



## Microscopic Agglutination Test (MAT) for Leptospirosis in Association with Acute Renal Failure in Dogs

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### ABSTRACT

The present investigation was carried out in the Department of Veterinary Medicine, College of Veterinary and Animal Sciences, GBPUA&T, Pantnagar, Uttarakhand, India. Total of 100 sera samples from azotemic dogs were subjected to Microscopic Agglutination Test (MAT) using *Leptospira* culture in *Leptospira* lab, Department of Veterinary Bacteriology and Mycology, IVRI, Izatnagar, Bareilly (U.P.) All the sera samples were tested against *L. interrogans*: Serovars icterohaemorrhagiae, pomona, canicola, autumnalis, grippotyphosa and javanica. Out of 100 sera samples, 20 (20%) were found positive (+) for different serovars of *Leptospira interrogans* and 3 (3%) were doubtful (±) at an antibody titre  $\geq 1:100$ . The serovars identified were *L. autumnalis* (17/100), *L. icterohaemorrhagiae* (8/100), *L. gryppotyphosa* (4/100), *L. canicola* (2/100), *L. javanica* (2/100) and *L. Pomona* (1/100). The highest prevalence of *Leptospira* was observed for serovars *L. autumnalis* (17%), followed by *L. icterohaemorrhagiae* (8%) and least for *L. pomona* (1%).

**Keywords:** Dog, Leptospirosis, MAT, Prevalence, Renal failure.

Renal failure is a common clinical manifestation in 2-5% of dogs and third leading cause of deaths in canine (Bronson, 1982; Lund *et al.*, 1999). The mean age of diagnosis is 6.5 years with 45% of cases reported over 10 years of age (Srivastava *et al.*, 2011). About 30.77% acute kidney injuries in dogs caused by leptospirosis (Segev *et al.*, 2008). Acute renal failure (ARF) is a multi-systemic disease involving usually more than one organ and causes rapid decline in renal function, resulting in acute uraemia, fluid retention, acid-base and electrolyte imbalances (Francey and Cowgill, 2002; Patias, 2002).

Dogs become infected by exposure to contaminated urine from shedding wild animals, mainly by rodents that act as a maintenance host for several *Leptospira* serovars. The *Leptospira* organisms penetrate the mucus membranes, reached to the liver and kidneys and damage these organs (Goldstein, 2010). The most common clinical signs observed in dogs consisted of icterus and haemorrhagic diathesis, but acute renal failure is the most prevalent clinical signs attributed now a days.

Microscopic agglutination test (MAT) and polymerase chain reaction (PCR) is most commonly used diagnostic techniques for leptospirosis (Goldstein, 2010). However, detection of *Leptospira* antibodies using the MAT has been the most common (Greene *et al.*, 2006). Keeping in view the present investigation was carried out at College of Veterinary and Animal Sciences, GBPUA&T, Pantnagar, Uttarakhand, India with the objective of serological investigation of leptospiral acute renal failure in dogs.

### MATERIALS AND METHODS

In the present investigation sera samples from 100 azotemic dogs belonging to different categories like vaccinated and unvaccinated, were subjected to Microscopic Agglutination Test (MAT) using *Leptospira* culture in *Leptospira* lab, Department of Veterinary Bacteriology and Mycology,

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IVRI, Izatnagar, Bareilly (U.P.). The results obtained are expressed in Table 1 and Fig 1. All the sera samples were tested against six serovars of *Leptospira* spp., most likely to cause disease in dogs in this region which are *L. interrogans*: Serovars icterohaemorrhagiae, pomona, canicola, autumnalis, grippotyphosa and javanica. The MAT was performed according to the method of Faine (1982). A homologous, high titred antiserum was included in each testing session. Serum dilutions were prepared in 8-well “U” bottomed disposable micro-liter plates. A serial two-fold dilution of each serum was made with phosphate buffered saline (pH 7.2) starting with an initial dilution of 1:100. An equal volume (i.e. 10 µl) of culture was added to each well, mixed by gentle rocking, and incubated at 37°C for 3 hours after sealing with polyethylene sheet. The MAT titer was the reciprocal of the highest dilution of the serum in which >50% of the antigen was agglutinated. A minimum titer of 1:100 and above was taken as the positive agglutination reaction for leptospirosis (Cole, 1973; O’Keefe *et al.*, 2002).

**Table 1:** *Leptospira* serovars identified by Microscopic Agglutination Test (MAT) in dogs affected with renal failure (+ indicates positive, ± indicates doubtful)

Sl. No.	Titre ≥ 1:800	Reacting Serovars
1	+	Autumnalis, Icterohaemorrhagiae, Javanica
2	+	Autumnalis, Canicola, Icterohaemorrhagiae, Pomona
3	+	Autumnalis, Grippotyphosa
4	+	Autumnalis, Icterohaemorrhagiae
5	+	Autumnalis
6	+	Autumnalis
7	+	Autumnalis, Grippotyphosa
8	+	Autumnalis
9	+	Autumnalis
10	+	Autumnalis
11	+	Autumnalis, Grippotyphosa, Icterohaemorrhagiae
12	+	Autumnalis, Grippotyphosa
13	+	Autumnalis
14	+	Autumnalis, Icterohaemorrhagiae
15	+	Autumnalis
16	+	Autumnalis, Icterohaemorrhagiae
17	±	Canicola
18	+	Canicola

19	±	Autumnalis
20	+	Icterohaemorrhagiae, Javanica
21	±	Autumnalis
22	±	Autumnalis
23	+	Autumnalis
24	+	Icterohaemorrhagiae



**Fig. 1:** Microphotograph showing positive titre (≥1:100) of microscopic agglutination test (MAT) for *Leptospira* in a dog affected with renal failure

## RESULTS AND DISCUSSION

Out of 100 sera samples, 20 (20%) were found positive (+) for different serovars of *Leptospira interrogans* and 3 (3%) were doubtful (±) at an antibody titre ≥1:100 (Table 1). Several antigenically distinct serovars of *Leptospira interrogans* are responsible for disease in dogs, were identified in the present investigation (Table 2 and Fig 2). The serovars most commonly incriminated in canine were *L. autumnalis* (17/100), *L. icterohaemorrhagiae* (8/100), *L. gryppotyphosa* (4/100), *L. canicola* (2/100), *L. javanica* (2/100) and *L. Pomona* (1/100). In this study most of the dogs were found vaccinated against *L. canicola* and *L. icterohaemorrhagiae* and it may be responsible for positive titre for these two serovars. The highest prevalence of *Leptospira* was observed for serovars *L. autumnalis* (17%), followed by *L. icterohaemorrhagiae* (8%) and least for *L. pomona* (1%).

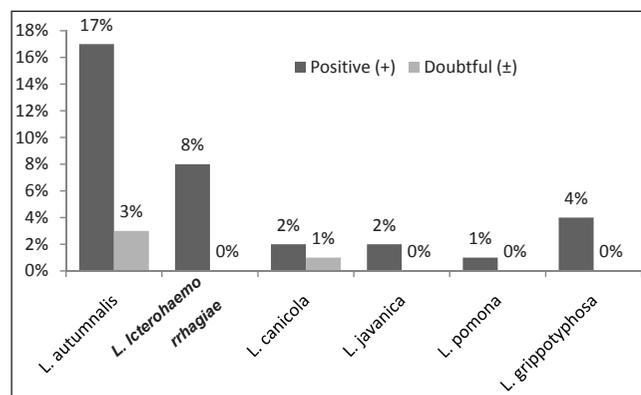
Leptospirosis is a significant cause of acute tubulointerstitial nephritis before the widespread use of antileptospiral bacterins (Robertson, 1986).

The prevalence of canine leptospirosis has increased in recent years but prevalence varies by region. Results of one study in Michigan indicated that >20% of healthy, client-owned dogs were exposed to *Leptospira* serovars (Stokes *et al.*, 2007).

**Table 2:** Seroprevalence of *Leptospira* spp. in dogs affected with renal failure.

Sl. No.	Serovars	Positive (+)	Doubtful (±)
1	<i>L. interrogans</i> serovar autumnalis	17/100 (17%)	3/100 (3%)
2	<i>L. interrogans</i> serovar icterohaemorrhagiae*	8/100 (8%)	—
3	<i>L. interrogans</i> serovar grippityphosa	4/100 (4%)	—
4	<i>L. interrogans</i> serovar canicola*	2/100 (2%)	1/100 (1%)
5	<i>L. interrogans</i> serovar javanica	2/100 (2%)	—
6	<i>L. interrogans</i> serovar Pomona	1/100 (1%)	—

\**Leptospira* vaccine in India contains *Leptospira* serovars Canicola and Icterohaemorrhagiae.



**Fig. 2:** Seroprevalence of leptospirosis in dogs affected with renal failure

In another study, 8.2% of dogs were shedding pathogenic leptospires irrespective of the health status (Harkin *et al.*, 2003). Acute kidney disease is the most commonly recognized disease in dogs, accounting for more than 90% of reported cases of leptospirosis (Levy *et al.*, 1996). The present findings are in accordance with the findings of many authors (Miller *et al.*, 2008; Senthil *et al.*, 2013).

It was concluded that leptospirosis is the most common cause of acute renal failure in canines resulting in death. MAT was highly reliable and rapid diagnostic method of leptospirosis in canine. It is therefore, suggested that regular serological testing (MAT) for leptospirosis in canine can save the life of valuable dogs by early detecting the disease and proper medical care.

## CONCLUSION

It is concluded that leptospirosis is a serious problems in dogs particularly in young animals and most commonly occurs in wet and humid climate. It causes serious acute renal failure (ARF) and resulted into multi-systemic organ failure. Microscopic agglutination test (MAT) is a reliable and highly sensitive serological test for leptospirosis in dogs. Vaccinated animals may show positive reaction against vaccinated serovars. So, regular MAT testing of dogs against this disease is must to save the life of valuable dogs even in early stage and also to prevent infection to the dog owner as the disease has zoonotic importance.

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