Research in Veterinary Science 206 (2026) 106160

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Evaluation of urinary amylase to creatinine ratio alone and in combination with urinary electrophoresis as a marker of renal damage in dogs with leishmaniosis undergoing conventional anti-*Leishmania* treatment

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ARTICLE INFO

Keywords:
Creatinine
Mixed proteinuria
Sodium dodecyl-sulphate agarose gel
Urinary protein-to-creatinine ratio

ABSTRACT

The aim of this study was to evaluate urinary amylase-to-creatinine ratio (uAm/Cr) alone and in combination with urinary sodium dodecyl-sulphate agarose gel electrophoresis (SDS-AGE) as a potential marker of renal damage in dogs with leishmaniosis at the time of diagnosis and post-treatment with meglumine antimoniate and allopurinol or miltefosine and allopurinol. Thirty-six healthy dogs and 31 dogs with leishmaniosis were enrolled in this prospective longitudinal study. Based on the initial creatinine (Cr) and urinary protein to creatinine ratio (UPC) values, leishmaniotic dogs were divided in: Group 1 if Cr was <1.4 mg/dl and UPC ≤ 0.5 ($n = 14$), Group 2 if Cr was <1.4 mg/dl and UPC > 0.5 ($n = 11$), and Group 3 if Cr was >1.4 mg/dl and a UPC > 0.5 ($n = 6$). According to SDS-AGE, 3 dogs had albuminuria, 4 tubular, and 20 mixed proteinuria at diagnosis. Post-treatment, 4 dogs had albuminuria, 5 tubular, 5 glomerular and 9 mixed proteinuria. At diagnosis the uAm/Cr was significantly different between all groups ($P < 0.001$) and after treatment decreased significantly only in Group 2 (269.7 [150.8–945] versus 8 [5–49.1], $P = 0.001$). At diagnosis and post-treatment, the uAm/Cr was significantly higher in dogs with mixed proteinuria ($n = 20$) compared to dogs with albuminuria and tubular proteinuria ($n = 7$) ($P < 0.0001$ and $P = 0.003$, respectively). The results suggest that uAm/cr could be a marker to evaluate renal damage at the time of diagnosis and eventual recovery after anti-*Leishmania* treatment, especially in dogs with proteinuria without azotemia.

1. Introduction

Canine leishmaniosis (CanL) due to *Leishmania infantum* is a protozoan disease that can cause different clinical signs and laboratory abnormalities as well as a wide spectrum of disease severity depending on the organs and tissues involved (Solano-Gallego et al., 2009). Renal disease may be one or the sole clinical manifestation of CanL and it can progress from mild proteinuria to nephrotic syndrome or in some cases to end stage renal disease (Solano-Gallego et al., 2011), and the main cause of mortality in dogs with leishmaniosis is represented by chronic renal failure (Roura et al., 2013, 2021; Solano-Gallego et al., 2011). Therefore, it is important in CanL to have an early diagnosis of renal damage.

Serum creatinine (Cr), symmetric dimethylarginine (SDMA) and urinary protein-to-creatinine ratio (UPC) are the biomarkers traditionally recommended by the International Renal Interest Society (IRIS) to evaluate and monitor renal damage/dysfunction (IRIS Staging of CKD, 2023). However, other markers have been investigated to better assess renal function such as urinary cystatin C (García-Martínez et al., 2015), plasma glomerular filtration rate (Cortadellas et al., 2008), serum cystatin C (de Oliveira Frazilio et al., 2018; Pasa et al., 2009), glomerular renal damage such as urinary ferritin (García-Martínez et al., 2015), urinary immunoglobulin-G to creatinine ratio (uIgG/Cr), urinary C-reactive protein to creatinine ratio (uCRP/Cr), and urinary ferritin to-creatinine ratio (uFerr/Cr) (Pardo-Marín et al., 2017) and tubular renal damage such as urinary gamma-glutamyl transpeptidase, *N*-acetyl-β-D-glucosaminidase and urinary β-glucuronidase (Palacio et al., 1995), urinary gamma-glutamyl transferase to creatinine ratio (uGGT/Cr) (de Oliveira Frazilio et al., 2018; Ibba et al., 2016; Paltrinieri et al., 2018), urinary retinol binding protein to creatinine ratio (uRBP/Cr), urinary *N*-

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https://doi.org/10.1016/j.rvsc.2026.106160
Received 6 June 2025; Received in revised form 16 January 2026; Accepted 23 March 2026
Available online 28 March 2026
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acetyl-β-D-glucosaminidase to creatinine ratio (uNAG/Cr) (Pardo-Marín et al., 2017) and urinary neutrophil-gelatinase associated lipocalin in dogs with leishmaniosis (de Lima Ruy Dias et al., 2020; Peris et al., 2020; Proverbio et al., 2024; Ruiz et al., 2024).

Three urinary glomerular markers such as uIgG/Cr, uCRP/Cr, and uFerr/Cr, and three urinary tubular markers such as uGGT/Cr, uRBP/Cr and uNAG/Cr were evaluated in dogs with leishmaniosis at IRIS stage I with proteinuria treated with meglumine antimoniate and allopurinol (Pardo-Marín et al., 2017). Interestingly, only dogs with a reduction in the UPC after treatment had a significant reduction of the urinary glomerular and tubular markers tested (Pardo-Marín et al., 2017).

Sodium dodecyl-sulphate agarose gel electrophoresis (SDS-AGE) separates low molecular weight proteins of tubular origin from high molecular weight proteins of glomerular origin and correlates well with the results of renal biopsy (Zini et al., 2004). Different authors have evaluated renal histologic lesions and urinary electrophoresis in dogs with leishmaniosis and displayed mainly a pattern of glomerular proteinuria or mixed proteinuria which was confirmed by the histopathological finding (Zaragoza et al., 2003; Zatelli et al., 2003). Furthermore, only a few studies have performed several urinary electrophoretic methods in dogs with leishmaniosis to identify glomerular and/or tubular damage at the time of diagnosis (Ibba et al., 2016; Navarro et al., 2022; Paltrinieri et al., 2016; Zaragoza et al., 2003; Zatelli et al., 2003).

The association between uGGT/Cr and urinary electrophoresis in leishmaniotic dogs with proteinuria was used to determine what type of renal damage was expressed by uGGT/Cr at diagnosis (Ibba et al., 2016; Paltrinieri et al., 2018). To the best knowledge of the authors, urinary electrophoresis has never been assessed in dogs with leishmaniosis with paired samples at the time of diagnosis and during treatment follow-up.

To date, proteinuria remains the most important clinicopathological finding to identify renal damage in the early stage of CanL (Koutinas and Koutinas, 2014; Paltrinieri et al., 2016), and therefore, further research is needed to identify new biomarkers that can identify early renal damage before renal failure develops.

Canine α-amylase is a ~ 54 kDa enzyme (Carne and Scheele, 1982; Hudson and Strombeck, 1978). It is freely filtered by the glomerulus and partially reabsorbed by renal tubules, resulting in little to no detectable urinary amylase under normal conditions (Hudson and Strombeck, 1978; Jacobs, 1988). Serum amylase activity increases with pancreatic acinar cell damage and hepatic neoplasia, but not with intestinal disease, "as the intestine is not a known source of increased serum α-amylase" (Stockham and Scott, 2025). Although urinary amylase has been investigated as an alternative diagnostic marker for pancreatitis, inconsistent increases in dogs with pancreatitis and marked hyperamylasemia limit their diagnostic value (Eto et al., 1969). Jacobs (1988) proposed that polymerization or complex formation between amylase and serum proteins increases its effective molecular size, reducing glomerular filtration. A later study showed a linear relationship between urinary amylase activity and proteinuria, indicating that urinary amylase primarily reflects glomerular damage (Jacobs, 1989).

A recent study showed, for the first time, that the urinary amylase-to-creatinine ratio (uAm/Cr) is a potential biomarker of renal damage for monitoring treatment response and for assessing the severity of CanL at the time of diagnosis (Pantaleo et al., 2024a). A strong positive correlation was found between uAm/Cr and UPC in dogs with leishmaniosis highlighting the potential role of uAm/Cr as a good marker of renal damage (Pantaleo et al., 2024a, 2024c). To the best knowledge of the authors, no studies have previously evaluated the uAm/Cr with urinary electrophoresis as a method to identify tubular and/or glomerular injury.

The aims of the present study were: 1) to determine the uAm/Cr in healthy dogs and in dogs with leishmaniosis with different degrees of disease severity at diagnosis and after conventional anti-*Leishmania* treatment, 2) to assess the urinary electrophoresis, based on SDS-AGE and the uAm/Cr at the same time in all dogs with leishmaniosis with different degrees of disease severity at diagnosis and after conventional anti-*Leishmania* treatment, 3) to evaluate the correlation between uAm/Cr and various enzymes, renal and urinary biomarkers in healthy dogs and in dogs with leishmaniosis with different degrees of disease severity at the time of diagnosis.

2. Material and methods

2.1. Dogs

This cross-over study includes 67 client-owned dogs that were admitted to the San Marco Veterinary Clinic (Veggiano, Italy) between October 2022 and January 2023.

Two study groups were defined as dogs with leishmaniosis (n = 31) and healthy dogs (n = 36). The diagnosis of clinical leishmaniosis was based on compatible clinical signs, clinicopathological findings, a positive *L. infantum* ELISA serology, and a positive *Leishmania* real-time polymerase chain reaction (q-PCR) in the bone marrow (Solano-Gallego et al., 2011, 2017). The following additional inclusion criteria were required for dogs with leishmaniosis: (1) routine laboratory tests including CBC, serum biochemistry, coagulation profile, urinalysis and abdominal ultrasound; (2) absence of *Dirofilaria immitis* antigen (Filarcheck 96, biopronix by Agrolabo, Italy), absence of *Anaplasma phagocytophilum*, *Ehrlichia canis* and *Rickettsia conorii* antibodies (semi-quantitative immunofluorescence by MegaFLUO *Anaplasma ph.* MEGACOR; MegaFLUO *Ehrlichia canis* MEGACOR; MegaFLUO *Rickettsia conorii* MEGACOR; Hörbranz, Austria); (3) inactive urine sediment; (4) no other concurrent diseases; (5) no medication administration in the previous 3 months with the exception of repellent or antiparasitic drugs. The following inclusion criteria were required to be considered healthy dogs: 1) unremarkable physical examination, 2) normal results in all laboratory tests including complete blood count (CBC), serum biochemistry, coagulation profile and urinalysis, 3) a negative *L. infantum* serology, 4) a negative *D. immitis*, *A. phagocytophilum*, *E. canis* and *R. conorii* serology in the last 6 months 5) no history of recent illness, and 6) no drug administration at the time of evaluation and in the previous 6 months with the exception of repellent or antiparasitic drugs.

2.2. Blood pressure measurement, sample collection and grouping

During the physical exam, after an adequate acclimatization period to the environment, in all dogs enrolled in the study, systolic blood pressure (SBP) was measured with the automated blood pressure monitor for companion animals SunTech Vet 20 (SunTech Medical Inc., USA), and after discarding the first measurement, the average value of 5 consecutive measurements was recorded (Acierno et al., 2018). All blood and urine tests were carried out in the morning after 12 h of fasting without any sedation at the time of diagnosis for all dogs, and after conventional anti-*Leishmania* treatment only for dogs with leishmaniosis.

All dogs diagnosed with leishmaniosis were divided into one of the following 3 groups based on their initial Cr and UPC values: Group 1 included dogs with a Cr < 1.4 mg/dl and a UPC ≤ 0.5, Group 2 dogs with a Cr < 1.4 mg/dl and a UPC > 0.5 and Group 3 dogs with a Cr > 1.4 mg/dl and a UPC > 0.5.

2.3. Treatment protocol

Based on the clinician's choice, two different protocols were used. Twenty-three dogs were treated with 50 mg/kg of meglumine antimoniate (Glucantime, Boehringer Ingelheim, Germany) subcutaneously every 12 h for 28 days and 10 mg/kg of allopurinol (Allopurinol DOC, Italy) orally twice daily for 6 months, and 8 dogs with 2 mg/kg of miltefosine (Milteforan, VIRBAC, France) orally every 24 h for 28 days and 10 mg/kg of allopurinol orally twice daily for 6 months (Solano-Gallego et al., 2011). All the diagnostic tests were carried out at diagnosis and on day 30 if dogs were treated with meglumine antimoniate and allopurinol

(Noli and Auxilia, 2005) or on day 60 if treated with miltefosine and allopurinol (Iarussi et al., 2020).

Of the 23 dogs treated with meglumine antimoniate and allopurinol 13 dogs belonged to Group 1, 9 to Group 2 and 1 to Group 3. Of the 8 dogs treated with miltefosine and allopurinol 1 dog belonged to Group 1, 2 to Group 2 and 5 to Group 3.

2.4. Blood tests

All tests were performed at the San Marco Veterinary Laboratory (Veggiano, Italy). A blood sample was collected by cephalic or saphenous or jugular venipuncture in a 10 ml sterile plastic syringe. Two ml of blood was transferred to plastic tubes containing K_3-EDTA for a CBC performed on an automated hematology analyzer (ADVIA 2120i, Siemens, Erlangen, Germany) with a blood smear reading. Four ml of blood was placed in serum glass tubes for chemistry analysis performed in an automated biochemical analyzer (Atellica® Solution, Siemens, Germany). Symmetric-dimethylarginine (SDMA) was measured with a canine SDMA ELISA according to manufacturer's instruction (Eurolyser Diagnostica GmbH, Salzburg, Austria). Ferritin (Ft) was measured and internally validated for dogs with a human two-site sandwich immunoassay using direct chemiluminometric technology (Atellica® IM Ferritin assay, Siemens Healthcare Diagnostic Inc., USA). A similar Ft human assay was previously validated for dogs (Martinez-Subiela et al., 2014). The variables ferritin (Ft), albumin (Alb), globulins (Glob), amylase, lipase, 1,2-*o*-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin) ester (DGGR) lipase, glucose (Glu), gamma-glutamyl transferase (GGT), urea, Cr, and SDMA were measured at diagnosis in healthy dogs and in dog with leishmaniosis, and at day 30 or 60 only in dogs with leishmaniosis depending on the conventional anti-*Leishmania* treatment chosen.

2.4.1. Leishmania serology

To detect *L. infantum* antibodies, a *Leishmania* ELISA test was performed following the manufacturer's instructions (VetLine *Leishmania*, *Leishmania* ELISA test, NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany) (Iatta et al., 2023). The result of the *Leishmania* ELISA test was considered positive if the antibody level was >11%.

2.5. Urine examination

Urine was collected by free catch in a sterile container in all dogs. A total volume of 10 ml of urine was obtained during spontaneous urination. Seven ml of urine was used for urinalysis and urinary chemistry performed on an automated urine analyzer (CLINITEK Novus®, Siemens, Germany) and on an automated biochemical analyzer (Atellica® Solution, Siemens, Germany) respectively.

Whole urine was used for urine analysis with test strips (CLINITEK Novus Pro12 Urinalysis Cassette, Siemens, Germany), urine specific gravity measurement (USG) (CLINITEK Novus Pro12 Urinalysis Cassette, Siemens, Germany), UPC determination, and urinary chemistry. Urine proteins and urinary creatinine were measured using pyrogallol red (Atellica CH Urinary/Cerebrospinal Fluid Protein (UCFP), Siemens Healthcare Diagnostics Inc., Tarrytown, USA) (Rossi et al., 2016), and a modified Jaffe method (Siemens Healthcare Diagnostics Inc., USA) respectively, and UPC was determined by calculating the ratio between the urine proteins and urinary creatinine.

Urinary sediment was examined by a clinical pathologist with an optical microscope and only dogs with inactive urine sediment (< 5 white blood cells per high power field [hpf], < 5 red blood cells/hpf or no visible bacteria or sperm) were included in the study. No casts were detected in any urine sediment. The uAm/Cr (IU/g) was calculated using the formula of uAm (IU/l) divided by urine creatinine concentration converted to the units g/l (Maruhn et al., 1977; Terui et al., 2013; Werner et al., 1970). The following parameters USG, UPC, uAm/Cr, urinary glucose to creatinine ratio (uGlu/Cr), uGGT/Cr, and uFerr/Cr were measured at diagnosis in healthy dogs and dogs with leishmaniosis, and after treatment in dogs with leishmaniosis, at day 30 or 60 based on the treatment protocol chosen.

2.6. Urinary electrophoresis

2.6.1. Sample selection and SDS-AGE procedure

Qualitative assessment of proteinuria was performed by SDS-AGE (Hydragel 5 proteinuric, Sebia Italia SRI, Italy) using a semiautomated system (Hydrasys analyzer, Sebia Italia SRI, Italy) on left over urine samples of all dogs with leishmaniosis stored at −20 °C until analyzed within 7 days of urine collection and following manufacturer's instruction.

Since SDS-AGE can show positive bands in non proteinuric dogs with concentrated urine (Giori et al., 2011), to reduce false positive results, SDS-AGE was performed only in dogs with a UPC ≥ 0.2. Eighty µL of the final urine supernatant were mixed with 20 µl of the additive provided by the manufacturer. Five µL of the treated urine were loaded on the gels (agarose 50 g/l), and the migration, staining with acid-violet and drying were conducted with the semi-automated system. The limit of sensitivity was 15 mg/l per band. Each gel contained 5 wells: the fifth one being filled with the manufacturer's molecular markers solution (Molecular mass control, Sebia) to facilitate protein identification. This solution contained lysozyme (14.3 kDa), triose-phosphate isomerase (26.6 kDa), bovine albumin (66 kDa) and human IgG (150 kDa).

2.6.2. Gel interpretation and proteinuria patterns

Gels were visually analyzed by the same operator, and 4 bands could be identified. Those bands migrating at the same distance as lysozyme and triose-phosphate isomerase, low molecular weight protein bands, were considered of tubular origin. A weak albumin band at the same distance as bovine albumin was considered albuminuria. An intense albumin band alone or with a band at the same distance as human IgG, high molecular weight band, was considered of glomerular origin and recorded as such. When a low molecular weight protein band and a high molecular weight protein or a low molecular weight protein band and an intense albumin band were detected, proteinuria was considered of mixed origin (Fig. 1). If no bands or a single tubular band of approximately 25 kDa in entire males (compatible with arginine esterase prostatic protein) (Hokamp et al., 2018) were identified, the pattern was considered normal with no proteinuria.

Urinary electrophoresis was performed at diagnosis and on day 30 or 60 depending on the conventional anti-*Leishmania* treatment chosen for the dogs with leishmaniosis.

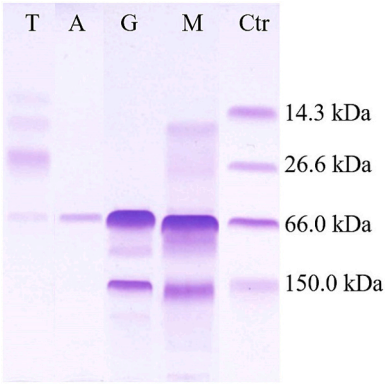

Fig. 1. Sodium dodecyl sulfate–agarose gel electrophoresis patterns.
The first lane represents tubular proteinuria (T), the second lane albuminuria (A), the third lane glomerular proteinuria (G), the fourth lane mixed proteinuria (M), and the last lane shows the control (Ctr) sample having a marker with known molecular weight (MW), corresponding to lysozyme (14.3 kDa), triose phosphate isomerase (26.6 kDa), albumin (66 kDa) and IgG (150 kDa).

Based on SDS-AGE, dogs with leishmaniosis could have no proteinuria, tubular proteinuria, albuminuria, glomerular proteinuria or mixed proteinuria (tubular and glomerular proteinuria) (Giori et al., 2011; Schultze and Jensen, 1998; Zini et al., 2004).

2.7. Evaluation of Leishmania parasitic load

2.7.1. Bone marrow collection and sampling procedure

Leishmania q-PCR was measured in the bone marrow of all dogs with leishmaniosis at the time of diagnosis. Bone marrow aspirates were obtained from costochondral junctions using an 18-gauge needle connected to a 10 ml syringe according to the protocol described by Paparcone et al. for the diagnosis of CanL (Paparcone et al., 2013). The procedure was performed in each dog in lateral recumbency or standing (depending on the position preferred by the dog) in the presence of the owner, without any local anesthesia or analgesia and lasted less than 2 min as previously described (Pantaleo et al., 2024a).

2.7.2. DNA extraction, real time-PCR protocol and interpretation

DNA extraction was performed using a High Pure PCR Template Preparation Kit (Roche Science Applied) and carried out according to the manufacturer's protocol. Real-time PCR was performed using LightCycler FastStart DNA Master^{PLUS} hybridisation probes (Roche, Mannheim, Germany), using a LightCycler version 3.5.17 instrument (Roche, Mannheim, Germany). *Leishmania infantum* primers and a hybridization Taqman MGB probe which amplified a fragment of the kinetoplast minicircle were used. The thermal profile was 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. Positive and negative controls were used in all the q-PCR runs as previously reported (Galletti et al., 2011).

The results were considered positive if >100 copies of kinetoplast/ml were detected. The limit of detection was established according to the definition proposed by the Clinical Laboratory Standards Institute (Forootan et al., 2017).

2.8. Sample size

The sample size and power for the two-sample Mann-Whitney *U* Test were calculated according to Noether (Noether, 1987). With a significant level of 0.05, the two sample sizes per group allow to reach a power of 0.94 with effect size 0.75. Therefore, the sample size used in the present study is sufficient.

2.9. Statistical analysis

Qualitative data were expressed as counts and percentages. Quantitative variables were reported as mean and standard deviation (SD) or median and interquartile range (IQR), according to Shapiro-Wilk's test for normality. Statistical differences between two groups were analyzed with Student's *t*-test, under the hypotheses of normality and homoscedasticity, or with the Mann-Whitney rank test for non-normal distributed variables. For more than two groups, under the hypotheses of normality parametric ANOVA was applied, otherwise the Kruskal-Wallis nonparametric ANOVA was applied considered. Post-hoc analyses were performed through pairwise comparisons between group levels with Holm correction for multiple tests.

Associations between qualitative variables were assessed with the Pearson Chi-Square test. The relationships between quantitative variables were evaluated using Spearman correlation plots. Finally, the receiver-operating characteristic (ROC) curve and the area under the ROC curve (AUC) were used to assess the ability of quantitative parameters to identify early renal damage between healthy dogs and dogs in Group 1 at the time of diagnosis. Logistic regression analysis was performed to determine whether the combined use of UPC and uAm/Cr provides additional diagnostic value compared with UPC alone.

The accuracy of the test was classified as excellent (AUC > 0.9), good (0.8 < AUC ≤ 0.9), fair (0.7 < AUC ≤ 0.8), poor (0.6 < AUC ≤ 0.7) or failed (0.5 < AUC ≤ 0.6) (Swets, 1988). The significance level was set at $P < 0.05$. The data were analyzed using statistical software R, version 4.3.2.

3. Results

3.1. Clinical data of healthy dogs and dogs with leishmaniosis at diagnosis

A summary of clinical data of healthy dogs and dogs with leishmaniosis at the time of diagnosis is listed in Table 1. There was a weak association between sex and group ($p = 0.021$). Healthy dogs and dogs in Group 1 had a similar sex distribution, in Group 2 there was a prevalence of male dogs and in Group 3 of female dogs.

3.2. Serum and urinary markers in healthy dogs and dogs with leishmaniosis at diagnosis

Serum Ft, Alb, Glob, Glu, GGT are reported in the supplementary material (Table S1). Serum amylase, urea, Cr and SDMA were significantly different between the healthy dogs and the three groups of dogs with leishmaniosis ($p < 0.001$, Table 2). Serum amylase concentration increased significantly going from healthy dogs to dogs in Groups 1, 2, and 3 ($p_a = 0.04$; $p_b < 0.001$; $p_c < 0.001$, respectively, Table 2).

A significant increase in uAm/Cr was observed in Group 1 compared to healthy dogs ($p_a < 0.001$) and in Group 2 compared to Group 1 ($p_d < 0.001$, Table 2).

The uAm/Cr was higher in Group 3 compared to healthy dogs, Group 1 and Group 2 ($p_c < 0.001$; $p_e < 0.001$; $p_f = 0.03$, respectively, Table 3). The uFerr/Cr increased significantly from healthy dogs to Group 1 ($p_a = 0.008$, Table 3), from Group 1 to Groups 2 and 3 ($p_d = 0.02$; $p_e = 0.03$, respectively, Table 2), but no significant difference was observed between Groups 2 and 3 ($p = 0.96$).

3.3. Serum and urinary markers in dogs with leishmaniosis post-treatment

Serum Ft, Alb, Glob, Glu, GGT values are reported in the supplementary material (Table S2). Serum amylase was higher in Groups 2 and 3 compared to Group 1 ($p_a = 0.008$; $p_b = 0.005$, respectively, Table 3), and serum Cr was significantly increased in Group 3 compared to Groups 1 and 2 ($p_b = 0.03$; $p_c = 0.03$, respectively, Table 3).

After treatment, UPC was significantly higher in Group 3 compared to Groups 1 and 2 ($p_b = 0.002$; $p_c = 0.003$, respectively, Table 3), and no difference was found between Groups 1 and 2 ($p = 0.8$).

The uAm/Cr was higher in Group 3 compared to Groups 1 and 2 ($p_b = 0.002$; $p_c = 0.004$, respectively) and in Group 2 compared to Group 1 ($p_a = 0.01$, Table 3). The uFerr/Cr was significantly greater in Group 3 compared to Group 1 ($p_b = 0.02$, Table 3) and in Group 2 compared to Group 1 ($p_a = 0.01$, Table 3), but not in Group 3 compared to Group 2 even if there was an increasing trend in Group 3 ($p_c = 0.05$).

The variation of various serum and urinary parameters within the same group of dogs with leishmaniosis (Groups 1, 2 and 3) between diagnosis and post-treatment is listed in Table 4.

Serum amylase and Cr decreased significantly in Group 3 ($p = 0.03$, respectively) post-treatment. The USG, UPC, uGGt/Cr and uFerr/Cr decreased significantly in Group 2 post-treatment ($p = 0.020$; $p = 0.004$; $p = 0.039$; $p = 0.036$, respectively, Table 4) as well as uAm/Cr ($p = 0.001$, Table 4, Fig. 2).

Table 1
Clinical and molecular data of healthy dogs and dogs with leishmaniosis.

Parameter (units)	Healthy dogs	Dogs with leishmaniosis ($n = 31$)			Statistic and p-value
		Group 1	Group 2	Group 3	
	n = 36	n = 14	$n = 11$	n = 6	
Female/male	17/19	6/8	1/10	5/1	$\chi^2 = 9.39$, $p = 0.021$*,
Breed/ mixed breed	7/29	6/8	8/3	1/5	$\chi^2 = 12.21$, $p = 0.005$*,
Castrated/intact	20/16	5/9	5/6	3/3	$\chi^2 = 1.66$, $p = 0.66$
Age (months)	68.4 ± 36.1	70.1 ± 31.8.2	64.9 ± 36.1	87.6 ± 37.8	$F = 0.07$, $df = 3$, $p = 0.98$
Body weight (kg)	24.0 ± 13.1	22.5 ± 9.6	19.2 ± 10.9	18.2 ± 8.4	$F = 0.75$, $df = 3$, $p = 0.53$
Heart rate (bpm)	100	100	120	110	KW = 6.053, $df = 3$, $p = 0.11$
	(87–110)	(97–120)	(102–146)	(98–126)	
Respiration rate (rpm)	30	30	32	31	KW = 2.998, $df = 3$, $p = 0.39$
	(24–33)	(25–40)	(29–44)	(28–32)	
Systolic blood pressure (mmHg)	142	147	142	184	KW = 8.02, $df = 3$, $p = 0.05$
	(130–150)	(140–159)	(135–155)	(158–189)	
Bone marrow *Leishmania* q-PCR (k/ml)	–	1.3×10^7 (3.01×10^6–1.25×10^8)	1.9×10^7 (1.84×10^6–9.22×10^8)	2.57×10^8 (1.12×10^8–8.28×10^8)	KW = 3.70, $df = 2$, $p = 0.16$

Data are expressed as numbers, mean and standard deviation or median and interquartile range. Group 1, dogs with creatinine <1.4 mg/dl and UPC ≤ 0.5; Group 2, dogs with Cr < 1.4 mg/dl and UPC > 0.5; Group 3, dogs with Cr > 1.4 mg/dl and UPC > 0.5. Kg, kilograms; bpm, beats per minute; rpm, breaths per minute; mmHg, millimeters of mercury; k/ml, kinetoplast copies per milliliter; χ^2, Chi-square test; KW, Kruskal-Wallis test; *df*, degrees of freedom; F, ANOVA non parametric test. * $p < 0.05$.

Table 2
Serum and urinary markers of healthy dogs and dogs with leishmaniosis at diagnosis.

Parameter (units)	Healthy dogs	Dog with leishmaniosis (n = 31)			Statistic and p-value
		Group 1	Group 2	Group 3	
(Reference interval)	n = 36	n = 14	$n = 11$	n = 6	
Amylase (IU/l) (176–764)	569 (463–762)	922 (708–1190)	1220 (1080–1412)	2358 (2228–2504)	KW = 32.42, $df = 3$, $p < 0.001$; Post-hoc, $p_a = 0.04$, $p_{b,\ c,\ f} < 0.001$, $p_e = 0.007$
Lipase (IU/l) (65–635)	316 ± 125.8	262.9 ± 188.3	204.5 ± 108	187.7 ± 164.3	$F = 2.7$, $df = 2$, $p = 0.053$
DGGR lipase (IU/l) (29–143)	78 (68–97)	99.5 (81.2–144)	85 (72–108)	122.5 (87.5–171)	KW = 8.51, $df = 3$, $p = 0.037$
Urea (mg/dl) (20–48)	33 (27–38)	28 (25–36)	23 (20–35)	151 (112–184)	KW = 19.25, $df = 3$, $p < 0.001$; Post-hoc, $p_c < 0.001$, $p_e = 0.003$, $p_f = 0.004$
Cr (mg/dl) (0.7–1.4)	1.1 (1–1.2)	0.93 (0.8–1.2)	0.78 (0.6–0.82)	2.9 (2.1–4.6)	KW = 34.74, $df = 3$, $p < 0.001$; Post-hoc, $p_{b,\ c} < 0.001$, $p_d = 0.03$, $p_e = 0.002$, $p_f = 0.003$
SDMA (µg/dl) (0–15)	8.4 (7.4–11.5)	12 (10.2–14)	12 (10–14.5)	27 (16–44.5)	KW = 22.81, $df = 3$, $p < 0.001$; Post-hoc, $p_a = 0.03$, $p_b = 0.007$, $p_c = 0.004$
USG (1015–1050)	1042 (1034–1052)	1039 (1029–1044)	1037 (1030–1048)	1022 (1018–1028)	KW = 8.94, $df = 3$, $p = 0.03$; Post-hoc, $p_c = 0.02$
UPC (0.1–0.5)	0.2 (0.17–0.2)	0.3 (0.2–0.4)	1.4 (1.1–3.4)	11.7 (7.9–14.2)	KW = 44.24, $df = 3$, $p < 0.001$; Post-hoc, $p_{a,f} = 0.008$, $p_{b,\ c,\ d} < 0.001$, $p_e = 0.002$
uAm/Cr (IU/g) (0.1–4.5)	0.7 (0.4–1.1)	4.15 (1.5–10.9)	269.7 (150.8–945)	1568 (1317–1762)	KW = 44.66, $df = 3$, $p < 0.001$; Post-hoc, $p_{a,\ b,\ c,\ d} < 0.001$, $p_e < 0.002$, $p_f = 0.03$
uGlu/Cr (2–8.5)	3.1 (2.8–3.6)	4.6 (4.3–5.1)	5.1 (4.2–7.9)	5.2 (2.7–17.6)	KW = 21.67, $df = 3$, $p < 0.001$; Post-hoc, $p_a = 0.001$, $p_b < 0.001$
uGGT/Cr (IU/g) (13−22)	15.8 (12.3–19.9)	24.2 (15.6–72.7)	55.3 (43–116)	81.4 (73.7–90.6)	KW = 27.34, $df = 3$, $p < 0.001$; Post-hoc, $p_a = 0.04$, $p_b < 0.001$, $p_c = 0.001$
uFerr/Cr (0–25)	1 (0–3)	8 (1.2–25.6)	36 (25–59.5)	41.5 (28–53.5)	KW = 37.30, $df = 3$, $p < 0.001$; Post-hoc, $p_a = 0.008$, $p_{b,\ c} < 0.001$, $p_d = 0.02$, $p_e = 0.03$

Data are expressed as median and interquartile range. Group 1, dogs with creatinine <1.4 mg/dl and UPC ≤ 0.5; Group 2, dogs with Cr < 1.4 mg/dl and UPC > 0.5; Group 3, dogs with Cr > 1.4 mg/dl and UPC > 0.5. DGGR, 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin) ester; Cr, creatinine; SDMA, symmetric-dimethylarginine; USG, urine specific gravity; UPC, urinary protein to creatine ratio; uAm/Cr, urinary amylase to creatinine ratio; uGlu/Cr, urinary glucose to creatinine ratio; uGGT/Cr, urinary gamma-glutamyl transferase to creatinine ratio; uFerr/Cr, urinary ferritin to creatinine ratio; KW, Kruskal Wallis test; *df*, degrees of freedom.

p_a statistically significant differences between healthy dogs and Group1.
p_b statistically significant differences between healthy dogs and Group 2.
p_c statistically significant differences between healthy dogs and Group 3.
p_d statistically significant differences between Group 1 and Group 2.
p_e statistically significant differences between Group 1 and Group 3.
p_f statistically significant differences between Group 2 and Group 3.

3.4. Urinary electrophoresis and uAm/Cr in dogs with leishmaniosis at diagnosis and post-treatment

At diagnosis, of the 31 dogs with leishmaniosis, 4 (13%) had no proteinuria and a median value and IQR for uAm/Cr of l.6 (1.2–2.75), 3 (10%) albuminuria with a median value and IQR for uAm/Cr of 2.7 (1.7–18.5), 4 (13%) tubular proteinuria with a median value and IQR for uAm/Cr of 4.9 (1.6–8.8) and 20 (64%) mixed proteinuria with a median value and IQR of 387.4 (176.9–1509.2).

After treatment, 8 (26%) dogs had no proteinuria with a median value and IQR for uAm/Cr of 0.6 (0.6–2.5), 4 (13%) albuminuria with a median value and IQR for uAm/Cr of 9.4 (3.8–18.1), 5 (16%) tubular proteinuria with a median value and IQR for uAm/Cr of 3 (2.4–3.6), 5 (16%) glomerular proteinuria with a median value and IQR for uAm/Cr of 154.5 (68.3–164.7), and 9 (29%) mixed proteinuria with a median value and IQR for uAm/Cr of 193.1 (11.3–1222).

At diagnosis, dogs with mixed proteinuria had a median value and IQR for uAm/Cr significantly higher compared to dogs with albuminuria and tubular proteinuria together [387.4 (176.9–1509.2) versus 2.7 (1.35–9.85), $p < 0.0001$].

After treatment, dogs with glomerular proteinuria had a median value and IQR for uAm/Cr significantly higher compared to dogs with albuminuria and tubular proteinuria together [154.5 (68.3 - 164.5) versus 3.6 (2.4 - 5.8), $p = 0.002$]; a significant difference was found between dogs with mixed proteinuria and dogs with albuminuria and tubular proteinuria together [193.1 (11.3 - 1222) versus 3.6 (2.4 - 5.8), $p = 0.003$]; and no significant difference was found between dogs with mixed and glomerular proteinuria ($p = 0.898$).

The SDS-AGE was evaluated in the three groups of dogs with leishmaniosis at diagnosis and post treatment (Tables 5 and 6). At the time of diagnosis, mixed proteinuria was present in 3/14 dogs in Group 1 and in all dogs in Groups 2 and 3. The uAm/Cr was significantly higher in dogs with mixed proteinuria in Group 3 (1568 [1317.2–1762]) compared to Group 2 (269.7 [150.8–945], $p = 0.027$). After treatment 2/11 dogs in Group 2 and 5/6 dogs in Group 3 had still mixed proteinuria (Table 6).

3.5. Relationship between uAm/Cr and various parameters in healthy dogs and dogs with leishmaniosis at diagnosis

The correlations between the uAm/Cr and various enzymes, renal and urinary markers in healthy dogs and dogs with leishmaniosis at diagnosis are reported in Table 7. In healthy dogs there was a moderate correlation between the uAm/Cr and UPC ($r = 0.50$, $p < 0.001$), and in dogs with leishmaniosis a very strong correlation between the uAm/Cr and UPC in Groups 1, 2 and 3 ($r = 0.98$, $p < 0.001$; $r = 0.99$, $p < 0.001$; $r = 0.94$, $p < 0.001$, respectively). In healthy dogs there was a weak positive correlation between uAm/Cr and uferr/Cr ($r = 0.39$, $p = 0.002$, Table 7).

A strong and moderate positive correlation was detected between uAm/Cr and uFerr/Cr in dogs in Groups 1 and 3 ($r = 0.79$, $p = 0$ 0.02; $r = 0.45$, $p = 0.02$, respectively, Table 7). The correlation between uAm/Cr and uFerr/Cr in Group 2 was weak ($r = 0.21$, $p = 0.02$, Table 7). ROC curves for UPC and uAm/Cr were calculated to identify renal damage between healthy dogs and dogs in Group 1 at the time of diagnosis. The UPC showed fair accuracy (AUC = 0.74, 95% CI = [0.57–0.91]; $p = 0.0025$, Fig. 3) and the uAm/Cr good accuracy (AUC = 0.83, 95% CI = [0.70–0.97]; $p = 0.0001$, Fig. 3). A multivariable logistic regression model including both variables was fitted to assess their combined diagnostic value. The uAm/Cr remained independently associated with the outcome ($p = 0.040$), whereas UPC was not ($p = 0.57$), indicating that uAm/Cr accounts for most of the discriminatory information. The combined model showed high discriminative ability (AUC = 0.88, 95% CI: 0.79–0.98) but performed only marginally better than uAm/Cr alone, with no significant difference between ROC curves ($p = 0.19$).

Table 3
Comparison of serum and urinary renal markers of dogs with leishmaniosis (Groups 1, 2 and 3) post-treatment.

Parameter (units) (Reference interval)	Group 1 n = 14	Group 2 n = 11	Group 3 n = 6	Statistic and *p*-value
Amylase (IU/l) (176–764)	797.5 (699–1131)	1469 (1028–1669)	1701 (1325–2019)	KW = 12.86, $df = 2$, $p = 0.0016$; Post-hoc, $p_a = 0.008$, $p_b = 0.005$ (176–764)
Lipase (IU/l) (65–635)	263.3 ± 163.1	206.5 ± 93.7	195.5 ± 131.6	$F = 32.42$, $df = 2$, $p = 0.47$
DGGR lipase (IU/l) (29–143)	122.50 (97.25–156.75)	95 (85–99.45)	122.50 (95–158.3)	KW = 3.28, $df = 2$, $p = 0.19$
Urea (mg/dl) (20–48)	28 (23.2–29)	28 (24–37.5)	62.5 (48–170.8)	KW = 7.80, $df = 2$, $p = 0.02$; Post-hoc, $p_b = 0.02$
Cr (mg/dl) (0.7–1.4)	0.96 (0.74–1.1)	0.76 (0.66–0.93)	1.8 (1.1–3.7)	KW = 8.65, $df = 2$, $p = 0.011$; Post-hoc, $p_{b,\ c} = 0.03$
SDMA (µg/dl) (0–15)	11.5 (11–14.7)	14 (11–16.5)	21 (15.5–36.2)	KW = 9.05, $df = 2$, $p = 0.016$, Post-hoc, $p_b = 0.02$
USG (1015–1050)	1038 (1026–1040)	1019 (1015–1041)	1018 (1015–1020)	KW = 8.23, $df = 2$, $p = 0.08$
UPC (0.1–0.5)	0.3 (0.2–0.47)	0.3 (0.15–0.6)	7.6 (3.8–14.4)	KW = 13.54, $df = 2$, $p = 0.001$, Post-hoc, $p_b = 0.002$, $p_c = 0.003$
uAm/Cr (IU/g) (0.1–4.5)	2.2 (0.6–4.7)	8.0 (5–49.1)	729 (204–1794)	KW = 16.77, $df = 2$, $p < 0.001$; Post-hoc, $p_a = 0.01$, $p_b = 0.002$, $p_c = 0.004$
uGlu/Cr (2–8.5)	4.4 (3.8–7.4)	4.8 (2.6–5.7)	3.9 (3.1–8.3)	KW = 0.77, $df = 2$, $p = 0.68$
uGGT/Cr (IU/g) (13–22)	27.5 (17.1–58.2)	44.8 (37.1–53.2)	53.4 (39.6–66.3)	KW = 2.61, $df = 2$, $p = 0.27$
uFerr/Cr (0–25)	8 (3.2–24.7)	14 (9.5–38.5)	47 (34.2–71)	KW = 8.93, $df = 2$, $p = 0.011$; Post-hoc, $p_a = 0.01$, $p_b = 0.02$

Data are expressed as median and interquartile range. Group 1, dogs with creatinine <1.4 mg/dl and UPC ≤ 0.5; Group 2, dogs with Cr < 1.4 mg/dl and UPC > 0.5; Group 3, dogs with Cr > 1.4 mg/dl and UPC > 0.5. DGGR, 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin) ester; Cr, creatinine; SDMA, symmetric-dimethylarginine; USG, urine specific gravity; UPC, urinary protein to creatine ratio; uAm/Cr, urinary amylase to creatinine ratio; uGlu/Cr, urinary glucose to creatinine ratio; uGGT/Cr, urinary gamma-glutamyl transferase to creatinine ratio; uFerr/Cr, urinary ferritin to creatinine ratio; KW, Kruskal Wallis test; *df*, degrees of freedom

p_a statistically significant differences between Group 1 and Group 2.
p_b statistically significant differences between Group 1 and Group 3.
p_c statistically significant differences between Group 2 and Group 3.

Table 4
Variation of various serum and urinary parameters among dogs with leishmaniosis (Groups 1, 2 and 3) between time at diagnosis and after treatment.

Parameter	Group 1			Group 2			Group 3		
	Diagnosis	Tx	Statistic *p*-value	Diagnosis	Tx	Statistic *p*-value	Diagnosis	Tx	Statistic *p*-value
Amylase (IU/l)	922 (708–1190)	797.5 (699–1131)	$t = -0.73$ $p = 0.480$	1220 (1080–1412)	1469 (1028–1669)	$t = 1.45$ $p = 0.177$	2358 (2228–2504)	1701 (1325–2019)	$t = -3.06$ $p = 0.028$*
Lipase (IU/l)	262.9 ±188.3	263.3 ±163.1	$t = 0.04$ $p = 0.969$	204.5 ±108	206.5 ±93.7	$t = 0.19$ $p = 0.851$	187.7 ±164.3	195.5 ±131.5	$t = 0.37$ $p = 0.729$
DGGR lipase (IU/l)	99.5 (81.2–144)	122.5 (92.25–156.75)	$V = 84.5$ $p = 0.048$*	85 (72–108)	95 (85–99.45)	$V = 65$ $p = 0.005$	122.5 (87.5–171)	122.5 (95–158.3)	$V = 13.5$ $p = 0.599$
Urea (mg/dl)	28 (25–36)	28 (23.2–29)	$t = -0.46$ $p = 0.677$	23 (20–35)	28 (24–37.5)	$t = -0.91$ $p = 0.382$	151 (112–184)	62.5 (48–170.8)	$t = -2.56$ $p = 0.051$
Cr (mg/dl)	0.93 (0.8–1.2)	0.96 (0.74–1.1)	$V = 43$ $P = 0.583$	0.78 (0.6–0.82)	0.76 (0.66–0.93)	$V = 46$ $p = 0.266$	2.9 (2.1–4.6)	1.8 (1.1–3.7)	$V = 0$ $p = 0.035$*
SDMA (μg/dl)	12 (10.2–14)	11.5 (11–14.7)	$t = 0.37$ $p = 0.715$	12 (10–14.5)	14 (11–16.5)	$t = 0.58$ $p = 0.574$	27 (16–44.5)	21 (15.5–36.2)	$t = -2.05$ $p = 0.953$
USG	1039 (1029–1044)	1038 (1026–1040)	$t = -1.185$ $p = 0.257$	1037 (1030–1048)	1019 (1015–1041)	$t = -2.76$ $p = 0.020$*	1022 (1018–1028)	1018 (1015–1020)	$t = -2.45$ $p = 0.058$
UPC	0.3 (0.2–0.4)	0.3 (0.2–0.47)	$V = 21$ $p = 0.268$	1.4 (1.1–3.4)	0.3 (0.15–0.6)	$V = 0$ $p = 0.004$*	11.7 (7.9–14.2)	7.6 (3.8–14.4)	$V = 3$ $p = 0.156$
uAm/Cr (IU/g)	4.15 (1.5–10.9)	2.2 (0.6–4.7)	$V = 22$ $p = 0.108$	269.7 (150.8–945)	8.0 (5–49.1)	$V = 0$ $p = 0.001$*	1568 (1317–1762)	729 (204–1794)	$V = 3$ $p = 0.156$
uGlu/Cr	4.6 (4.3–5.1)	4.4 (3.8–7.4)	$V = 61$ $p = 0.615$	5.1 (4.2–7.9)	4.8 (2.6–5.7)	$V = 9$ $p = 0.067$	5.2 (2.7–17.6)	3.9 (3.1–8.3)	$V = 13$ $p = 0.687$
uGGT/Cr (IU/g)	24.2 (15.6–72.79	27.5 (17.1–58.2)	$t = -0.33$ $p = 0.746$	55.3 (43–116)	44.8 (37.1–53.2)	$t = -2.37$ $p = 0.039$*	81.4 (73.7–90.6)	53.4 (39.6–66.3)	$t = -1.71$ $p = 0.147$
uFerr/Cr	8 (1.2–25.6)	8 (3.2–24.7)	$V = 68$ $p = 0.344$	36 (25.59.5)	14 (9.5–38.5)	$V = 9$ $p = 0.036$*	41.5 (28–53.5)	47 (34.2–71)	$V = 178$ $p = 0.219$

Data are expressed as mean and standard deviation or median and interquartile range. Differences are expressed using t-test (*t*) or Wilcoxon signed-rank test (V) and *p*-Value (*p*).
Group 1, dogs with creatinine <1.4 mg/dl and UPC ≤ 0.5; Group 2, dogs with Cr < 1.4 mg/dl and UPC > 0.5; Group 3, dogs with Cr > 1.4 mg/dl and UPC > 0.5. DGGR, 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin) ester; Cr, creatinine; SDMA, symmetric-dimethylarginine; USG, urine specific gravity; UPC, urinary protein to creatine ratio; uAm/Cr, urinary amylase to creatinine ratio; uGlu/Cr, urinary glucose to creatinine ratio; uGGT/Cr, urinary gamma-glutamyl transferase to creatinine ratio; uFerr/Cr, urinary ferritin to creatinine ratio, Tx: treatment.

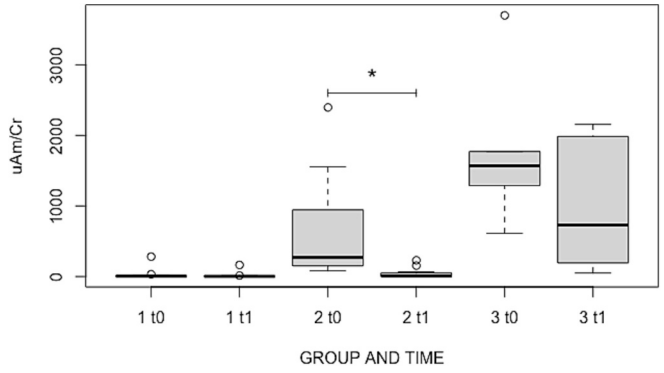

Fig. 2. Urinary amylase-to-creatinine ratio in dogs with leishmaniosis.
Data shows the uAm/Cr in Groups 1, 2 and 3 at diagnosis and post-treatment. The bottom and top of the box indicate the 25th and the 75th percentile, and the horizontal line inside the box the median.

Table 5
Urinary electrophoresis and uAm/Cr in all dogs with leishmaniosis at diagnosis.

Type of SDS-AGE and uAm/Cr at diagnosis	Group 1	Group 2	Group 3
	n = 14	n = 11	n = 6
No proteinuria	*n* = 4	–	–
uAm/Cr	1.6 (1.55)		
Albuminuria	n = 3	–	–
uAm/Cr	2.7 (16.45)		
Tubular proteinuria	n = 4	–	–
uAm/Cr	4.85 (7.17)		
Glomerular proteinuria	–	–	–
Mixed proteinuria	n = 3	n = 11	n = 6
uAm/Cr	11.2 (136.4)	269.7 (794.1)	1568 (444.8)

Data are expressed as median and interquartile range. Group 1, dogs with creatinine <1.4 mg/dl and UPC ≤ 0.5; Group 2, dogs with Cr < 1.4 mg/dl and UPC > 0.5; Group 3, dogs with Cr > 1.4 mg/dl and UPC > 0.5. SDS-AGE, sodium dodecyl-sulphate agarose gel electrophoresis; uAm/Cr, urinary amylase to creatinine ratio; n, number; Cr, creatinine.

4. Discussion

This study described, for the first time, the evaluation on uAm/Cr in conjunction with the urinary electrophoresis, in all dogs with leishmaniosis with different degrees of disease severity at the time of diagnosis and following conventional anti-*Leishmania* treatment. At diagnosis, the uAm/Cr was significantly increased in dogs with leishmaniosis compared to healthy dogs with increasing values from leishmaniotic dogs with no azotemia and no proteinuria to dogs with azotemia and proteinuria.

Half of the non-azotemic and non-proteinuric dogs with leishmaniosis exhibited uAm/Cr values above the reference interval, suggesting subclinical renal involvement. In this group, uAm/Cr showed good accuracy for discriminating renal damage, whereas UPC demonstrated only fair accuracy, indicating a potential advantage of uAm/Cr for early detection when UPC remains within the reference interval. This result is particularly relevant, as proteinuria is considered a key marker of early renal damage in CanL (Koutinas and Koutinas, 2014; Paltrinieri et al., 2016), and the increase in uAm/Cr may precede its onset.

These findings differ from previous observations in dogs with pyometra, where increased urinary amylase was always associated with proteinuria (de Schepper et al., 1989). Although uAm/Cr has been previously proposed primarily as a marker of disease severity in CanL (Pantaleo et al., 2024a, 2024c), the current results support its role as an earlier indicator of renal damage than proteinuria.

At diagnosis, dogs with proteinuria and dogs with azotemia and

Table 6
Urinary electrophoresis and uAm/Cr in all dogs with leishmaniosis post-treatment.

Type of SDS-AGE and uAm/Cr post-treatment	Group 1	Group 2	Group 3
	n = 14	n = 11	*n* = 6
No proteinuria	n = 6	*n* = 2	–
uAm/Cr	0.6 (0)	5 (3)	
Albuminuria	n = 2	n = 2	–
uAm/Cr	7.8 (6.4)	17.3 (12.7)	
Tubular proteinuria	n = 3	n = 2	–
uAm/Cr	3.3 (1.6)	4.1 (1.65)	
Glomerular proteinuria	n = 1	n = 3	n = 1
uAm/Cr	164.7	68.3 (63.35)	236.6
Mixed proteinuria	n = 2	n = 2	*n* = 5
uAm/Cr	8.25 (3.2)	117.75 (112.35)	1222 (1701.5)

Data are expressed as median and interquartile range. Group 1, dogs with creatinine <1.4 mg/dl and UPC ≤ 0.5; Group 2, dogs with Cr < 1.4 mg/dl and UPC > 0.5; Group 3, dogs with Cr > 1.4 mg/dl and UPC > 0.5. SDS-AGE, sodium dodecyl-sulphate agarose gel electrophoresis; uAm/Cr, urinary amylase to creatinine ratio; n, number; Cr, creatinine.

proteinuria in addition to an increase in uAm/Cr, had a significant increase in uGGT/Cr indicating tubular damage (Ibba et al., 2016) and an increase in uFerr/Cr indicating glomerular damage (García-Martínez et al., 2015) at the same time. Amylasuria has been reported to reflect, through various mechanisms, alterations in glomerular and/or tubular integrity (Stockham and Scott, 2025); consequently, increased urinary amylase excretion may be considered an indicator of renal damage at different levels.

After anti-*Leishmania* therapy, there was a decrease in uAm/Cr in all 3 groups enrolled in the current study, but the decrease was significant only in dogs with leishmaniosis with only proteinuria at the time of diagnosis. These findings are partially in accordance with the ones of a previous study in which all dogs with leishmaniosis (regardless of the severity of the disease) after conventional anti-*Leishmania* treatment had a significant decrease in uAm/Cr (Pantaleo et al., 2024a). The discrepant results between studies may be explained by differences in dog classification (creatinine and UPC in the present study versus LeishVet clinical staging in the other study) and by the low number of dogs with azotemia and proteinuria in the present cohort. This may have limited the detection of post-treatment changes in uAm/Cr in more severely affected dogs. After treatment, significant reductions in uAm/Cr, UPC, uGGT/Cr, and uFerr/Cr were observed only in dogs with proteinuria at diagnosis, suggesting greater potential for recovery from tubular and/or glomerular damage.

In non-azotemic and non-proteinuric dogs with leishmaniosis, urinary electrophoresis revealed normal patterns as well as albuminuria, tubular, and mixed proteinuria. The presence of tubular proteinuria suggests that tubular damage can be present as an indicator of early renal damage. In a recent study, SDS-polyacrylamide agarose gel electrophoresis (PAGE) showed in non-azotemic dogs with leishmaniosis, and border line proteinuria (UPC between 0.2 and 0.5) the presence of distal tubular injury. This finding supports the concept that tubular damage can lead to early detection of renal damage in absence of proteinuria (Daza et al., 2024). More than 60% of dogs with leishmaniosis had mixed proteinuria at the time of diagnosis. These findings are consistent with previous studies showing mixed proteinuria in dogs with leishmaniosis, either in all dogs by SDS-PAGE and Western blot (Zaragoza et al., 2003), or in the majority by SDS-AGE (Ibba et al., 2016), likely reflecting tubulointerstitial lesions commonly complicating glomerular disease as explained by Zatelli et al. (2003). Interestingly, all dogs with proteinuria or azotemia and proteinuria at diagnosis showed mixed proteinuria, in contrast to a previous study describing albuminuria, tubular, and glomerular proteinuria in some proteinuric dogs (Ibba et al., 2016). The differences between the two

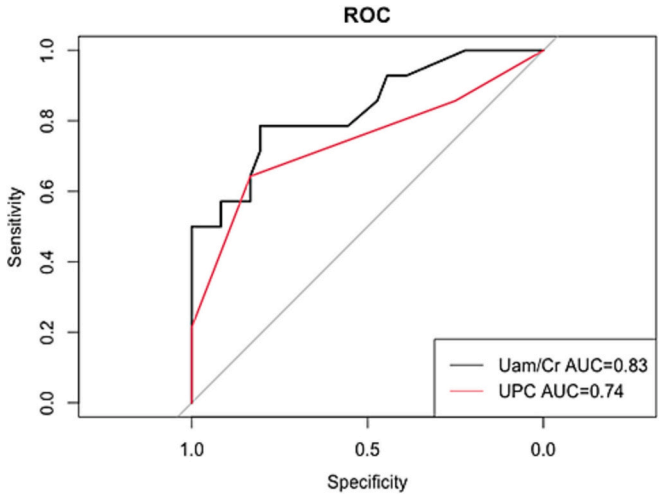

Fig. 3. Receiver operating characteristic curve (ROC) and area under the curve (AUC).
The ROC and the area under the curve differentiate between healthy dogs and dogs with leishmaniosis in Group 1 using uAm/Cr and UPC. UAm/Cr, urinary amylase to creatinine ratio; UPC, urinary protein to creatinine ratio.

Table 7
Correlations between the uAm/Cr and various enzymes, renal and urinary markers in healthy dogs and dogs with leishmaniosis at diagnosis.

Markers	Healthy dogs		Group 1		Group 2		Group 3	
	n = 36		n = 14		n = 11		n = 6	
Statistical test	*r*	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
uAm/Cr x amylase	0.18	0.83	0.62	0.17	0.67	0.06	0.37	0.50
uAm/Cr x lipase	−0.15	0.96	−0.18	0.26	−0.34	0.32	−0.62	0.14
uAm/Cr x DGGR lipase	−0.08	0.75	0.01	0.66	0.46	0.65	−0.52	0.14
uAm/Cr x urea	−0.09	0.50	0.75	0.07	0.22	0.35	−0.24	0.71
uAm/Cr x Cr	−0.29	0.16	0.49	0.90	0.06	0.89	−0.33	0.56
uAm/Cr x SDMA	−0.03	0.35	0.23	0.40	−0.08	0.87	−0.21	0.65
uAm/Cr x USG	**−0.36**	**0.03**	−0.04	0.03	−0.2	0.03	−0.16	0.03
uAm/Cr x UPC	**0.5**	**< 0.0001**	**0.98**	**< 0.0001**	**0.99**	**< 0.0001**	**0.94**	**< 0.0001**
uAm/Cr x uGlu/Cr	0.15	0.39	−0.23	0.39	0.58	0.39	−0.08	0.39
uAm/Cr x uGGT/Cr	0.18	0.29	0.51	0.29	−0.03	0.29	0.25	0.29
uAm/Cr x uFerr/Cr	**0.39**	**0.002**	**0.79**	**0.02**	0.21	0.02	**0.45**	**0.02**

Spearman test, Bold: when *P*-value and *r* are significant in each group. Group 1, dogs with creatinine <1.4 mg/dl and UPC ≤ 0.5; Group 2, dogs with Cr < 1.4 mg/dl and UPC > 0.5; Group 3, dogs with Cr > 1.4 mg/dl and UPC > 0.5. uAm/Cr, urinary amylase-to-creatinine ratio; DGGR, 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin) ester; Cr, creatinine; SDMA, symmetric-dimethylarginine; USG, urine specific gravity; UPC, urinary protein to creatinine ratio; uGlu/Cr, urinary glucose to creatinine ratio; uGGT/Cr, urinary gamma-glutamyltransferase to creatinine ratio; uFerr/Cr, urinary ferritin-to-creatinine ratio.

studies are difficult to explain, especially in dogs that had only proteinuria and therefore could have albuminuria, glomerular or tubular damage. Furthermore, pure tubular proteinuria is infrequent in dogs with leishmaniosis (Ibba et al., 2016), and could be identified only when free light chain proteinuria was present (Bonfanti et al., 2004).

At diagnosis, the uAm/Cr was increased in some dogs with albuminuria or tubular proteinuria and in all dogs with mixed proteinuria. After treatment, the uAm/Cr remained above the reference interval in all dogs with glomerular or mixed proteinuria. These findings suggest that an increase in uAm/Cr can identify renal damage at various levels and that dogs with mixed or glomerular proteinuria have higher values of uAm/Cr compared to dogs with albuminuria and tubular proteinuria together, with no difference between glomerular and mixed patterns. These findings support uAm/Cr as a marker of glomerular damage in CanL, as previously reported in other diseases (de Schepper et al., 1989; Jacobs, 1989). Given that leishmaniosis is primarily a glomerular disease in dogs (Costa et al., 2003; Zatelli et al., 2003), progressive tubulointerstitial involvement (Zatelli et al., 2003) may contribute to further loss of urinary amylase.

Mixed proteinuria is common in advanced stages of leishmaniosis (Zatelli et al., 2003) but does not indicate irreversible glomerular and/or tubular damage. Consistently, the present study shows that proteinuric dogs, all with mixed proteinuria at diagnosis, can exhibit variable recovery of glomerular and/or tubular damage after conventional anti-*Leishmania* treatment.

In this study, correlation analysis was performed to assess the association between uAm/Cr and various enzymatic, renal, and urinary markers in healthy dogs and dogs with leishmaniosis. A strong positive correlation was observed between uAm/Cr and UPC in all dogs with leishmaniosis, regardless of disease severity. This relationship, previously reported in dogs with hereditary glomerulopathy and hospitalized dogs with proteinuria, suggests that urinary amylase is closely linked to glomerular damage (Jacobs, 1989). As uAm/Cr may increase in non-azotemic and non-proteinuric dogs, a combined model including uAm/Cr and UPC demonstrated high discriminative ability, but only marginally improved performance compared with uAm/Cr alone. Therefore, uAm/Cr appears to be the primary driver of diagnostic performance in multivariable analysis for early renal damage, with limited additional value from the inclusion of UPC in dogs with no evidence of renal disease.There was also a positive correlation between the uAm/Cr and uFerr/Cr in healthy dogs and dogs with leishmaniosis. No clear explanation was found for the low correlation between the uAm/Cr and uFerr/Cr in dogs with proteinuria. The correlation between these two markers suggests that the uAm/Cr is associated with glomerular damage. No correlation was found between uAm/Cr and functional renal markers such as Cr, SDMA and USG in dogs with leishmaniosis. The lack of a significant association between USG and urinary amylase activity was already described (Jacobs, 1989). The uAm/Cr appears to reflect structural renal damage rather than function, distinguishing it from conventional markers and supporting its potential role as an early indicator of renal damage, particularly glomerular injury, rather than a marker of reduced glomerular filtration that occurred in advanced leishmaniosis.

Amylase and lipase were assessed in dogs with leishmaniosis at diagnosis and after anti-*Leishmania* treatment. Consistent with previous findings, amylase but not lipase was significantly increased at diagnosis compared with healthy dogs (Pantaleo et al., 2024b). The current study confirmed these results with a greater increase of amylase in dogs with renal disease as shown in dogs with chronic kidney disease (Polzin et al., 1983). After treatment, amylase remained elevated, while lipase showed no significant change.

To our knowledge, this is the first report of DGGR lipase activity in dogs with leishmaniosis at diagnosis and after treatment. This enzyme remained within the reference interval at diagnosis and did not increase significantly after therapy, despite a mild upward trend. Although dogs with leishmaniosis, particularly those with advanced LeishVet clinical stages, treated with meglumine antimoniate have been reported to be at increased risk of pancreatitis (Viñeta et al., 2024), no dogs in the present study was suspected to develop acute pancreatitis. Differences between studies may be related to diagnostic criteria and treatment protocols, as most dogs with advanced disease in the current study received miltefosine plus allopurinol rather than meglumine antimoniate.

Overall, the results of this study show the clinical utility of uAm/Cr in CanL for the detection of renal damage in early stages of the disease and for monitoring renal recovery after conventional anti-*Leishmania* treatment. Further studies are warranted to evaluate the role of uAm/Cr, either in combination with or as an alternative to UPC, as an early diagnostic marker of renal damage and as a prognostic indicator during long-term follow-up.

This study has several limitations. The low number of dogs studied could have reduced statistical power to detect significant differences between the different groups. A second limitation is represented by the lack of kidney biopsy in healthy dogs and in dogs with leishmaniosis. Kidney biopsy is mandatory to confirm or rule out the presence of renal damage and in case of proteinuria allows the localization of renal lesions (Lees et al., 2005). Even if SDS-AGE differentiates the origin of urinary proteins based on their molecular weight (Schultze and Jensen, 1998) and correlates with the results of renal histopathological lesions, the specificity is low (Zini et al., 2004). Another limitation of this study is the inability to adjust the analyses for treatment effects due to the highly unbalanced distribution of the two therapeutic protocols and the small number of dogs in several treatment–group combinations, resulting in sparse data and unstable estimates; consequently, the potential influence of treatment on uAm/Cr across and within groups could not be reliably evaluated and should be interpreted with caution.

5. Conclusions

The present study demonstrated that the uAm/Cr was higher in dogs with leishmaniosis compared to healthy dogs. Dogs with proteinuria and dogs with azotemia and proteinuria, all with mixed proteinuria, had higher uAm/Cr compared to dogs with no azotemia and no proteinuria. The uAm/Cr can be a useful marker to identify early renal damage at diagnosis and to monitor renal recovery after anti-*Leishmania* treatment, especially in dogs with only proteinuria.

CRediT authorship contribution statement

Valeria Pantaleo: Writing – original draft, Validation, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Tommaso Furlanello:** Writing – review & editing, Investigation. **Laura Ventura:** Writing – review & editing, Software, Formal analysis. **Laia Solano-Gallego:** Writing – review & editing, Validation, Supervision, Conceptualization.

Institutional review board statement

All collection procedures were performed solely for the dogs' benefit and for standard diagnostic purposes. Written informed consent was obtained from all owners of the dogs. No anesthesia or euthanasia was required in any part of the study. All procedures complied with the European legislation on the protection of animals used for scientific purposes (Directive 2010/63/EU) and with the ethical requirement of the Italian law (*Decreto legislativo* 04/03/2014, n. 26). Accordingly, the study did not require ethical approval.

Funding

The research receives no external funding.

Declaration of competing interest

The authors declare that they have not known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2026.106160.

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