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Original article**Comparison of clinicopathological patterns of renal tubular damage in dogs with acute kidney injury caused by leptospirosis and other aetiologies**

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Highlights

- Increased magnitude and frequency of glucosuria characterise leptospirosis in dogs.
- Renal wasting of potassium frequently occurs in canine leptospirosis.
- Urinary neutrophil gelatinase-associated lipocalin is similar in leptospirosis and other causes of acute kidney injury.

Abstract

In humans, leptospiral acute kidney injury (AKI) is characterised by tubulointerstitial involvement and renal electrolyte losses, impacting clinical presentation and case management. The aim of this study was to evaluate urine chemistry findings in dogs with leptospirosis in order to identify characteristic patterns of tubular damage associated with this disease. Dogs with intrinsic AKI caused by leptospirosis and by other aetiologies were prospectively enrolled. Clinical and clinicopathological variables, including serum and urine chemistry, fractional excretion (FE%) of electrolytes, and urinary neutrophil gelatinase-associated lipocalin (NGAL), were evaluated in both groups and compared statistically.

Dogs with leptospirosis ($n = 38$) had significantly higher serum creatinine concentration than dogs with AKI caused by other aetiologies ($n = 37$). Serum potassium and

glucose concentrations were comparable between groups. Dogs with leptospiral AKI had significantly higher FE of potassium (median 100%, range 20-480 vs. median 68%, range 5-300; $P = 0.048$), as well as higher magnitude of glucosuria (urine glucose to creatinine ratio, median 0.64, range 0-26 vs. median 0.22, range 0-13; $P = 0.023$) and frequency of positive glucose dipstick reaction (59% vs. 18%; $P = 0.002$), than dogs with AKI of other aetiologies. Additional markers of tubular damage considered in this study, including FE of other electrolytes and urinary NGAL, did not differ between groups. In conclusion, when compared to other aetiologies of intrinsic AKI, canine leptospirosis was characterised by increased glucosuria and kaliuresis.

Keywords: Electrolyte fractional excretion; Kaliuresis; NGAL; Urine chemistry; Urine glucose

Introduction

Leptospirosis is a multiorgan disease and a common cause of acute kidney injury (AKI) in humans and dogs (Brown et al., 2015; Schuller et al., 2015; Troia et al., 2018a). Leptospiral AKI typically causes structural parenchymal damage, leading to intrinsic AKI (iAKI), associated with acute interstitial nephritis with proximal tubular involvement (Nally et al., 2004; De Brito et al., 2006; Schuller et al., 2015). Glucosuria, proximal renal tubular acidosis and impaired sodium reabsorption have been described in human and experimental animal infections (Seguro et al., 1990; Magaldi et al., 1992; Wu et al., 2004). Hypokalaemia due to increased kaliuresis, and less commonly, dysfunction of the thick ascending limb of the Loop of Henle, causing renal magnesium and phosphate wasting, have also been documented (Seguro et al., 1990; Abdulkader et al., 1996; Daher Ede et al., 2004; Khositseth et al., 2008).

Various electrolyte disturbances have been described in spontaneous canine leptospirosis, including hyponatraemia, hypochloraemia and hypokalaemia (Navarro et al., 1981; Mastroianni et al., 2007; Knöpfler et al., 2017) and a case report describing severe refractory hypokalaemia associated with inappropriate kaliuresis has been published (Allen et al., 2016). Despite the clinical utility of urine chemistry and fractional excretion (FE) of electrolytes in the characterisation of AKI and the early differentiation of iAKI from transient volume-responsive AKI (vrAKI; Brown et al., 2015; Segev et al., 2015; Troia et al., 2018a; Monari et al., 2020), comparisons of these laboratory parameters in AKI of various aetiologies have not previously been investigated. Additionally, published reports of complete urine chemistry and urinary electrolyte analysis in canine leptospirosis are currently lacking.

The aim of this study was to compare urine chemistry findings in dogs with leptospiral iAKI and those with iAKI caused by other aetiologies, focusing on urine glucose and renal electrolyte excretion. We hypothesised that, as for published human studies, leptospirosis would lead to the development of characteristic patterns of tubular damage and urine electrolyte wasting in dogs.

Materials and methods

Study design, inclusion criteria and grouping

This was a prospective study conducted at the veterinary university hospital of the University of Bologna (January 2015–January 2017) and approved by the local Scientific and Ethical Committee (Approval number, ID 751; Approval date, 0 November 2014). Dogs with spontaneously occurring iAKI were enrolled; iAKI was defined as acute (<7 days) onset of clinical signs, persistent azotaemia (serum creatinine [sCr] >1.6 mg/dL) >48 h and/or

persistent oliguria/anuria over 6 h despite appropriate fluid therapy, once euvolaemia was achieved, as previously reported (¹; Troia et al., 2018a). Signalment, history, clinical, clinicopathological and imaging findings at the time of admission were recorded for each enrolled dog. Exclusion criteria included historical, clinical, laboratory and imaging findings suggestive of chronic kidney disease (CKD; e.g. reported signs of polyuria/polydipsia or weight loss; palpable kidney abnormalities; previously reported azotaemia or persistent proteinuria; ultrasound evidence of kidney abnormalities of chronic duration), AKI on CKD, vrAKI (evidence of increase in urine production >1 mL/kg/h over 6 h of adequate fluid therapy, or decrease in serum creatinine concentrations to baseline over 48 h; Troia et al., 2018a), postrenal AKI (e.g. uroabdomen, obstruction), endocrinopathies associated with the potential to promote hyperglycaemic glucosuria or inappropriate electrolyte wasting (e.g. diabetes mellitus, Addison disease, Cushing disease), and finally, administration of drugs known to increase urinary electrolyte excretion (e.g. diuretics, hypertonic saline, glucose) or interfere with tests used to measure urine glucose (e.g. ascorbic acid, beta-lactam antimicrobials).

Dogs were tested to confirm or exclude leptospiral infection as the underlying cause of iAKI with a combination of specific tests. Leptospirosis was diagnosed by: (1) a positive microagglutination test (MAT) at the time of hospital admission (titre $\geq 1:800$); (2) a four-fold increase in the convalescent MAT titre performed on serum sample collected 7–14 days after admission; (3) a positive real-time quantitative PCR (qPCR) on blood, urine, or both; (4) a positive in-clinic rapid test detecting circulating IgM (WITNESS Lepto, Zoetis). Dogs vaccinated for leptospirosis within 15 weeks of admission were excluded (Troia et al.,

¹ See: International Renal Interest Society (IRIS), 2016. Guidelines: IRIS grading of acute kidney injury. <http://www.iris-kidney.com/guidelines/grading.html> (Accessed 30 October 2020).

2018b). Positive results on rapid testing were further confirmed by qPCR, MAT, or both. Dogs with at least one positive test result were diagnosed with leptospirosis and assigned to the iAKI-Lepto group. Dogs with negative leptospiral infection results and an alternative recognised cause of iAKI were assigned to the iAKI-Other group. Dogs that recovered and were discharged alive from the hospital were classified as survivors, while dogs that died despite medical treatment or were euthanised were classified as non-survivors.

Clinical and clinicopathological data

Blood sampling and complete clinical and laboratory data were collected and analysed at the time of hospital admission (simultaneously with AKI diagnosis); clinical monitoring was performed and recorded during hospitalisation. Clinical data included bodyweight, body temperature, heart rate, respiratory rate, non-invasive systolic blood pressure measurement, hydration and volume status, and urinary output (UO). For the purposes of the study, UO was measured to make the diagnosis of AKI commencing at the time of hospital admission. Once fluid resuscitation had been performed and fluid therapy instituted for at least 6 h, UO was used to classify AKI type (vrAKI vs. iAKI), as previously reported (Troia et al., 2018a), and was then monitored during hospitalisation. Dogs were graded according to the IRIS AKI grading system¹ at the time of enrolment, and were treated based on the assessment by attending clinicians and nephrologists. No renal replacement therapies were performed. Blood samples were collected by standard venepuncture using a blood vacuum collection system; concurrent spot urine samples were obtained by cystocentesis, catheterisation or spontaneous voiding prior to the administration of fluid therapy. All specimens were processed and analysed within 1 h after sampling.

At the time of admission in the study, the following analyses were performed in all dogs: venous or arterial blood gas analysis; complete blood count (CBC) and blood smear microscopic evaluation; chemistry profile; urinalysis, including urine specific gravity (USG), dipstick examination, microscopic sediment evaluation, and urine chemistry including the measurement of urinary creatinine (uCr), proteins, electrolytes, urea, glucose (uGlu), and urinary neutrophil gelatinase-associated lipocalin (NGAL). Pigmented urine specimens were excluded from analyses. If there was pyuria (>5 white blood cells/high-power field) on fresh urine sediment evaluation, specimens were excluded from urinary NGAL analysis, as previously reported (Monari et al., 2020).

Laboratory methods

Diagnosis of leptospirosis was achieved as previously reported (Troia et al., 2018b). Blood gas analysis was determined by a blood gas analyser (ABL 800 Flex, Radiometer Medical ApS). CBC was obtained using an automated haematology system (ADVIA 2120, Siemens Healthcare Diagnostics). Serum and urine chemistry were determined using an automated chemistry analyser (AU 480, Olympus/Beckman Coulter). C-reactive protein was measured using immunoturbidimetric assays (CRP OSR6147, Olympus/Beckman Coulter), previously validated in our laboratory for dogs (Gentilini et al., 2005). Urine dipstick analysis was performed using a commercially available method (Combur-Test 10 UX, Roche); glucose was detected at a minimum concentration of 50 mg/dL. Dipstick analysis was performed using an automated reader (URISYS 1100, Roche) and results were confirmed by visual inspection. Microscopic urine sediment examination was performed at 100X (low-power field, LPF) and 400X (high-power field, HPF). Tubular granular casts were considered as present or absent and graded as follows: >2 at LPF, 1+; >6 LPF 2+; and >10 LPF, 3+. Urinary proteins (pyrogallol red), uGlu (hexokinase) and uCr (Jaffe's reaction) were

measured using commercially available colorimetric methods (Urinary/CSF Protein OSR6170, Glucose OSR6121 and Creatinine OSR6178, Olympus/Beckman Coulter). The following ratios were calculated: uCr to sCr (uCr:sCr), urine proteins to uCr (UPC), and uGlu to uCr (uGlu:uCr). FE of electrolytes including FE of sodium (FENa), potassium (FEK), chloride (FECl), calcium (FECa), phosphate (FEP), magnesium (FEMg), and urea were calculated according to a previously reported equation and expressed as % (Brown et al., 2015).

Aliquots of the urine supernatant were stored at -80°C and used for urinary NGAL evaluation. NGAL was measured by using a commercial ELISA sandwich assay according to the manufacturer's instructions (Dog NGAL ELISA Kit, BIOPORTO Diagnostics), as previously reported (Monari et al., 2020). The results were expressed as absolute urinary NGAL concentration (uNGAL; pg/mL) and uNGAL to uCr ratio (uNGAL:uCr, pg/mg).

Statistical methods

Assuming that there would be 40% dogs in the iAKI-Other group and 80% in the iAKI-Lepto group with glucosuria, respectively, and a mean increase of 25% in FEK in the iAKI-Lepto group compared to iAKI-Other group (Troia et al., 2018a), to achieve 80% statistical power based on the assumption of a 1:1 case ratio and a 5% type I error rate (α), a minimum of 24 dogs was to be enrolled in each group. After checking for normality (graphical evaluation and Shapiro–Wilk test results), data were evaluated using standard descriptive statistics and presented as mean \pm standard deviation (SD) or median and range (minimum–maximum), if normally or not normally distributed, respectively. Results were compared between iAKI-Lepto and iAKI-Other groups using Student's *t* test or Mann-Whitney *U* test, based on data distribution. Categorical data were compared using Fisher

exact or Chi-square tests (frequency of granular cast). Receiver operating characteristic (ROC) curves were used to determine optimal cut-off values (OCV) with the maximal sum of sensitivity (Se) and specificity (Sp) for variables discriminating between iAKI-Lepto and iAKI-Other groups. ROC curve analysis was used to separately evaluate the accuracy of FEK, uGlu:uCr and dipstick glucosuria (negative vs. positive dipstick reaction). Results of the area under the ROC curve (AUC) were reported with their 95% confidence intervals (CI) and accuracy was classified as weak (0.6–0.7), moderate (0.7–0.8), or strong (0.8–1.0). Variables of interest that differed in the iAKI-Lepto and iAKI-Other group were entered into a multivariate model (stepwise approach removing factors with $P \geq 0.05$) to correctly identify iAKI-Lepto cases. To avoid collinearity, in the case of collinear variables, only the most significant variable at the initial statistical comparison was entered into the model. Binary logistic regression results were presented as odds ratio (OR) and 95% CI. Results of all statistical tests were considered significant at $P < 0.05$. Statistical analysis was performed using statistical software available online (MedCalc Statistical Software version 19.0.7, MedCalc Software bvba).

Results

AKI was suspected during the study period in 181 dogs (excluding post-renal cases); 22 cases were excluded because they were diagnosed with AKI on CKD, eight cases were excluded because of underlying endocrinopathies and 18 dogs were excluded due to the administration of drugs known to interfere with the results of the analytes of interest (furosemide, hypertonic saline, mannitol and beta-lactam antimicrobials). Of the remaining 133 dogs with AKI, 45 dogs had vrAKI, five dogs did not have a clear classification of vrAKI vs. iAKI, and in eight dogs, no underlying condition that could be associated with AKI could be identified. Seventy-five dogs with iAKI were enrolled in the study: 38/75 (51%)

were diagnosed with leptospirosis and assigned to the iAKI-Lepto group, while 37/75 (49%) were assigned to the iAKI-Other group. Summary results for the tests used in the diagnosis of leptospirosis in dogs in the iAKI-Lepto group are reported in Table 1. Underlying aetiologies and comorbidities diagnosed in the iAKI-Other group were as follows: non-infectious inflammatory disease ($n=14/37$); toxic ingestion ($n=8/37$); sepsis ($n=8/37$); trauma ($n=4/37$); and neoplasia ($n=3/37$). None of these dogs tested positive for leptospiral infection using the criteria reported above.

In the iAKI-Lepto group, median age was 6 years (3 months–12 years), median bodyweight was 24 kg (range, 4.1–49.9), and there were 25 (65.8%) males (one castrated) and 13 (34.2%) females (seven spayed). In this group, there were 12 mixed breed, five Dachshunds, four Labrador retrievers, three German shepherds, two Jack Russell terriers, and 12 other breeds. Dogs in the iAKI-Other group had a median age of 7 years (range, 3 months–14 years) and a median bodyweight of 12.2 kg (range, 1.3–42.6); 15 (40.5%) were males (three castrated) and 22 (59.5%) were females (eight spayed). In the iAKI-Other group, there were 13 mixed breed, two Rottweilers, two Poodles, two Segugio Italianos, two Pinschers, and 16 other breeds. Median length of hospital stay was 6 days (range, 1–20) for the iAKI-Lepto group and 5 days (range, 1–14) for the iAKI-Other group. Among dogs in the iAKI-Lepto group, 21 (55%) survived, and 17 (45%) did not. In the iAKI-Other group, 18 (49%) dogs survived, and 19 (51%) did not. Age was not different between groups ($P = 0.65$), but bodyweight was significantly higher in the iAKI-Lepto group than the iAKI-Other group ($P = 0.034$); there were more males (including castrated) in the iAKI-Lepto group than in the iAKI-Other group ($P = 0.038$). Duration of hospital stay and survival frequency were not different between groups ($P = 0.79$ and $P = 0.07$, respectively).

Major clinical and clinicopathological findings detected in the study populations are reported in Table 2. Dogs in the iAKI-Lepto group had significantly higher sCr, IRIS AKI grade, serum total bilirubin and alanine transaminase concentrations than those in the iAKI-Other group. Dogs in the iAKI-Lepto group had significantly increased FEK than those in the iAKI-Other group ($P = 0.048$; Table 2; Fig. 1). In addition, both the magnitude (uGlu:uCr) and the frequency of dipstick glucosuria (positive reaction) were significantly higher in the iAKI-Lepto group than the iAKI-Other group (0.64, 0-26 vs. 0.22, 0-13, $P = 0.023$ and 59% vs. 18%, $P = 0.002$, respectively; Table 2; Figs. 2 and 3). Frequency and severity of granular cast detection were not statistically different between the iAKI-Lepto group and the iAKI-Other group ($P = 0.2$ and $P = 0.4$, respectively; Table 2; Fig. 4). Urinary NGAL did not differ between groups when evaluated both as uNGAL or uNGAL:uCr ($P = 0.18$ and $P = 0.86$, respectively; Table 2). According to the ROC curve analysis, FEK had 71.9% Se and 56.7% Sp (OCV >77%; AUC = 0.646, 95% CI = 0.514–0.763; $P = 0.04$); uGlu:uCr had 64% Se and 80.8% Sp (OCV >0.34; AUC = 0.685, 95% CI = 0.540–0.808; $P = 0.02$); and dipstick glucosuria had 59.4% Se and 82.1% Sp (OCV = positive glucose reaction; AUC = 0.708, 95% CI = 0.595–0.820; $P = 0.0003$) to correctly differentiate dogs in the iAKI-Lepto group from those in the iAKI-Other group.

Multivariate binary logistic regression analysis was performed with sCr, FEK and dipstick glucosuria as independent variables to correctly identify dogs to be assigned to the iAKI-Lepto group. Positive dipstick glucosuria was the only variable retained by the model that correctly predicted occurrence of leptospirosis ($P = 0.003$), with an OR of 6.2 (95% CI, 1.8-21.1).

Discussion

Proximal tubular damage is the most prevalent form of renal injury in human leptospirosis, as demonstrated by normoglycaemic glucosuria, hyperphosphaturia and bicarbonate loss (Seguro et al., 1990; Magaldi et al., 1992; Liamis et al., 2000; Wu et al., 2004; Khositseth et al., 2008). Inappropriate kaliuresis leading to hypokalaemia occurs in 20–45% of people with leptospirosis (Abdulkader et al., 1996; Liamis et al., 2000; Khositseth et al., 2008), while hyperuricosuria and excessive urinary magnesium wasting causing hypomagnesaemia have been reported less frequently (Visith and Kearkiat 2005; Khositseth et al., 2008). In veterinary medicine, leptospirosis has been inconsistently associated with serum electrolyte depletion (Navarro et al., 1981; Knöpfler et al., 2017). However, studies evaluating the pattern of urinary electrolytes during the disease are lacking. A case report documenting severe symptomatic hypokalaemia requiring mechanical ventilation attributed to severe kaliuresis (FEK, 31%) in a dog with leptospirosis was recently published (Allen et al., 2016); this was, however, an unusual presentation for canine leptospirosis (Allen et al., 2016).

Increased urinary potassium excretion in the iAKI-Lepto group might reflect similar molecular mechanisms investigated in human and animal models of the disease, such as Na-K ATPase inhibition, increased plasma aldosterone and cortisol concentration, and impairment of Na-K-Cl co-transport in the thick ascending limb of the Loop of Henle (Abdulkader et al., 1996; Visith and Kearkiat 2005; Khositseth et al., 2008). Of note, dogs in the iAKI-Lepto group in our study also had higher sCr and IRIS grade than dogs in the iAKI-Other group. Hence, the higher kaliuresis documented here might reflect the presence of a more severe tubular impairment in dogs with leptospirosis. However, there were no differences in additional urinary markers of tubular damage, including urinary NGAL and

granular casts in the urine sediment, suggesting that AKI severity is not the only reason for elevated kaliuresis in the iAKI-Lepto group.

Another unexpected finding in iAKI-Lepto group compared to the iAKI-Other group was the significantly greater magnitude and frequency of glucosuria. Positive dipstick glucosuria correctly predicted the occurrence of leptospirosis in our population in multivariate binary logistic regression analysis. Comparable serum glucose concentrations between groups further corroborates the possibility of inappropriate renal glucose wasting during canine leptospiral infection. Mild to moderate normoglycaemic glucosuria is a well-known feature of acute tubular damage and has been reported in dogs with AKI of various aetiologies including leptospirosis (Ross, 2011; Schuller et al., 2015). Comparisons of the occurrence and severity of glucosuria have not been previously addressed in canine leptospirosis and should be confirmed in larger studies.

It is possible that there is a link between the pathophysiology of renal potassium and glucose wasting in dogs with leptospirosis. Most of the filtered potassium is reabsorbed in the proximal convoluted tubule and in the thick ascending limb of the Loop of Henle; thereafter, approximately 10% of the filtered load reaches the distal tubule, where it is secreted into the lumen for final excretion (DiBartola, 2012; Bailey et al., 2015). Similarly, almost all filtered glucose is reabsorbed in the proximal tubule (65–70%) and in the ascending limb of Henle (10–20%), but the role of the distal nephron in renal glucose handling remains unclear (Mather and Pollok 2011). This suggests selective damage to the proximal tubular segments involved in potassium and glucose reabsorption in canine leptospirosis. Glomerular hyperfiltration, increasing distal tubular flow and enhancing potassium and glucose concentration at the distal tubule level, as demonstrated in other conditions (e.g., human

pregnancy; Mather and Pollok 2011; DiBartola, 2012), is less likely, considering the pathogenesis of leptospiral AKI, but cannot completely be ruled out. However, due to the lack of additional markers to localise tubular damage in our study population, no further conclusions regarding the pathogenesis of increased kaliuresis and glucosuria during canine leptospirosis can be drawn. According to the ROC curve analysis, the accuracy of both kaliuresis and glucosuria (uGlu:uCr) to discriminate between the iAKI-Lepto and iAKI-Other groups was weak; hence, clinicians in practice cannot rely on these findings when estimating the likelihood of leptospirosis in an individual dog. Nevertheless, based on our results, renal glucosuria in a dog with AKI could increase the suspicion of leptospirosis.

Despite the growing interest in the clinical utility of NGAL in dogs with AKI, to the best of the author's knowledge, data regarding NGAL concentrations during canine leptospirosis are not available. Both circulating and urinary NGAL values can increase during inflammatory and septic states in dogs, challenging its specificity to diagnose and prognosticate AKI in such settings (Cortellini et al., 2015; Monari et al., 2020). In the present study, white blood cell count was significantly higher in the iAKI-Lepto group than the iAKI-Other group, but serum CRP concentrations were similar in both groups. Indeed, multiple inflammatory or septic conditions were diagnosed in the iAKI-Other group. Urinary NGAL was significantly increased in all dogs with AKI in our study, but did not differ between the iAKI-Lepto and iAKI-Other groups. Hence, urinary NGAL was not a predictor of leptospirosis over other possible AKI aetiologies.

Some limitations should be acknowledged when interpreting our results. Because of the intrinsic limitations in the diagnosis of canine leptospirosis (Schuller et al., 2015), dogs with false-negative leptospirosis results might have been assigned to the iAKI-Other group.

However, all dogs in this group were diagnosed with a non-leptospirosis disease that could plausibly have caused iAKI, reducing the potential for a missed diagnosis of leptospirosis. Our decision to exclude dogs with iAKI of unknown aetiology (as they did not fit in the iAKI-Lepto or in iAKI-Other groups) aimed to increase the accuracy of our inclusion criteria and the value of the comparisons performed. This approach, however, could make the study less generalisable to clinical settings where iAKI without an identified underlying disease are sometimes managed. Furthermore, urine chemistry was only assessed at the time of presentation; the development of specific features of tubular damage or unexpected urinary electrolyte wasting might have occurred later during the course of disease, and might not have been recognised. Finally, no histopathological data were available to assess the severity of AKI and the degree of tubular damage.

Conclusions

Dogs with intrinsic AKI due to leptospirosis had increased glucosuria and kaliuresis compared to other causes of iAKI, probably due to the type of renal tubular damage. The diagnostic potential of these abnormalities, however, is only fair, and specific serologic and molecular diagnostic tests are required to confirm the infection.

Conflicts of interest

None of the authors has financial or personal relationships that could inappropriately influence or bias the content of the paper.

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References

- Abdulkader, R.C., Seguro, A.C., Malheiro, P.S., Burdmann, E.A., Marcondes, M., 1996. Peculiar electrolytic and hormonal abnormalities in acute renal failure due to acute leptospirosis. *The American Journal of Tropical Medicine and Hygiene* 54, 1-6.
- Allen, A.E., Buckley, G.J., Schaer, M., 2016. Successful treatment of severe hypokalemia in a dog with acute kidney injury caused by leptospirosis. *Journal of Veterinary Emergency and Critical Care* 26, 837-843.
- Bailey, M.A., Shirley, D.G., Unwin, R.J., 2015. Renal Physiology. In: *Comprehensive Clinical Nephrology*. 4th Edn. Elsevier Saunders, Philadelphia, PA, USA, pp 14-29.
- Brown, N., Segev, G., Francey, T., Kass, P., Cowgill, L.D., 2015. Glomerular filtration rate, urine production and fractional clearance of electrolytes in acute kidney injury in dogs and their association with survival. *Journal of Veterinary Internal Medicine* 29, 28–34.
- Cortellini, S., Pelligand, L., Syme, H., Chang, Y.M., Adamantos, S., 2015. Neutrophil gelatinase-associated lipocalin in dogs with sepsis undergoing emergency laparotomy: A prospective case-control study. *Journal of Veterinary Internal Medicine* 29, 1595-1602.
- Daher Ede, F., Zanetta, D.M., Abdulkader, R.C., 2004. Pattern of renal function recovery after leptospirosis acute renal failure. *Nephron Clinical Practice* 98, 8-14.
- De Brito, T., Menezes, L.F., Lima, D.M., Lourenco, S., Silva, A.M., Alves, V.A., 2006. Immunohistochemical and in situ hybridization studies of the liver and kidney in human leptospirosis. *Virchows Archive: European Journal of Pathology* 448, 575-583.
- DiBartola, S.P., 2012. Disorders of Potassium: Hypokalemia and Hyperkalemia. In: *Fluid, Electrolyte and Acid-Base Disorders in Small Animal Practice*. 4th Edn. Elsevier Saunders, St. Louis, MO, USA, pp. 92-119.
- Gentilini, F., Mancini, D., Dondi, F., Ingrà, L., Turba, M.E., Forni, M., Famigli Bergamini, P., 2005. Validation of a human immunoturbidimetric assay for measuring canine C-reactive protein. *Veterinary Clinical Pathology* 34, Supplement, 318.
- Khositseth, S., Sudjaritjan, N., Tananchai, P., Ong-ajyuth, S., Sitprija, V., Thongboonkerd, V., 2008. Renal magnesium wasting and tubular dysfunction in leptospirosis. *Nephrology Dialysis Transplantation* 23, 952-958.
- Knöpfler, S., Mayer-Scholl, A., Luge, E., Klopfleisch, R., Gruber, A.D., Nöckler, K., Kohn B., 2017. Evaluation of clinical, laboratory, imaging findings and outcome in 99 dogs with leptospirosis. *Journal of Small Animal Practice* 58, 582-588.
- Liamis, G., Rizos, E., Elisaf, M.S., 2000. Reversible proximal tubular dysfunction in a patient with acute febrile illness and normal renal function: An evidence towards leptospirosis. *Clinical Nephrology* 53, 316.

- Magaldi, A.J., Yasuda, P.N., Kudo, L.H., Seguro, A.C., Rocha, A.S., 1992. Renal involvement in leptospirosis: A pathophysiologic study. *Nephron* 62, 332-339.
- Makris, K., Spanou, L., 2016. Acute kidney injury: Diagnostic approaches and controversies. *The Clinical Biochemistry Reviews* 37, 153-175.
- Mastrorilli, C., Dondi, F., Agnoli, C., Turba, M.E., Vezzali, E., Gentilini F., 2007. Clinicopathologic features and outcome predictors of *Leptospira interrogans* Australis serogroup infection in dogs: A retrospective study of 20 cases (2001-2004). *Journal of Veterinary Internal Medicine* 21, 3-10.
- Mather, A., Pollock, C., 2011. Glucose handling by the kidney. *Kidney International* 79, Supplement 120, S1-S6.
- Monari, E., Troia, R., Magna, L., Gruarin, M., Grisetti, C., Fernandez, M., Balboni, A., Giunti, M., Dondi, F., 2020. Urine neutrophil gelatinase-associated lipocalin to diagnose and characterize Acute Kidney Injury in dogs. *Journal of Veterinary Internal Medicine* 34, 176-185.
- Nally, J.E., Chantranuwat, C., Wu, X.Y., Fishbein, M.C., Pereira, M.M., Da Silva, J.J., Bianco, D.R., Lovett, M.A., 2004. Alveolar septal deposition of immunoglobulin and complement parallels pulmonary haemorrhage in a guinea pig model of severe pulmonary leptospirosis. *The American Journal of Pathology* 164, 1115-1127.
- Navarro, C.E., Kociba, G.J., Kowalski, J.J., 1981. Serum biochemical changes in dogs with experimental *Leptospira interrogans* serovar icterohaemorrhagiae infection. *American Journal of Veterinary Research* 42, 1125-1129.
- Ross, L., 2011. Acute Kidney Injury in dogs and cats. *Veterinary Clinics of North America Small Animal practice* 41, 1-14.
- Schuller, S., Francey, T., Hartmann, K., Hugonnard, M., Kohn, B., Nally, J.E., Sykes, J., 2015. European consensus statement on leptospirosis in dogs and cats. *Journal of Small Animal Practice* 56, 159-179.
- Segev, G., Daminet, S., Meyer, E., De Loor, J., Cohen, A., Aroch, I., Bruchim, J., 2015. Characterization of kidney damage using several renal biomarkers in dogs with naturally occurring heatstroke. *Veterinary Journal* 206, 231-235.
- Seguro, A. C., Lomar, A.V., Rocha, A.S., 1990. Acute renal failure of leptospirosis: non-oliguric and hypokalemic forms. *Nephron* 55, 146-151.
- Troia, R., Gruarin, M., Grisetti, C., Serafini, F., Magna, L., Monari E., Giunti, M., Dondi, F., 2018a. Fractional excretion of electrolytes in volume-responsive and intrinsic acute kidney injury in dogs: Diagnostic and prognostic implications. *Journal of Veterinary Internal Medicine* 32, 1372-1382.

- Troia, R., Balboni, A., Zamagni, S., Frigo, S., Magna, L., Perissinotto, L., Battilani, M., Dondi, F., 2018b. Prospective evaluation of rapid point-of-care tests for the diagnosis of acute leptospirosis in dogs. *Veterinary Journal* 237, 37-42.
- Visith, S., Kearkiat, P., 2005. Nephropathy in leptospirosis. *Journal of Postgraduate Medicine* 51, 184-188.
- Wu, M.S., Yang, C.W., Pan, M.J., Chang, C.T., Chen, Y.C., 2004. Reduced renal Na-K-Cl cotransporter activity and inhibited NKCC2 mRNA expression by *Leptospira shermani*: From bed-side to bench. *Nephrology Dialysis Transplantation* 19, 2472-2479.

Table 1

Diagnostic test results for the diagnosis of leptospirosis in dogs with intrinsic acute kidney injury and leptospirosis (iAKI-Lepto).

Diagnostic test	Sample	<i>n</i> (%)	Serogroup	<i>n</i> (%)	Antibody titre			
					1:6400	1:3200	1:1600	1:800
MAT (admission)	Serum	24/38 (63)	Australis	23/24 (96)	7/23	7/23	5/23	4/23
			Icterohaemorrhagiae	5/24 (21)			3/5	2/5
			Gryppotypophosa	5/24 (21)		1/5	3/5	1/5
			Pomona	1/24 (4)				1/1
MAT (convalescent)	Serum	15/38 (39)	Australis	15/15 (100)	7/15	4/15	3/15	1/15
			Gryppotypophosa	9/15 (60)	1/9	2/9	5/9	1/9
			Pomona	2/15 (13)				2/2
qPCR	Blood/urine	9/38 (24)						
Rapid ICT	Serum	30/38 (79)						

MAT, microagglutination test; qPCR, real-time PCR; *n*, number of positive dogs to the test; ICT, immunochromatographic test.

Table 2

Results of clinical and clinicopathological evaluation and their comparison between dogs with intrinsic acute kidney injury and leptospirosis (iAKI-Lepto) and dogs with intrinsic acute kidney injury and other diseases (iAKI-Other). Numerical data are reported as mean \pm SD or median and range (minimum–maximum value) based on their distribution.

Variable	iAKI-Lepto (<i>n</i> = 38)	iAKI-Other (<i>n</i> = 37)	Reference Interval	<i>P</i>
Clinical variables				
AKI IRIS grading	4 (2–5)	3 (1–5)	-	0.005
UO ^a (mL/kg/h)	0.99 (0–3.50)	1 (0–13)	-	0.2
Haematology				
HCT (%)	40 \pm 10	43 \pm 12	37–55	0.24
WBC (/mm ³)	21080 (8830–76410)	13835 (3140–79730)	6000–17000	0.003
Neutrophils	16380 (7280–68740)	10460 (2330–69390)	3000–12000	0.005
Monocytes	1280 (470–4910)	763 (173–3986)	100–1400	0.003
Lymphocytes	1830 (570–4298)	1029 (346–9060)	1000–4800	0.009
Platelets (/mm ³)	169280 \pm 104152	305382 \pm 194182	160000–500000	0.002

Serum chemistry

CRP (mg/dL)	7.86 (1.34-40.38)	4.86 (0.44-45.48)	0-0.85	0.09
Glucose (mg/dL)	107 \pm 25	117 \pm 34	65-115	0.59
Potassium (mEq/L)	3.9 (2.5-7.6)	4.5 (2.8-7.8)	3.8-5	0.11
Sodium (mEq/L)	141 \pm 6	142 (114-158)	143-151	0.57
Chloride (mEq/L)	107 (72-119)	99 \pm 15	108-118	0.19
Total calcium (mg/dL)	9.6 (5.5-12.6)	9.5 \pm 1.3	9.0-11.8	0.71
Ionised calcium (mmol/L)	1.10 (0.50-1.30)	1.16 (0.60-1.33)	1.22-1.35	0.46
Phosphorus (mg/dL)	15.4 \pm 8.1	11.1 (3.1-36.4)	2.6-4.9	0.33
Magnesium (mg/dL)	3.3 \pm 0.9	3.1 \pm 1.0	1.6-3.2	0.24
Creatinine (mg/dL)	7.17 (1.99-28.70)	4.19 (1.41-21.39)	0.75-1.40	0.006
Urea (mg/dL)	320 (61-729)	211 (30-784)	17-48	0.02
Total protein (g/dL)	6.34 \pm 1.07	6.36 \pm 1.59	5.60-7.30	0.84
Albumin (g/dL)	2.64 \pm 0.48	2.77 \pm 0.76	2.75-3.85	0.19
Albumin:Globulin	0.715 (0.420-1.160)	0.855 (0.210-1.37)	0.75-1.35	0.03
ALT (U/L)	185 (4-12410)	75 (15-4120)	15-65	0.01

Total Bilirubin (mg/dL)	0.85 (0.18–36.17)	0.30 (0.01–10.17)	0.07–0.34	0.0001
Urinalysis and urine chemistry				
USG	1014 (1008–1040)	1014 (1008–1050)	<1030	0.96
pH	5.5 (5–7)	6 (5–9)	/	0.17
Dipstick glucose (mg/dL)	50 (0–300)	0 (0–300)	absent	0.002
Granular casts (+/LPF)	2 (0–3)	2 (0–3)	absent	0.2
UPC	2.14 (0.40–184)	2.38 (0.09–72)	0–0.5	0.85
uCr (mg/dL)	76 (12–422)	67 (8–307)	44–563	0.92
uCr:sCr (mg:mg)	12 (0–87)	12 (0–218)	37–547	0.46
uGlu:uCr (mg:mg)	0.64 (0–26)	0.22 (0–13)	0–0.6	0.023
FEK (%)	100 (20–480)	68 (5–300)	2.3–23.8	0.048
FENa (%)	2.70 (0.20–18)	1.80 (0.04–69)	0–0.69	0.37
FECl (%)	3.70 (0.20–83)	1.90 (0.06–66)	0–1.09	0.14
FEca (%)	4.2 (0.6–70)	3.9 (0.1–56)	0–0.33	0.31
FEP (%)	33 (14–125)	37 ± 27)	2.2–27.2	0.21
FEMg (%)	11 (3–141)	8 (1–81)	0–4	0.29

uNGAL (pg/mL)	156038 ± 99276	35920 (637–1241800)	0–2600	0.18
uNGAL:uCr (pg:mg)	238862 ± 220938	69770 (511–3873232)	0–1200	0.86

AKI, acute kidney injury; IRIS, International renal interest society; UO, urine output; HCT, haematocrit value; WBC, white blood cell count; Albumin:Globulin, albumin to globulin ratio; ALT, alanine transaminase; USG, urine specific gravity; LPF, low power field; UPC, urine protein to creatinine ratio; uCr, urine creatinine; uCr:sCr, urine creatinine to serum creatinine ratio; uGlu, urine glucose; uGlu:uCr, urine glucose to creatinine ratio; FEK, fractional excretion of potassium; FENa, fractional excretion of sodium; FECl, fractional excretion of chloride; FECa, fractional excretion of calcium; FEP, fractional excretion of phosphate; FEMg fractional excretion of magnesium; uNGAL, urine NGAL; uNGAL:uCr, urine NGAL to creatinine ratio.

^a UO results reported in the table refer to UO measured after fluid resuscitation and after at least 6 h of fluid therapy.

Fig. 1. Dot plot showing results of fractional excretion of potassium (FEK) in dogs with intrinsic acute kidney injury and leptospirosis (iAKI-Lepto; $n = 38$) and dogs with intrinsic acute kidney injury of different aetiology (iAKI-Others; $n = 37$). Results were significantly different between groups ($P = 0.048$). Thin bars represent minimum and maximum values, while the central horizontal line indicates median value. Horizontal dotted lines represent upper and lower limits of the reference interval.

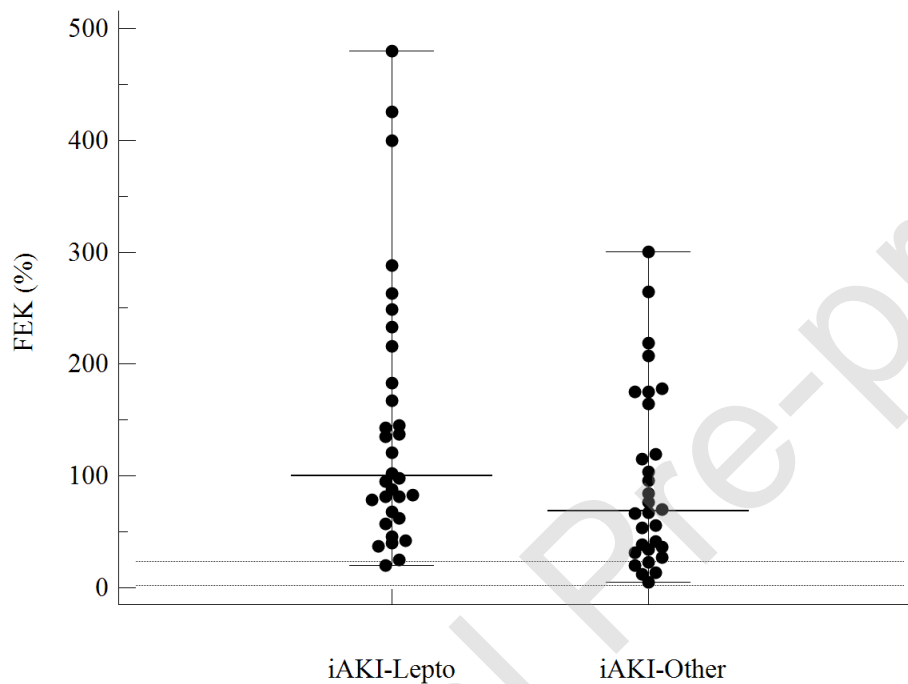


Fig. 2. Dot plot showing results of urine glucose to creatinine ratio (uGlu:uCr) in dogs with intrinsic acute kidney injury and leptospirosis (iAKI-Lepto; $n = 38$) and dogs with intrinsic acute kidney injury of different aetiology (iAKI-Others; $n = 37$). Results were significantly different between groups ($P = 0.023$). Thin bars represent minimum and maximum values, while the central horizontal line indicates median value. Horizontal dotted line represents the upper limit of the reference interval.

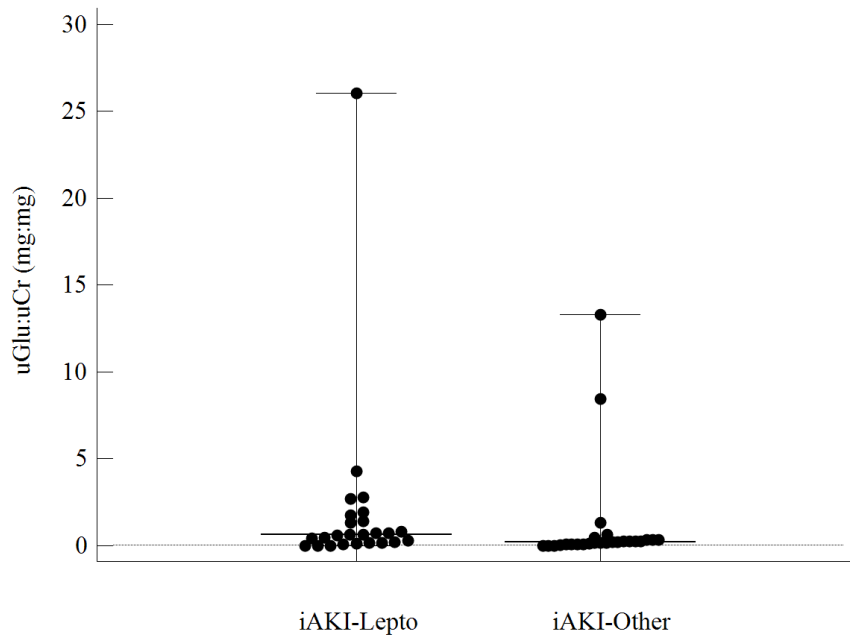


Fig. 3. Frequency of dipstick glucosuria; in dogs with intrinsic acute kidney injury and leptospirosis (iAKI-Lepto; $n = 38$) and dogs with intrinsic acute kidney injury of different aetiology (iAKI-Others; $n = 37$). Results were significantly different between groups ($P = 0.002$). Black boxes represent the percentage of positive results.

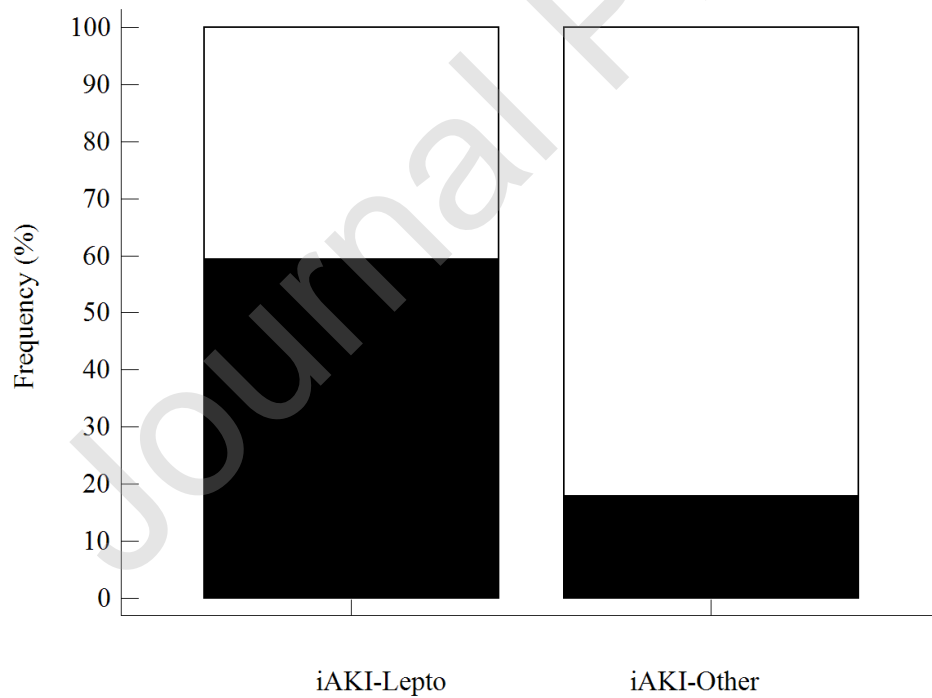


Fig. 4. Frequency and severity of cylindruria (granular casts in the urine sediment) in dogs with intrinsic acute kidney injury and leptospirosis (iAKI-Lepto; $n = 38$) and dogs with intrinsic acute kidney injury of different aetiology (iAKI-Other; $n = 37$). Results were not different between groups ($P = 0.2$).

Severity of cylindruria was graded as number of granular casts at low-power field (LPF) as follows: >2 LPF, light grey box; >6 LPF, dark grey box; >10 LPF, black box.

