RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2023.54.4.921-926

Assessment of acute kidney injury in dogs suffering from babesiosis and leptospirosis#

Asma Idrees[1], P.M. Deepa[2], K. Vinodkumar[3], R. L. Rathish[3] and M. Pradeep[4]
Department of Veterinary Epidemiology and Preventive Medicine
College of Veterinary & Animal Sciences, Pookode, Wayanad, Kerala, 673 576
Kerala Veterinary and Animal Sciences University
Kerala, India

Citation: Idrees A., Deepa P.M., Vinodkumar K., Rathish R.L. and Pradeep M. 2023. Assessment of acute kidney injury in dogs suffering from babesiosis and leptospirosis.
J. Vet. Anim. Sci. **54**(4):921-926
DOI: https://doi.org/10.51966/jvas.2023.54.4.921-926

Received: 20.12.2022 *Accepted: 25.01.2023* *Published: 31.12.2023*

Abstract

Acute kidney injury (AKI) is a serious complication of infectious diseases like babesiosis and leptospirosis in dogs. Both the diseases show similar clinical manifestations, due to which timely differential diagnosis often gets delayed. The present study focused on diagnosis of major infectious agents causing AKI and its grading in dogs. Peripheral blood smear examination and PCR were used for the diagnosis of babesiosis. Leptospirosis was screened using PCR and MAT and was later confirmed by MAT on paired sera sample. Diagnosis of AKI was made based on the increase in serum creatinine levels ≥0.3 mg/dL within 48 h of presentation. Staging of animals was carried out as per International Renal Interest Society (IRIS) guidelines. All the dogs with confirmed leptospirosis and 15 per cent of dogs with babesiosis were diagnosed with AKI. The timely diagnosis of AKI in dogs during their initial stages helps to regain normal renal function within short-term treatment itself.

Keywords: *Acute kidney injury, babesiosis, leptospirosis, MAT*

Renal dysfunction is one among the most common ailments found in dogs and is the third most leading cause of death in dogs (Komal *et al*., 2020). Over all case fatality rate due to acute kidney injury (AKI) is as high as 45 to 60 per cent in dogs managed medically or even with haemodialysis (Segev *et al.,* 2008). Acute kidney injury can be defined as the rapid loss of excretory function of kidney, leading to accumulation of nitrogen metabolism end products (azotemia), fluid, electrolyte and acid-base abnormalities (Ross, 2022). Pathophysiology of AKI in dogs is multifactorial and

 # Part of M. V. Sc. thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala, India

1. *M. V. Sc. Scholar*
2. *Associate Professor & Head*
3. *Assistant Professor*
4. *Assistant Professor, Department of Veterinary Pathology*

**Corresponding author: asmaidrees1994@gmail.com, Ph. 9895985902*

characterising them into several stages will aid in a more accurate diagnosis and better treatment (Rohini *et al*., 2022). Hemodynamic decline (hypotension and hypovolemia), infectious diseases (leptospirosis, babesiosis and pyelonephritis), exposure to nephrotoxic substances (nonsteroidal anti-inflammatory drugs) and urinary tract obstruction (urolithiasis) are the most frequently reported causes of AKI.

Acute kidney injury is one of the most serious complications of infectious diseases like leptospirosis and babesiosis in dogs. Hypoxia and inflammatory response are comparably linked to the mechanisms of renal damage in babesiosis (Kules *et al*., 2018) and leptospirosis (Dias *et al*., 2021). Acute kidney injury is categorised into five levels by the guidelines of International Renal Interest Society (IRIS) based on serum creatinine level. The dogs in the initial stages of AKI (grade I and II) would show the serum creatinine and BUN (which are the currently used functional biomarkers) within the normal range at the time of presentation of animals, making them very difficult to be relied upon. Azotemia, oliguria/anuria and creatinine progressively worsen as a result of parenchymal degeneration in Stages III to V (Mannucci *et al*., 2019).

Therefore, in veterinary practice, the diagnosis of AKI is usually checked after the onset of the disease and practically never during the first two phases, since the dogs will still be non-azotemic. Thus, renal failure in dogs is usually detected in the later stages, when irreversible renal damage has already occurred (Ross, 2011). Hence the present study aimed in the identification and grading of AKI in dogs and detection of the major causative infectious agents.

Materials and methods

A total of 221 blood and sera samples were collected from dogs presented with clinical signs such as inappetence/ anorexia, pyrexia, vomiting, dehydration, pale/icteric/ congested mucus membrane, lethargy, oliguria/ anuria/ haematuria/ haemoglobinuria and tick infestation at Teaching Veterinary Clinical Complex, Pookode from December 2021 to August 2022.

Peripheral blood smear examination for detection of Babesia spp.

Peripheral blood was taken from the ear tips of the dogs for preparing fresh thin blood smears. The blood smears were fixed using methanol for 5 min and stained using 10 per cent Giemsa to detect the intraerythrocytic piroplasm of *Babesia* species. The blood samples were examined under oil immersion objective of the microscope and the results were compared to those of the PCR assay.

Polymerase chain reaction

Blood samples were collected into EDTA-coated vacutainer tubes and genomic DNA was extracted from 200 µL of each blood sample using a commercial DNA extraction kit (Origin ™ DNA Kit) and subjected to PCR targeting *18S rRNA* gene of *B. gibsoni* and *B. vogeli* and *lipL32* gene of *Leprospira* (Table 1).

The PCR for *B. gibsoni* was performed in 25*µ*L volume in a PCR thermal cycler (Biorad, USA), with the following conditions: initial denaturation at 95ºC for 5 min, followed by 40 cycles of denaturation at 95ºC for 30 s, annealing at 56ºC for 30 s, extension at 72ºC for 1.45 min and a final extension at 72ºC for 5 min. The PCR for *B. vogeli* was performed with the following conditions: initial denaturation at 94ºC for 10 min, followed by 40 cycles of denaturation at 94ºC for 30 s, annealing at 60ºC for 30 s, extension at 72ºC for 30 s and a final extension at 72ºC for 5 min. The PCR for leptospirosis was performed with the following conditions: initial denaturation at 94ºC for 5 min, followed by 30 cycles of denaturation at 94ºC for 1 min, annealing at 55ºC for 45 s, extension at 72ºC for 30 s and a final extension at 72ºC for 6 min. The PCR products obtained were subjected to electrophoresis on a 1.5 per cent agarose gel and visualised using gel documentation system (Biorad, USA).

Microscopic Agglutination Test (MAT)

Thirteen serovars of Leprospira were used in this study as reference strains. Reference serovars maintained were *Leptospira*

Table 1. Primers used for the amplification of various genes

Organism	Target gene	Primer sequence 5'-3'	Product size	Reference
Leptospira interrogans	*lipL 32*	F-5′-GTC GAC ATG AAA AAA CTT TCG ATT TTG-3′ R-5′-CTG CAG TTA CTT AGT CGC GTC AGA AGC-3′	756 bp	Cheema *et al*. (2007)
Babesia gibsoni	*18S rRNA*	Gib599: 5′ – CTC GGC TAC TTG CCT TGT C-3′ Gib1270: 5′-CCG AAA CTG AAA TAA CGG C3′	662 bp	Inokuma *et al.* (2004)
Babesia vogeli	*18S rRNA*	PIRO-A1: 5′-AGG GAG CCT GAG AGA CGG CTA CC-3′ PIRO-B: 5′-TTA AAT ACG AAT GCC CCC AAC -3′	450 bp	Foldvari *et al*. (2005)

Table 2. IRIS AKI Grading Criteria (Cowgill *et al*., 2016)

AKI STAGE	sCr	Urine Output	Clinical description
Stage I	<1.6 mg/dL	UOP<0.5 mL/kg/h x 6 hour	Non azotemic AKI
Stage II	1.7-2.5 mg/dL	UOP<0.5 mL/kg/h x 12 hour	Mild AKI / progressive azotemia
Stage III	2.6-5.0 mg/dL	UOP<0.3 mL/kg/h x 24 hour Or Anuria x 12 hour	• Moderate to severe AKI • Increasing severities of azotemia • Functional renal failure
Stage IV	5.1-10.0 mg/dL		
Stage V	>10.0 mg/dL		

interrogans serovar Icterohaemorrhagiae, Hebdomadis, Hardjo, Djasiman, Autumnalis, Canicola, Australis, Bataviae, Grippotyphosa, Pomona, Pyrogenes, Tarassovi and Javanica. Microscopic agglutination test was carried out using live leptospira serovars as described by Faine *et al.* (1998). In the first step, 1:100 serum dilutions were prepared in PBS, 50µL of which was taken and mixed with 50µL of each of the four-to-six-day old live leptospiral serovars separately. Antigen controls were set with 50µL PBS and 50µL of different live leptospiral serovars and the plates were incubated at 37°C for two hours. After incubation, the result was read by examining a drop of serum-antigen mixture from each well under 10X objective of DFM for agglutination of leptospires. Samples positive at 1:100 dilution were again serially diluted up to 1:1200. The combination at which 50 per cent or more leptospiral organisms were seen agglutinated were taken as positive. The MAT is frequently carried out for serovar identification and diagnosis (Divya *et al*., 2021).

Grading of AKI in dogs

Serum creatinine and BUN were estimated using semi-automatic serum biochemical analyser (MISPAVIVA 2578-10/17). Commercially available biochemical kits of serum creatinine (sCr) and Blood Urea Nitrogen (BUN) were used for estimation. Estimation of sCr was done at the time of presentation and 48 h after presentation. Acute kidney injury was graded based on IRIS guidelines (Cowgill *et al*., 2016) (Table 2).

Results and discussion

Polymerase chain reaction amplification targeting the *18S rRNA* gene resulted in a product of ~662 bp for *B. gibsoni* and ~450 bp for *B. vogeli,* respectively (Fig. 1). Polymerase chain reaction amplification targeting the *lipL32* gene resulted in a product of ~756 bp from all the tested leptospira serovars (Fig. 2).

In the present study, 221 sera samples from AKI suspected dogs were tested for leptospirosis and confirmed by paired serum sample examination during acute and convalescent phase of infection. Seventeen samples (30.9 %) showed fourfold rise in antibody titre to different *Leptospira* serovars. Babesiosis was confirmed by peripheral blood smear examination and PCR.

Pathophysiology of AKI in dogs are multifactorial. All the dogs with confirmed leptospirosis were having AKI (n=17,100 per cent), whereas 19 (15 per cent) dogs among 129 confirmed cases of babesiosis turned positive for AKI. The most common infectious causes of AKI include leptospirosis and babesiosis. Renal hypoxia due to anaemia and systemic hypotension are the primary cause of renal damage and so is tissue hypoxia which has a nephrotoxic effect. According to Yang (2018) AKI due to leptospira infection, could develop into CKD if the condition is not effectively managed. All babesiosis infected dogs experienced comparable kidney damage regardless of uncomplicated or complicated babesiosis (Defauw *et al*., 2020). Leukocytosis, neutrophilia, increased sCr, increased BUN, haemolytic anaemia, thrombocytopenia and proteinuria were observed. Clinical manifestations of dogs with AKI either infected with leptospirosis or babesiosis is depicted in Table 3.

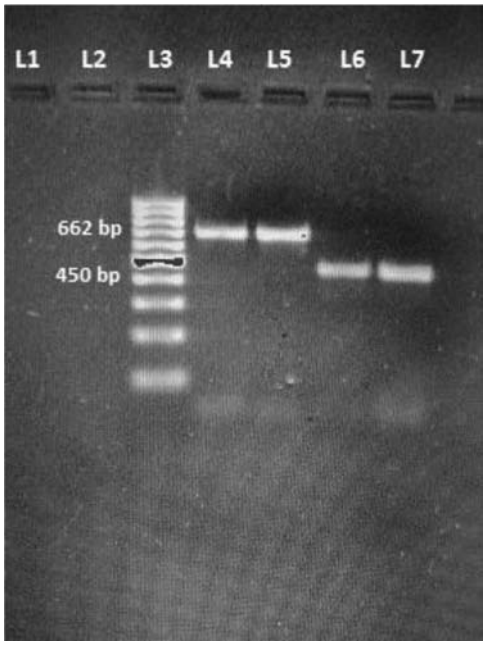
Table 3. Clinical manifestations of dogs with AKI (n=36)

Clinical signs	No. of animals	Percentage (%)
Inappetence	36	100
Oliguria	30	83
Tick infestation	25	69
Pyrexia	23	64
Dehydration	21	58
Pale mucous membrane	18	50
Lethargy	12	33
Vomiting	11	30
Icterus	10	27
Diarrhoea	9	25
Haemoglobinuria	3	8

These 36 dogs which were either infected with leptospirosis or babesiosis were identified as having AKI based on serum creatinine values. Estimation of sCr was done at the time of presentation and also 48 h after presentation. Those animals with an increase of sCr ≥0.3 mg/dL were considered as having AKI. These dogs were divided according to IRIS guidelines into five stages comprising of 18 case (50 %) in stage I, nine cases (25 %) in stage II, five in stage III and two each in stage IV and V (Table 4). It showed that 75 per cent of the cases were included in stage I and stage II of AKI even though the serum creatinine values were in normal range for these animals at the time of presentation. The different stages in the IRIS classification of AKI indicates a specific point in the disease progression and is expected to vary if the diseases gets improved, becomes worse, or progresses to chronic kidney disease. Animals diagnosed and treated with IRIS AKI Stage I and II may recover adequate renal function in a few days, preventing potentially fatal azotemia and electrolyte imbalances, and only requiring short-term assistance (Cowgill *et al*., 2016). Kidney disease most often, goes unrecognised till damage to the kidney exceeds approximately 75 per cent (Polzin, 2011). Thus diagnosing AKI in early stages have more importance than in later stages.

Table 4. Grading of AKI dogs on the basis of serum creatinine as per IRIS guidelines

STAGE	Leptospirosis	Babesiosis	Total no. of dogs (n=36)
Stage I	10	8	18
Stage II	3	6	9
Stage III	3	2	5
Stage IV	1	1	2
Stage V	0	2	2
TOTAL	17	19	36



Lane 1- negative control for *Babesia gibsoni*

Lane 2- negative control for *Babesia vogeli*.

Lane 3- 100 bp ladder marker

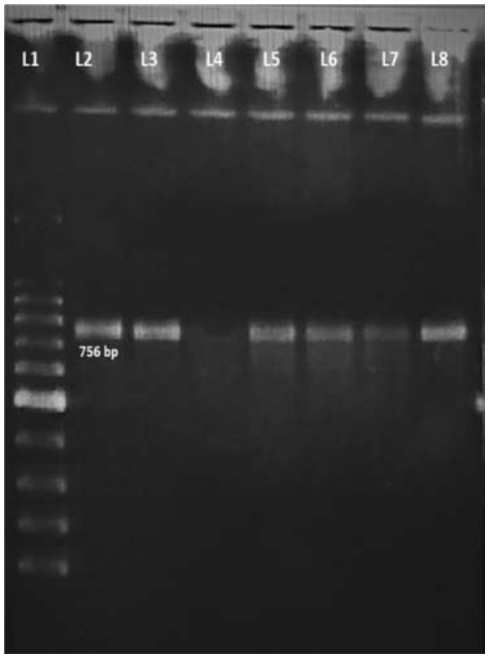
Lane 4- positive control for *Babesia gibsoni.*

Lane 5- sample 1

Lane 6- positive control for *Babesia vogeli.*

Lane 7- sample 2

Fig. 1. PCR assay based on *18srRNA* primer, showing 662 bp and 450 bp amplification products specific for *Babesia gibsoni* and *Babesia vogeli*, respectively



Lane 1- 100 bp ladder marker

Lane 2- positive control

Lane 4- negative control

Lane 3- sample 1

Lane 5- sample 2

Lane 6- sample 3

Lane 7- sample 4

Lane 8- sample 5

Fig. 2. PCR amplicons of *lipl32* gene of *Leptospira*

Conclusion

Dogs which were either infected with leptospirosis or babesiosis were identified as having AKI based on serum creatinine values. All the dogs with confirmed leptospirosis were having acute kidney injury (n=17,100 per cent), whereas 19 (15 per cent) dogs among 129 confirmed cases of babesiosis were having AKI. Diagnosis of early stages of AKI is difficult by mere evaluation of serum creatinine at the time of presentation alone. Therefore, the rise in sCr at the rate of ≥0.3 mg/dL within 48 h should be taken as a criterion for the diagnosis of AKI.

Upon AKI staging, revealed 75 per cent of the cases were included in stage I and stage II of AKI even though the serum creatinine values were in normal range for these animals at the time of presentation.

Acknowledgement

The authors are thankful to Kerala Veterinary and Animal Sciences University for providing all facilities for the completion of this work.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Cheema, P. S., Srivastava, S. K., Chaudhuri, P., Amutha, R., Singh, S., Sankar, M. and Thangapandian, E. 2007. Cloning and sequencing of a 21 kDa outer membrane protein gene of Leptospira interrogans serovar Canicola strain Hond Utrecht IV. *Vet. Res. Commun.* **31**: 521.

Cowgill, L. D., Polzin, D. J., Elliott, J., Nabity, M. B., Segev, G., Grauer, G. F., Brown, S., Langston, C. and van Dongen, A. M. 2016. Is progressive chronic kidney disease a slow acute kidney injury? *Vet. Clinic. Small Anim. Pract.* **46**: 995-1013.

Defauw, P., Schoeman, J. P., Leisewitz, A. L., Goddard, A., Duchateau, L., Aresu, L., Meyer, E. and Daminet, S. 2020. Evaluation of acute kidney injury in dogs with complicated or uncomplicated *Babesia rossi* infection. *Ticks tick-borne. Dis.* **11**: 101406.

Dias, C. S., Paz, L. N., Solcà, M. S., Portela, R.W.D., Bittencourt, M. V. and Pinna, M. H. 2021. Kidney Injury Molecule-1 in the detection of early kidney injury in dogs with leptospirosis. *Comp. Immunol. Microbiol. Infect. Dis.* **76**: 101637.

Divya, D., Siju Joseph., Mini, M., Sreeja Nair, R. and Justin Davis, K. 2021. Multi-locus sequence typing for species/ serovar identification of clinical isolates of Leptospira. *J. Vet. Anim. Sci.* **52**: 238-244.

Faine, S. 1982. Guidelines for the control of leptospirosis. **67**:171.

Foldvari, G., Hell, E. and Farkas, R., 2005. *Babesia canis canis* in dogs from Hungary: detection by PCR and sequencing. *Vet. Parasitol.***127**: 221-226.

Inokuma, H., Yoshizaki, Y., Matsumoto, K., Okuda, M., Onishi, T., Nakagome, K., Kosugi, R. and Hirakawa, M., 2004. Molecular survey of Babesia infection in dogs in Okinawa, Japan. *Vet. Parasitol.* **121**: 341-346.

Komal, S., Jain, T. and Singh, M. 2020. Chronic renal failure in dogs: A case report. *J. Entomol. Zool. Stud.* **8**: 470-471

Kules, J., Bilic, P., Ljubic, B. B., Gotic, J., Crnogaj, M., Brkljacic, M. and Mrljak, V. 2018. Glomerular and tubular kidney damage markers in canine babesiosis caused by *Babesia canis*. *Ticks tick-borne. Dis.* **9**: 1508-1517.

Mannucci, T., Lippi, I., Rota, A. and Citi, S. 2019. Contrast enhancement ultrasound of renal perfusion in dogs with acute kidney injury. *J. Small Anim. Pract.* **60**: 471-476.

Polzin, D. J. 2011. Chronic kidney disease in small animals. *Vet. Clin. Small Anim. Pract.* **41**:15-30.

Rohini, B. G., George, A., Usha, N. P.,MadhavanUnny, N., Ambily, R. and Gleeja, V.L. 2022. A retrospective study of acute kidney injury in dogs with renal resistive index as a prognostic indicator. *J. Vet. Anim. Sci.* **53**: 583-591

Ross, L. 2011. Acute kidney injury in dogs and cats. *Vet. Clin. Small Anim. Pract***. 41**: 1-14.

Ross, L. 2022. Acute kidney Injury in dogs and cats. *Vet. Clin. Small Anim. Pract.* **52**: 659-672.

Segev, G., Kass, P. H., Francey, T. and Cowgill, L. D. 2008. A novel clinical scoring system for outcome prediction in dogs with acute kidney injury managed by hemodialysis. *J. Vet. Intern. Med.* **22**: 301-308.

Yang, C. W. 2018. Leptospirosis renal disease: emerging culprit of chronic kidney disease unknown etiology. *Nephron*. **138**:129-136. ■