina, and ocular adnexa. Among the most common findings are keratoconjunctivitis, uveitis, blepharitis, and ocular discharge, generally associated with parasitic dissemination and immune-mediated inflammatory responses triggered by infection (Peña et al., 2000; Solano-Gallego et al., 2009).

Diagnosis of VL is based on a combination of clinical evaluation, serological tests, parasitological methods, and molecular techniques. Among these, real-time polymerase chain reaction (qPCR) has demonstrated high sensitivity and specificity for detecting parasitic DNA, enabling identification even in samples with low parasitic load or in tissues less frequently investigated (Mary et al., 2004; Solcà et al., 2014).

Molecular approaches have also contributed to studies on tissue tropism, infection dynamics, and the genetic diversity of circulating *Leishmania* strains (Schönian et al., 2003; Boité et al., 2012).

Parasitological culture and isolation remain important tools for confirming active infection, as they allow recovery of viable parasites for subsequent biological, molecular, and epidemiological characterization (Solano-Gallego et al., 2011).

Isolation of *L. (L.) infantum* for VL diagnosis has been described from tissues such as lymph nodes, bone marrow, spleen, skin, and blood, using culture media such as biphasic Novy-MacNeal-Nicolle (NNN) medium and liquid Schneider's medium supplemented with fetal bovine serum. Although culture methods are generally less sensitive and more time-consuming than molecular techniques, they are considered highly specific and remain essential for strain characterization and parasite maintenance in research laboratories (Maia; Campino, 2008).

Furthermore, while ophthalmic samples such as conjunctival swabs and aqueous humor have been investigated in VL, studies involving vitreous humor remain scarce. Detection and isolation of *L. (L.) infantum* in vitreous humor are poorly described in the literature, particularly in association with evident clinical ophthalmological manifestations.

## Case report

This report describes the molecular detection and parasitological isolation of *L. (L.) infantum* from different biological samples obtained from a dog in an endemic municipality for VL in the northwestern region of São Paulo State, Brazil. Clinical evaluation and sample collection were performed as part of routine activities of municipal zoonosis surveillance and control services.

An adult male, mixed-breed dog was examined. Clinical assessment revealed progressive weight loss, generalized lymphadenomegaly, ulcerative lesions on the distal region of the ear, and bilateral ophthalmological changes compatible with chronic uveitis associated with vision loss **(**Figure 1). The combination of systemic clinical manifestations, ocular involvement, and epidemiological history raised strong suspicion of CVL.

Following clinical diagnosis, immunological testing was performed using the Dual Path Platform rapid test (TR-DPP®) and ELISA (Bio-Manguinhos/Fiocruz, Brazil), in accordance with the diagnostic protocol recommended for VL in Brazil (Grimaldi Junior et al., 2012; Brasil, 2026). Both tests were reactive, supporting a serological diagnosis of CVL. The ELISA O.D. was 0.529, above the cut-off value.

Due to the unfavorable prognosis associated with advanced disease, the animal was referred for euthanasia in accordance with Brazilian Ministry of Health guidelines (Brasil, 2026), with formal consent from the owner. Clinical evaluation, euthanasia, and sample collection were performed as part of routine activities of the municipal Zoonoses Control Program, in accordance with the Brazilian Ministry of Health guidelines for canine visceral leishmaniasis (Brasil, 2026).




**Figure 1.** a) General clinical presentation of the dog at admission: bilateral ocular opacity with corneal edema and cutaneous/auricular lesion. The images document the general clinical condition and do not represent a detailed ophthalmological examination. b) Ulcerative lesions affecting the distal auricular region.

Laboratory analyses were approved by the Scientific Technical Council of the Adolfo Lutz Institute (CTC-IAL; approval no. 33-R/2025). No experimental procedures were performed, and CEUA or CEP approval was not required.

Prior to euthanasia, the dog was deeply sedated and anesthetized with 2% xylazine hydrochloride (2 mg/kg) and 10% ketamine hydrochloride (10 mg/kg), both administered by the intramuscular route. Anesthetic depth was assessed and confirmed by the loss of the palpebral and interdigital (pedal withdrawal) reflexes and by the absence of motor response to noxious stimuli prior to any procedure, indicating an adequate depth of anesthesia before sample collection. Pre-mortem samples were obtained for parasitological and molecular investigation, including popliteal lymph node aspirate and bone marrow collected by iliac crest puncture.

Euthanasia was then performed by intravenous administration of embutramide, mebezonium iodide, and tetracaine hydrochloride (T-61®, MSD, Brazil), in accordance with ethical guidelines (Conselho Federal de Medicina Veterinária, 2013). Post-mortem, spleen and liver fragments were collected for parasitological culture. Given the ophthalmological impairment observed, cornea, lens, and vitreous humor samples were also obtained. Vitreous humor was collected by sterile ocular puncture, with careful aspiration of approximately 500 μL of intraocular contents. All samples were immediately transferred to saline solution supplemented with gentamicin and sent to the laboratory for processing.

For parasitological culture, 200 μL of each saline suspension was inoculated into biphasic medium consisting of blood agar supplemented with Brain Heart Infusion (BHI), Schneider's medium supplemented with sterile urine, and fetal bovine serum, following established protocols for *Leishmania* isolation (Evans et al., 1989; Maia; Campino, 2008). Cultures were maintained at 25 °C in a Biochemical Oxygen Demand (BOD) incubator and monitored microscopically for parasite growth and promastigote development.

Initial growth was observed after 24 hours in lymph node cultures. Spleen, liver, and vitreous humor cultures showed growth after 48 hours. Positive isolates progressed to exponential and stationary phases. Cultures from lymph nodes and vitreous humor reached stationary phase after approximately seven days, while spleen and liver isolates reached this phase after eight days. Corneal and lens samples remained negative throughout the 28-day observation period. No parasitological culture was performed on the bone marrow sample (Table 1).

**Table 1.** Results of parasitological culture and qPCR performed on different tissues and ocular samples in the dog naturally infected with *L. infantum*.

| Clinical sample | Parasitological (Culture) | Time to first observed growth | Time to Stationary phase (in days) | qPCR (*L. infantum*) | Ct (*L. infantum*) | Ct (GAPDH) |
|---|---|---|---|---|---|---|
| Lymph node | Positive | 24 h | 7 | Not performed | – | – |
| Bone marrow | Non performed | – | – | Positive | 35 | 20 |
| Spleen | Positive | 48 h | 8 | Not performed | – | – |
| Liver | Positive | 48 h | 8 | Not performed | – | – |
| Vitreous humor | Positive | 48 h | 7 | Positive | 36 | 20 |
| Cornea | Negative | Absent | Not reached | Negative | – | 23 |
| Lens | Negative | Absent | Not reached | Negative | – | 22 |

Extraction and purification of DNA samples were performed using the QIAamp DNA Mini Kit (Qiagen®, Hilden, Germany), following the manufacturer's instructions. DNA concentration was determined from absorbance at 260 nm and purity was assessed using the 260/280 ratio using a NanoDrop One spectrophotometer, and samples were diluted to 100 ng/μL. Reactions were conducted by qPCR as previously described (Colombo et al., 2011; Carneiro et al., 2026). Reactions contained 10 μL of 2X TaqMan Universal PCR Master Mix and 1.25 μL of assay mix (18 μM primers, 5 μM probe). The primers identified *L. (L.) infantum* (belonging to the L. donovani complex) and they were: Ldon37act-F (5'AAGTGCGACATTGATGTGCGC3'), Ldon114act-R (5'AAGGTTGAGGAACATGGTCGAC3'), and probe Ldon66act-PB (5'CCGGACAGCACGATGTTCCCGTAC3') labeled with FAM at the 5' end and BHQ-1 quencher at the 3' end. The canine GAPDH gene served as endogenous control, and ROX dye was used as a passive reference for fluorescence normalization.

Reactions were performed on the Applied Biosystems 7500 Fast Real-Time PCR System, with positive and negative controls included in all runs. Amplification conditions consisted of an initial step at 50 °C for 2 min, denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 sec and annealing/extension at 60 °C for 1 min.

qPCR was positive for *L. (L.) infantum* in bone marrow and vitreous humor samples. Corneal and lens samples remained negative throughout amplification cycles. GAPDH amplification confirmed the presence of amplifiable canine DNA **(**Table 1).

## Discussion

CVL is a systemic infectious disease with diverse clinical manifestations, including ocular involvement. Ophthalmic alterations such as uveitis, conjunctivitis, keratoconjunctivitis, blepharitis, and corneal opacity are frequently reported in naturally infected dogs, underscoring the relevance of ocular changes in CVL (Peña et al., 2008; Solano-Gallego et al., 2011).

The dog analyzed in this case presented bilateral ocular opacity compatible with chronic uveitis, associated with parasite detection in vitreous humor by both parasitological culture and qPCR. The concomitant positivity in axenic culture and qPCR suggests parasite viability in the intraocular compartment. These findings highlight the clinical relevance of parasites when interpreted alongside compatible ophthalmic signs and complementary diagnostic methods (Mary et al., 2004). Amplification of the endogenous GAPDH gene in vitreous humor confirmed sample integrity, efficient DNA extraction, and absence of significant qPCR inhibition, technically validating the molecular results (Magalhães-Cunha et al., 2026).

Literature evidence indicates that ocular changes in CVL may result from both direct hematogenous dissemination of parasites and immune-mediated inflammatory mechanisms (Peña et al., 2000; El Goulli et al., 2023). The occurrence of ophthalmic lesions associated with parasite identification reinforces the eye as a relevant site of involvement in CVL (Magalhães-Cunha et al., 2026).

Parasites were not detected in cornea and lens samples by culture or qPCR. The absence of parasites in cornea and lens samples, as shown by culture and qPCR, likely reflects the avascular nature of these structures, which may hinder parasite establishment compared to highly vascularized tissues or those of the mononuclear phagocytic system. This pattern aligns with the classic tissue tropism of *L. (L.) infantum* (Solcà et al., 2014).

Beyond tissue tropism, the diagnostic rationale for sampling the vitreous humor itself also deserves consideration. In clinical veterinary ophthalmology, vitreous humor sampling is not a routine diagnostic procedure. Vitreocentesis is invasive and carries a substantial risk of ocular complications, including uveitis, intraocular hemorrhage, and retinal detachment; therefore, its indication in vivo is restricted to selected situations, mainly non-visual eyes or eyes scheduled for enucleation, in the investigation of endophthalmitis, intraocular neoplasia, or uveitis of undetermined etiology. When performed, vitreous analysis may provide cytological, microbiological, and molecular information relevant for diagnosis.

In the present case, however, the vitreous humor was not sampled in vivo: collection was performed post-mortem, after euthanasia indicated on clinical and sanitary grounds. This approach eliminates the procedural risks described above and, given the anatomical isolation and relative sterility of the vitreous compartment, supports it as a safe and accessible sample for molecular investigation of *L. (L.) infantum*.

The combined use of qPCR and parasitological isolation strengthens confirmation of infection in vitreous humor. While molecular analysis enabled specific identification of *L. (L.) infantum*, culture demonstrated parasite viability in the sample. Together, these methods enhance diagnostic robustness and support evidence of parasite presence and viability in this compartment. In clinical practice, CVL diagnosis is predominantly based on lymphoid tissue samples (lymph nodes, spleen, bone marrow), which may underestimate parasite presence in less accessible compartments. This study indicates that vitreous humor may function as an auxiliary diagnostic matrix in cases exhibiting ophthalmic manifestations, especially when alternative samples demonstrate low parasite loads or yield inconclusive outcomes.

## Conclusion

Molecular detection and parasitological isolation of *L. (L.) infantum* in vitreous humor confirm intraocular involvement in CVL, reiterating the parasite's presence in different tissue compartments. These findings expand knowledge of tissue tropism and contribute to understanding the dissemination of *L. (L.) infantum* in ocular tissues of naturally infected dogs.

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## Ethics statement

This study describes clinical cases managed as part of routine veterinary care. No experimental procedures or interventions were performed for research purposes. Written informed consent for the publication of clinical information and images was obtained from the animal owners. Therefore, ethical approval was not required.

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## Conflict of interests

DMBB, GMDB, FMTB, ABPS, ICBB, RG, VLPC and JET – No conflict of interest.

## Authors' contributions

DMBB - Study design; analysis of the results; manuscript writing; veterinary clinical care; collection of the biological samples. FMTB, ICBB and RG - Contributed to the laboratory processing; molecular analysis; critical review of the manuscript. ABPS - Contributed to the laboratory processing of the samples as a FAPESP scholarship holder. VLPC and JET - Participated in the scientific supervision; interpretation of the results; final review of the study; manuscript writing. JET - Project administration; funding acquisition. GMDB - Veterinary clinical care; collection of the biological samples. All authors read and approved the final version of the manuscript.

## Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

## Use of Artificial Intelligence (AI)

The authors used Microsoft Copilot for language revision, grammar correction, and text organization. All outputs were reviewed, verified, and approved by the authors, who assume full responsibility for the content of the manuscript.

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