ACTA VETERINARIA INDONESIANA

Vol. 14, No. 2: 220-225, July 2026
DOI : 10.29244/avi.14.2.220-225
P-ISSN 2337-3202, E-ISSN 2337-4373

Research Article

OPEN ACCESS

The Role of Rats as a Source of Leptospirosis Transmission: Detection of the LipL32 Gene

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Article history:
Received: 29 September 2025
Revised : 17 May 2026
Accepted: 2 June 2026

Keywords:
rat
cattle
leptospirosis
PCR
LipL32 gene

ABSTRACT

Background: Leptospirosis is an infectious disease that can cause fatal infections in a wide range of animal species and humans. Rats are recognized as the primary reservoir of *Leptospira* spp., capable of shedding the bacteria throughout their lifetime without exhibiting clinical signs. The presence of rats in cattle farming environments may increase the risk of *Leptospira* transmission to livestock and the surrounding ecosystem.

Aims: This study aimed to detect pathogenic *Leptospira* in rats captured around leptospirosis-seropositive cattle farms and to evaluate the potential role of rats as a primary reservoir of leptospirosis in cattle.

Methods: Rats were trapped in ten cattle farm areas where leptospirosis-seropositive cattle had previously been identified. Leptospirosis in cattle was diagnosed using the Microscopic Agglutination Test (MAT) with a positive cutoff titer of 1:100. Rats captured during a two-week trapping period were euthanized, and kidney samples were collected for DNA extraction. Detection of pathogenic *Leptospira* was performed by polymerase chain reaction (PCR) targeting the *LipL32* gene fragment (506 bp). PCR products were analyzed by electrophoresis at 100 V for 45 min and visualized under ultraviolet illumination.

Results: Serological examination revealed that leptospirosis-seropositive cattle were infected with one or two *Leptospira* serovars, predominantly Hardjo, Icterohaemorrhagiae, Rachmati, and Bataviae. A total of 32 house rats (*Rattus tanezumi*) were captured, yielding a trapping success rate of 2.3%. PCR analysis detected the *LipL32* gene (506 bp) in seven of the 32 kidney samples (21.87%), confirming the presence of pathogenic *Leptospira* in rats inhabiting the vicinity of leptospirosis-seropositive cattle farms.

Conclusion: Pathogenic *Leptospira* were detected in rats captured around leptospirosis-seropositive cattle farms, supporting the role of rats as an important reservoir of leptospirosis. These findings indicate that rats may contribute to the dissemination of pathogenic *Leptospira* to the environment, livestock, and humans, highlighting the importance of integrated surveillance and control measures in cattle farming areas.

INTRODUCTION

Leptospirosis is an infectious disease caused by pathogenic *Leptospira* spp. that affects almost all mammals and is zoonotic in nature. The disease poses a significant public health problem and causes considerable economic losses in livestock production (Karpagam et al., 2020). *Leptospira* spp. are bacteria capable of genomic rearrangement during adaptation to new hosts, making prevention and control of leptospirosis challenging (Picardeau, 2017). *Leptospira* spp. comprise over 300 serovars grouped into 25 serogroups (Levett, 2001; Lekhal et al., 2022) and 69 species (NCBI, 2021). These are highly motile, obligate

aerobic bacteria that grow optimally at temperatures between 28°C and 30°C and at a pH range of 6.8–7.4 (Adler and Moctezuma, 2010; Rajapakse, 2022).

The Microscopic Agglutination Test (MAT) is the gold standard for diagnosing leptospirosis due to its ability to detect specific antibodies against *Leptospira* species in serum samples (Vijayachari et al., 2007). However, Ahmed et al. (2012) suggested that PCR techniques are preferable because they are more specific, faster, and do not require maintaining live *Leptospira* cultures of different serovars. Satbige et al. (2020) explained that *Leptospira* detection by PCR can target: (1) ribosomal RNA genes such as 16S rRNA and 23S rRNA; (2) outer membrane components such as *LipL21*, *LipL32*, *LipL41*, and *OmpL1*; (3) immunoglobulin-like genes (*LigA*, *LigB*, *LigC*); and (4) flagellar antigens such as *flaA* and *flaB*. PCR targeting the *LipL32* gene specifically detects pathogenic *Leptospira* belonging to the *L. interrogans* group. The LipL32 lipoprotein is the most abundant surface protein in *Leptospira* and plays a key role in the pathogenesis, diagnosis, and prevention of leptospirosis (Haake et al., 2010).

In cattle and other ruminants, acute or severe forms of leptospirosis are rare, with most cases occurring sporadically in calves due to accidental exposure to specific strains (Loureiro and Lilenbaum, 2020). According to Mulyani et al. (2021), leptospirosis-seropositive cattle in Kulon Progo Regency, Indonesia, showed no clinical symptoms even when infected with more than one serovar. Suprayoga et al. (2020) reported that 33.3% of cattle slaughtered in Yogyakarta abattoirs tested seropositive for leptospirosis. The risk of leptospirosis infection in cattle in Kulon Progo Regency was found to be 2.67 times higher in barns where rats were present (Mulyani et al., 2016).

Leptospirosis is spread through direct contact with the infected animals' urine of kidney as well as indirectly through a contaminated environmental resource such as water and soil (Bierque et al., 2020). Rodents are the most important reservoirs of pathogenic *Leptospira* spp. because of their close proximity to humans and domestic animals (Plank and Dean, 2000). Infection in animals and humans occurs primarily through direct contact with infected animals or indirectly through exposure to soil or water contaminated with *Leptospira* spp. (Ko et al., 2009). Leptospira seroprevalence in rats captured near pig farms in Colombia was 13.8% among the 555 captured rats, and the positivity for pathogenic Leptospira spp. was 4% (López-Osorio et al., 2022).

Leptospira are bacteria with a unique cell structure compared to other gram-negative bacteria, particularly in their distinctive periplasmic membrane and flagella. The membrane structure of *Leptospira* plays a crucial role in pathogenesis, adhesion, and resistance to the host environment (Picardeau, 2017). The outer membrane of *Leptospira* contains lipopolysaccharide (LPS), lipoproteins, and outer membrane proteins (OMPs). Surface lipoproteins, such as LipL32, LipL41, OmpL1, LigA, and LigB, are important proteins for adhesion and virulence (Haake et al., 2010; Koizumi & Watanabe, 2023).

Proteomic studies have shown that approximately 75% of Leptospira's outer surface proteins are lipoproteins, with LipL32 accounting for 38–50% of the total outer membrane proteins (Haake et al., 2000; Cullen et al., 2002). The lipL32 gene is found conservatively in all strains of *Leptospira interrogans*, L. kirschneri, and L. borgpetersenii, and used as a target in molecular detection, particularly in lipL32 gene-based PCR. This gene is a specific diagnostic marker for pathogenic *Leptospira* because it is not found in non-pathogenic strains, many real-time PCR protocols for leptospirosis diagnosis use the lipL32 target as a determinant of the presence of pathogenic *Leptospira* (Podgoršek et al., 2020).

MATERIALS AND METHODS

The study focused on rats captured in cattle barns where cattle had tested seropositive for leptospirosis. The diagnosis of leptospirosis in cattle was established based on the MAT, considered positive at a dilution of 1:100. Ten rat traps baited with roasted coconut were placed in ten leptospirosis-positive cattle barns for two weeks. Captured rats were euthanized, and their kidney samples were homogenized and processed for DNA extraction using a commercial DNA extraction kit (Geneaid).

Detection of *Leptospira* was performed by targeting the *LipL32* lipoprotein, a major component of the *Leptospira* outer membrane. The forward primer (21 bases) used was 5'-TGG ATC TGA TCA ACT ATT ACG-3' with a GC content of 38.1% and Tm of 57.2°C, while the reverse primer (22 bases) was 5'-CAC TTC ACC TGG TTT GTA GGT-3' with a GC content of 45.5% and Tm of 62.1°C. The amplification reaction used 10.5 µL of extracted DNA as the template (excluding dH_2O).

The PCR conditions for *LipL32* gene amplification were as follows: initial denaturation for 5 minutes at 95°C; denaturation for 30 seconds at 95°C; annealing for 30 seconds at 55°C; and extension for 1 minute at 72°C. Amplification was carried out for 40 cycles, followed by a final extension at 72°C for 10 minutes. Electrophoresis was performed at 100 V for 45 minutes or until the tracking dye migrated two-thirds of the gel length. The

gel was visualized under a UV trans-illuminator and documented for analysis.

RESULTS AND DISCUSSION

Trapping was conducted in ten cattle barns where leptospirosis-seropositive cattle were identified in Kulon Progo Regency, Yogyakarta Special Region Province, Indonesia. The trapping was carried out for two weeks using roasted coconut bait. The trapping sites are shown in Figure 1. A total of 32 house rats (*Rattus tanezumi*) were trapped across ten cattle barns during the two-week trapping period. The trapping data and PCR results are presented in Table 1.

Leptospirosis diagnosis in rats was conducted using PCR on all 32 trapped samples. DNA extracted from rat kidney tissues was used as the template for amplification. PCR was performed using an Infinigen thermocycler with KAPA 2G reagents. The electrophoresis results observed under a UV transilluminator are shown in Figure 2.

The number of rats trapped during the 2-week period of trap deployment was 32.The trapping success rate reached 2.3%, calculated by dividing the number of rats captured by the number of traps installed per day. According to Ramadhani and Yunianto (2012), the average trapping success rate is approximately 7% in indoor environments and 2% in outdoor or plantation habitats. The success rate observed in this study, being above the typical outdoor range, indicates a relatively large rat population in the study areas. Htwe et al. (Htwe et al., 2012), R. tanezumi can be found both indoors and outdoors. These rats frequently inhabit roof spaces, wall voids and ceilings, which can cause damage to the structure of the building (Barnett, 2001). As omnivores, their diet consists primarily of plant matter and they are active primarily at night, particularly at dusk and dawn.

The leptospirosis prevalence among rats was 7 out of 32 (21.9%), demonstrating that rats can serve as reservoirs for their environment, other animals, and humans. Rat species previously reported as *Leptospira* carriers include *Rattus norvegicus*, *Rattus diardii*, *R. bartelsi*, *R. argentiventer*, and *R. tanezumi* (Gunawan et al., 2020). Ristiyanto (2020) reported that *Rattus tanezumi* is the most common rat species infected with leptospirosis in Indonesia. The Rattus norvegicus showed more prevalence of Leptospira spp in the kidney

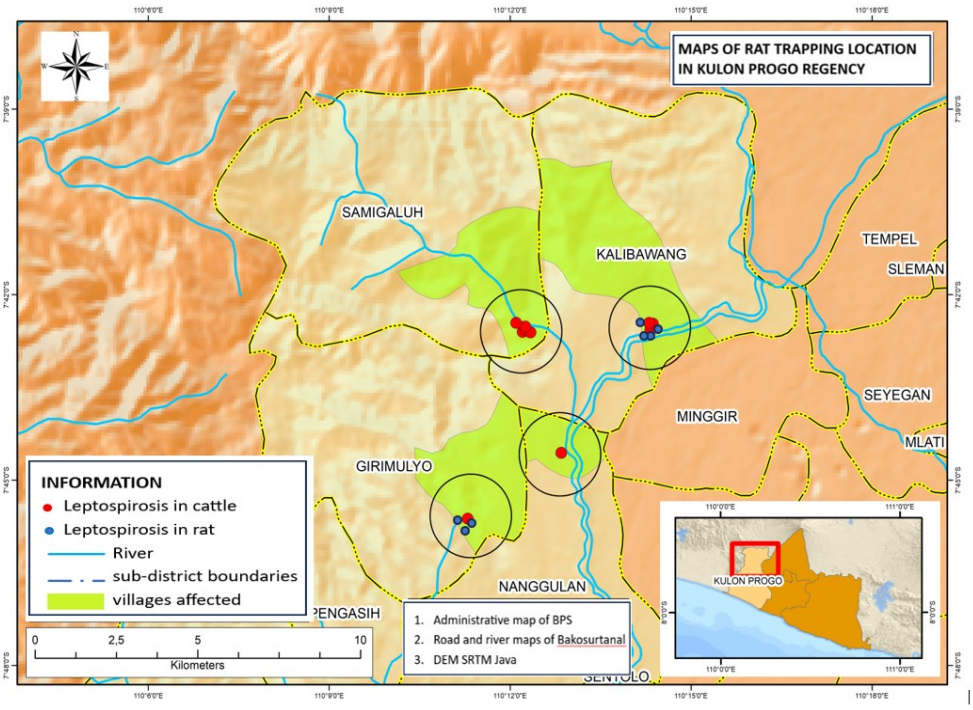

Figure 1. Trapping locations of rats in Kalibawang District (3 traps), Girimulyo District (3 traps), Samigaluh District (3 traps), and Nanggulan District (1 trap).

tissue, this might have been because of the fact that *Leptospira* spp. has more affinity on specific receptor (PRR) present in the kidney of Rattus norvegicus and hence lesser immune complex activation to clear *Leptospira* spp. in Rattus norvegicus (Akira et al., 2006). López-Osorio et al. (2022) detected pathogenic *Leptospira* in rats around pig farms, where the dominant serovars were Icterohaemorrhagiae (66.67%), Pyrogenes (16.66%), and other pathogenic *Leptospira* serovars (16.66%).

After penetrating the epithelium, *Leptospira* enter the bloodstream (bacteremia), it can reach many tissues. Leptospires reach the kidneys via two reported routes: through the glomerulus (via blood filtration) or through the peritubular capillaries and then attach to the tubular epithelium (Hakee and Matsunaga, 2010). Upon *Leptospira* invasion, bacterial components such as lipopolysaccharides, peptidoglycans, and outer membrane proteins (glycoproteins) activate toll-like receptors (Gomes et al., 2020). Renal tubular damage occurs due to the direct binding of the bacterial outer membrane protein LipL32 to toll-like receptor-2 (TLR2), expressed in renal tubular epithelial cells, which triggers intracellular inflammatory signaling pathways (Hung et al., 2005). Numerous in vitro and in vivo studies have shown that leptospires can form aggregates/biofilms on the renal surface (seen in reservoir mice and other models). These biofilms/aggregates are thought to contribute to resistance to local immune responses and maintain long-term shedding (Santos et al., 2021). Colonization of the kidneys by *Leptospira* leads to persistent, asymptomatic infections, serving as a source of transmission to animals and humans. Wild rodents have been identified as asymptomatic reservoirs that shed *Leptospira* spp. in their urine (Udechukwu et al., 2021).

Pathogenic leptospires can survive and remain virulent for several weeks in the water and soil environment. Environmental survival capacities depend on the species and strains (Parker and Walker, 2011). *Leptospira* may also interact with other members of soil communities such as Free-Living Amoebas. Amoebas,

Table 1. Results of rat trapping at ten locations and PCR detection of *Leptospira* using the *LipL32* gene.

Location	Address	Number of Rats Trapped	PCR Result (Positive Samples)
1	Kisik, Banjarasri, Kalibawang (3 sites)	18	4
2	Duwet, Purwoharjo, Samigaluh (3 sites)	0	0
3	Krikil, Pendoworejo, Girimulyo (3 sites)	12	3
4	Wiyu, Kembang, Nanggulan (1 site)	2	0
Total		**32**	**7**

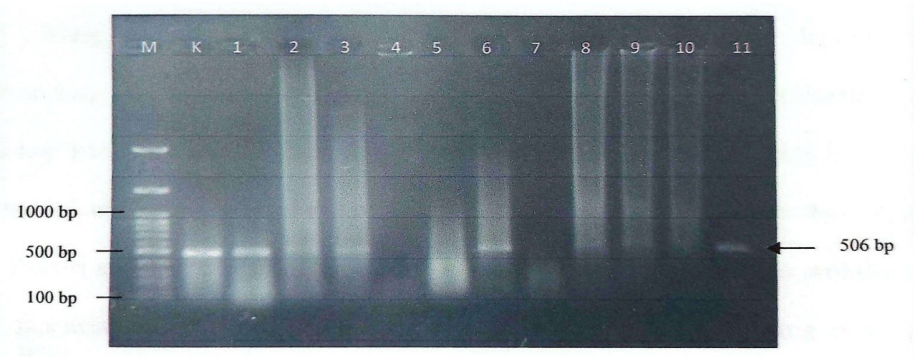

Figure 2. Electrophoresis results of *Leptospira LipL32* gene amplification from rat kidney samples collected around leptospirosis-seropositive cattle barns. M: marker; K: control positive; 1–11: samples.

which are one of the main colonizers of drinking water networks, are known to be possible reservoirs of potentially pathogenic bacteria (Delafont et al., 2016). *Leptospira* has a cell wall consisting of 3 layers, the outer layer plays a role in antigen recognition by the immune system and functions as protection against environmental stress (Ricaldi & Fouts, 2019).

CONCLUSION

This study provides information on the presence of leptospirosis cases in rats in a seropositive cattle pen. Several limitations were encountered during the study, including the limited number of traps and the insufficiently long trapping time, resulting in a small sample size. This prevented inferential statistical analysis, which could impact prevalence estimates. The study did not identify the serovars that infect both cattle and rats, thus hindering the conclusion that rats are indeed the source of leptospirosis infection in cattle. Other sources of infection are possible, although the possibility of rats in the pen area cannot be ruled out. A larger sample size would be better for epidemiological standards, and the results would be more representative of the broader population. Molecular analysis down to the serovar level could provide more valid data and serve as a basis for concluding the role of rats as a source of infection for cattle.The results of this study indicate that *Leptospira* pathogenic have been found in rats living in the areas surrounding leptospirosis-seropositive cattle farms. Rats can play a role in the spread of pathogenic *Leptospira* to the environment, other animals, and humans. Therefore, efforts are recommended to break the life cycle of *Leptospira* in the environment and promptly treat animals that test positive for leptospirosis.

AUTHORS CONTRIBUTION

G.T.M. conceived and designed the study, conducted field sampling, performed laboratory analyses, analyzed the data, and prepared the initial manuscript draft. A.H. contributed to the study supervision, methodology development, data interpretation, and critical revision of the manuscript. Both authors read and approved the final version of the manuscript.

ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to the veterinarians from Puskeswan in Kalibawang, Samigaluh, Girimulyo, and Nanggulan Districts, Kulon Progo Regency, Yogyakarta Special Region, for their valuable assistance during field sampling. The authors also thank the Department of Biochemistry, Faculty of Veterinary Medicine, Universitas Gadjah Mada, for permitting the use of laboratory facilities.

"The author declares that there is no conflict of interest with the parties involved in this research".

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