The Pharma Innovation Journal 2023; 12(4): 1613-1615

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(4): 1613-1615

www.thepharmajournal.com
Received: 18-01-2023
Accepted: 22-02-2023

M Roshni
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala Veterinary and Animal Sciences University, Kerala, India

R Ambily
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala Veterinary and Animal Sciences University, Kerala, India

M Mini
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala Veterinary and Animal Sciences University, Kerala, India

BK Mani
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala Veterinary and Animal Sciences University, Kerala, India

T Naicy
Base Farm, Kolahalamedu, Idukki, Kerala Veterinary and Animal Sciences University, Kerala, India

PS Reshma
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala Veterinary and Animal Sciences University, Kerala, India

Corresponding Author:
M Roshni
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala Veterinary and Animal Sciences University, Kerala, India

Diagnosis of leptospirosis in a cat by *loa22* gene specific PCR

M Roshni, R Ambily, M Mini, BK Mani, T Naicy and PS Reshma

Abstract
The present study involves molecular diagnosis of leptospirosis in a cat. A tomcat aged one year was presented to the clinics with symptoms of anorexia and vomiting. Blood (in EDTA) and serum samples were submitted to the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Kerala, suspecting leptospirosis. The serum sample was subjected to Microscopic Agglutination Test (MAT) employing a panel of 12 live reference serovars of Leptospira as antigen. The sample was found to be negative. Blood sample was subjected to Polymerase Chain Reaction (PCR) targeting loa22 gene of Leptospira and amplicon of 525 bp was obtained, thus confirming it as a case of leptospirosis.

Keywords: Leptospira, cat, MAT, loa22, PCR, Kerala

1. Introduction
Leptospirosis is one of the most dreadful bacterial zoo noses, worldwide. The disease is caused by spirochaetes of the genus *Leptospira*. Leptospirosis remains a problem in tropical and subtropical countries, where humid climate, animal rearing practices and environmental conditions favour the survival of *Leptospira*. Susceptible animals and humans can get infected either by direct contact with contaminated urine of carrier animals or indirect contact through environmental sources (Levett, 2001) [7]. All mammals can get infected by leptospirosis. Although studies on leptospirosis in dogs, cattle and pigs are many, the same on cats are limited.
Cats are regarded as resistant to leptospirosis. It has been reported that prevalence of leptospirosis in household cats is low when compared with outdoor cats as they may not be exposed to environment harbouring leptospires or rodents carrying infections as frequent as a feral cat (Jamshidi *et al.*, 2009) [5]. A clinical infection of leptospirosis in cats is usually mild or inapparent. If present, they can range from fever to anorexia, vomiting, diarrhoea, polydipsia, polyuria, alterations in biochemical profiles and many more (Arbour *et al.*, 2012, Hartmann *et al.*, 2013) [1, 4].
Ko *et al.* (2009) [6] opined that *loa22* of pathogenic *Leptospira* was the only gene which fulfilled the Koch's molecular postulates formulated by Falkow for a virulence factor. The protein Loa22 encoded by this gene is a crucial determinant for pathogenesis. Ristow *et al.* (2007) [8] produced a mutant loa22^{-} strain of *Leptospira* and tried to produce infection in animal disease models but, it was found to be ineffective, suggesting its importance in virulence. Varadarajan *et al.* (2016) [10] suggested that sensitivity of *loa22* gene was higher than *lipl32* or *lipl21*, which are more routinely used for molecular diagnosis of leptospirosis.
The present study involves diagnosis of leptospirosis in a household cat by MAT and *loa22* gene specific PCR.

2. Materials and Methods
A tomcat was presented to the University Veterinary Hospital, Mannuthy with symptoms of sudden onset of anorexia and vomiting. Blood sample was collected both in EDTA and clot activator vials and brought to the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Kerala, to rule out leptospirosis.
Microscopic agglutination test with the separated serum was carried out (WHO, 2007) [11] using 12 live *Leptospira* reference serovars as antigen. The serovars were *Leptospira interrogans* serovars Australis, Autumnalis, Bataviae, Canicola, Djasiman, Hardjo, Hebdomadis, Icterohaemorrhagiae, Pomona and Pyrogenes, *Leptospira kirschneri* serovar

Grippotyphosa and *Leptospira borgpetersenii* serovar Javanica. A serum dilution of 1:20 was prepared using sterile Phosphate Buffered Saline (PBS) (HiMedia). Fifty µL of the diluted serum was taken in a 96-well microtitre plate (Tarsons) and it was mixed with an equal quantity of each of the *Leptospira* serovars, separately. A negative control was also kept using PBS and different serovars. After mixing, the plate was incubated at 37 °C for two hours. After incubation, the results were read by examining a drop of serum-antigen mixture from each well under low power objective of a dark field microscope for agglutination of leptospires. The end point was recorded as the serum showing 50% agglutination or reduction in number of organisms in comparison to the respective antigen control.

Deoxyribonucleic acid (DNA) was extracted from the blood collected in EDTA vial using HiMedia Hipur A multi-sample DNA kit. Afterwards, the DNA was subjected to PCR targeting *loa22* gene of pathogenic *Leptospira* employing primers designed using Primer3 software. The primer sequences were 5' - AGAGGAGAATTCAGCTCCTGAGC - 3' and 5' - TGGTGCCTGCAGCGCAAAACGGA - 3'. The components of the reaction and PCR protocol are given in the tables 1 and 2, respectively. Deoxyribonucleic acid extracted from liquid cultures of *Leptosira interrogans* serovar Australis and *Leptospira biflexa* serovar Patoc were also kept as positive and negative controls, respectively. After PCR, submarine agarose gel electrophoresis of the PCR products was done using two% agarose gel at a voltage of 80V. A 100 bp DNA ladder was also run along with PCR products to analyse the size of amplicons. The gel was visualized and documented in a gel documentation system (Bio rad).

Table 1: Components of the PCR

Components	Volume (µL)
Nuclease free water	1.25
Forward primer (10 p*M*/ µL)	1
Reverse primer (10 p*M*/ µL)	1
PCR master mix	6.25
Template DNA	3
Total	12.5

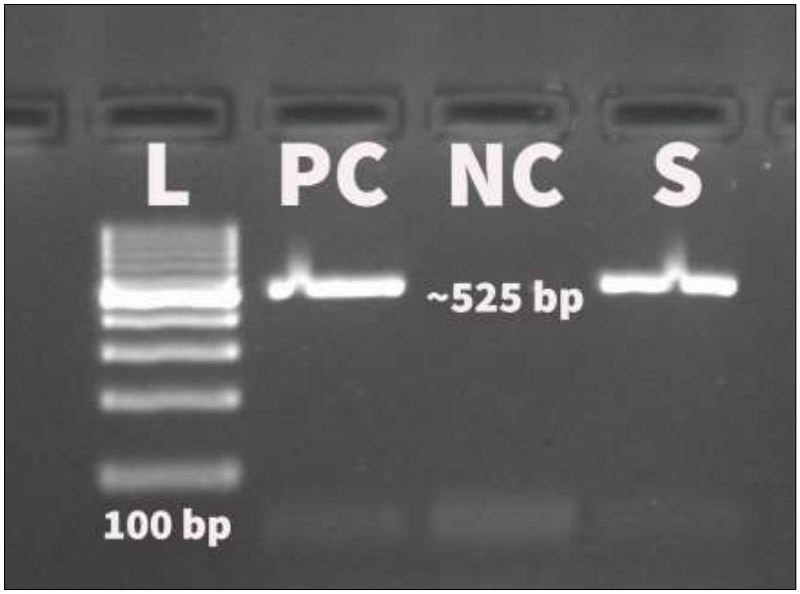
Table 2: PCR protocol

Step		Temperature (°C)	Time
Initial denaturation		94	4 min.
30 cycles	Denaturation	94	1 min.
	Annealing	60	45 sec.
	Extension	72	2 min.
Final extension		72	10 min.

3. Results

After incubation, a drop from each antigen and serum mixture was taken and examined for agglutination. No agglutination was observed by MAT.

The PCR revealed an amplicon of approximately 525 bp, indicating *loa22* gene (Fig. 1).



(Lane L-100 bp DNA ladder, PC-Positive control, NC-Negative control, S-Sample)

Fig 1: Amplicons of *loa22* genes of *Leptospira*

4. Discussion

Leptospirosis is one of the most dreadful bacterial zoonoses. The disease can affect all mammals. Leptospires survive in alkaline soil and humid conditions making places like Kerala ideal for its survival and persistence. The disease is prevalent in dogs, pigs and cattle, but the information on leptospirosis in cats is limited. As popularity of cats being pets is on the rise, it is noteworthy that, apart from being a suitable companion, they can potentially transmit many diseases. The present study identifies leptospirosis in a household cat through *loa22* gene specific PCR.

Clinical infection of leptospirosis in cats is rare. When acutely infected, they can be presented with signs such as polydipsia, polyuria, haematuria, anorexia and many more (Arbour *et al.*, 2012, Hartmann *et al.*, 2013) [1, 4]. In the present case, the animal had symptoms of anorexia, vomiting and weakness. The animal was kept indoor with no access to rodents, as reported by the owner.

Microscopic Agglutination Test could not detect anti-leptospiral antibodies in the serum. On PCR, DNA extracted from blood amplified *loa22* gene, confirming the case as leptospirosis.

According to Faine (1982) [3], antibodies will be produced after the leptospiraemic stage. Thus, MAT has certain

limitations in detecting an active infection. Molecular tools like PCR have an upper hand in diagnosing leptospirosis in terms of rapidity, precision and sensitivity.

The present study detected leptospiral DNA by *loa22* gene specific PCR. Most studies on leptospirosis utilise primers targeting genes such as *lipl32*, *lipl21* or *lipl41* of pathogenic *Leptospira*. However, Ko *et al.* (2009) [6] described that *loa22* gene was the only one in *Leptospira* which met with Koch's molecular postulates formulated by Falkow for a virulence factor. In a study conducted in Chennai, *loa22* gene was found to be highly conserved among both the reference serovars and clinical isolates (Varadarajan *et al.,* 2016) [10]. Hence, *loa22* gene can be utilized as a more reliable molecular marker for leptospirosis instead of other genes used in molecular diagnosis.

As per the available literature, in India, only two previous reports of feline leptospirosis could be identified. In 2001, Natarajaseenivasan *et al.* (2002) [9] reported an overall seroprevalence of 66.6% in Salem, Tamil Nadu where six out of nine animals tested were positive. Other study was by Divya *et al.* (2021) [2] in Thrissur, Kerala, where an overall seroprevalence of 9.01% was reported. The authors tested 11 animals suspected for leptospirosis and, one was positive. These studies reveal that leptospirosis is prevalent among cats.

Tests for leptospirosis is not regularly carried out in cats as the disease is thought to be infrequent in them. However, the present study detected leptospiral DNA from blood of a cat, suggesting an active infection. Although the disease is less reported in cats, its occurrence need not be overlooked. This study suggests that while assessing possible morbidities in a cat, leptospirosis should also be considered.

5. Conclusion

The present study reports the occurrence of leptospirosis in a cat by PCR targeting *loa22* gene in Thrissur, Kerala. No agglutination was observed in MAT. The presence of leptospiral DNA in whole blood confirms the case as leptospirosis. In acute leptospirosis, leptospiral DNA can be detected in blood, even before seroconversion. Thus, the present study reports a case of acute leptospirosis in a household cat. The study reveals the need for conducting tests for leptospirosis in cats, which is often overlooked during infectious disease investigations.

6. Acknowledgment

The authors are thankful to the Dean, College of Veterinary and Animal Sciences, Mannuthy for providing all facilities for work.

7. References

1. Arbour J, Blais MC, Carioto L, Sylvestre D. Clinical leptospirosis in three cats (2001-2009). Journal of the American Animal Hospital Association. 2012;48(4):256-260.
2. Divya D, Joseph S, Mini M, Nair SR, Davis KJ. Seroprevalence of leptospirosis in animals in Thrissur district of Kerala. International Journal of Current Microbiology and Applied Sciences. 2021;10(3):1616-1620.
3. Faine S. Guidelines for the Control of Leptospirosis. WHO offset Publication, Geneva. 1982;27:171.
4. Hartmann K, Egberink H, Pennisi MG, Lloret A, Addie D, Belák S, *et al*. *Leptospira* species infection in cats: ABCD guidelines on prevention and management. Journal of Feline Medicine and Surgery. 2013;15(7):576-581.
5. Jamshidi S, Akhavizadegan MA, Bokaei S, Maazi N, Ghorbanali A. Serologic study of feline leptospirosis in Tehran, Iran. Iranian Journal of Microbiology. 2009;1(2):32-36.
6. Ko AI, Goarant C, Picardeau M. Leptospira: The dawn of the molecular genetics era for an emerging zoonotic pathogen. Nature Reviews Microbiology. 2009;7(10):36-747.
7. Levett PN. Leptospirosis. Clinical Microbiology Reviews. 2001;14(2): 296-326.
8. Ristow P, Bourhy P, McBride FWDC, Figueira CP, Huerre M, Ave P, *et al*. The OmpA-like protein Loa22 is essential for leptospiral virulence. PLoS pathogens. 2007;3(7):e97.
9. Natarajaseenivasan K, Boopalan M, Selvanayaki K, Suresh SR, Ratnam S. Leptospirosis among rice mill workers of Salem, South India. Japanese journal of infectious diseases. 2002;55(5):170-173.
10. Varadarajan MT, Gopalakrishnan R, Govindan B, Rajendiran A, Kathaperumal K. Virulence Gene *loa22*-The Molecular Diagnostic Beacon of Canine Leptospirosis. International Journal of Chemical, Environmental & Biological Sciences. 2016;3(1):21-24.
11. WHO [World Health Organization] country office for India. Leptospirosis: Laboratory manual; c2007. p. 60.