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Research Article

INVESTIGATION ON CARRIER STATUS OF LEPTOSPIROSIS IN SHELTERED AND CLIENT-OWNED DOGS IN HEBBAL, BENGALURU

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ABSTRACT

Leptospirosis, is now reemerging as an infectious threat to both canines and humans. Dogs can carry pathogenic *Leptospira* in their renal tubules and shed the bacteria in urine for prolonged periods, contributing to environmental transmission. This study aimed to evaluate the seroprevalence and urinary shedding of *Leptospira* in healthy sheltered and house owned dogs. A total of 92 dogs, including both sheltered and house owned, were included in the study and categorized into two groups: unvaccinated and vaccinated over 1 year ago. Sero-positivity was determined using immunochromatographic assay. Among the 64 blood samples collected from sheltered dogs, 73.43 per cent tested positive for anti-Leptospiral antibodies. Similarly, among the 28 samples from the house owned dogs presented to the Department of Veterinary Medicine at Veterinary College Hospital, Hebbal, Bengaluru, 71.42 per cent showed antibodies against *Leptospira* organisms. PCR analysis of urine samples was conducted to detect leptospiruria. Out of the 64 urine samples collected from sheltered animals, 7.81 percent were found to be shedding *Leptospira* organisms, while out of 28 urine samples from dogs that were house owned, 7.14 percent exhibited leptospiruria. No changes were noticed in the hematobiochemical parameters.The study emphasizes dogs as *Leptospira* carriers, even if not showing any symptoms. Their shedding of *Leptospira* organisms in urine poses a threat of environmental contamination, increasing the risk of transmission to both dogs and humans.

Keywords: Carrier status, *Leptospira* shedding, Seroprevalence, Sheltered dogs, Urine PCR

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Leptospirosis, an infectious disease caused by Leptospira bacteria, affects humans and animals globally. In the endemic areas, dogs are often asymptomatic carriersof virulent strains and contribute to environmental contamination, posing zoonotic risks (Sant'Anna *et al.*, 2021). Dogs and cats in shelters often act as indicators for numerous zoonotic illnesses, possibly attributable to unhygienic environments, crowded living spaces, stress, and encounters with rodents and other carriers of diseases (Smith *et al.*, 2022). Stray dogs and dogs housed in shelters are considered more prone to Leptospira infection because of their heightened exposure to the pathogen in their surroundings. (Jittapalapong *et al.*, 2009). Asymptomatically infected dogs can be unknowingly admitted and adopted in dog shelters, potentially increasing the risks of zoonotic transmission (Miotto *et al.*, 2018).

Both unvaccinated and vaccinated dogs release pathogenic strains of Leptospira in their urine, indicating their significant contribution to environmental contamination (Athapattu *et al.*, 2022). Leptospiral shedding in dogs usually commences approximately 7 to 10 days after infection and persists for a duration of 4 to 6 weeks, occasionally even lasting for multiple years (Bal *et al.*, 1994). Efficient management of chronically infected dogs is crucial to minimize environmental contamination.

Leptospirosis can be diagnosed using direct methods such as visualizing leptospires, culturing the organism, or detecting its DNA through PCR. Indirect methods include the identification of specific antibodies using serological tests (Adler, 2014). In the present study, seropositivity was assessed using a rapid test kit based on the sandwich lateral flow immunochromatographic assay and urinary shedding of Leptospira was evaluated by PCR targeting the *lipL*32 gene.

This study aimed to identify leptospiral antibodies in healthy, vaccinated and unvaccinated sheltered and house-owned dogs and to detect pathogenic leptospiral organisms in their urine. It also assessed hematobiochemical parameters in carrier dogs and examined potential risk factors for carrier status.

MATERIALS AND METHODS

Source of samples

A total of 92 animals were selected for the study, comprising those presented to the Department of Veterinary Medicine at Veterinary College Hospital, Hebbal, Bengaluru (n=28), as well as animals housed in the animal shelter (n=64). Apparently healthy animals showing no signs of illness, regardless of age, breed, or gender, were selected for the study. Dogs with a history of having received antibiotics four weeks prior to sample collection were excluded from the study.

The study population comprised two distinct groups of animals.

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Group-I: Animals which were never vaccinated for leptospirosis

Group-II: Animals that have not been inoculated with the vaccine for leptospirosis1-year preceding sampling.

Sample collection

Five ml of blood was drawn by venipuncture to the cephalic/saphenous vein. Separation of serum was done by centrifuging at 3000 rpm for 5 minutes and the serum sample was stored at -20º C for further use. Urine samples were collected in a sterile manner by catheterization.

Sample processing

The EDTA blood samples were processed for assessment of the haematological parameters viz., Total Leucocyte Count (TLC) ($x10^3$/µl), Total Erythrocyte Count (TEC) (x 10^6/µl), Haemoglobin (Hb) (g/dl), Packed Cell Volume (PCV) (%), Differential leuckocyte count (DLC) (%), Platelet Count (x 10^3/µl) using BC-2800 Vet, Auto Haematology Analyzer. The serum biochemical parameters that were estimated were ALT, ALP and creatinine.

Leptospira spp. Antibody Rapid Test (Lepto Ab) manufactured by J&G Biotech Ltd. (London, England) was used to carry out the identification of antibodies against *Leptospira* spp. in serum and the test was conducted according to manufacturer's instructions. The test follows the principle of Sandwich lateral flow immunochromatographic assay. *lipL*32 gene has been used in the test kit for recombinant protein expression. Ten µL serum sample was placed in the sample hole of the test card. Then, 3 drops of the assay buffer were added into the sample hole. Results were interpreted within 5 to 10 minutes.

Interpretation:

- **Positive:** The presence of both C line and zone T line, no matter T line is clear or vague.
- **Negative:** Presence of only C line with no T line.
- **Invalid:** Absence of coloured line in the C zone.

Urine samples were stored at 4º C for a maximum of 6 hours after collection and then transferred to 1.5 ml Eppendorf tubes and centrifuged at 14000×g for 15 minutes. The supernatant was then discarded and the sediment was stored in phosphate buffered saline (pH=7.2) for a maximum of 48 hours and extraction of DNA was done using instructions given in QIAamp DNA Micro kit.

PCR assay targeted the *lipL*32 gene (present majority in pathogenic leptospires) using the primers *lipL*32_F 5' CATATGGGTCTGCCAAGCCTAAA 3' and *lipL*32_R 5' CTCGAGTTACTTAGTCGCGTCAGAA 3' which produced an amplicon of size 756 bp. Amplification condition was performed in a 25 µl reaction volume and consisting of 6.5 µL nuclease free water, 12.5 µL PCR master mix, 2 µL (10 pmol/µl) forward primer, 2 µL (10 pmol/µl) reverse primer and 2 µL DNA template. All reactions were carried out in the thermocycler (Eppendorf, Germany) and the temperatures used are mentioned in Table 1. PCR products were examined by electrophoresis at 90 V for 45 minutes in a 2 per cent (w/v) agarose gel in 10x TBE buffer. A DNA ladder 100 bp was used as marker. After electrophoresis, the gel bands were visualized at 300 nm wave length using a UV trans-illuminator and recorded in a gel documentation unit (Geldoc unit (M/s Bio-rad, USA).

Statistical analysis: For statistical analysis, the animals were categorized into 3 groups.

- ***Seronegative healthy animals:*** Animals that tested seronegative and demonstrated the absence of *Leptospira* organisms in urine PCR. These animals were considered control animals as they were apparently healthy, seronegative and showed no presence of *Leptospira* organisms in their urine.
- ***Seropositive non-leptospiruric animals:*** Animals that tested positive for antibodies but did not exhibit organism shedding in urine samples.
- ***Seropositive leptospiruric animals:*** Animals which showed both seropositivity and the presence of *Leptospira* organisms in urine.

The data obtained were subjected to statistical analysis using Graphpad Prism Software. To compare the mean values of continuous variables, ANOVA was used. Whereas, to know the association between categorical variables, Pearson Chi square test/Fisher's exact test was used.

RESULTS AND DISCUSSION

High prevalence observed in developing countries like India could be attributed to the prevalent climatic condition. It often experiences high temperatures and a tropical climate, coupled with an extended rainy season. Such conditions create a conducive environment for leptospires to survive in the environment and facilitate the transmission of the infection (Soo *et al.*, 2020). In the present study, a seroprevalence of 73.43% was observed among shelter dogs and 71.42% among client-owned dogs, supporting the likelihood of widespread environmental exposure. The warm and humid climate of Bengaluru during the study period may have contributed to the high antibody prevalence detected by the immunochromatographic assay.

Table 1. PCR cycle conditions for amplification of *lipL*32 gene of *Leptospira* spp.

Step	Temperature	Time	No. of cycles
Initial denaturation	95° C	5 minutes	1
Denaturation	95° C	1 minute	34
Annealing	55° C	1 minute	
Extension	72° C	1 minute	
Final extension	72° C	10 minutes	1

Table 2. Effect of Vaccination status on seropositivity and leptospiruria

S. No.	Vaccination status	Total no. of samples collected	Seropositive		Leptospiruric & seropositive	
			N	(%)	N	(%)
1.	Not vaccinated	73	55	75.34	7	9.58
2.	Vaccinated more than 12 months ago	19	12	63.15	0	0

p value = 0.264

Table 3. Mean ± S.E. values of hematological parameters of seronegative, seronegative and leptospiruric animals

S.No.	Parameter	Seronegative dogs	Seropositive dogs	Dogs with leptospiruria	*p value*
1.	Haemoglobin (g/dL)	14.86 ± 0.58	15.2 ± 0.29	13.6 ± 0.87	0.262
2.	TLC (10^3/µL)	14.89 ± 0.78	14.61 ± 0.79	13.88 ± 2.41	0.916
3.	TEC (10^6/µL)	5.23 ± 0.15	5.23 ± 0.09	4.81 ± 0.29	0.350
4.	PCV (%)	45.12 ± 1.62	47.04 ± 1.02	41.64 ±2.44	0.183
5.	PLT (10^3/µL)	234.2 ±33.00	199.42 ±14.52	220.14 ±59.73	0.538
6.	MCV (fL)	64.70 ± 1.00	64.47 ± 0.64	62.84 ± 6.32	0.685
7.	MCH (pg)	21.03 ± 0.34	21.23 ± 0.30	20.54 ± 1.66	0.706
8.	MCHC (%)	32.54 ± 0.16	32.99 ± 0.39	32.82 ± 0.53	0.618
9.	Neutrophils (%)	66.96 ± 2.30	66.09 ± 1.81	65.74 ± 4.73	0.957
10.	Lymphocytes (%)	22.22 ± 2.00	21.65 ± 1.42	23.17 ± 6.02	0.932
11.	Monocytes (%)	5.45 ± 0.28	5.29 ± 0.19	5.75 ± 0.96	0.730
12.	Eosinophils (%)	6.72 ± 0.68	6.52 ± 0.58	6.12 ± 1.30	0.944
13.	Basophils (%)	0.36 ± 0.95	0.50 ± 0.06	0.62 ± 0.18	0.336

Table 4. Mean±S.E.values of serum biochemical parameters of seronegative, seropositive and leptospiruric animals

S. No.	Parameter	Seronegative dogs	Seropositive dogs	Dogs with leptospiruria	*p value*
1.	ALT (U/L)	26.56 ± 5.25	33.75 ± 2.36	26.18 ± 3.08	0.113
2.	ALP (U/L)	146.34 ±5.12	153.80 ± 3.39	152.52 ± 6.15	0.472
3.	Creatinine (mg/dL)	1.29 ± 0.54	1.35 ± 0.03	1.19 ± 0.10	0.220

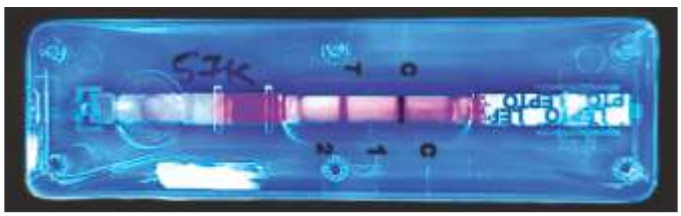

Fig. 1. The presence of both C line and zone T line in the immunochromatographic assay indicating positive results

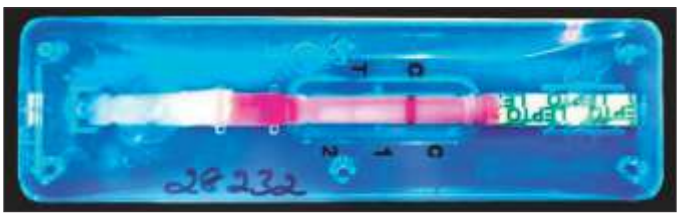

Fig. 2. Presence of only C line with no T line in the immunochromatographic assay indicating negative results

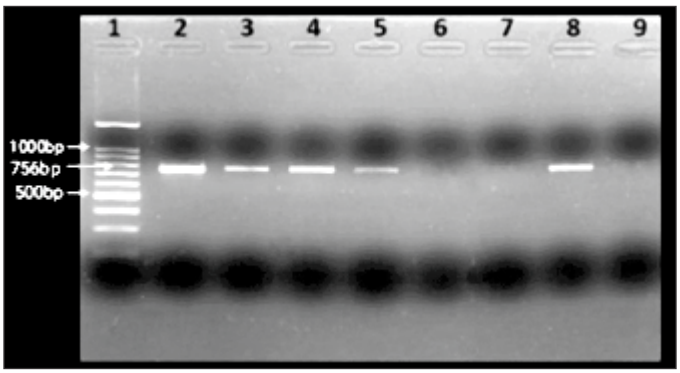

Fig. 3. Agarose gel electrophoresis of urine PCR using *lip*L32 primer
Lane 1: 100 base pair DNA ladder marker; Lanes 2-5: Urine samples positive for leptospires; Lanes 6: Urine samples negative for leptospires; Lane 7: Negative template control; Lane 8: Positive control; Lane 9: Negative control

Indeed, Gautam *et al.* (2010) stated that the prevalence of leptospirosis varies according to geographic location. This observation is also supported by the meta-analysis conducted by Ricardo *et al.* (2020), which found higher estimates of seroprevalence in the region of South Asia, represented by studies from India, Pakistan, and Nepal. In addition to these factors, variations in prevalence seen across studies worldwide can be linked to several methodological factors. These include using different diagnostic tests, surveying during different times of the year, variations in sample sizes, characteristics of the animals sampled, differences in samples collection and variations in the criteria used to identify an animal as a

positive serological reactor (Azevedo *et al.*, 2005).

Five out of 64 urine samples of sheltered animals (7.81%) tested positive for Leptospira. Among 28 dogs presented to the College Veterinary Hospital 2 urine samples (7.14%) were positive. A total shedding prevalence of 7.6 per cent in the present study appears rather low but the true shedding prevalence may be underestimated because 7 out of 73 unvaccinated dogs were shedding, while 55 of them had anti-leptospiral antibodies. Therefore, nearly three-quarters of the dogs had experienced infection at least once in their lifetime, given that they had never been vaccinated against Leptospira. Given the low occurrence of shedding alongside a high seroprevalence, this finding indicates that these animals may not have been currently infected at the time of sampling. Instead, they might have been infected in the past or were possibly in a latent phase of infection. The lack of leptospiruria in numerous dogs could be due to the higher antibody titers present in those animals, indicating that they may have successfully cleared the infection. However, the intermittent shedding of leptospires commonly seen in maintenance hosts can result in false-negative PCR results. Relying solely on a single PCR evaluation for identifying leptospiruric dogs may limit the ability to account for occasional, intermittent or persistent urinary shedding of the pathogen (Miotto *et al.*, 2018). Given that human leptospirosis is endemic in India, a higher shedding prevalence can be anticipated in the present survey, considering the country's hot and humid climate. However, several factors could explain the observed prevalence. Leptospirosis may exhibit seasonal patterns, with increased transmission during specific times of the year (Smith *et al.*, 2019). Additionally, the absence of natural disasters, such as flooding (Azocar-Aedo and Monti, 2016), which is known to facilitate leptospirosis outbreaks, particularly during the rainy season, at the time of sampling for the present study, could have influenced the prevalence rates.

Among the 73 unvaccinated dogs, 75.34 per cent (55 dogs) displayed seropositivity, while among the 19 dogs vaccinated more than 1ago, 63.15 per cent (12 dogs) were found to be seropositive (Table 2). It is noteworthy that all the dogs detected with leptospiruria had not received vaccination against leptospirosis (7 out of 73). Statistical analysis indicated a non-significant ($p > 0.05$) difference between seronegative healthy animals, seropositive non leptospiruric animals and seropositive leptospiruric animals regarding vaccination status.

Unvaccinated animals showing antibodies in their serum may suggest prior infection with *Leptospira* spp., leading to the development of antibodies as a response. This indicates a continuous exposure of the animals to the pathogens. In accordance to the results of this study, the seroprevalence of leptospirosis among vaccinated dogs in the study conducted by Pratt *et al.* (2017) was lower (56.5%) compared to non-vaccinated dogs (69.2%).

Although the difference is not statistically significant, it is interesting that all the dogs which were leptospiruric were unvaccinated. In a study carried out by Bouvet *et al.* (2016) no instances of urinary shedding of Leptospira organisms were observed among the vaccinated animals which is similar to the present study. But the findings contradict those of the study conducted by Athapattu *et al.* (2022), where it was demonstrated that both unvaccinated and vaccinated dogs excrete various pathogenic *Leptospira* spp. in their urine.

There was no significant difference observed in any of the hematobiochemical parameters among the seronegative animals, seropositive animals and leptospiruric animals (Table 3 and IV). A study by Sant' Anna *et al.* (2021) similarly reported no hematobiochemical alterations in carrier dogs during their investigation. However, the literature supporting these findings is very limited. A key limitation of this study lies in the potential for false-negative PCR results due to the intermittent shedding of Leptospira Since only a single PCR evaluation was performed per animal, occasional or transient shedding may have gone undetected. This reliance on one-time sampling may have underestimated the true prevalence of leptospiruric dogs, due to its occasional or intermittent urinary shedding (Miotto *et al.*, 2018).

Carrier animals of leptospirosis may have normal hematobiochemical parameters because leptospirosis can often present as a subclinical or asymptomatic infection in carrier animals. While these animals may harbor the Leptospira bacteria and shed it in their urine, they may not show any outward signs of illness. As a result, routine hematobiochemical parameters may remain within normal ranges.

This study reveals that both sheltered and client-owned dogs can serve as asymptomatic carriers of Leptospira, with high seroprevalence and low but notable urinary shedding. Leptospiruria was found only in unvaccinated dogs, highlighting the importance of vaccination in reducing transmission risk. Despite the absence of clinical signs or hematobiochemical changes, the detection of Leptospira DNA in urine underscores the potential zoonotic threat. These findings emphasize the need for routine screening, improved hygiene in shelters and vaccination to control leptospirosis in endemic regions.

REFERENCES

Athapattu, T., Fernando, R., Abayawansha, R., Fernando, P., Fuward, M., Samarakoon, N., Koizumi, N. and Gamage, C. (2022). Carrier status of *Leptospira* spp. in healthy companion dogs in Sri Lanka. *Vector-Borne and Zoonotic Dis.* **22(2)**: 93-100.

Azevedo, S.S.D., Batista, C.S.A., Vasconcellos, S.A., Aguiar, D.M.D., Ragozo, A.M.A., Rodrigues, A.A.R., Alves, C.J. and Gennari, S.M.(2005). Seroepidemiology of *Toxoplasma gondii* and *Neospora caninum* in dogs from the state of Paraíba, Northeast region of Brazil. *Res. Vet. Sci.* **79(1)**: 51-56.

Azócar-Aedo, L. and Monti, G.(2016). Meta-analyses of factors associated with leptospirosis in domestic dogs. *Zoonoses Public Health.* **63(4)**: 328-336.

Bal, A.E., Gravekamp, C., Hartskeerl, R.A., De Meza-Brewster, J., Korver, H. and Terpstra, W.J. (1994). Detection of leptospires in urine by PCR for early diagnosis of leptospirosis. *J. Clinical Microbial.* **32(8)**: 1894-1898.

Bouvet, J., Cariou, C., Valfort, W., Villard, S., Hilaire, F., Oberli, F., Cupillard, L. and Guigal, P.M. (2016). Efficacy of a multivalent DAPPi-L multi canine vaccine against mortality, clinical signs, infection, bacterial excretion, renal carriage and renal lesions caused by *Leptospira* experimental challenges. *Vax. Rep.* **6**: 23-28.

Gautam, R., Wu, C.C., Guptill, L.F., Potter, A. and Moore, G.E. (2010). Detection of antibodies against *Leptospira* serovars via microscopic agglutination tests in dogs in the United States, 2000–2007. *J. Am. Vet. Med. Ass.* **237(3)**: 293-298.

Jittapalapong, S., Sittisan, P., Sakpuaram, T., Kabeya, H., Maruyama, S. and Inpankaew, T. (2009). Coinfection of *Leptospira* spp. and *Toxoplasma gondii* among stray dogs in Bangkok, Thailand. *S.E. Asian J. Trop. Med and Public Health.* **40(2)**: 247.

Karpagam, K.B. and Ganesh, B. (2020). Leptospirosis: a neglected tropical zoonotic infection of public health importance- an updated review. *Eur. J. Clin. Microbiol. Infect. Dis.* **39(5)**: 835-846.

Miotto, B.A., Guilloux, A.G.A., Tozzi, B.F., Moreno, L.Z., da Hora, A.S., Dias, R.A., Heinemann, M.B., Moreno, A.M., Filho, A.F.D.S., Lilenbaum, W. and Hagiwara, M.K. (2018). Prospective study of canine leptospirosis in shelter and stray dog populations: Identification of chronic carriers and different *Leptospira* species infecting dogs. *PloS one*, **13(7)**: p.e0200384.

Pratt, N., Conan, A. and Rajeev, S. (2017). *Leptospira* seroprevalence in domestic dogs and cats on the Caribbean Island of Saint Kitts. *Vet. Med. Int.* pp. 1-6.

Rojas, P., Monahan, A.M., Schuller, S., Miller, I.S., Markey, B.K. and Nally, J.E. (2010). Detection and quantification of leptospires in urine of dogs: a maintenance host for the zoonotic disease leptospirosis. *Eur. J. Clin. Microbiol. Infect. Dis.* **29**: 1305-1309.

Ricardo, T., Previtali, M.A. and Signorini, M. (2020). Meta-analysis of risk factors for canine leptospirosis. *Prev. Vet. Med.* **181**: 105037.

Sant'Anna da Costa, R., N. Di Azevedo, M.I., dos Santos Baptista Borges, A.L., Carvalho-Costa, F.A., Martins, G. and Lilenbaum, W. (2021). Persistent high leptospiral shedding by asymptomatic dogs in endemic areas triggers a serious public health concern. *Animals.* **11(4)**: 937.

Smith, A.M., Arruda, A.G., Evason, M.D., Weese, J.S., Wittum, T.E., Szlosek, D. and Stull, J.W. (2019). A cross-sectional study of environmental, dog, and human-related risk factors for positive canine leptospirosis PCR test results in the United States, 2009 to 2016. *BMC Vet. Res.* **15(1)**: 1-12.

Smith, A.M., Stull, J.W. and Moore, G.E. (2022). Potential drivers for the re-emergence of canine leptospirosis in the United States and Canada. *Trop. Med. Infect. Dis.* **7(11)**: 377.

Soo, Z.M.P., Khan, N.A. and Siddiqui, R.(2020). Leptospirosis: Increasing importance in developing countries. *Acta tropica*. **201**: p.105183.

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