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Assessment of Renal Functions and Lesions in Dogs with Serological Diagnosis of Canine Visceral Leishmaniasis

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ABSTRACT

***Background*:** Visceral leishmaniasis is a complex vector-borne disease caused by the protozoan *Leishmania infantum*. In urban centers of South America, where this zoonotic cycle occurs, dogs seem to be the main reservoirs and infection sources. Animals with canine visceral leishmaniasis (CVL) may have a wide clinical spectrum, and dogs are usually classified as asymptomatic, oligosymptomatic, and symptomatic. Several organs are affected in canine CVL, and renal involvement is often a determining factor in dog prognosis. Nevertheless, serum markers are slow to indicate loss of renal function. The aim of this study was to evaluate kidney impairment in dogs diagnosed with CVL.

***Materials, Methods & Results*:** Blood and urine samples were collected from 45 dogs from Barra Mansa-RJ, and used for urinalysis, urine protein/creatinine (UPC) ratio, and serum concentrations of urea and creatinine. The animals were classified as symptomatic (42.2%), oligosymptomatic (37.8%), and asymptomatic (20.0%). Some alterations were found in the urine samples; pale-yellow color in 17.8%, low specific gravity in 6.7%, turbidity in 51.1%, proteinuria in 80%, occult blood in 46.7%, bilirubin in 8.89%, and glucose in 6.7% of the samples. According to the UPC ratio, 60% of dogs were proteinuric, and UPC > 2.0 was high in symptomatic dogs. Azotemia was observed only in three dogs with CVL.

***Discussion*:** The majority of dogs presented one or more symptoms of CVL, as expected in an endemic area from Brazil. Pale-yellow urine was observed in some samples, and this change, when accompanied by the decreased urine specific gravity in dogs with CVL, suggests some degree of kidney disease. The presence of epithelial and red blood cells, leukocytes, bacteria, suspended mucus, and phosphate crystals that precipitate in alkaline urines could be associated, to some degree, with the urine turbidity found in the present study. The alkaline urine identified in some dogs could be related to the animals' diet, but renal tubular acidosis (RTA) is another possible cause when referring to animals with CVL. The abnormal presence of bilirubin and glycosuria can be justified by liver damage and glomerular and tubular damage, respectively. Occult blood was found in the urine of almost half of the tested dogs, which occurred because of the presence of red blood cells in the urine sediment and hematuria in some animals, could be caused by tubular and glomerular lesions. The presence of granular and hyaline casts found in the samples reinforce the possibility of tubular injury. We found different levels of proteinuria; it was an important result, possibly caused by immune complex deposition in addition to tubular disease. Most tested dogs, including animals without clinical manifestation, were classified as proteinuric or borderline proteinuric, showing that the renal disease could be the only clinical manifestation of CVL and that it could progress from slight proteinuria to end-stage renal disease, resulting in chronic renal failure, which is the main cause of death. The UPC ratio > 2.0 was significantly the more frequent finding in this study, mainly in symptomatic dogs. This result indicates a glomerular disease in these animals, reinforcing that the progression of renal disease follows the clinical progression of CVL. A few serum samples showed increased urea and creatinine levels, proving that azotemia is an uncommon finding in CVL-infected dogs. In conclusion, urinalysis helped in the early identification of renal injury in CVL-infected dogs, highlighting elements that reinforce the presence of tubular or glomerular lesions, or both, even in non-azotemic dogs. The high frequency of symptomatic dogs with UPC ratio > 2.0 suggests a relationship between the progression of renal disease and the clinical progression of CVL.

Keywords: *Leishmania infantum*, kidney disease, urinalysis, urine protein/creatinine ratio, serum biochemistry.

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INTRODUCTION

Visceral leishmaniasis is a zoonosis caused by the protozoan *Leishmania infantum* through the bite of phlebotomine sand flies such as *Lutzomyia longipalpis* [28,30]. In South America, the disease has expanded from rural to urban centers where the zoonotic cycle occurs, and dogs seem to be the main reservoirs and infection sources [17,22]. These animals with canine visceral leishmaniasis (CVL) may have a wide clinical spectrum and are usually classified as asymptomatic (without clinical signs), oligosymptomatic (with up to three clinical signs), and symptomatic (with four or more clinical signs). CVL lesions may involve any body fluid, tissue or organ, including the kidneys, and renal involvement usually occurs because of immune complex deposition [6,13,17,22,30]. Clinical symptoms and laboratory evidence depend on the degree of organ injury and may appear only when there is extensive tissue damage [6,11,13,18]. Therefore, proteinuria initially may not be accompanied by azotemia in infected dogs [7]. In this context, other tools that help in the early diagnosis of urinary system alterations can be used [29]. Urinalysis is a simple, inexpensive three-step routine test that may assist in the identification of systemic and other diseases that compromise the urinary tract [29,32]. The urine protein/creatinine (UPC) ratio is another useful tool in assessing kidney disease in dogs [5,7,11,14].

Given the importance of renal disorders in the clinical condition of dogs with CVL and the need for early diagnosis of these disorders, the aim of this study was to identify the occurrence of urinalysis and UPC abnormalities in a dog population previously diagnosed with CVL.

MATERIALS AND METHODS

Sample collection

A prospective study was performed on CVL-infected dogs from Barra Mansa (22°32′39″ S, 44°10′17″ W), a Brazilian municipality in the south of the state of Rio de Janeiro (RJ), within the Vale do Paraíba region. CVL diagnosis was based on positive results from the dual-path platform immunochromatographic rapid test (DPP®)[1] and the enzyme-linked immunosorbent assay (ELISA) for CVL (EIE-LVC®)[1]. The EIE-LVC test was performed at the Noel Nutels Central Laboratory (LACEN) from Rio de Janeiro, Brazil.

The dogs in this study were referred to the Laboratório de Pesquisa Clínica em Dermatozoonoses de Animais Domésticos (LAPCLIN-DERMZOO/ Fundação Oswaldo Cruz), where they were chemically restrained using a mixture of 10 mg/kg of ketamine (Cetamin®)[2] and 0.2 mg/kg of acepromazine (Apromazin 1%®)[2] for physical examination and collection of blood and urine (cystocentesis) samples. Classification, according to the presence of typical CVL clinical signs, was performed as previously suggested [17]. The collected blood was stored in anticoagulant-free tubes for serum extraction.

Laboratory tests

Renal function tests (determination of serum concentrations of urea and creatinine) were conducted in an automated analyzer (Labmax 240 Premium®)[3], using commercial kits (Labtest®)[4] according to the manufacturer's instructions.

To perform urinalyses, the minimum volume of urine obtained by cystocentesis in each dog was 5.0 mL. Physical examination of the urine was based on a qualitative evaluation of the color, odor, and transparency (clear or cloudy) of the samples. Urine specific gravity was determined by refractometry (ATAGO SZJ-D Refractometer®)[3], and urine chemical analysis was performed using a dipstick test (Uriquest Plus®)[4]. For the microscopic sediment examination, a fresh urine sample was centrifuged in suitable tube; the supernatant was then decanted, and the pellet was resuspended, transferred to a coverslipped glass slide, and observed under a microscope at 400× magnification.

Moreover, UPC was calculated in all dogs. Urinary protein was determined by a colorimetric method with a pyrogallol-molybdate red complex, and urinary creatinine was measured by the Jaffé reaction using picric acid.

Statistical analysis

Qualitative variables were initially evaluated descriptively and later, in groups according to their CVL clinical status using the G-test. Quantitative variables were presented by their means and standard deviation, first in all dogs and, later, in groups according to their CVL clinical status. Kruskal-Wallis and Mann-Whitney tests with independent samples and 95% confidence interval were used to compare the clinical groups.

On the basis of UPC ratio and according to the International Renal Interest Society (IRIS), dogs were considered as non-proteinuric (UPC < 0.2), borderline proteinuric (UPC 0.2 - 0.5), and proteinuric (UPC > 0.5). Subsequently, proteinuric dogs were stratified by UPC ratio into three categories, as proposed by Lees et al. [14].

RESULTS

Clinical status

For this study, blood and urine were obtained from 45 dogs in the LAPCLIN-DERMZOO. The animals were unvaccinated against CVL and presented positive serological reaction in both tests that were applied (DPP® and ELISA). According to the physical examination, we observed 42.2% of symptomatic dogs (19/45), 37.8% of oligosymptomatic dogs (17/45), and 20.0% of asymptomatic dogs (9/45).

Urinalysis, UPC and serum biochemistry

The frequencies of urinalysis results obtained for all animals with CVL, as well as for each clinical group, are described in Table 1.

Examined urine samples showed predominantly a normal color (82.2%; 37/45). The abnormalities observed were found in 17.8% (8/45) of the samples. Among the samples from symptomatic dogs, the altered colors were pale yellow (10.5%; 2/19); dark yellow (5.3%; 1/19); and amber (5.3%; 1/19). Pale-yellow urine was found in 17.6% (3/17) of the oligosymptomatic dogs' samples, and amber urine was found in 11.1% (1/9) of samples from asymptomatic dogs. There was no significant difference in the frequency of normal or altered color of the urine from the evaluated clinical groups. In all animals, the odor was sui generis. Regarding urine transparency, 51.1% (23/45) of the samples were slightly or completely cloudy, while 48.9% (22/45) were totally clear. Among the urine samples from symptomatic animals, 47.4% (9/19) were cloudy or slightly cloudy (Table 1). The altered aspect of the urine represented 52.9% (9/17) of the oligosymptomatic dogs' samples and 55.6% (5/9) of the asymptomatic ones. However, there was no significant difference regarding urine turbidity between the evaluated clinical groups.

We found 93.3% (42/45) of dog samples with normal urine specific gravity and 6.7% (3/45) of CVL-infected dogs' samples with urine specific gravity below 1,015 (from two symptomatic and one oligosymptomatic dog), with no statistical difference between the clinical groups. In most animals (60.0%; 27/45), urine pH results were within the expected range; however, 26.7% (12/45) and 13.3% (6/45) of the animals presented alkaline and slightly acid urine, respectively regarding the clinical groups, alkaline pH was identified in 26.3% (5/19), 23.5% (4/17), and 33.3% (3/9) urine of symptomatic dogs, oligosymptomatic, and asymptomatic dogs, respectively (Table 1). There was no significant difference between urinary pH and clinical status of the animals with CVL.

The presence of proteins was a remarkable finding in the urine chemical examination. In 80.0% (36/45) of dogs with CVL, proteinuria of 1+ or more classification was observed, especially among symptomatic animals (89.5%; 17/19), followed by asymptomatic (77.8%; 7/9) and oligosymptomatic dogs (70.6%; 12/17). It was also observed that 15.6% (7/45) of the dogs presented protein traces in their urine, and only in 4.4% (2/45) of the cases, the sample was negative for the presence of proteins (Table 1). There was no significant difference in the clinical groups evaluated.

Results greater than 1+ of bilirubin in the urine were identified in 15.8% (3/19) of symptomatic dogs and in 11.1% (1/9) of the asymptomatic dogs. The occurrence of occult blood and glucose was identified, respectively, in 46.7% (21/45) and 6.7% (3/45) of the samples from dogs with CVL (Table 1). There was no significant difference according to the animals' clinical status and these variables.

Leukocytes and red blood cells were detected in 77.8% (35/45) and 40.0% (18/45) of the urinary sediment samples, respectively. The presence of leukocytes and red blood cells was higher than normal in 8.9% (4/45) and 17.8% (8/45) of cases, respectively, with no significant difference between clinical groups. Regarding the cellularity, 48.9% (22/45) of the samples presented epithelial cells of the lower urinary tract, which was classified as moderate or intense in 15.6% (7/45) of the dogs' samples. In addition, 4.4% (2/45) and 6.7% (3/45) of the evaluated animals presented, respectively, renal pelvis cells and renal cells in the urinary sediment (Table 1). These animals were in the symptomatic and oligosymptomatic groups, although no significant difference was observed between them.

Table 1. Frequencies of physical, chemical and urinary sediment results of dogs with serological diagnosis of canine visceral leishmaniasis from Barra Mansa, RJ - Brazil according to clinical groups.

Variable	Dogs	Symptomatic	Oligosymptomatic	Asymptomatic	Reference*
Color			n (%)		
Normal	37 (82.2)	15 (78.9)	14 (82.4)	8 (88.9)	Yellow
Altered	8 (17.8)	4 (21.1)	3 (17.6)	1 (11.1)	
Transparency			n (%)		
Clear	22 (48.9)	10 (52.6)	8 (47.1)	4 (44.4)	Clear
Cloudy	23 (51.1)	9 (47.4)	9 (52.9)	5 (55.6)	
Urine specific gravity			n (%)		
Normal	42 (93.3)	17 (89.5)	16 (94.1)	9 (100)	1,015 to 1,045
< 1,015	3 (6.7)	2 (10.5)	1 (5.9)	0	
pH			n (%)		
< 5.5	6 (13.3)	3 (15.8)	1 (5.9)	2 (22.2)	5.5 – 7.5
5.5 - 7.5	27 (60.0)	11 (57.9)	12 (70.6)	4 (44.4)	
> 7.5	12 (26.7)	5 (26.3)	4 (23.5)	3 (33.3)	
Protein			n (%)		
Negative	2 (4.4)	1 (5.3)	1 (5.9)	0	Negative to trace
Trace	7 (15.6)	1 (5.3)	4 (23.5)	2 (22.2)	
≥ 1+	36 (80.0)	17 (89.5)	12 (70.6)	7 (77.8)	
Bilirubin			n (%)		
Negative	28 (62.2)	9 (47.4)	12 (70.6)	7 (77.8)	Negative to weak positive
1+	13 (28.9)	7 (36.8)	5 (29.4)	1 (11.1)	
> 1+	4 (8.9)	3 (15.8)	0	1 (11.1)	
Occult blood			n (%)		
Negative	24 (53.3)	11 (57.9)	9 (52.9)	4 (44.4)	Negative to trace
≥ 1+	21 (46.7)	8 (42.1)	8 (47.1)	5 (55.6)	
Glucose			n (%)		
Negative	42 (93.3)	18 (94.7)	16 (94.1)	8 (88.9)	Negative
≥ 1+	3 (6.7)	1 (5.3)	1 (5.9)	1 (11.1)	
Leukocytes			n (%)		
Absent	10 (22.2)	4 (21.1)	3 (17.6)	3 (33.3)	< 3/hpf
1 - 3/hpf	31 (68.9)	14 (73.7)	12 (70.6)	5 (55.6)	
> 3/hpf	4 (8.9)	1 (5.3)	2 (11.8)	1 (11.1)	
Red Blood Cells			n (%)		
Absente	27 (60.0)	13 (68.4)	10 (58.8)	4 (44.4)	0 - 3/hpf
1 - 3/hpf	10 (22.2)	4 (21.1)	3 (17.7)	3 (33.3)	
> 3/hpf	8 (17.8)	2 (10.5)	4 (23.5)	2 (22.2)	
Epithelial cells			n (%)		
Absent	23 (51.1)	9 (47.4)	9 (52.9)	5 (55.6)	Few
Discreet 1+	15 (33.3)	8 (42.1)	5 (29.4)	2 (22.2)	
Mild 2+	6 (13.3)	2 (10.5)	2 (11.8)	2 (22.2)	
Intense 3+	1 (2.2)	0	1 (5.9)	0	
Pelvis cells			n (%)		
Absent	43 (95.6)	18 (94.7)	16 (94.1)	9 (100)	0
Present	2 (4.4)	1 (5.3)	1 (5.9)	0	
Renal cells			n (%)		
Absent	42 (93.3)	18 (94.7)	15 (88.2)	9 (100)	0
Present	3 (6.7)	1 (5.3)	2 (11.8)	0	
Granular casts			n (%)		
Absent	22 (48.9)	7 (36.8)	11 (64.7)	4 (44.4)	Absent
1+	22 (48.9)	12 (63.2)	6 (35.3)	4 (44.4)	
2+	1 (2.2)	0	0	1 (11.1)	
Hyaline casts			n (%)		
Absent	38 (84.4)	15 (78.9)	15 (88.2)	8 (88.9)	Absent
Present	7 (15.6)	4 (21.1)	2 (11.8)	1 (11.1)	
Crystals (amorphous phosphates)			n (%)		
Absent	27 (60.0)	12 (63.2)	9 (52.9)	6 (66.7)	Absent
Present	18 (40.0)	7 (36.8)	8 (47.1)	3 (33.3)	
Crystals (struvite)			n (%)		
Absent	39 (86.7)	17 (89.5)	14 (82.3)	8 (88.9)	Absent
Present	6 (13.3)	2 (10.5)	3 (17.7)	1 (11.1)	
Crystals (calcium oxalate)			n (%)		
Absent	43 (95.6)	18 (94.7)	17 (100)	8 (88.9)	Absent
Present	2 (4.4)	1 (5.3)	0	1 (11.1)	
Bacteria			n (%)		
Absent	27 (60.0)	9 (47.4)	11 (64.7)	7 (77.8)	Absent
Present	18 (40.0)	10 (52.6)	6 (35.3)	2 (22.2)	
Total	45 (100)	19 (100)	17 (100)	9 (100)	-

*Chew *et al*. [5]; Stockham & Scott [32]. hpf: high-power field.

Granular casts were detected in 51.1% (23/45) of the studied animals' urine, with the discrete quantity being predominant (63.2% of symptomatic animals, 35.3% of oligosymptomatic animals, and 44.4% of asymptomatic animals). Hyaline casts in the samples were observed in 15.6% (7/45) of dogs with CVL, always in discrete quantities (21.1% of symptomatic dogs, 11.8% of oligosymptomatic dogs, and 11.1% of asymptomatic dogs).

The crystals found in the urinary sediments were predominantly characteristic of alkaline pH, totaling 40.0% (18/45) of amorphous phosphate and 13.3% (6/45) of struvite crystals. We also found 4.4% (2/45) of samples containing calcium oxalate. The presence of bacteria, in different degrees, was seen in 40.0% (18/45) of samples from dogs with CVL. This finding was more frequent among symptomatic animals (52.6%), followed by oligosymptomatic animals (35.3%), and asymptomatic animals (22.2%). There was no significant difference between the presence of casts, crystals, or bacteria, and the clinical status of the animal.

The mean values obtained for urinary protein and creatinine are shown in Table 2. No significant difference was observed between the urinary proteins and creatinine in the clinical groups (Table 2). According to UPC ratio values (Table 3), dogs in the present study were considered predominantly proteinuric (60.0%; 27/45). Of the evaluated animals, 22.2% (10/45) were considered borderline proteinuric, and 17.8% (8/45) were classified as non-proteinuric. Stratification of proteinuric dogs (n = 27) revealed 51.9% of UPC ratio > 2.0; 29.6% of UPC ratio from 1.0 to 2.0; and 18.5% of UPC ratio from 0.5 to 1.0. The presence of dogs with UPC ratio above 2.0 was significantly higher ($P = 0.0020$) than the others, being more marked in symptomatic dogs.

Although the increase in serum urea concentration has been found in 51.1% of the evaluated dogs, the simultaneous increase in serum urea and serum creatinine levels was observed only in 6.7% (3/45) of dogs with CVL, and this azotemia was observed in one animal from each clinical group, without significant difference.

Table 2. Mean ± standard deviation for urinary values of protein and creatinine, and of urine protein/creatinine ratio for dogs with serological diagnosis of canine visceral leishmaniasis from Barra Mansa, RJ - Brazil according to clinical groups.

Variable	Dogs	Symptomatic	Oligosymptomatic	Asymptomatic	Statistical Analysis
	Mean ± SD				
UPr (mg/dL)	185.6 ± 238.3	142.2 ± 141.6	241.9 ± 339.5	170.6 ± 158.9	Not significant
UCr (mg/dL)	120.9 ± 80.2	119.9 ± 104.0	112.0 ± 60.9	139.8 ± 55.1	Not significant
UPC ratio	2.4 ± 3.3	2.2 ± 2.6	3.2 ± 4.5	1.1 ± 0.8	Not significant

SD: standard deviation; UPr: Urine protein; UCr: Urine creatinine; UPC: urine protein/creatinine.

Table 3. Frequencies of urine protein/creatinine ratio categories in dogs with serological diagnosis of canine visceral leishmaniasis from Barra Mansa, RJ - Brazil according to clinical groups.

Variable	Dogs	Symptomatic	Oligosymptomatic	Asymptomatic
	UPC ratio			
< 0.2	8 (17.8)	4 (21.05)	3 (17.7)	1 (11.1)
0.2 - 0.5	10 (22.2)	4 (21.05)	4 (23.5)	2 (22.2)
> 0.5	27 (60.0)	11 (57.9)	10 (58.8)	6 (66.7)
Total	45 (100)	19 (100.0)	17 (100.0)	9 (100)
	Stratification of UPC ratio in proteinuric dogs			
> 0.5 to 1.0	5 (18.5)	1 (9.1)	4 (40.0)	0
> 1.0 to 2.0	8 (29.6)	3 (27.3)	0	5 (83.3)
> 2.0	14 (51.9)	7 (63.6)	6 (60.0)	1 (16.7)
Total	27 (100)	11 (100)	10 (100)	6 (100)

*UPC: urine protein/creatinine.

DISCUSSION

In our study, we observed a high frequency of dogs with one or more symptoms of CVL (42.2% of symptomatic dogs and 37.8% of oligosymptomatic dogs) and a low frequency of asymptomatic dogs (20.0%) at the time of sampling. This result is consistent with that of another study in an endemic area for CVL in Brazil where 71% of symptomatic dogs are detected [4].

Pale yellow was the most frequent color observed in the physical examination of urine, followed by amber. Different authors have reported that pale-yellow urine may be associated with decreased urine specific gravity, which may suggest inability in renal tubular resorption and progression of chronic kidney disease [5,21,29]. Another investigation regarding dogs with CVL showed amber color in 70% of urine samples. According to the authors, the concentration of urochrome depends on the urine specific gravity and the pathological state of the animal [15].

More than half of the animals evaluated (51.1%) presented some degree of turbidity in their urine samples, which was similar among the three clinical groups. Cloudy urine may be associated with the presence of epithelial cells, red blood cells, leukocytes, bacteria, and suspended mucus [21]. Besides, the presence of phosphate crystals that precipitate in alkaline urine is another common cause for the appearance of cloudy urine [29]. Since all the above-mentioned components were found in samples from animals with CVL, a high occurrence of cloudy urine was expected in the present study.

The total concentration of solutes determines the urine specific gravity, which remained predominantly within the normal range in our study. In 6.7% of dogs' samples, the urine specific gravity was considered low, and dogs that presented this abnormality were symptomatic and oligosymptomatic. This finding was consistent with the occurrence of pale-yellow color in these groups and may suggest the inability of the kidneys to concentrate urine, a feature that has been reported by other authors, even in dogs with CVL [15,21,29].

The urine pH values remained within the expected range for most animals, and alkaline urine was the most frequent change observed in this study (26.7% of dogs). Variations in urinary pH are mainly related to the composition of the animals' diet. However, patients with renal tubular acidosis (RTA) also have an inability to acidify their urine, keeping its pH in the alkaline range [21]. The RTA can occur secondary to other diseases, being a transient or a permanent condition; and, despite the abundance of RTA reports in humans, only a few studies of such disorder in veterinary patients have been published [26].

Small amounts of bilirubin are occasionally present in dog urine, and this event is considered non-pathological [19]. In our study, bilirubin values greater than 1+ were found both in symptomatic and asymptomatic dogs. The progression of CVL may trigger activation of Kupffer cells, in addition to an inflammatory reaction (capsular or portal) and intralobular formation of granulomas, causing liver damage [24], which may justify increased bilirubin in this work.

Glycosuria was identified in 6.7% of dogs with CVL. Blood glucose is known to be filtered by the glomeruli and reabsorbed by the proximal tubules. Thus, glycosuria may be present when the glomerular filtration rate exceeds resorption capacity (due to hyperglycemia); or when tubular resorption is deficient [21,29]. Although the occurrence of hyperglycemic disorders (such as diabetes mellitus or Cushing's syndrome) has not been investigated in the present study, we cannot overlook the fact that *L. infantum* infection causes glomerular and tubular damage [8], justifying the presence of glucose in the urine samples.

The dipstick test can detect peroxidase activity in erythrocytes, characterizing the existence of blood in the urine sample. However, both hemoglobin and myoglobin catalyze this reaction, which can be distinguished by the observation of erythrocytes in the microscopic examination of the urine sediment [29]. In our study, 46.7% of dogs had occult blood in the urine, 40.0% of dogs had red blood cells in the urine sediment, and 17.8% of dogs had hematuria. The cause of hematuria can be in the kidneys (renal hematuria; glomerular or non-glomerular) or in the low urinary tract. Renal hematuria is distinguished from other etiologies by the frequent presence of proteinuria and erythrocyte casts [1,29]. Researchers believe that tubular and glomerular lesions that occur in CVL [20,25,36] may have been the cause of occult blood and hematuria in the animals of the present study.

Although the presence of leukocytes in the urinary sediment was frequent, only 8.9% of dogs had high counts in their samples, characterizing pyuria. Eryth-

rocytes and leukocytes have been commonly found in urinalysis of humans and dogs with visceral leishmaniasis [9,10,27]. Pyuria may contribute to urine turbidity, as well as being a strong indicator of pyelonephritis, glomerulonephritis and renal inflammatory processes, disorders identified in dogs with CVL [20,25,36].

The presence of small amounts of epithelial cells in the urine sediment may be considered normal [19,21,29]. However, the occurrence of these cells in moderate and excessive quantities, along with the presence of both renal pelvis and renal tubular cells requires attention, as it may be directly associated with marked renal damage [29].

The existence of granular and hyaline casts in the present study was expected and is in agreement with the results of other studies of dogs with CVL [2,12,25]. Casts are formed by mucoproteins, which precipitate and acquire a cylindrical shape as they pass through the renal tubules [11,23]. The occurrence of cylindruria suggests tubular injury in dogs with CVL, as proposed before [9,11,23,25]. Amorphous phosphate, struvite and calcium oxalate crystals were also observed in the present study. The presence of crystalluria in dogs infected with *Leishmania* spp. was also previously described; apparently, this finding is primarily related to changes in urinary pH and not to the presence of the parasite itself. In general, the amount of crystals is more significant than their shape, because crystals are a frequent finding in routine urine examinations [19,31,33]. It is noteworthy that struvite crystals may appear in cases of bacterial infection [29]. In addition to the increased urinary pH, our study also found bacteria in sediment samples, which may have favored the formation of struvite crystals along with the alkaline medium. Similar findings were previously described in dogs with CVL [15]. Unfortunately, more detailed techniques, including Gram staining and culture, were not performed on the sampled animals.

Proteinuria ≥ 1+ was identified in 80.0% of the samples by the urine dipstick test; this was the most frequently found abnormality in our study. Using the UPC ratio as a tool to assess proteinuria, the results were also high, as 60.0% of dogs with CVL were classified as proteinuric, and 22.2% of dogs were classified as borderline proteinuric. Interestingly, dipstick and UPC ratio demonstrated the occurrence of proteinuria even in asymptomatic animals. Proteinuria in dogs with CVL has also been reported in previous studies [12,16]. Renal disease may be the sole clinical manifestation of CVL; it can progress from slight proteinuria to the end-stage renal disease, resulting in chronic renal failure, which is the main cause of death due to CVL [30]. Stratification of the UPC ratio revealed that values higher than 2.0 were significantly more frequent, particularly among symptomatic dogs. According to the literature, this result (UPC ratio > 2.0) is a strong indication of glomerular disease [14]. Therefore, our findings are as expected and reinforce that the progression of renal disease follows the clinical progression of CVL.

Proteinuria may be caused by modification of glomerular vascular permeability, immune complex deposition, vascular inflammation, intraglomerular hypertension, and tubulointerstitial dysfunction [14]. In dogs with visceral leishmaniasis, immune complex deposition is a frequent abnormality [2,3,10,34,35] and, in addition to tubular disease, justifies the results of the present study.

The increase in serum urea and creatinine levels were observed only in 6.7% of the dogs sampled, which was expected, as these markers can only detect renal disease late, requiring the loss of approximately 66% of nephrons [11]. On the other hand, azotemia is an uncommon finding in CVL-infected dogs, even in those with a high prevalence of kidney lesions [30]. The occurrence of proteinuric and non-azotemic CVL-infected dogs has also been reported before [7].

CONCLUSION

In the present study, urinalysis was a useful tool for identifying renal abnormalities in dogs with CVL, although significant differences were not important between the clinical groups of the disease, reinforcing the fact that renal injury arises even before clinical signs become apparent. Variation in color, turbidity, glycosuria, increased cellularity, and presence of erythrocytes and leukocytes were relevant elements detected in the animals infected by *Leishmania infantum* in our study. The high occurrence of occult blood, proteins, and casts, together with hematuria, confirmed the presence of tubular or glomerular lesions, or both, in CVL. The high frequency of symptomatic dogs with UPC ratio > 2.0 indicates that the progression of renal disease follows the clinical progression of CVL. The sole usage of serum markers (urea and creatinine) may underestimate the presence of kidney disease in dogs with CVL.

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