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Polymerase chain reaction-driven detection of *Leptospira spp.* in urban rat populations of Surabaya, Indonesia

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ABSTRACT

Objectives: Leptospirosis is a zoonotic disease caused by *Leptospira spp.,* with rodents as the main reservoir. This study aims to detect *Leptospira spp.* in the urban rat population in Surabaya using the polymerase chain reaction (PCR) method and analyze its phylogenetic relationship.
Methods: This laboratory-based surveillance study used a molecular approach to detect *Leptospira spp.* in the urban rat population from Genteng Village, Surabaya using PCR for detection and subsequent phylogenetic analysis of the positive isolates based on the partial *lipL32* gene sequence. A total of 30 rat traps were set up, and 26 rats were successfully captured.
Results: The PCR results showed that 22 of 25 *Rattus norvegicus* (88 %) were positive for *Leptospira spp.*, whereas the single *Rattus tanezumi* and three other *Rattus norvegicus* were negative. With a prevalence rate of 84.6 %, these findings indicate a high risk of leptospirosis transmission in Surabaya. Phylogenetic analysis revealed that *Leptospira* DNA from *Rattus norvegicus* are closely related to *Leptospira interrogans*, a species known to be pathogenic to humans.
Conclusions: These results strengthen the evidence that *Rattus norvegicus* plays a major role as a vector in the spread of leptospirosis in urban environments. Therefore, mitigation efforts, such as rodent population control, improved environmental hygiene, and community education, are needed to reduce the risk of leptospirosis infection.

Introduction

Leptospirosis, a zoonotic disease caused by the pathogenic bacterium *Leptospira spp.*, is a global health threat with the highest incidence in the tropics and subtropics, including Indonesia [1,2]. This organism spreads through the urine of infected animals, especially rodents such as rats (*Rattus spp.*), which act as the main reservoir [3]. In Indonesia, cases of leptospirosis are reported to have increased significantly, with Surabaya as one of the epicenters due to a combination of population density, rapid urbanization, and poor drainage systems [4]. In urban areas of East Java, 34 % of rats carry *Leptospira spp.*, and these bacteria can infect humans through contact with contaminated water or soil [5]. In addition to posing a risk to humans, leptospirosis also affects livestock, which can serve as secondary reservoirs and contribute to transmission. A recent study in Southern Thailand detected *Leptospira* infection in meat goats using molecular approaches, reinforcing the relevance of polymerase chain reaction (PCR)–based surveillance in animal and human populations [6].

Surabaya, as the second largest metropolitan city in Indonesia, faces unique challenges in controlling the rat population. The Surabaya Health Office Report (2022) recorded 217 cases of leptospirosis in humans in the last 3 years, of which 68 % of victims reported direct exposure to environments identified as having high rat infestations. Unplanned urbanization, accumulation of waste, and waterlogging due

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to the long rainy season create ideal conditions for the breeding of rats and the persistence of *Leptospira* [7]. The dominant *Leptospira interrogans* (*serovar Icterohaemorrhagiae* and *Copenhageni*) strain has high pathogenicity to humans [8]. Conventional detection of *Leptospira* through serological tests (e.g. microscopic agglutination test) or bacterial cultures is often not optimal in the field. The *Leptospira* culture requires 4–12 weeks and has a sensitivity of only 30–40 %. Although serological tests such as the microscopic agglutination test are considered standard, they are limited by factors such as timing of sample collection, delayed antibody response, early antibiotic use, limited serogroup panels, and the inability to distinguish current infections from past exposures. PCR has been proven as a fast and specific molecular diagnostic tool for the identification of *Leptospira* DNA in environmental and clinical samples [9]. The *lipL32* gene-based PCR (pathogenicity marker) achieves 95 % sensitivity and 100 % specificity in detecting *Leptospira* in rat kidneys, far surpassing conventional methods [9].

In Indonesia, the use of PCR for *Leptospira* surveillance in rodents is still limited. Rats in Jakarta slums tested positive for *Leptospira* DNA by PCR assay [10]. Surabaya's distinct ecological characteristics such as rainfall patterns, urban layout, and human-rat interactions can influence the dynamics of the spread of this pathogen. The risk of leptospirosis is closely related to rat density and waste management practices—the two critical issues in Surabaya [11,12]. Another challenge is the lack of laboratory capacity, compounded by low community awareness of leptospirosis symptoms and the continued reliance of local health facilities on rapid tests with less than 60 % accuracy [4]. This study aims to detect *Leptospira* spp. in the kidneys of rats from Surabaya using PCR methods targeting the *lipL32* genes. Using this molecular approach, this study will not only identify the prevalence of pathogenic leptospires in local rat populations but also provide important information regarding the potential risk of zoonoses in urban areas of Indonesia. The findings of this study are expected to support leptospirosis prevention and control efforts through rat population control programs and increased public awareness of the importance of environmental sanitation.

Methodology

Design, location, and time of research

This laboratory-based surveillance study used a molecular approach to detect *Leptospira* spp. in urban rat population from Genteng village, Surabaya, using PCR and subsequent phylogenetic analysis of the positive isolates. The research was conducted in Genteng Village, Surabaya, which is an area with a high population density and varied sanitation systems, see Figure 1. Samples were collected during the research period which lasted from January to March 2023.

Sampling of rats

A total of 30 mousetraps were set up at several strategic points, such as traditional markets, waterways, landfills, and densely populated areas. The captured rats were then classified by species, weight, and physical condition before kidney organ sampling was performed.

Identification and sampling process

After the rats were captured, they were humanely sacrificed in accordance with the animal research ethics protocol. Kidney organs were taken under aseptic conditions and stored in microcentrifuge tubes containing RNAlater solution to prevent bacterial DNA degradation. The samples were then stored at −20 °C before DNA extraction.

DNA extraction

DNA extraction was carried out using a commercial kit according to the protocol recommended by the manufacturer. The kidney sample was homogenized, then DNA was extracted through the stages of lysis, purification, and elution using a special buffer. The concentration and purity of the DNA obtained were analyzed using a NanoDrop spectrophotometer.

Polymerase chain reaction

DNA amplification was performed using specific primers for the *lipL32* gene, which is a specific marker for *Leptospira spp.* The PCR process was carried out in a total reaction of 25 µl consisting of 12.5 µl PCR Master Mix (New England Biolabs, NEB), 1 µl forward primer (10 µM; sequence: 5′-AAG CAT TAC CGC TTG TGG TG-3′), 1 µl reverse primer (10 µM; sequence: 5′-GAA CTC CCA TTT CAG CGA TT-3′), 5 µl DNA template, and 5.5 µl nuclease-free water. The PCR cycle included initial denaturation: 95 °C for 5 min, denaturation: 95 °C for 30 s, annealing: 55 °C for 30 s, elongation: 72°C for 45 s, final elongation: 72 °C for 5 min, and the number of cycles: 35 cycles.

DNA samples were subjected to PCR amplification targeting the *lipL32* gene, which is conserved among pathogenic *Leptospira* species. The primers used were those described by Stoddard et al. [13], which amplify a 423 bp fragment specific to *lipL32*. PCR conditions followed the optimized protocol, with a final reaction volume of 25 µl [13]. To validate assay specificity, sequences from known pathogenic reference strains, including *Leptospira interrogans serovar* Icterohaemorrhagiae and *Leptospira borgpetersenii*, were included in the phylogenetic analysis and aligned with positive sample sequences. These reference sequences were retrieved from GenBank. The analytical sensitivity of the *lipL32* assay, based on Stoddard et al. [13], is reported at 10 genome equivalents per reaction, ensuring high sensitivity for detecting pathogenic strains without cross-reactivity with intermediate or saprophytic species.

Agarose gel electrophoresis

The DNA amplification result was analyzed using 1.5 % agarose gel electrophoresis with GelRed dye. The sample was then run at a voltage of 100 V for 45 min and visualized using a ultraviolet transilluminator.

Data analysis

The PCR data were analyzed descriptively by calculating the prevalence of *Leptospira spp.* infection in the rat population. The prevalence was calculated as the ratio of the number of positive samples to the total number of samples tested, then expressed as a percentage. In addition, to determine the phylogenetic relationship of the *Leptospira spp.* isolates found, DNA sequencing of positive PCR products was performed using the Sanger sequencing method. PCR products were purified using a commercially available purification kit and sequenced in forward and reverse directions. The sequencing reactions were carried out with the BigDye Terminator v3.1 Cycle Sequencing Kit, and the resulting sequences were analyzed using an ABI 3130xl Genetic Analyzer. The sequencing results were compared with the GenBank database using the BLAST software. The phylogenetic tree was constructed using the neighbor-joining method with bootstrap 1000 replicates using MEGA X software.

Ethical of research

This study was approved by the Ethics Committee of the Laboratory of Animal Biochemistry, Faculty of Medicine, Universitas Airlangga and had passed the ethical test number 655/HRECC.FODM/VIII/2022. All procedures carried out in this study have been in accordance with the

Figure 1. Sampling location in Surabaya City, Indonesia.

principles of animal welfare and international guidelines in laboratory animal research.

Result

Prevalence of Leptospira spp. in Surabaya, Indonesia urban rat populations

Table 1 shows the results of the presence of *Leptospira spp.* bacteria in the urban rat population in Surabaya using the PCR method and the prevalence rate of infection. Of the total of 30 traps set up in various high-risk locations (dense settlements, traditional markets, and waterways), 26 rats were caught, consisting of 25 (96.2 %) *Rattus norvegicus* and one (3.8 %) *Rattus tanezumi.* These results show the dominance of *R. norvegicus* as an urban rat species in Surabaya, which is in line with its characteristics as an invasive species that is adaptive in densely populated environments [14]. Of the 26 rat kidney samples analyzed, 22 (84.6 %) samples tested positive for *Leptospira spp.* DNA through *lipL32* gene amplification using PCR. All positive results came from *R. norvegicus*, whereas three *R. norvegicus* and one *R. tanezumi* showed negative results (Figure 2). The prevalence of infection in *R. norvegicus* reached 88 % (22 of 25), much higher than the global prevalence which generally ranges from 30 % to 60 % in urban rats [2]. This indicates that Surabaya has a significant risk of contracting leptospirosis through contact with rats or environments contaminated with rat urine.

Table 1
Prevalence and detection results of *Leptospira spp.* in Surabaya, Indonesia urban rat populations.

Parameters	Amount	Description
Total mousetraps	30	Number of traps installed
Number of rats caught	26	The rat that was successfully caught
Rat species		
Rattus norvegicus	25	The majority of species found
Rattus tanezumi	1	Minority species found
Detection result *Leptospira spp.*		
Positive *Leptospira spp.* rat	22	All come from *Rattus norvegicus*
Negative *Leptospira spp.* rat	4	*Rattus norvegicus* (3), *Rattus tanezumi* (1)
Prevalence rate	84.6 %	The risk of contracting leptospirosis is high

The dominance of *R. norvegicus* as the main reservoir of *Leptospira spp.* is thought to be related to its habitat close to humans, such as dirty waterways and waste piles, which are ideal media for bacterial growth.

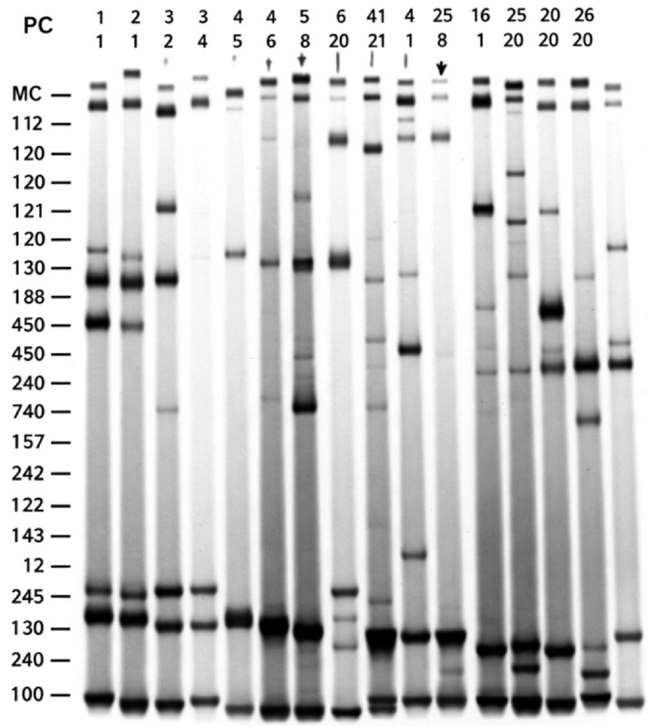

Figure 2. Polymerase chain reaction of *Leptospira spp.*

In addition, the negative results for *R. tanezumi* may be due to sample limitations (only one animal) or differences in ecological factors, such as habitat preferences that are more isolated from human activity. Positive PCR results in 84.6 % of samples also indicate that most rats in Surabaya are infected with *Leptospira spp*. Therefore, further phylogenetic analysis is needed to identify the dominant strain and its potential association with leptospirosis cases in humans.

Phylogenetic analysis of Leptospira spp. in Surabaya, Indonesia urban rat populations

The phylogenetic tree shown in Figure 3 shows the evolutionary relationship between *Leptospira* isolates found in sewer rats (*Rattus norvegicus*) from various samples, as well as several reference *Leptospira* species. This tree was constructed based on the sequence analysis of the *lipL32* gene, a commonly used marker in bacterial phylogenetic studies. The tree illustrates the clustering of isolates based on their genetic similarity, providing insights into their phylogenetic relationships. The phylogenetic analysis shows that most of the *Rattus norvegicus* samples that tested positive for *Leptospira* tend to cluster together, indicating horizontal transmission of *Leptospira* between individuals in the rat population. Some sequences derived from rat samples clustered within the pathogenic *Leptospira* clade, forming distinct branches in proximity to reference strains of *Leptospira interrogans* and *Leptospira borgpetersenii*. Although these species are phylogenetically distinct, the grouping indicates that the detected strains are likely pathogenic and share evolutionary lineage characteristics with these reference species. This indicates that *Rattus norvegicus* can act as a reservoir for these pathogenic *Leptospira* species.

The finding of 22 of 25 *Rattus norvegicus* samples positive for *Leptospira spp*. indicates a high prevalence (88 %) in this rat population. This supports the hypothesis that *Rattus norvegicus* is the main reservoir of leptospirosis in urban environments. This could be caused by differences in *Leptospira* strains, differences in detection methods, or other factors that affect test results, such as low bacterial load in the tissue, sample degradation, infection at an early stage, or the presence of PCR inhibitors. Although one sample of *Rattus tanezumi* was negative for *Leptospira spp.*, indicating a potential variability in susceptibility, the small sample size of this species limits our ability to draw definitive conclusions regarding its susceptibility to *Leptospira* infection.

Discussion

The high prevalence (84.6 %) of *Leptospira spp.* in urban rats in Surabaya is a critical indicator of the risk of zoonosis in this region. This figure far exceeds global reports on urban rats (30–60 %) and similar studies in Manila but is in line with findings in Jakarta (92 %) [15–17]. Environmental factors such as poor sanitation, waterlogging, and suboptimal waste management are thought to be the main triggers [18]. These conditions create an ideal habitat for rats and *Leptospira* bacteria, which can survive for up to a month in a damp environment [19]. The dominance of *Rattus norvegicus* as the main reservoir (88 % positive) is consistent with its role as an invasive species that is adaptive in urban environments [20]. *R. norvegicus* accounts of human leptospirosis cases through contact with contaminated water [21,22]. In Surabaya, intense human-rat interactions in dense settlements and traditional markets increase the risk of transmission. Although *Rattus tanezumi* has been identified as a *Leptospira* reservoir in previous studies from Thailand, the single negative result in this study should be interpreted with caution. Its preference for more closed habitats (e.g. secondary forests) may reduce exposure in urban areas [23,24]. However, this remains speculative due to the limited sample size (n = 1), and further sampling is needed to clarify its epidemiologic role in this setting.

Leptospirosis is a zoonotic disease that can cause symptoms ranging from mild to severe, including kidney failure and bleeding. Workers who frequently interact with contaminated environments, such as cleaners and drainage workers, are at high risk of exposure. A prevalence of > 80 % in rats also has the potential to trigger leptospirosis outbreaks, especially in flood-prone areas. There is a significant correlation between the prevalence of *Leptospira* in rats and the incidence of human cases [25,26]. Leptospirosis is a significant health problem in Southeast Asia, especially in areas with poor sanitation [27]. In Surabaya, the lack of public awareness about leptospirosis prevention and limited health facilities can make the situation worse. One health approach integrating rodent control, community education, and environmental surveillance is recommended [28]. Integrated surveillance framework consisting of (1) rodent surveillance using community reporting and trapping in high-risk urban zones, (2) environmental monitoring of standing water and flood-prone areas for *Leptospira* detection, and (3) syndromic surveillance in health care centers to identify early warning signals of human leptospirosis cases. Intersectoral coordination among the municipal health office, waste management authority, academic researchers, and local communities is vital for timely interventions.

Phylogenetic analysis of *Leptospira* isolates from *Rattus norvegicus* shows an interesting clustering pattern, providing important information on the epidemiology and evolution of this bacterium. The resulting phylogenetic tree indicates genetic diversity among *Leptospira* isolates circulating in the rat population. Most of the positive *Leptospira* isolates from *Rattus norvegicus* are grouped together, indicating active horizontal transmission among the rat population [29]. This clustering is in line with previous research by Costa et al., showing that rats play an important role in the spread of *Leptospira* in urban environments [15]. The phylogenetic proximity between these isolates also suggests the possibility of a common source of infection or a similar transmission route. Interestingly, several *Leptospira* positive isolates from *Rattus norvegicus* clustered within the pathogenic clade and demonstrated phylogenetic similarity to reference strains of either *Leptospira interrogans* or *Leptospira borgpetersenii*, both of which are known pathogenic species and represent distinct species with significant genomic and ecological differences (Figure 3).

This finding confirms the role of *Rattus norvegicus* as an important reservoir for pathogenic *Leptospira* serovars, which can be a source of

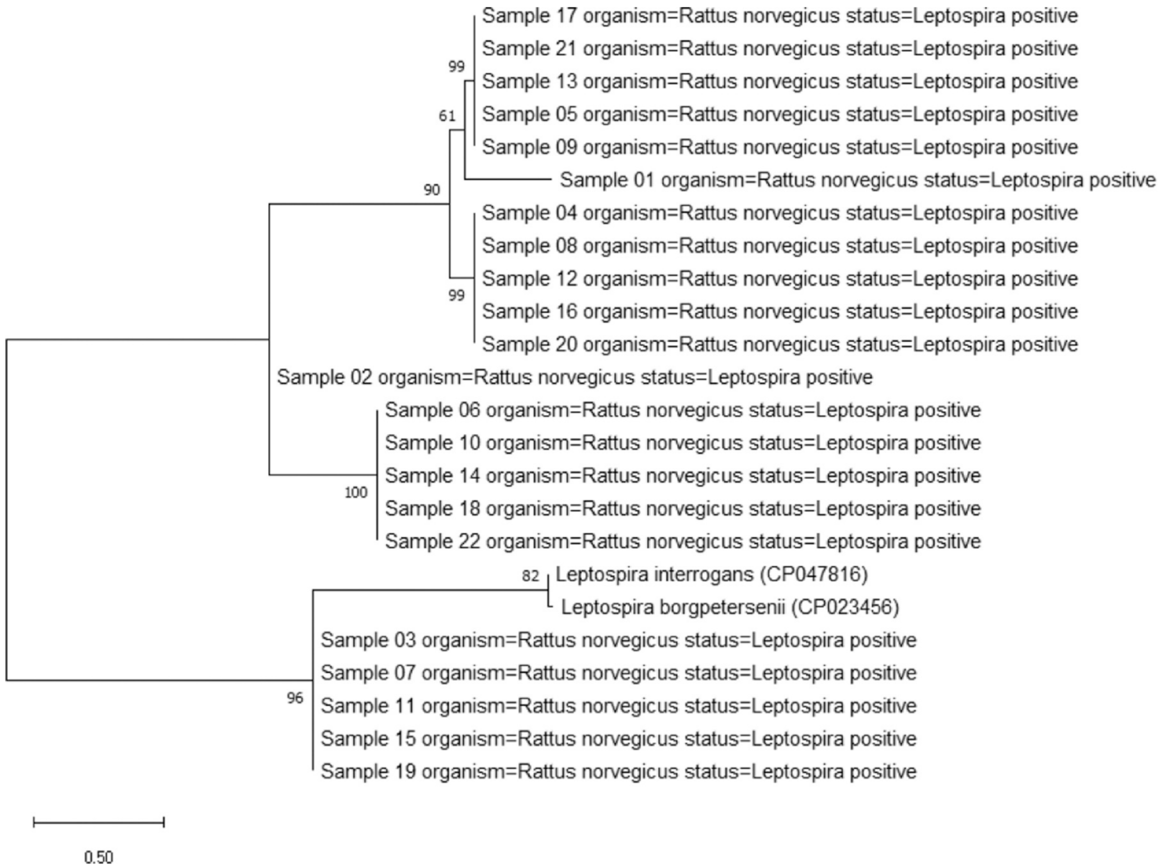

Figure 3. Results of phylogenetic analysis of *Leptospira spp*. in Surabaya, Indonesia urban rat populations.

infection for humans and other animals [30]. Only samples positive for *Leptospira spp.* based on *lipL32* PCR amplification were included in the phylogenetic analysis. *Rattus norvegicus* specimens that tested negative did not yield sufficient target DNA and were, therefore, excluded from tree construction. This can be caused by various factors, such as differences in the detection methods used, geographical variations, or differences in the immunity of individual rats. Although *Rattus tanezumi* was represented by only one subject in the study and tested negative for *Leptospira*, its absence of infection may still suggest possible host-specific differences in susceptibility or exposure compared to other rodent species and further sampling is necessary to draw definitive conclusions [31]. Overall, this phylogenetic analysis provides strong evidence for the genetic diversity of *Leptospira* in *Rattus norvegicus* and its role as a reservoir for pathogenic serovars. A more nuanced understanding of these transmission dynamics is essential for the design of integrated leptospirosis surveillance systems under a One Health framework. Further research should focus on longitudinal surveillance of rodent and environmental samples, along with health data integration, to enable predictive modeling and early outbreak detection.

Conclusion

This study successfully identified a very high (84.6 %) prevalence of *Leptospira spp.* infection in the urban rat population in Surabaya, with *Rattus norvegicus* as the dominant reservoir (88 % positive). These results indicate that urban rats in Surabaya are a potential source of leptospirosis transmission to humans, especially in areas with poor sanitation and high population density. The adaptive dominance of *R. norvegicus* in urban environments, such as waterways and slums, reinforces its role as the main vector of *Leptospira* bacteria. In addition, the negative result for *R. tanezumi* (one sample) may be related to its preference for more closed habitats, although further studies with larger samples are needed for confirmation. Phylogenetic analysis revealed that the detected *Leptospira* DNA sequence shares a genetic relationship with pathogenic strains such as *Leptospira interrogans,* which are widely reported in Southeast Asia [27]. This indicates the potential for the same strain to circulate between rodents and humans, especially in areas with a history of flooding or standing water. The high prevalence in rodents also reflects the risk of uncontrolled environments, such as garbage piles and poor drainage, which support the survival of the bacteria. Numerous studies have identified key risk factors associated with the spread of *Leptospira* among rodent populations and its transmission to humans, further research is warranted to elucidate the relative importance, interactions, and local context-specific dynamics of these factors, particularly, in light of emerging environmental and ecological changes. Research limitations, such as limited geographical scope and small sample size, form the basis for recommendations for further studies using a whole-genome sequencing approach for strain mapping and more in-depth environmental risk analysis. Thus, this study provides a scientific basis for strengthening leptospirosis prevention policies in Indonesia.

Author contributions

EAW worked on data curation, formal analysis, conceptualization, software, writing – original draft. JUR worked on data curation, formal analysis, methodology, software, writing – review & editing. AYPA worked on investigation, methodology, supervision. TSS worked on investigation, formal analysis, methodology.

Ethical approval

This study has been approved by the Ethics Committee of the Laboratory of Animal Biochemistry, Faculty of Medicine, Universitas

Airlangga and has passed the ethical test number 655/HRECC.FODM/VIII/2022.

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Data availability

The corresponding author's data supporting this study findings are available upon reasonable request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] Antima BS, Banerjee S. Understanding the dynamics and drivers of leptospirosis in tropical and subtropical regions: a mathematical and epidemiological approach. Nonlinear Dyn 2025;113:10705–27. https://doi.org/10.1007/s11071-024-10767-9.
[2] Boey K, Shiokawa K, Rajeev S. Leptospira infection in rats: a literature review of global prevalence and distribution. PLoS Negl Trop Dis 2019;13:e0007499. https://doi.org/10.1371/journal.pntd.0007499.
[3] Gomard Y, Dellagi K, Goodman SM, Mavingui P, Tortosa P. Tracking animal reservoirs of pathogenic Leptospira: the right test for the right claim. Trop Med Infect Dis 2021;6:205. https://doi.org/10.3390/tropicalmed6040205.
[4] Gasem MH, Hadi U, Alisjahbana B, Tjitra E, Hapsari MM, Lestari ES, et al. Leptospirosis in Indonesia: diagnostic challenges associated with atypical clinical manifestations and limited laboratory capacity. BMC Infect Dis 2020;20:179. https://doi.org/10.1186/s12879-020-4903-5.
[5] Widayati A, Mulyono AM, Gardjito T, Handayani FD, Mujiyanto M, Hidajat MC, et al. Survey on Leptospira spp infection in rat species in Indonesia. Southeast Asian J Trop Med Public Health 2022;53:695–713.
[6] Chanchayanon B, Nilsuwan P, Petcharat S, Keawchana N, Yaprajan H, Suwannarat P, et al. Molecular detection of Leptospira infection in meat goat of southern, Thailand. Int J Vet Sci 2024;13:772–5.
[7] Rahelinirina S, Bourhy P, Andriamiaramanana F, Garin B, Rajerison M. High prevalence of Leptospira spp. in rodents in an urban setting in Madagascar. Am J Trop Med Hyg 2019;100:1079–81. https://doi.org/10.4269/ajtmh.18-0642.
[8] Arent Z, Gilmore C, Pardyak L, Dubniewicz K, McInerney B, Ellis W. The serological and genetic diversity of the Leptospira interrogans icterohaemorrhagiae serogroup circulating in the UK. J Vet Res 2023;67:529–36. https://doi.org/10.2478/jvetres-2023-0063.
[9] Sreevalsan TV, Chandra R. Relevance of polymerase chain reaction in early diagnosis of leptospirosis. Indian J Crit Care Med 2024;28:290–3. https://doi.org/10.5005/jp-journals-10071-24649.
[10] Susanna D, Nova RIT, Rozek L. The existence and characteristics of rats and shrews in endemic leptospirosis areas and types of ectoparasites: a case study of West Jakarta, Indonesia. F1000Research 2021;10:335. https://doi.org/10.12688/f1000research.47068.1.
[11] Notobroto HB, Mirasa YA, Rahman FS. Sociodemographic, behavioral, and environmental factors associated with the incidence of leptospirosis in highlands of Ponorogo Regency, Province of east Java, Indonesia. Clin Epidemiol Glob Health 2021;12:100911. https://doi.org/10.1016/j.cegh.2021.100911.
[12] Komalaningsih S, Juliansyah R. Identification of rodent species confirmed as the cause of leptospirosis disease, along with ecosystem distribution and environmental factors. J Character Environ 2024;2:69–82. https://doi.org/10.61511/jocae.v2i1.2024.800.
[13] Stoddard RA, Gee JE, Wilkins PP, McCaustland K, Hoffmaster AR. Detection of pathogenic Leptospira spp. through TaqMan polymerase chain reaction targeting the LipL32 gene. Diagn Microbiol Infect Dis 2009;64:247–55. https://doi.org/10.1016/j.diagmicrobio.2009.03.014.
[14] Utama MAH, Suhartono S, Budiyono B. Identification of captured rat species and detection of Leptospira bacteria: study at the gapura Surya Nusantara passenger terminal, Tanjung Perak Port, Surabaya. Maj Kesehat Indones 2023;4:89–94. https://doi.org/10.47679/makein.2023179.
[15] Costa F, Hagan JE, Calcagno J, Kane M, Torgerson P, Martinez-Silveira MS, et al. Global morbidity and mortality of leptospirosis: a systematic review. PLoS Negl Trop Dis 2015;9:e0003898. https://doi.org/10.1371/journal.pntd.0003898.
[16] Villanueva SYAM, Ezoe H, Baterna RA, Yanagihara Y, Muto M, Koizumi N, et al. Serologic and molecular studies of Leptospira and leptospirosis among rats in the Philippines. Am J Trop Med Hyg 2010;82:889–98. https://doi.org/10.4269/ajtmh.2010.09-0711.
[17] Koizumi N, Morita M, Nuradji H, Noor SM, Dharmayanti NI, Randusari P, et al. Comparative genomic analysis of Leptospira spp. isolated from Rattus norvegicus in Indonesia. Infect Genet Evol 2022;102:105306. https://doi.org/10.1016/j.meegid.2022.105306.
[18] Fajriyah SN, Udiyono A, Saraswati LD. Environmental and risk factors of leptospirosis: a spatial analysis in Semarang city. IOP Conf Ser Earth Environ Sci 2017;55:12013. 10.1088/1755-1315/55/1/012013.
[19] Costa F, Ribeiro GS, Felzemburgh RDM, Santos N, Reis RB, Santos AC, et al. Influence of household rat infestation on Leptospira transmission in the urban slum environment. PLoS Negl Trop Dis 2014;8:e3338. https://doi.org/10.1371/journal.pntd.0003338.
[20] Koizumi N, Miura K, Sanai Y, Takemura T, Ung TT, Le TT, et al. Molecular epidemiology of Leptospira interrogans in Rattus norvegicus in Hanoi, Vietnam. Acta Trop 2019;194:204–8. https://doi.org/10.1016/j.actatropica.2019.02.008.
[21] Felzemburgh RDM, Ribeiro GS, Costa F, Reis RB, Hagan JE, Melendez AX, et al. Prospective study of leptospirosis transmission in an urban slum community: role of poor environment in repeated exposures to the Leptospira Agent. PLoS Negl Trop Dis 2014;8:e2927. https://doi.org/10.1371/journal.pntd.0002927.
[22] Soro S, Groud K, Fafournoux A, Monchatre-Leroy E, Kodjo A, Lefebvre S, et al. Dynamics of leptospirosis transmission within Urban Norway rat (Rattus norvegicus) populations in densely populated French areas: implications for public health. Transbound Emerg Dis 2025;2025:3451406. https://doi.org/10.1155/tbed/3451406.
[23] Cosson J-F, Picardeau M, Mielcarek M, Tatard C, Chaval Y, Suputtamongkol Y, et al. Epidemiology of Leptospira transmitted by rodents in Southeast Asia. PLoS Negl Trop Dis 2014;8:e2902. https://doi.org/10.1371/journal.pntd.0002902.
[24] Meerburg BG, Singleton GR, Kijlstra A. Rodent-borne diseases and their risks for public health. Crit Rev Microbiol 2009;35:221–70. https://doi.org/10.1080/10408410902989837.
[25] Baharom M, Ahmad N, Hod R, Ja'afar MH, Arsad FS, Tangang F, et al. Environmental and occupational factors associated with leptospirosis: a systematic review. Heliyon 2024;10:e23473. https://doi.org/10.1016/j.heliyon.2023.e23473.
[26] Desvars-Larrive A, Smith S, Munimanda G, Bourhy P, Waigner T, Odom M, et al. Prevalence and risk factors of Leptospira infection in urban brown rats (Rattus norvegicus), Vienna, Austria. Urban Ecosyst 2020;23:775–84. https://doi.org/10.1007/s11252-020-00957-9.
[27] Douchet L, Goarant C, Mangeas M, Menkes C, Hinjoy S, Herbreteau V. Unraveling the invisible leptospirosis in mainland Southeast Asia and its fate under climate change. Sci Total Environ 2022;832:155018. https://doi.org/10.1016/j.scitotenv.2022.155018.
[28] Hayman DTS, Adisasmito WB, Almuhairi S, Behravesh CB, Bilivogui P, et al. One Health High-Level Expert Panel (OHHLEP). Dev One Health Surveill Syst One Health 2023;17:100617. https://doi.org/10.1016/j.onehlt.2023.100617.
[29] Levett PN. Leptospirosis. Clin Microbiol Rev 2001;14:296–326. https://doi.org/10.1128/CMR.14.2.296-326.2001.
[30] Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, Lovett MA, et al. Leptospirosis: a zoonotic disease of global importance. Lancet Infect Dis 2003;3:757–71. https://doi.org/10.1016/S1473-3099(03)00830-2.
[31] Lau CL, Smythe LD, Craig SB, Weinstein P. Climate change, flooding, urbanisation and leptospirosis: fuelling the fire? Trans R Soc Trop Med Hyg 2010;104:631–8. https://doi.org/10.1016/j.trstmh.2010.07.002.