Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Wild Rodents as Reservoirs of Pathogenic *Leptospira*Following an Outbreak in Pangandaran

Rina Marina

rina028@brin.go.id

National Research and Innovation Agency https://orcid.org/0000-0003-4041-2897

Herjuno Ari Nugroho

National Research and Innovation Agency

Sugiyono Saputra

National Research and Innovation Agency

Endang Puji Astuti

National Research and Innovation Agency

Mutiara Widawati

National Research and Innovation Agency

Ristiyanto Ristiyanto

National Research and Innovation Agency

Tri Wahono

National Research and Innovation Agency

Agus Ari Wibowo

Indonesian Institute for Public Health Laboratories, Jakarta, Ministry of Health of Indonesia

Yuneu Yuliasih

National Research and Innovation Agency

Muhammad Umar Riandi

Pangandaran Public Health Laboratory, Ministry of Health of Indonesia

Andri Ruliansyah

Pangandaran Public Health Laboratory, Ministry of Health of Indonesia

Dyah Widiastuti

National Research and Innovation Agency

Fitrine Ekawasti

National Research and Innovation Agency

Pandji Wibawa Dhewantara

National Research and Innovation Agency

Research Article

Keywords: Leptospira, leptospirosis, wild rodents, zoonotic diseases, 16S rRNA, Indonesia

Posted Date: April 23rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9491922/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: The authors declare no competing interests.

Abstract

Following a leptospirosis outbreak in 2022, this study aimed to characterize circulating pathogenic *Leptospira* spp. in wild rodents in rural Pangandaran, West Java, Indonesia.

Wild rodents were captured using live traps at four sampling sites. Kidney tissues were collected and analyzed by PCR targeting the 16S rRNA gene, followed by sequencing and phylogenetic analysis of positive samples. A total of 142 rodents were captured, predominantly *Rattus tanezumi* (93.7%) and *Rattus tiomanicus* (6.3%). Seven kidney samples (4.9%) tested positive for *Leptospira*, with a prevalence of 4.5% (6/133) in *R. tanezumi* and 11.1% (1/9) in *R. tiomanicus*. Sequence analysis identified two pathogenic species, *Leptospira interrogans* and *Leptospira borgpetersenii*. Phylogenetic analysis based on partial 16S rRNA sequences showed that most isolates clustered within the *L. interrogans* clade, forming two subclades closely related to reference strains representing the Bataviae–Pomona and Icterohaemorrhagiae/Copenhageni lineages, while one isolate grouped with *L. borgpetersenii*. Due to the conserved nature of the 16S rRNA marker, species-level identification was supported, whereas serovar-level classification could not be definitively resolved.

The detection of pathogenic *Leptospira* in wild rodents highlights their role as reservoir hosts and underscores the importance of strengthened One Health–based surveillance to mitigate the risk of future leptospirosis outbreaks in the region and potential zoonotic transmission to humans.

1. Introduction

Leptospirosis is a life-threatening but neglected zoonotic disease caused by pathogenic *Leptospira* species (Haake and Levett, 2015). Globally, it is estimated to affect more than one million people annually, resulting in approximately 58,000 deaths and posing a major public health concern, particularly in tropical and subtropical regions (Costa et al., 2015). The global burden of leptospirosis has been estimated at 2.90 million Disability Adjusted Life Years (DALYs), with approximately 1.03 million cases reported each year (Torgerson et al., 2015). In addition to human infections, *Leptospira* spp. also affect animals, particularly livestock such as cattle and sheep, leading to significant economic losses due to increased treatment costs, abortions, stillbirths, and reduced productivity (Zarantonelli et al., 2018).

Transmission occurs through direct or indirect contact with infected animals or environments contaminated with the urine of infected hosts. Rodents are key reservoirs in the transmission cycle of *Leptospira*, serving as persistent carriers that facilitate the maintenance and dissemination of the bacteria in various environments. Wild rodents including *Rattus rattus*, *R. tanezumi*, *R. exulans*, and *R. tiomanicus*, are commonly found in urban, semi-urban, and rural areas, and have been identified as important reservoirs responsible for maintaining *Leptospira* in multiple endemic regions (Azhari et al., 2018; Blasdell et al., 2019; Boey, Shiokawa and Rajeev, 2019; Shafie et al., 2022; Sunaryo and Priyanto, 2022; Darlan et al., 2025).

Based on their genetic characteristics, the genus *Leptospira* comprises a highly diverse group of bacteria that can be classified into pathogenic, intermediate, and saprophytic bacterial species (Picardeau, 2017). The most common pathogenic *Leptospira* species responsible for human infection include *Leptospira interrogans, L. borgpetersenii*, and *L. kirschneri* (Stuart, Prescott and Singleton, 2012; Blasdell et al., 2019; Philip et al., 2020; Komalaningsih and Juliansyah, 2024). Although these species are pathogenic, they differ in transmission pathways and environmental persistence. *L. interrogans* can survive longer in soil and water, increasing its likelihood of environmental transmission (Plata-Luis et al., 2016).

Despite often being overshadowed by other febrile illnesses like dengue and malaria, leptospirosis can lead to severe health consequences. Severe cases may result in life-threatening complications, including acute kidney injury, pulmonary haemorrhage syndrome, and multi-organ failure (Haake and Levett, 2015). Furthermore, the disease is often underdiagnosed and underreported in many endemic areas due to limited access to diagnostic facilities and the non-specific clinical manifestations (Esteves et al., 2025). These challenges highlight the urgent need for improved surveillance and diagnostic strategies to better understand the disease burden and the global distribution of *Leptospira* species.

In Indonesia, leptospirosis is endemic and has been reported in various provinces, with outbreaks frequently associated with flooding events (van Bavel et al., 2019; Sutiningsih et al., 2024; Darlan et al., 2025). Recently, a leptospirosis outbreak was reported in Pangandaran regency, resulting in 30 deaths (Widawati et al., 2023). This highlights the importance of identifying local reservoirs of *Leptospira*, particularly rodents, which play a central role in maintaining transmission in both urban and rural settings (Pellizzaro et al., 2019). Currently, there is no evidence regarding the prevalence of pathogenic *Leptospira* spp. in wild rodent populations in Pangandaran area.

This study aimed to detect pathogenic *Leptospira* in rodent populations in the outbreak area of Pangandaran, West Java. Understanding the diversity of *Leptospira* in rodent populations will provide valuable insights into the potential sources of infection and contribute to the development of targeted interventions for preventing future human leptospirosis outbreaks.

2. Materials and Methods

1.1 Ethics statement

Ethical approval

for the study was granted by the Indonesia Research Ethics Committee of the National Research and Innovation Agency (No. 039/KE.03/SK/04/2023).

1.2 Study area

The study was conducted from June 2023 to June 2024 in Pangandaran District, West Java Province [7.615S, 108.498E]. The district comprises 10 sub districts and 93 villages with a total area of 1,011 km2.

The total population of 423,670 people, ranged from 1641 people in Mekarwangi village to 12,365 people in Babakan village (Central Bureau of Statistics, 2022). Sampling was carried out in two sub-districts: Cijulang (Kondangjajar Village) and Cimerak (Cimerak, Legokjawa, and Cigugur Villages) (Fig. 1). The sites were selected as these areas were identified as hotspot for leptospirosis transmission during the outbreak in 2022 (Widawati et al., 2023).

Figure 1. Sample collection sites in Pangandaran

1.3 Rodents collection

A total 100 live-traps (30 cm x 20 cm x 14 cm) were placed in residential areas, including household premises (indoor and outdoor), surrounding vegetation, and rice fields to capture wild rodents. Traps were baited with roasted coconut and set in the evening. Rat collection was conducted for three consecutive nights in each survey sites, with the baits and successful traps replaced each morning. Cages containing captured rodents were promptly transferred to a bag. Captured rats were then euthanized by intraperitoneal administration of an overdose of a ketamine - xylazine mixture, following approved athical procedures. Species identification was performed based on phenotypic characterization and morphometric measurements. Sex, body weight, and head-body length, tail length, hind foot length, and ear length were recorded for each specimen.

1.4 Kidney collection

Following species identification and morphometric measurement, rodents were dissected under aseptic condition. Both kidneys were aseptically excised using sterile instruments and placed into sterile tubes containing 70% ethanol. The samples were transported to Public Health Laboratory of Pangandaran and stored at 2–8°C until further analysis.

1.5 DNA Extraction

DNA was extracted from kidney samples using the Quick-DNA Miniprep Kit (Zymo Research, USA, Cat. No D4300), following the manufacturer's instructions. Briefly, 20 mg of kidney tissue was homogenized in lysis solution containing 95 µL ddH2O, 95 µL Solid Tissue Buffer, and 10 µL Proteinase K, and incubated at 55°C for 3 hours. DNA was then purified through a series of binding and wash steps, and finally eluted with 50 µL of DNA Elution Buffer. The extracted DNA was stored at − 20°C until further PCR analysis.

1.6 PCR Amplification

The extracted DNA was screened for pathogenic *Leptospira* spp. using PCR targeting 16S rRNA gene (expected amplicon size ~ 330 bp). PCR amplification was performed following a previously described protocol with minor modifications (Zulkifli et al., 2018), using primers 16s-F: (5'-GGCGGCGCGTCTTAAACATG-3') and 16s-R (5'-TTCCCCCCATTGAGCAAGATT-3'). Briefly, each PCR reaction was carried out in a total volume of 25 µL containing 12.5 µL of MyTaq™ HS Red Mix (2x) (Meridian-Bioscience, Cat. No. BIO-25048), 0.4 µM of each primer, 3 µL of template DNA, and nuclease-

free water to the final volume. Thermal cycling was conducted under the following conditions: initial denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 2 min, followed by final extension at 72°C for 10 min.

Positive samples obtained from 16S rRNA amplification were subjected to sequencing for species identification. In each PCR run, positive control (Leptospira DNA obtained from previously confirmed positive sample provided by the Ministry of Health of Indonesia) and non-template control (nuclease-free water) were included. All amplifications were performed using the Applied Biosystems Veriti™ Thermal Cycler (Thermo Fisher Scientific, USA). PCR products were separated by electrophoresis on 1.5% agarose gel stained with SYBR Safe DNA Gel Stain (Thermo Fisher Scientific, USA), and visualized under UV illumination.

1.7 Phylogenetic analysis

PCR-positive amplicons were purified and submitted to 1st Base Laboratory (Selangor, Malaysia) for Sanger sequencing. Chromatograms were inspected for quality, trimmed, and assembled into consensus sequences using BioEdit software version 7.2 (Informer Technologies, Inc., USA). The consensus sequence were compared with reference sequences in the GenBank database using the Basic Local Alignment Search Tool for nucleotides (BLASTn) to determine sequence similarity and preliminary species identification. Multiple sequence alignment was performed using ClustalW implemented in MEGA version 12. Phylogenetic relationships were inferred using Maximum Likelihood approach under the Kimura 2-parameter model with 1,000 bootstrap replicates to assess node support.

3. Results

3.1. Morphometric characteristics of captured rodents

A total of the 142 rodents were captured during the study period (Table 1). Two species were identified based on morphological assessment. *Rattus tanezumi* (Asian house rat) was the most common species (n = 133; 93.7%), followed by *R. tiomanicus* (Malayan tree rat) (n = 9; 6.3%).

Table 1
Species composition and morphometric characteristics of rodents captured in Pangandaran Regency, Indonesia

Species	Total (%)	Sex		Median Weight (g)	Median Length (cm)			
		Male	*Female*		Head/body	Tail	Ear	Foot
R. tanezumi	133 (93.7)	77 (57.9)	56 (42.1)	92	15	15.5	1.9	3
R. tiomanicus	9 (6.7)	7 (77.7)	2 (22.3)	89	15.5	17.5	2	3
Total	142 (100)	84 (59.2)	58 (40.8)	91	15	15.5	1.9	3

Among *R. tanezumi*, maled accounted for a higher proportion (57.9%) than female. The median body weight was 92 g, with median head-body and tail lengths of 15 cm and 15.5 cm respectively.

The *R. tiomanicus* group consisted of seven males and two females. The median body weight was 89 g, with median head-body and tail lengths of 15.5 cm and 17.5 cm respectively.

Table 1. **Species composition and morphometric characteristics of rodents captured in Pangandaran Regency, Indonesia**

3.2. Leptospira detection by PCR from kidney samples

The highest number of rodents was captured in Legokjawa (n = 86; 60.6%), followed by Cimerak (n = 35, 24.6%), Kondangjajar (n = 14, 9.9%), and Bunisari (n = 7, 4.9%) (Table 2). Agarose gel electrophoresis revealed that 7 of 142 kidney samples (4.9%) were positive for the 16S rRNA gene, producing the expected amplicon size of approximately 330 bp (Fig. 2).

Table 2
Distribution of rodents by sampling sites and prevalence of *Leptospira* infection detected by PCR

Sampling site	N (%)	No. of rodents (%)		*Leptospira*-positive kidney samples n (%)		
		R. tanezumi	*R. tiomanicus*	*R. tanezumi*	*R. tiomanicus*	Total
Bunisari	7 (4.9)	7 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cimerak	35 (24.6)	30 (85.7)	5 (14.3)	1 (3.3)	1 (20.0)	2 (5.7)
Kondangjajar	14 (9.9)	14 (100.0)	0 (0.0)	3 (21.4)	0 (0.0)	3(21.4)
Legokjawa	86 (60.6)	82 (95.3)	4 (4.7)	2 (2.3)	0 (0.0)	2 (2.3)
Total	142 (100.0)	133 (93.7)	9 (6.3)	6 (4.5)	1 (11.1)	7 (4.9)

Figure 2. **Agarose gel electrophoresis of PCR amplification of the Leptospira 16S rRNA gene. Lane M: 100 bp DNA ladder. Lanes 2A-27E show the seven positive kidney samples with the expected amplicon size (~ 330 bp)**

The highest proportion of *Leptospira*-positive rodents was observed in Kondangjajar (21.4%, n = 3/14), followed by Cimerak (5.7%, n = 2/35), and Legokjawa (2.3%, n = 2/86). No positive samples were detected among rodents captured in Bunisari (Fig. 1).

Based on species, *R. tiomanicus* showed a higher prevalence (11.1%, n = 1/9) compared to *R. tanezumi* (4.5%, n = 6/133). The single positive *R. tiomanicus* specimen was captured in Cimerak. Among *R. tanezumi*, the highest infection rate was recorded in Kondangjajar (21.4%, n = 3/14), followed by Cimerak (3.3%, n = 1/30) and Legokjawa (2.3%, n = 2/82).

Table 2. **Distribution of rodents by sampling sites and prevalence of Leptospira infection detected by PCR**

3.3. Molecular characterization of leptospira in rodents

Phylogenetic analysis was performed using the maximum likelihood approach with 1,000 bootstrap replicates based on partial 16S rRNA sequences (260 nucleotides). Most sequences clustered within the *Leptospira interrogans* clade with strong bootstrap support, forming two distinct subclades associated with reference strains corresponding to serovars Bataviae–Pomona (3A, 16D, 21B, 27E) and Icterohaemorrhagiae/Copenhageni (7C, 12C).

One sequence (2A) clustered separately with *Leptospira borgpetersenii*, indicating the co-circulation of multiple pathogenic *Leptospira* species in the study area (Fig. 3).

Figure 3. A phylogenetic tree constructed using the maximum likelihood approach based on partial 16S rRNA sequences (260 nucleotides), with 1,000 bootstrap replicates, showing the relationships between the seven positive samples and reference Leptospira sequences. Bootstrap values are indicated at the nodes.

BLAST analysis supported the phylogenetic findings, revealing high nucleotide similarity (99.62–100%) with pathogenic *Leptospira* species (Table 3). Sample 2A showed 100% identity with *L. borgpetersenii* strain KB1105K (GenBank accession no. KC662453.1), whereas the remaining sequences exhibited 99.62–100% similarity to *L. interrogans* reference strains.

Table 3
Blast nucleotide analysis of partial 16S rRNA sequences compared with reference sequence in GenBank

No	ID Sampel	*Accesion Number*	BLAST Result	% Similarity
1	2A	KC662453.1	*Leptospira borgpetersenii* strain KB1105K 16S ribosomal RNA gene, partial sequence	100
2	3A	AY996800.1	*Leptospira interrogans* serovar Pomona strain Pomona 16S ribosomal RNA gene, partial sequence	99.62
3	21B	AY996800.1	*Leptospira interrogans* serovar Pomona strain Pomona 16S ribosomal RNA gene, partial sequence	99.62
4	12C	AY996800.1	*Leptospira interrogans* serovar Pomona strain Pomona 16S ribosomal RNA gene, partial sequence	100
5	16D	AY996800.1	*Leptospira interrogans* serovar Pomona strain Pomona 16S ribosomal RNA gene, partial sequence	99.62
6	7C	MK330632.1	*Leptospira interrogans* serovar Copenhageni strain C51/2015 16S ribosomal RNA gene, partial sequence.	100
7	27E	AY996800.1	*Leptospira interrogans* serovar Pomona strain Pomona 16S ribosomal RNA gene, partial sequence	99.62

Sequences derived from different rodent species clustered within the same clades, suggesting the absence of host-specific segregation. However, phylogenetic resolution was limited to species-level identification due to the conserved nature of the 16S rRNA marker.

Table 3. **Blast nucleotide analysis of partial 16S rRNA sequences compared with reference sequence in GenBank**

4. Discussion

Recent human leptospirosis outbreaks in Pangandaran in 2022 indicate the circulation of pathogenic *Leptospira* spp. among local reservoir hosts. In response to this public health concern, the present study was conducted in 2023 to investigate the occurrence of pathogenic *Leptospira* species among wild rodent populations in affected areas. Our findings provide the first molecular evidence of pathogenic *Leptospira* spp. circulating among wild rodents in four outbreak-associated locations in Pangandaran, with an overall prevalence of 4.9% (n = 7/142). The infection rate among the two rodent species (*Rattus tanezumi* and *Rattus tiomanicus*) varied across localities, ranging from 2.3% in Legokjawa village to 21.4% in Kondangjajar village. Phylogenetic analysis revealed three distinct clades belonging to the pathogenic species *L. interrogans* and *L. borgpetersenii*. The presence of these pathogenic *Leptospira* species among wild rodents highlights their role as important reservoirs and underscores the need for continuous rodent surveillance to support leptospirosis prevention and control efforts in the region. The present study provides insights into the molecular epidemiology of *Leptospira* spp. in the outbreak area of Pangandaran and highlights the importance of rodent monitoring for outbreak prevention and control.

The prevalence of *Leptospira* in rodent populations varied across sampling sites and species. Although the overall infection rate was relatively low (4.9%), it indicates that the pathogenic *Leptospira* is present and circulating in rodent population in the outbreak area. For instance, the absence and low prevalence of *Leptospira*-positive among captured rodents in Bunisari (0%) and Legokjawa (2.3%) suggesting low *Leptospira* transmission in the environment. This might be due to several factors, including low rodent density and environmental conditions that might be unfavourable for the survival of *Leptospira* (Krairojananan et al., 2025). In contrast, high prevalence of *Leptospira* observed among rodents population in Kondangjajar (21.4%), with all cases attributed to *R. tanezumi*, indicate a widespread distribution of the pathogen in the area. This finding emphasizes the need for better targeted rodent surveillance and intervention. Kondangjajar is characterized as a densely peri-urban populated area with poor waste management, lack of adequate sanitation facilities, and prone to flooding. These conditions might have contributed to the maintenance and growth of rodent population in the area. Studies have shown that flood-prone, impoverished areas with poor waste management and inadequate sanitation are at greater risk of leptospirosis outbreaks (Mohd Radi et al., 2018; Rehan et al., 2023). Differences in habitat, rodent density, and behaviour may influence exposure risk, as supported by studies highlighting the importance of species-specific ecology in zoonotic transmission (Estrada-Peña et al., 2014).

The presence of pathogenic *Leptospira* among the captured rodents in this study is consistent with previous studies elsewhere in Indonesia and in other tropical countries, which highlights its adaptability to human-modified environments and its role as a key reservoir for zoonotic pathogens (Komalaningsih and Juliansyah, 2024; Liu et al., 2025). Our study was conducted in areas primarily comprised of agricultural lands, coconut crops, and adjacent to human settlements, which is a common habitat for *R. tanezumi* (Asian rat) (Stuart, Prescott and Singleton, 2012). A study conducted in 29 provinces in Indonesia from 2015 to 2018 revealed that *R. tanezumi* is the predominant species hosted *Leptospira* spp (Ristiyanto et al., 2022). Similarly, a study in Yogyakarta also reported *Leptospira*-positive among *R. tanezumi* (4.8%) in addition to *R. norvegicus* population (Sunaryo and Priyanto, 2022). In the Mekong Delta region, *R. tanezumi* showed a notable seroprevalence of *Leptospira* spp, which found that 24.6% of

R. tanezumi tested positive for leptospiral antibodies, indicating a significant presence of infection among this species (Loan et al., 2015).

Our study detected one positive *R. tiomanicus* (Malayan tree rat) (20%, n = 1/5) in Cimerak village. This finding suggests a potential role of *R. tiomanicus* as a reservoir for *Leptospira* spp. in the area, which is predominantly characterized by forested landscapes and coconut plantation area. The prevalence of pathogenic *Leptospira* in this species is comparable to findings from a study conducted in Malaysia, which also reported a relatively high infection rate in *R. tiomanicus* population (18.2%, n = 4/22) (Sunaryo and Priyanto, 2022). Similarly, a study in Selangor reported that 12.5% (n = 2/16) of *R. tiomanicus* tested positive for pathogenic *Leptospira.* Given their wide distribution and close interaction with humans, particularly in rural environments, this species may play a significant role in the circulation and transmission of *Leptospira*, posing a risk to specific occupational groups such as farmers. This findings underscore the need to strengthen surveillance and public health promotion strategies to increase awareness of leptospirosis risk among rural communities.

Our study identified two pathogenic *Leptospira* species circulating in the study area, namely *L. interrogans* and *L. borgpetersenii*. These findings are consistent with previous studies conducted in Indonesia, including reports from North Sumatera that also detected *L. interrogans* in rodent populations (Sunaryo and Priyanto, 2022). Similar observations have been reported globally, where *L. interrogans* and *L. borgpetersenii* are recognized as predominant pathogenic species circulating among rodents in Nigeria (Udechukwu et al., 2025), China (Zhang et al., 2019; Xu et al., 2022), and several European countries, including Spain and France (Millán et al., 2017; Izquierdo-Rodriguez et al., 2020). Phylogenetic analysis based on partial 16S rRNA sequences (260 nucleotides) showed that the most sequences clustered within revealed three distinct clades within the pathogenic group. These clades clustered closely with reference strains representing *L. interrogans* serovar Bataviae-Pomona, Icterohaemorrhagiae and Copenhageni, as well as *L. borgpetersenii* strain KB1105K. This clustering pattern suggests the possible co-circulation of multiple pathogenic *Leptospira* lineages, including those related to the Bataviae–Pomona and Icterohaemorrhagiae serogroups, within the rodent population in the study area.

However, due to the relatively conserved nature of the 16S rRNA gene, the phylogenetic resolution in this study was limited to species-level identification, and definitive serovar determination cannot be inferred solely from this marker. Therefore, the clustering with reference strains representing particular serovars should be interpreted cautiously and considered indicative of phylogenetic relatedness rather than definitive serovar assignment.

Lineages related to *L. interrogans serovar Icterohaemorrhagiae* have been widely associated with severe human leptospirosis (Santos et al., 2018) and have been implicated in outbreaks across several Southeast Asian countries (Cosson et al., 2014). The detection of closely related lineages in rodents from the outbreak area suggests a potential epidemiological link and highlights the possible role of these rodents as reservoirs contributing to human infection (Cosson et al., 2014; Environmental Health Technology and Disease Control Agency, 2023). The presence of these pathogenic *Leptospira* species in

wild rodents indicates a continuing risk of transmission to humans as well as domestic animals and livestock in the area (Marder et al., 2008; Adler and de la Peña Moctezuma, 2010; Ellis, 2015). These findings emphasize the importance of continuous surveillance to monitor the diversity and spatial distribution of pathogenic *Leptospira* strains in wild rodents as well as in domestic animals such as cats, dogs, and cattle in these rural settings.

Characterizing circulating *Leptospira* species is essential for informing targeted control strategies. The results underscore the importance of considering both species-specific and location-specific factors when assessing *Leptospira* transmission risks. Public health interventions, such as rodent control and environmental management, should be tailored to the specific epidemiological context of each location. For example, Kondangjajar, with its high positivity rate, may require more intensive rodent control measures, while Bunisari may benefit from continued surveillance to ensure early detection of any changes in transmission risk. This approach aligns with recommendations from the World Health Organization (WHO) and other studies emphasizing the need for integrated, context-specific strategies to combat zoonotic diseases.

This study has several limitations. First, phylogenetic analysis was based on a partial 16S rRNA fragment, which provides reliable species-level identification but limited resolution for definitive serovar determination. Higher-resolution approaches such as MLST or additional gene targets would be needed to further characterize strain diversity. Second, the number of positive samples was relatively small, which may limit the representativeness of the detected diversity. Finally, molecular comparison with human isolates from the 2022 outbreak was not performed, preventing direct assessment of transmission linkage. Despite these limitations, this study provides important baseline data on pathogenic *Leptospira* circulating in wild rodents in the outbreak area.

5. Conclusion

This study provides the first molecular evidence of pathogenic *Leptospira* spp. circulating in wild rodents from outbreak-associated areas in Pangandaran, West Java. Two pathogenic species, *L. interrogans* and *L. borgpetersenii*, were detected in *Rattus tanezumi* and *Rattus tiomanicus*, supporting their role as important reservoir hosts. Phylogenetic analysis based on partial 16S rRNA sequences showed that most isolates clustered within *L. interrogans*, forming two subclades closely related to reference strains representing Bataviae–Pomona and Icterohaemorrhagiae/Copenhageni lineages, while one isolate grouped with *L. borgpetersenii*. These findings indicate the continued circulation of pathogenic *Leptospira* in study the area and highlight the need for strengthened One Health–based surveillance and higher-resolution molecular approaches to better understand the diversity and transmission dynamics of *Leptospira* in this region.

References

1. Adler, B. and de la Peña Moctezuma, A. (2010) 'Leptospira and leptospirosis', *Veterinary Microbiology*, 140(3), pp. 287–296. Available at: https://doi.org/https://doi.org/10.1016/j.vetmic.2009.03.012.
2. Azhari, N.N. *et al.* (2018) 'Molecular characterization of pathogenic Leptospira sp. in small mammals captured from the human leptospirosis suspected areas of Selangor state, Malaysia.', *Acta tropica*, 188, pp. 68–77. Available at: https://doi.org/10.1016/j.actatropica.2018.08.020.
3. van Bavel, B. *et al.* (2019) 'An Interdisciplinary Study of Leptospirosis Surveillance Systems in Three Regencies of East Java, Indonesia BT - Law, Public Policies and Complex Systems: Networks in Action', in R. Boulet, C. Lajaunie, and P. Mazzega (eds). Cham: Springer International Publishing, pp. 89–112. Available at: https://doi.org/10.1007/978-3-030-11506-7_5.
4. Blasdell, K.R. *et al.* (2019) 'Association of rodent-borne Leptospira spp. with urban environments in Malaysian Borneo.', *PLoS neglected tropical diseases*, 13(2), p. e0007141. Available at: https://doi.org/10.1371/journal.pntd.0007141.
5. Boey, K., Shiokawa, K. and Rajeev, S. (2019) 'Leptospira infection in rats: A literature review of global prevalence and distribution.', *PLoS neglected tropical diseases*, 13(8), p. e0007499. Available at: https://doi.org/10.1371/journal.pntd.0007499.
6. Cosson, J.F. *et al.* (2014) 'Epidemiology of Leptospira Transmitted by Rodents in Southeast Asia', *PLoS Neglected Tropical Diseases*, 8(6). Available at: https://doi.org/10.1371/journal.pntd.0002902.
7. Costa, F. *et al.* (2015) 'Global Morbidity and Mortality of Leptospirosis: A Systematic Review', *PLoS Neglected Tropical Diseases*, 9(9). Available at: https://doi.org/10.1371/journal.pntd.0003898.
8. Darlan, D.M. *et al.* (2025) 'Molecular and serological detection of Leptospira interrogans among wild rats in flood-prone residential areas of Indonesia.', *Open veterinary journal*, 15(1), pp. 437–445. Available at: https://doi.org/10.5455/OVJ.2025.v15.i1.39.
9. Ellis, W.A. (2015) 'Animal leptospirosis.', *Current topics in microbiology and immunology*, 387, pp. 99–137. Available at: https://doi.org/10.1007/978-3-662-45059-8_6.
10. Environmental Health Technology and Disease Control Agency, M. of H. of I. (2023) *Epidemiological Investigation Report on Leptospirosis in Pangandaran. Environmental Health Technology and Disease Control Agency*. Jakarta.
11. Esteves, S.B. *et al.* (2025) 'Into the spotlight: A spatial study of potentially underreported leptospirosis among dengue-negative patients in São Paulo city, Brazil', *PLoS neglected tropical diseases*, 19(3), p. e0012888. Available at: https://doi.org/10.1371/journal.pntd.0012888.
12. Estrada-Peña, A. *et al.* (2014) 'Effects of environmental change on zoonotic disease risk: An ecological primer', *Trends in Parasitology*, 30(4), pp. 205–214. Available at: https://doi.org/10.1016/j.pt.2014.02.003.
13. Haake, D.A. and Levett, P.N. (2015) 'Leptospirosis in humans.', *Current topics in microbiology and immunology*, 387, pp. 65–97. Available at: https://doi.org/10.1007/978-3-662-45059-8_5.
14. Izquierdo-Rodriguez, E. *et al.* (2020) 'Pathogenic Leptospira Species in Rodents From Corsica (France)', *Plos One*, 15(6), p. e0233776. Available at: https://doi.org/10.1371/journal.pone.0233776.

15. Komalaningsih, S. and Juliansyah, R. (2024) 'Identification of rodent species confirmed as the cause of leptospirosis disease, along with ecosystem distribution and environmental factors', *Journal of Character and Environment*, 2(1), pp. 69–81. Available at: https://doi.org/10.61511/jocae.v2i1.2024.800.
16. Krairojananan, P. *et al.* (2025) 'Leptospirosis Risk Assessment in Rodent Populations and Environmental Reservoirs in Humanitarian Aid Settings in Thailand', *Microorganisms*. Available at: https://doi.org/10.3390/microorganisms13010029.
17. Liu, R.J. *et al.* (2025) 'Mite Infestation on Rattus tanezum rats in southwest China concerning risk models', *Frontiers in Veterinary Science*, 12(March), pp. 1–16. Available at: https://doi.org/10.3389/fvets.2025.1519188.
18. Loan, H.K. *et al.* (2015) 'How important are rats as vectors of leptospirosis in the mekong delta of vietnam?', *Vector-Borne and Zoonotic Diseases*, 15(1), pp. 56–64. Available at: https://doi.org/10.1089/vbz.2014.1613.
19. Marder, G. *et al.* (2008) 'Prevalence of leptospirosis in sinantropic rodents from Corrientes City, Argentina. Period May 2005-June 2008', *Revista Veterinaria*, 19, pp. 150–153.
20. Millán, J. *et al.* (2017) 'Risk Factors of <i>Leptospira</I> Infection in Mediterranean Periurban Micromammals', *Zoonoses and Public Health*, 65(1). Available at: https://doi.org/10.1111/zph.12411.
21. Mohd Radi, M.F. *et al.* (2018) 'Leptospirosis Outbreak After the 2014 Major Flooding Event in Kelantan, Malaysia: A Spatial-Temporal Analysis', *The American Journal of Tropical Medicine and Hygiene*, 98(5), pp. 1281–1295. Available at: https://doi.org/10.4269/ajtmh.16-0922.
22. Pellizzaro, M. *et al.* (2019) 'Molecular detection of Leptospira spp. In rats as early spatial predictor for human disease in an endemic urban area', *PLoS ONE*, 14(5), pp. 1–11. Available at: https://doi.org/10.1371/journal.pone.0216830.
23. Philip, N. *et al.* (2020) 'Leptospira interrogans and Leptospira kirschneri are the dominant Leptospira species causing human leptospirosis in Central Malaysia.', *PLoS neglected tropical diseases*, 14(3), p. e0008197. Available at: https://doi.org/10.1371/journal.pntd.0008197.
24. Picardeau, M. (2017) 'Virulence of the zoonotic agent of leptospirosis: still terra incognita?', *Nature reviews. Microbiology*, 15(5), pp. 297–307. Available at: https://doi.org/10.1038/nrmicro.2017.5.
25. Plata-Luis, J. *et al.* (2016) 'Leptospira interrogans in Rodents from Cape Verde.', *Vector borne and zoonotic diseases (Larchmont, N.Y.)*, 16(11), pp. 731–733. Available at: https://doi.org/10.1089/vbz.2016.1989.
26. Rehan, S.T. *et al.* (2023) 'Urban flooding and risk of leptospirosis; Pakistan on the verge of a new disaster: A call for action', *International Journal of Hygiene and Environmental Health*, 248(October 2022), p. 114081. Available at: https://doi.org/10.1016/j.ijheh.2022.114081.
27. Ristiyanto *et al.* (2022) 'Survey on Leptospira Spp Infection in Rat Species in Indonesia', *Southeast Asian Journal of Tropical Medicine and Public Health*, 53(Supplement 2), pp. 659–713.

28. Santos, L.A. *et al.* (2018) 'Genomic Comparison Among Global Isolates of L. interrogans Serovars Copenhageni and Icterohaemorrhagiae Identified Natural Genetic Variation Caused by an Indel.', *Frontiers in cellular and infection microbiology*, 8, p. 193. Available at: https://doi.org/10.3389/fcimb.2018.00193.
29. Shafie, N.J. *et al.* (2022) 'Prevalence of Pathogenic Leptospira spp. in Non-Volant Small Mammals of Hutan Lipur Sekayu, Terengganu, Malaysia.', *Pathogens (Basel, Switzerland)*, 11(11). Available at: https://doi.org/10.3390/pathogens11111300.
30. Stuart, A.M., Prescott, C. V and Singleton, G.R. (2012) 'Natal nest locations of the Asian house rat (*Rattus tanezumi*) in lowland rice–coconut cropping systems: a coconut penthouse or rice bunds with water frontage?', *Wildlife Research*, 39(6), pp. 496–502. Available at: https://doi.org/10.1071/WR11197.
31. Sunaryo, S. and Priyanto, D. (2022) 'Leptospirosis in rats and livestock in Bantul and Gunungkidul district, Yogyakarta, Indonesia', *Veterinary World*, 15(6), pp. 1449–1455. Available at: https://doi.org/10.14202/vetworld.2022.1449-1455.
32. Sutiningsih, D. *et al.* (2024) 'Geospatial Analysis of Abiotic and Biotic Conditions Associated with Leptospirosis in the Klaten Regency, Central Java, Indonesia', *Tropical Medicine and Infectious Disease*, 9(10). Available at: https://doi.org/10.3390/tropicalmed9100225.
33. Torgerson, P.R. *et al.* (2015) 'Global Burden of Leptospirosis: Estimated in Terms of Disability Adjusted Life Years.', *PLoS neglected tropical diseases*, 9(10), p. e0004122. Available at: https://doi.org/10.1371/journal.pntd.0004122.
34. Udechukwu, C.C. *et al.* (2025) 'Isolation and Molecular Characterization of Pathogenic Leptospira spp. from Brown Rats (Rattus norvegicus) in Zaria and Environs, Kaduna, Nigeria, 2022.', *Journal of wildlife diseases*, 61(1), pp. 166–172. Available at: https://doi.org/10.7589/JWD-D-23-00159.
35. Widawati, M. *et al.* (2023) 'An investigation of geographical clusters of leptospirosis during the outbreak in Pangandaran, West Java, Indonesia', *Geospatial health*, 18(2), pp. 1–8. Available at: https://doi.org/10.4081/gh.2023.1221.
36. Xu, G. *et al.* (2022) 'Serological and molecular characteristics of pathogenic Leptospira in rodent populations in Fujian Province, China, 2018-2020.', *BMC microbiology*, 22(1), p. 151. Available at: https://doi.org/10.1186/s12866-022-02566-2.
37. Zarantonelli, L. *et al.* (2018) 'Isolation of pathogenic Leptospira strains from naturally infected cattle in Uruguay reveals high serovar diversity, and uncovers a relevant risk for human leptospirosis.', *PLoS neglected tropical diseases*, 12(9), p. e0006694. Available at: https://doi.org/10.1371/journal.pntd.0006694.
38. Zhang, C. *et al.* (2019) 'Genetic characteristics of pathogenic Leptospira in wild small animals and livestock in Jiangxi Province, China, 2002-2015.', *PLoS neglected tropical diseases*, 13(6), p. e0007513. Available at: https://doi.org/10.1371/journal.pntd.0007513.
39. Zulkifli, N.F. *et al.* (2018) 'Detection of leptospira species in environmental samples by amplification of 16S rRNA and rpoβ genes', *Sains Malaysiana*, 47(8), pp. 1795–1800. Available at:

https://doi.org/10.17576/jsm-2018-4708-18.

Figures

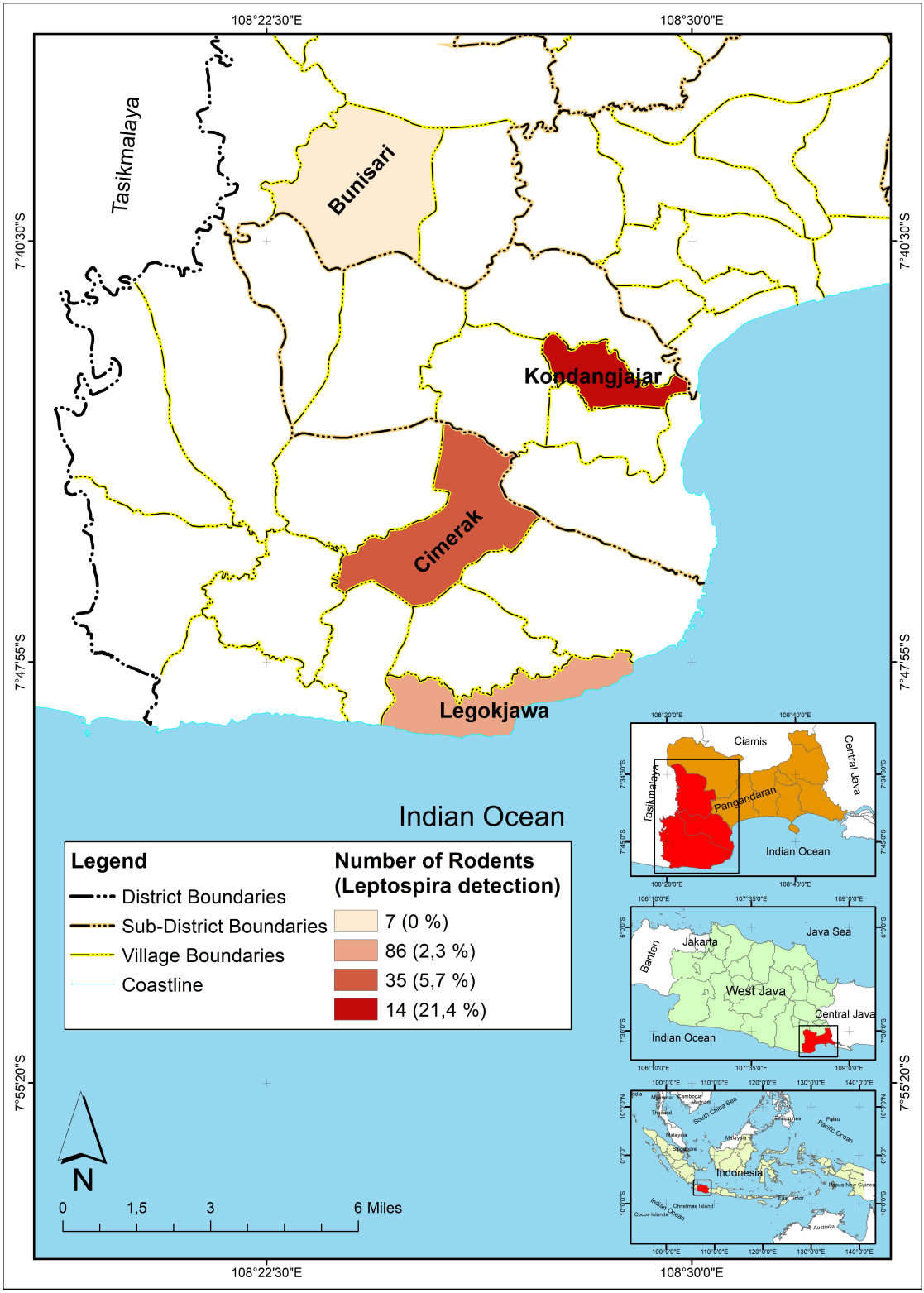


Figure 1

Sample collection sites in Pangandaran

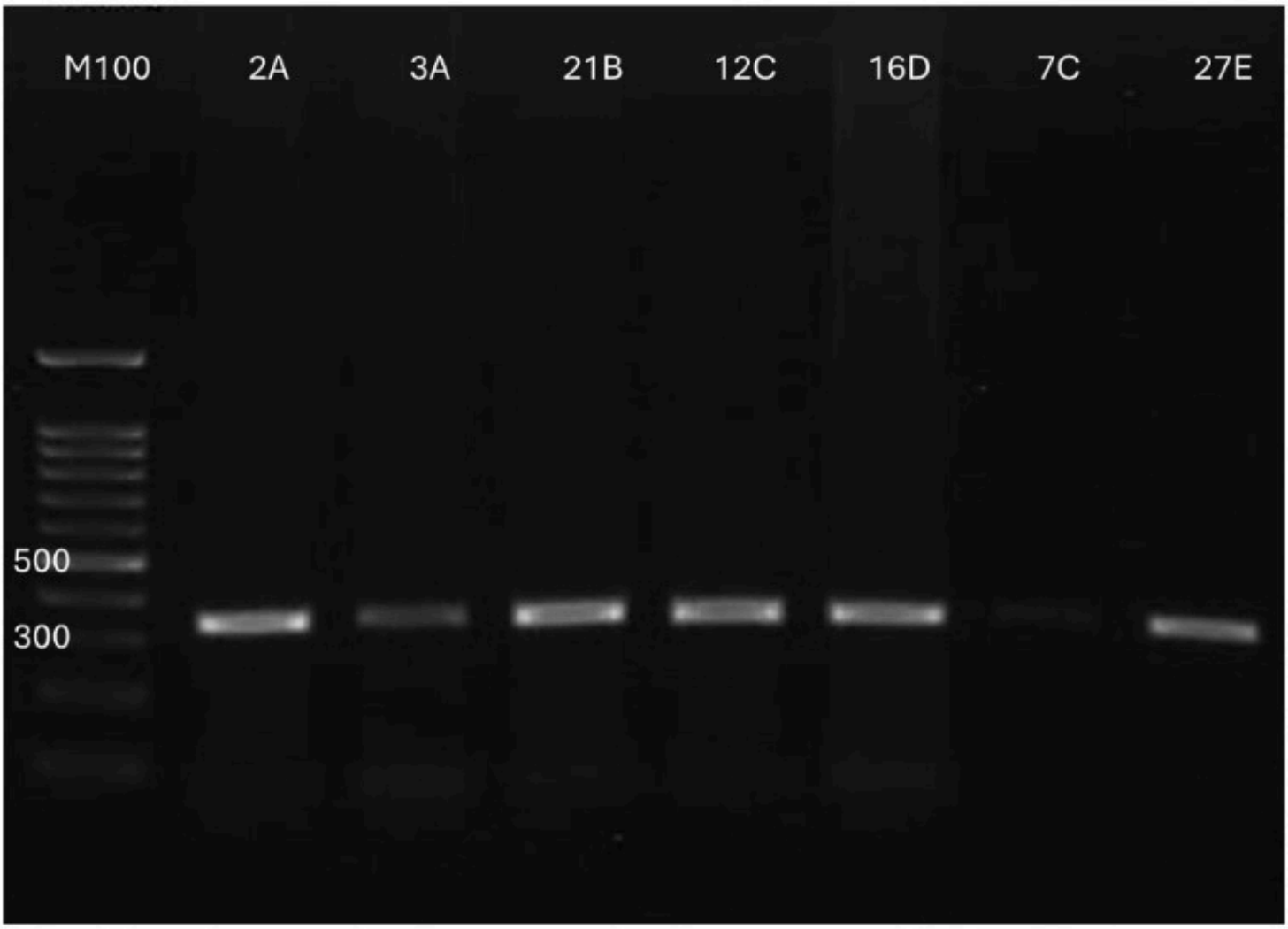


Figure 2

Agarose gel electrophoresis of PCR amplification of the Leptospira 16S rRNA gene. Lane M: 100 bp DNA ladder. Lanes 2A-27E show the seven positive kidney samples with the expected amplicon size (~330 bp)

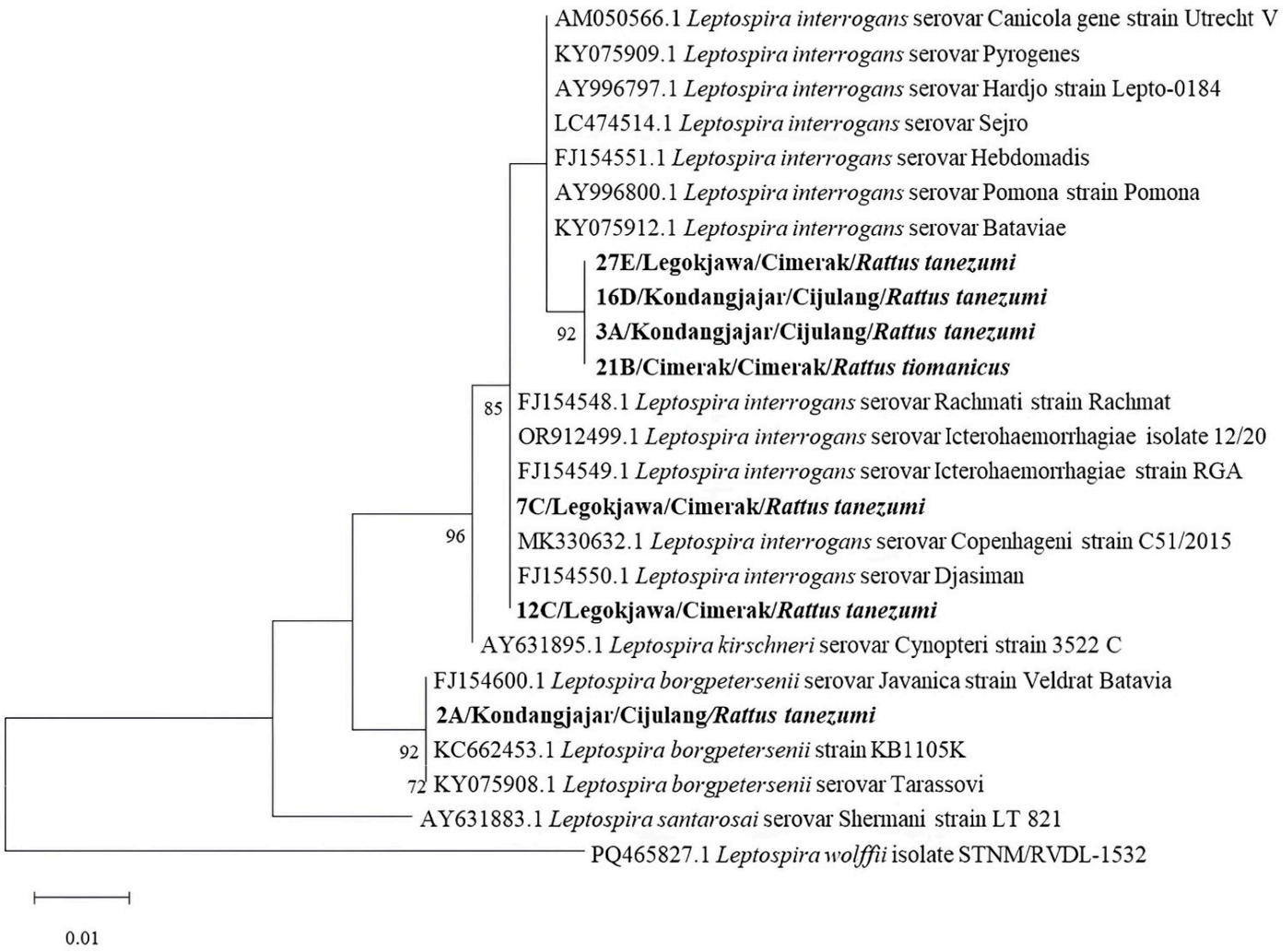


Figure 3

A phylogenetic tree constructed using the maximum likelihood approach based on partial 16S rRNA sequences (260 nucleotides), with 1,000 bootstrap replicates, showing the relationships between the seven positive samples and reference *Leptospira* sequences. Bootstrap values are indicated at the nodes.