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LABORATORY DIAGNOSIS AND CLINICAL SIGNS OF CANINE VISCERAL LEISHMANIASIS IN DOGS EXAMINED AT THE CENTER FOR ZOONOSIS CONTROL IN CAMPO GRANDE – MS, BRAZIL

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ABSTRACT: Visceral leishmaniasis is a type of zoonosis caused by several *Leishmania* species endemic to tropical, subtropical, and Mediterranean climate regions. Dogs are the primary source of infection in urban areas and can be symptomatic or asymptomatic. This study focused on the observation of clinical signs of leishmaniasis in dogs in Campo Grande, Mato Grosso do Sul, Brazil. Samples from affected animals were analyzed using indirect fluorescent antibody (IFA) tests, an enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR) assays to determine the optimal diagnostic tool for use on animals that present clinical symptoms. A predominance of clinical symptoms affecting the integumentary system was observed, and splenomegaly and hepatomegaly were the most important pathological signs. Among the diagnostic tests, the greatest agreement was seen between ELISA and IFA, followed by ELISA and PCR, and finally IFA and PCR. PCR diagnostic results showed the greatest extent of correlation with clinical signs, followed by ELISA and then IFA. When choosing a diagnostic method, veterinarians should consider the clinical signs and health status of the patient.
Key Words: ELISA; PCR; IFA; RV1/RV2 primers, Visceral leishmaniasis

DIAGNÓSTICO LABORATORIAL E SINAIS CLÍNICOS PARA LEISHMANIOSE VISCERAL CANINA EM CÃES EXAMINADOS NO CENTRO DE CONTROLE DE ZOONOSES DE CAMPO GRANDE – MS

RESUMO: A Leishmaniose Visceral é uma zoonose causada por várias espécies de *Leishmania*, sendo endêmica em paises de áreas Tropicais, Subtropicais e do Mediterrâneo. O cão é a principal fonte de infecção em regiões urbanas, podendo também desenvolver a doença. Neste trabalho fez-se a observação de sinais clínicos de cães da cidade de Campo Grande – MS, que foram posteriormente submetidos aos testes de RIFI, ELISA e PCR, objetivando avaliar qual deverá ser a técnica de diagnóstico a ser solicitada, quando o animal estiver apresentando determinados sinais clínicos. Pode-se observar um predomínio de sinais clínicos relacionados ao sistema tegumentar. Os sinais anatomopatológicos de maior importância foram a esplenomegalia e a hepatomegalia. Dentre os testes diagnósticos, houve maior concordância entre ELISA e RIFI, seguido por ELISA e PCR e, por fim, RIFI e PCR. Quanto à concordâncias com os sinais clínicos, o teste diagnóstico que apresentou maior correlação com cada um foi a PCR, logo depois o ELISA e, com menor correlação, apresentou-se a RIFI. Afirma-se que, o clínico veterinário, ao decidir por uma técnica de diagnóstico, deve considerar os sinais clínicos observados e o estado de saúde do paciente.
Palavras-chave: ELISA, PCR, IFA, iniciadores RV1/RV2, Leishmaniose Visceral

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INTRODUCTION

Visceral leishmaniasis (VL) is a zoonotic disease caused by several *Leishmania* species (Ross, 1903), all of which belong to the subgenus *Leishmania* and the *L. donovani* and *L. infantum* complexes (Thomaz-Soccol *et al.*, 1993). In the Americas and in Mediterranean regions, the species *L. (L.) infantum* is responsible for VL, which may affect humans who come into contact with the parasite transmission vectors. In the case of human transmission, the disease becomes an anthropozoonosis (Badaró, 1983).

VL is endemic to several countries in tropical, subtropical, and Mediterranean climate regions, including India, Sudan, Bangladesh, Nepal, and Brazil, all of which have economically underdeveloped areas and socially impoverished populations. These countries account for approximately 90% of reported VL cases (Gontijo and Melo, 2004; Chappuis *et al.*, 2007). Mammals, including humans and wild animals (e.g., foxes, skunks, and rodents), are susceptible to VL infection, and in urban areas, VL primarily affects dogs that are bitten by the female phlebotomine sand fly (*Lutzomia longipalpis*), which is the vector species (Brasil, 2006; Gontijo and Melo, 2004).

The first VL cases in Brazil were reported in 1934, and in 1936, Evandro Chagas described the first case *in vivo.* VL was recognized as endemic in 1953, with outbreaks most commonly occurring in Ceará, Bahia, Piauí, and Minas Gerais (Michalick, 2005; Genaro, 1993). However, the epidemiological profile of VL in Brazil has been changing over time. Initially, the incidence of the disease was associated with poverty as well as canine and human malnutrition in rural or wild areas, such as in the Northeast region of Brazil. Currently, in the Southeast and Midwest regions of Brazil, the disease occurs in urban and peri-urban areas, which is indicative of the urbanization of the disease (Brasil, 2006; Nunes *et al.*, 1988).

Although Campo Grande is a rapidly developing and expanding city, it nonetheless exhibits distinct rural features that facilitate the adaptation and subsequent urbanization of VL. For this reason, state and municipal health departments, particularly those with primary health care services and services for health care professionals responsible for educational programs, must actively search for and communicate with populations in high risk areas to reduce VL cases in humans and dogs (Borges *et al.*, 2008).

Canine visceral leishmaniasis (CVL) is a chronic disease and can be either symptomatic or asymptomatic. Symptomatic dogs may display apathy, alopecia, hair opacification, progressive weight loss, keratoconjuctivitis, facial dermatitis, nail stretching, nose and ear sores, swelling, and paresis of the hind paws. Asymptomatic animals are diagnosed through seropositivity. Depending on the stage of the disease and the immunological status of the animal, infected dogs can become important sources of *Leishmania* transmission (Gontijo and Melo, 2004; Silva *et al.*, 2005; Costa *et al.*, 2007).

The diagnosis of CVL in dogs can be performed based on the animals' clinical characteristics and can be confirmed by direct and indirect laboratory methods (Bonates, 2003). Direct methods of diagnosis include the visualization of the etiological agent in aspiration biopsies of lymphoid organs. Indirect methods are based on DNA testing and antibody detection using serological tests (Thomé, 1999; Feitosa *et al.*, 2000). Currently, the Ministry of Health recommends the use of two serological tests: an enzyme-linked immunoabsorbent assay (ELISA) and an indirect fluorescent antibody (IFA) assay. The IFA assay is the most commonly used routine diagnostic technique (Brasil, 2004).

The goals of this study were to classify the clinical symptoms of CVL and compare the diagnostic efficacy of the serological tests (IFA and ELISA) and a polymerase chain reaction (PCR) assay using peripheral blood samples from dogs examined at the Center for Zoonosis Control in Campo Grande, Mato Grosso do Sul, Brazil, from 2009 to 2010.

MATERIAL AND METHODS

Animals

This study was conducted using 200 dogs that were examined at the Center for Zoonosis Control (Centro de Controle de Zoonozes – CCZ) in the municipality of Campo Grande, Mato Grosso do Sul, Brazil. The animals' owners submitted them to the center as the result of an intense educational campaign in the municipality on the importance of controlling leishmaniasis in dogs. The animals were examined for clinical signs and were classified into three groups according to their symptoms: asymptomatic (dogs in which no alterations were identified during a physical examination or by microscopic examination performed during autopsy), oligo-symptomatic (dogs that presented with up to three clinical symptoms, as revealed by physical examination, or that had up to three compromised organs), and symptomatic (dogs with more than three clinical symptoms, as revealed by physical examination, or which had more than three compromised organs).

Indirect Immunofluorescence Test

The IFA method is the "gold standard" for human leishmaniasis diagnosis and is also used for the diagnosis of CVL in veterinary medicine. Briefly, the IFA assay is based on the reaction of sera suspected of infection with parasites fixed on microscope slides. The readout is performed using a microscope equipped for the detection of ultraviolet excitation light. Sera are considered positive when the parasites show fluorescent staining around the periphery, with a cutoff titer of 1:40 (Bio-Manguinhos/Fiocruz kit). The established standard titer range used here was 1:40 to 1:80.

Enzyme-Linked Immunosorbent Assay

The ELISA used for the diagnosis of CVL (*Leishmania donovani* complex) was developed by Avrameas *et al.* in 1992 and modified by Laurentino-Silva (Bio-Manguinhos/Fiocruz kit). The result is typically obtained via the visual observation of a color change without the need for absorbance measurements. In the tests performed here, a titration ranging from 1:40 to 1:80 was used.

Polymerase Chain Reaction Assays

PCR reproduces *in vitro* the natural process of DNA replication and can be repeated on a large scale. For the development of primers, this methodology requires, at a minimum, partial knowledge of the target DNA of a particular organism (Yang and Rothman, 2004).

Blood samples were collected at the CCZ from dogs with a clinical suspicion of CVL or that were determined to be seropositive by ELISA and IFA. For each sample, 100 µL of blood and 900 µL of DNAzol were placed in 1.5-mL microtubes. The tubes were mixed thoroughly by inversion and centrifuged at 10,000 x g for 10 minutes. The supernatant of each tube was transferred to a clean tube, 1 mL of pure ethanol was added, and the sample was centrifuged for 3 minutes at 4,000 x g. The precipitate was washed with 75% ethanol, centrifuged at 13,000 x g for 5 minutes, allowed to dry, and redissolved in 50 µL of water. The purity and concentration of the DNA were determined by measuring the optical density using a spectrophotometer (NanoDrop® ND-1000 UV-Vis) and by agarose gel electrophoresis.

A temperature curve was used to determine the optimal annealing temperature for the primers for the standardization of the PCR. The positive control, strain *L. chagasi* (MHOM/BR/74/PP/75), was provided by the Leishmaniasis Research Laboratory at the René Rachou/Fiocruz Research Center (Belo Horizonte, Brazil). Ultrapure water was used instead of DNA as the negative control. The following primers were used: RV1-CTTTTCTGGTCCCGCGGGTAGG and RV2- CACCTGGCCTATTTTACACCA. These primers were expected to generate a 145-bp product (Lachaud *et al.*, 2002).

The initial PCR was performed at a final volume of 25 µL containing 1 µL of DNA (40 – 100 ng/µL), 1x buffer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.16 pmol of each primer RV1 and RV2, 2 U Taq polymerase, and water up to the final volume. Samples were amplified using a Eppendorf Mastercycler® Personal with standard cycles. Verification of the 145-bp DNA fragment was performed using 10 µL of PCR product in a 4% agarose gel with tris-acetate-EDTA (TAE) pH 8.0. Gels were stained with ethidium bromide (0.5 mg/mL) and visualized using a UV transilluminator.

Statistical Analyses

The results of the diagnostic analyses and the clinical symptoms of the animals were tested for pair-wise agreement based on the frequency distribution of each test and each clinical symptom. The following criteria were used to conceptualize the results in terms of their agreement: values ≤ 40% were considered poor; 40.1 to 79.9% was regular; 80 to 89.9% was good; and ≥ 90% was considered excellent. The results obtained using the diagnostic techniques were analyzed using the Kappa coefficient test. The p values were calculated using the McNemar test to establish the significance ($p<0.05$) of the Kappa test.

RESULTS

Clinical symptoms and pathological changes

This study identified 37 asymptomatic dogs, 62 oligo-symptomatic dogs, and 101 symptomatic dogs. The primary symptoms were weight loss (40%), onychogryphosis (39%), pinna dermatitis (31%), and lymphadenopathy (29.0%). Less common symptoms were splenomegaly (18%), conjunctivitis (17.5%), peeling (15.5%), skin laceration (12%), myotrophies (11.5%), alopecia (11.5%), and dermatitis (10%). Other clinical symptoms occurred in less than 10% of the animals (Figure 1).

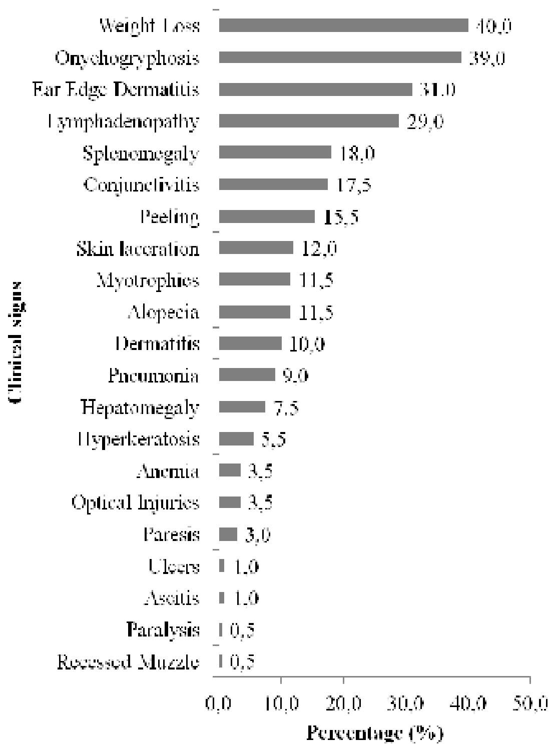

Figure 1 – Clinical symptoms observed in 200 dogs with canine visceral leishmaniasis in Campo Grande, MS, Brazil, in 2009.

Diagnostic tests

IFA assays identified 160 dogs (80%) that were positive for leishmaniasis, whereas 130 (65%) and 95 (47.5%) cases were identified using ELISA and PCR, respectively. According to all three diagnostic tests, 65 animals were positive (32.5%) and 25 were negative (12.5%).

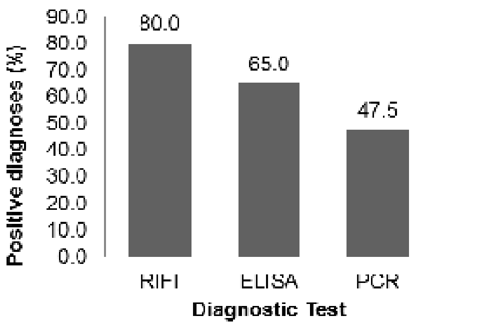

Figure 2 – Percentage of dogs positive for CVL according to serological tests (IFA and ELISA) and PCR.

The comparison of IFA, ELISA, and PCR diagnostic test results, including the percent agreement percentages, Kappa coefficients, and p values, are shown in Table 1.

Table 1 – Analysis of the agreement between IFA, ELISA and PCR results.

Statistics	Diagnostic Tests		
	IFA x ELISA	IFA x PCR	ELISA x PCR
Agreement	83%	53.50%	54.80%
Kappa	0.58	0.097	0.093
p Value	<0.0001[1]	<0.0001[1]	0.7

1 – p<0.05

Diagnostic results compared to clinical symptoms

The group of asymptomatic dogs had the lowest number of animals (37) and the least number of positive results according to all three tests: 29 (14.5%) cases by IFA, 26 (13%) by ELISA, and 17 (8.5%) by PCR. Among the group of 62 oligo-symptomatic dogs, 57 (28.5%) were diagnosed by IFA, 47 (23.5%) by ELISA, and 31 (15.5%) by PCR. The largest number of positive results (101 animals) was observed for the symptomatic group, of which 74 (37%) were seropositive by IFA, 57 (28.5%) were identified by ELISA, and 47 (23.5%) were indicated by PCR. This group also had the highest percentage of positive diagnoses of CVL.

The frequency of positive results for each diagnostic test among the asymptomatic, oligo-symptomatic, and symptomatic groups is shown in Table 2.

Table 2 – Frequency of positive diagnoses for CVL distributed among the symptomatic, oligo-symptomatic, and symptomatic groups for a total of 200 animals.

Clinical State	Diagnostic test		
	IFA N(%)	ELISA N(%)	PCR N(%)
Asymptomatic (n=37)	29 (14.5)	26 (13)	17 (8.5)
Oligo-symptomatic (n=62)	57 (28.5)	47 (23.5)	31 (15.5)
Symptomatic (n=101)	74 (37)	57 (28.5)	47 (23.5)
Total (n = 200)	160 (80)	130 (65)	95 (47.5)

N= number of positive animalia diagnosed

Asymptomatic Dogs

Table 3 shows the comparative data for the diagnostic tests (IFA, ELISA, and PCR) performed using the peripheral blood of asymptomatic dogs; the percent agreement, Kappa coefficient and p value results are reported. In this group, 13 (35%) of the animals were positive, and 5 (13.5%) were negative in all three diagnostic tests performed. Of the remaining animals, only a single type of test indicated a positive result in a total of 5 cases: 3 were positive only by IFA, 1 was only positive according to ELISA, and 1 was identified by PCR alone. Similarly, 1 animal was negative only according to IFA, whereas 2 and 11 cases were negative only according to ELISA and PCR results, respectively. The agreement within this group was 48.5% among the three tests.

Table 3 – Results for IFA, ELISA and PCR tests performed on the asymptomatic group of animals

Statistics	Diagnostic tests		
	IFA x ELISA	IFA x PCR	ELISA x PCR
Agreement	81.08%	56.76%	59.46%
Kappa	0.5085	0.1732	0.2150
p Value	0.2568	0.0027[1]	0.0201[1]

1 – p < 0.05

Oligo-symptomatic dogs

The comparison of IFA, ELISA, and PCR test results obtained using the peripheral blood of oligo-symptomatic dogs is presented in Table 4, which includes the percent agreement, Kappa coefficient, and p value results. In this group, 24 (38.7%) dogs were positive and 4 (6.4%) were negative according to the IFA, ELISA, and PCR tests. In addition, 4 animals were positive only by ELISA, and 1 was positive by PCR only; 6 were negative by ELISA only, and 23 were negative according to PCR only. In this group, the agreement among the three diagnostic tests was 45.1%.

Table 4 – Results for the IFA, ELISA, and PCR tests performed on the oligo-symptomatic animal group compared using the Kappa test.

Statistics	Diagnostic tests		
	IFA x ELISA	IFA x PCR	ELISA x PCR
Agreement	83.87%	54.84%	51.61%
Kappa	0.4312	0.0968	0.0323
p value	0.0016[1]	<0.0001[1]	0.0035[1]

1 – p < 0.05

Symptomatic dogs

The data comparing the results of the IFA, ELISA, and PCR diagnostic tests using peripheral blood collected from symptomatic dogs are shown in Table 4, including the percent agreement, kappa coefficient, and p value results. In this group, 28 (27.7%) of the animals were positive and 16 (15.8%) were negative for all three diagnostic tests. A total of 9 animals were positive only by IFA and 11 were positive only by PCR; 8 animals were negative only by ELISA and 29 were negative only by PCR. This group showed 43.5% agreement among the three diagnostic tests.

Table 5 – Results of the IFA, ELISA, and PCR tests performed on the symptomatic animal group compared using the Kappa test.

Statistics	Diagnostic test		
	IFA x ELISA	IFA x PCR	ELISA x PCR
Agreement	83.17%	51.48%	52.47%
Kappa	0.6419	0.0600	0.0579
p value	<0.0001[1]	0.0001[1]	0.1489

1 – p < 0.05

Diagnostic test results compared to clinical manifestations

The clinical symptoms most commonly observed during the clinical examinations of the animals are shown in Table 6. In this table, each clinical symptom is correlated with each of the three diagnostic tests in a pair-wise comparison. The percent agreement between each clinical symptom and the respective diagnostic test was calculated to determine the most accurate diagnostic test to be used when a clinical symptom is presented. The Kappa test indicates the magnitude of this correlation, and the p value indicates the significance of the Kappa test.

Table 6 – Agreement between positive diagnostic tests and the major symptoms present in the animals.

SYMPTOMS		DIAGNOSTIC METHOD		
		PCR	IFA	ELISA
Alopecia	Agreement	55%	29.5%	41.5%
	Kappa	0.064	0.036	0.050
	p value	<0.0001	<0.0001	<0.0001
Conjunctivitis	Agreement	50%	27.5%	34.5%
	Kappa	0.034	0.043	0.047
	p value	<0.0001	<0.0001	<0.0001
Dermatitis	Agreement	52.5%	26%	38%
	Kappa	0.010	0.000	0.000
	p value	<0.0001	<0.0001	<0.0001
Pinna Dermatitis	Agreement	51.5%	47%	51%
	Kappa	0.011	0.137	0.120
	p value	<0.0001	<0.0001	<0.0001
Peeling	Agreement	56%	33.5%	44.5%
	Kappa	0.089	0.059	0.080
	p value	<0.0001	<0.0001	<0.0001
Weight loss	Agreement	51.5%	41%	38%
	Kappa	0.089	0.059	0.080
	p value	<0.0001	<0.0001	<0.0001
Splenomegaly	Agreement	48.5%	33%	44%
	Kappa	0.064	0.031	0.060
	p value	<0.0001	<0.0001	<0.0001
Lymphadenopathy	Agreement	45%	45.5%	54.5%
	Kappa	0.124	0.134	0.194
	p value	0.0003	<0.0001	<0.0001
Onychogryphosis	Agreement	49.5%	46%	49%
	Kappa	0.021	0.046	0.043
	p value	0.091[1]	<0.0001	<0.0001

1 – p > 0.05

DISCUSSION

A predominance of clinical symptoms related to the integumentary system was observed, and 127 of 200 (63.5%) animals presented symptoms related to this system. Considering only those animals that showed clinical symptoms (163), the presence of clinical symptoms associated with the integumentary system was observed in 127 of 163 (78%) animals, which was a highly significant occurrence. Of these, 59 of 127 (46.5%) presented 1 clinical symptom, 42 of 127 (33.1%) presented 2 clinical symptoms, 21 of 127 (16.5%) had 3 clinical symptoms, and 5 of 127 (4%) had more than 3 clinical symptoms associated with the integumentary system. Thus, the observation of these symptoms by the veterinarian is of paramount importance in cases of suspicion of CVL and these symptoms are important for the differential diagnosis of demodicosis.

The most important pathological symptoms were splenomegaly and hepatomegaly, which were present in 36 (18%) and 15 (7.5%) of the 200 studied dogs, respectively. Of all the animals that showed hepatomegaly, only one did not present concurrent splenomegaly. Therefore, we believe that hepatomegaly occurs after splenomegaly and depends on disease progression.

Other commonly presented clinical symptoms were presented: weight loss in 80 of 200 (40%) dogs; lymphadenopathy in 58 of 200 (29.0%) dogs; and conjunctivitis in 35 of 200 (17.5%) of dogs. Other clinical symptoms were not as common. The great variability of clinical manifestations of CVL may be due to the genetic characteristics of each dog, which also determine the different immune responses seen in these animals. Additionally, some animals or breeds can be more resistant than others, thereby determining the disease susceptibility (Solano-Gallego *et al.*, 2000).

In the diagnostic assays, 90 of 200 dogs were positive for all three diagnostic tests with 45% agreement among the tests. According to the Kappa tests for the pairwise comparisons, there was a greater agreement between ELISA and IFA, followed by ELISA and PCR, and finally IFA and PCR.

Using only the ELISA diagnostic test, 130 (65%) samples were positive for CVL. When combined, ELISA and IFA increased the positive sample detection rate by 16% (32 of 200) for a total of 81% (162 of 200). If the PCR results were included, there was a 12.5% increase (25 of 200) for a total detection rate of 93.5% (187 of 200) of the CVL-positive samples. Therefore, the use of two or more techniques is recommended for the epidemiological control of canine leishmaniasis.

The symptomatic dog group, with a total of 101 animals (50.5%), comprised the largest group identified in this study. All three tests proved to be effective for this group. Accordingly, the symptomatic group showed the highest positive rate in the diagnostic tests, followed by the oligo-symptomatic and asymptomatic groups. Independent of the clinical group, IFA showed the highest positive rate for the diagnosis of CVL, followed by ELISA, and then PCR. The better performance of serological tests for diagnosing CVL can be explained by the high polyclonal stimulation of B lymphocytes caused by leishmaniasis, which leads to hypergammaglobulinemia and the extensive production of antibodies that facilitate diagnosis by these tests (Feitosa *et al.*, 2000).

ELISA is a relatively quick and simple test but requires trained personnel and specialized, expensive equipment. This test is sensitive; i.e., it allows for the detection of low titers of antibodies with a sensitivity of greater than 98% (Rey, 2001,Thomaz-Soccol *et al.*, 2009). IFA is now considered the test of choice by the Brazilian Ministry of Health in canine sero-surveys and exhibits 90% to 100% sensitivity and a specificity of approximately 80% in sera samples. Additionally, ELISA is a relatively easy test to perform with fast results and a low cost (Alves and Bevilacqua, 2004). However, there has been disagreement in the literature regarding the need for two or more diagnostic methods to increase rate of correct diagnosis of leishmaniasis (Szargiki *et al.*, 2009).

When compared to clinical symptoms, the PCR-based diagnostic test showed the greatest correlation, with 45% to 55% agreement with each clinical symptom, followed by ELISA, with 34.5% to 54.5% agreement. The lowest correlation was observed for IFA with 26% to 47% agreement with each clinical symptom. In other words, the test with the highest positive result rate, IFA, was the method with the lowest extent of correlation with clinical signs. In contrast, the test with the lowest positive rate, PCR, had the highest correlation with clinical signs. However, it is known that low levels of parasitemia in infected animals can contribute to low detection rates by PCR in blood samples, as reported by Fisa *et al.* (2001). The PCR technique could be improved if samples were collected from the popliteal lymph node or the leukocyte layer because these would house greater numbers of parasites, thereby increasing the sensitivity of detection.

The correlation results of the tests used in this study support those of other recent studies, such a report by as Lachoud *et al.* (2002), who motivated Gomes *et al.* (2007) to conduct a study to verify the ability of PCR (RV1/RV2) to diagnose CVL in different tissue samples from dogs in the State of São Paulo, Brazil. In another study conducted with 95 dogs in Italy, Manna *et al.* (2004) reported a 94% positive rate using PCR, with 4 animals positive by PCR that were negative according to other serological tests. In this present study, 13 of 200 (6.5%) animals were positive by PCR and negative by other diagnostic tests.

In this study, PCR proved to be the safest and most cost-effective test relative to the serological tests (IFA and ELISA), which have been shown to have several disadvantages, including the difficulty in differentiating between current and previous parasitic infections. Additionally, it is not possible to correlate the levels of circulating antibodies with the disease stage using IFA and ELISA, and the cost of producing specific purified antigens can be prohibitively high. Consequently, preparations with crude antigens are often used, thereby reducing the specificity and sensitivity of these tests (Green, 2006) and generating a relatively high false positive rate.

In veterinary medicine, the clinician is often confronted with cases suggestive of certain canine diseases, although diagnostic tests can indicate contradictory results (Francino *et al.*, 2006). In this study, PCR showed the lowest percentage of contradictory results when compared with observed clinical signs. Therefore, this article can serve as a tool to help veterinary doctors select a diagnostic technique while taking into account the observed symptoms and the patient status.

CONCLUSION

In this study, we used a relatively large number of dogs (200) for the clinical and laboratory evaluation of CVL. The objective of classifying dogs using clinical symptoms and the comparison of IFA, ELISA, and PCR tests was achieved. However, even with this representative sample number, the results support previous findings that demonstrate that no one diagnostic test is capable of properly identifying dogs with CVL when used alone. Although PCR showed the greatest correlation with the presence of clinical symptoms, our results clearly demonstrated that a negative result based on a single type of test can be misleading and may instead represent a false negative result. These results stress the need to employ a combination of diagnostic techniques. However, when a strong clinical suspicion is present, our results show that PCR is essential for reaching a definitive diagnosis. Finally, veterinarians should always consider the clinical symptoms and health status of the patient when selecting a diagnostic test.

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