Insights in Public Health Journal

https://doi.org/10.20884/1.iphj.2021.2.1.4979

Detection of Pathogenic Leptospires in Rat and Shallow Populations and Its Spatial Distribution in Bakaran Kulon Village, Pati District

Dyah Widiastuti.[1*], Nova Pramestuti.[1], Zumrotus Sholichah.[1], Endang Setiani.[1], Raden Ludhang P. Rizki.[2]

[1] Banjarnegara-Health Research and Development, Central Java, Indonesia
[2] Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia

Corresponding Author: Dyah Widiastuti. Jl. Selamanik no 16 A Banjarnegara. E-mail: dyahwidi1981@gmail.com Phone: +6285291221842

ABSTRACT

Background In tropical areas, Leptospirosis is still considered a public health problem, particularly in the event of heavy rainfall and flooding. Transmission of leptospira bacteria from infected animals, such as rats, takes place through the urine or blood. Different serovar leptospires can be found in different reservoir animals in the field. However, research on the presence of pathogenic leptospires in rats in Pati Regency, one of the areas with leptospirosis cases in Central Java, is very limited.
Objectives: The purpose of this research is to identify Leptospira characteristics. Spp in rat and its distribution in Pati Regency.
Methods: Rat capture was carried out in Bakaran Kulon Village in Pati Regency. Detection of pathogenic leptospires was carried out using the ropB gene and followed by phylogenetic analysis. As additional details, a buffer analysis was carried out to measure the distance between the positions of cases of leptospira and positive rats positions.
Results: Pathogenic were found in 11.76% (2/17) of rats and shallows in the area of study. Based on DNA leptospires sequencing, Leptospira found in Bakaran Kulon Village Pati Regency were *Leptospira interrogans* serovar Bataviae and *Leptospira borgpetersenii* serovar Ballum, both of which are pathogenic. Based on a buffer analysis, Leptospira-positive rat was found around cases of leptospirosis at distances of 30 and 60 m.
Conclusion: This study shows that *Leptospira spp*. in rats found around the location of leptospirosis cases, which indicates that rats have great potential as transmitters of leptospirosis to humans.

Keywords: ***Leptospira*, pathogenic, rat**

INTRODUCTION

Leptospirosis is an important infectious disease caused by bacteria, found mainly in areas affected by floods. This zoonotic disease is often considered a neglected disease, although the cases continue to occur in various areas of the world. It is estimated that in different areas of the world there are 873,000 cases of leptospirosis, with 48,600 deaths (1). In 2018, 895 cases of leptospirosis were reported in humans with a Case Fatality Rate (CFR) of 17.8 percent in Indonesia. Nevertheless, this number of cases may be underestimated in Indonesia because there are still obstacles in the detection of this disease in various regions (2). Leptospirosis is caused by leptospires, which are spiral-shaped bacteria of the Leptospiraceae family and the Leptospira genus. These bacteria are long, small, and motile spirochetes that can be either free to live in the environment or found as parasites in animal hosts.

These bacteria, which are around 6-20 μm long, have anchor-shaped ends. Leptospira are part of the Spirochaeta group and have motile movements. Motility in Spira can occur due to the presence of two periplasmic flagella organelles. Pathogenic leptospires have more active motility than saprophytic leptospires. This is since saprophytic leptospires do not have endophlagella as in pathogenic leptospires. Leptospires require a moist environment for their survival. These bacteria can survive in contaminated water environments such as lakes and salt water environments for several hours (3). The occurrence of leptospirosis is often associated with several natural disasters such as floods and storms in tropical regions (4).

Transmission of this disease generally occurs from animals infected with the leptospira bacteria through the soil, water contaminated with bacteria, or urine. Hence this disease, referring to the mode of transmission, is a risk to certain occupations that often come into contact with the source of infection (5). The spread of this disease occurs through direct contact with infected hosts, usually through soil, water, or urine contaminated with bacteria. Risk factors for this disease are often associated with certain occupations such as farmers, veterinarians, butchers, sewers, slaughterhouse workers, and others. The clinical manifestations of leptospirosis are often asymptomatic or begin with flu-like symptoms. The non-specific symptoms of this disease pose a challenge in the diagnosis of Leptospirosis. Several patients develop more severe clinical manifestations such as jaundice, bleeding, and acute renal failure and severe pulmonary haemorrhagia (6).

The transmission of this disease is correlated with the role of rats as the source of bacterial infection with Leptospira. The source of infection is rat urine that can last for a long period when secreted into the environment, and it can be a source of transmission to susceptible humans. Such bacteria enter the body as open sores through the skin, dry skin, or the body's mucous lining (such as the eyes, nose, or mouth). Typically humans may develop leptospirosis when exposed to floods where the water has been polluted with the urine of leptospira-infected animals(7). Bacteria of the Leptospira genus have a very diverse population, consisting of thousands of serovars and genetic forms living in various habitat types. Some strains selectively pick other hosts in this genus where they can live. The genus Leptospira consists of two species, the pathogenic ones: *Leptospira interrogans* and *Leptospira biflexa* as the saprophytic species.

Based on molecular methods, leptospira bacteria are classified into 17 groups of genomospecies. Based on this classification, pathogenic leptospires are divided into 8 species namely *Leptospira borgpetersenii, Leptospira weilii, L. interrogans sensu stricto*, *Leptospira noguchii*, *Leptospira santarosai*, *Leptospira alexanderi, Leptospira kirschneri* (formerly known as *Leptospira alstoni*) and Leptospira genospesies. The grouping determination of these species is based on phylogenetic analysis of the rrs gene encoding the 16S rRNA gene. However, there are still limitations in distinguishing one species from another species from this rrs gene because there is no high level of polymorphism in that gene, even though the complete gene is used. The classification of Leptospira bacteria based on their genetic variation can support information from epidemiological research. The rpoB gene is considered useful for differentiating bacterial species, including the population of spirochaetes (8, 9).

Data on the epidemiology of leptospirosis in Indonesia is still minimal, as this disease is still overlooked. Work on the epidemiology of leptospirosis is very important for a greater understanding of this disease and to allow effective prevention efforts. Pati Regency is one of the districts that have leptospirosis problems in Central Java. Since 2010, 11 cases of

leptospirosis have increased to 22 cases in 2011 and two of them have died. There was a drop in 2 cases in 2012 and a further increase to 14 cases in 2013 and one of them died. On 19 January 2014, a major flood began in the district of Pati, flooding 15 sub-districts and 152 villages. After the major flood, the cases of leptospirosis rose significantly. Twenty-seven cases of leptospirosis were reported until 3 March 2014 and eight of them died. Most cases of leptospirosis occurred at the location of the former flood (75.7%). Cases of leptospirosis found in the Pati District during January-February 2014 have been distributed in eleven districts. The majority of cases were located in the Juwana District, 7 cases. Data on cases of leptospirosis are secondary data collected from the local health office.

The presence of persistent cases of leptospirosis in Pati Regency requires in-depth and systematic research. Epidemiological studies related to Leptospirosis are important in Pati Regency. This research aims to classify the serovar of Leptospira bacteria in the Pati Regency rat population using a genetic relationship analysis based on polymorphism of rpoB gene and to explain the spatial overview of positive Pati Regency leptospira rats. We use Geographic Information System (GIS) methods, to visualize the epidemiological data. Using GIS, the pattern of the disease and the source of its transmission can be described in such a way that efforts to control the transmission chain can be made appropriately. The results of this study can provide information on the actual risks associated with the transmission of leptospirosis in the study area.

METHODS

Design and Location of Study

This is a descriptive study located in Bakaran Kulon Village, Pati Regency. The selection of survey locations is based on the location of the most leptospirosis cases found in the District of Juwana. Rat capture was carried out in Bakaran Kulon Village, Juwana District, Pati Regency in March 2014. Traps are designed for capturing rats in residential areas near positive cases of leptospirosis. This work was received permission from the local village government and also obtained ethical approval from the ethics committee of the Ministry of Health.

Rat Catching and Collection of Kidney Organ Samples

In Bakaran Kulon Village, a total of 130 single live traps were installed 3 days, laying traps inside and outside the house around the latest cases of leptospirosis. We took the kidney from the captured rat and put it in a tissue lysis buffer that was mounted in the tube of a microsenter. The kidney sample is processed at 40C before the detection process for Leptospira is performed.

DNA Isolation

DNA isolation was conducted for sample purification. A Genomic DNA Mini Kit (Tissue) reagent is used to isolate DNA. The phases are performed according to the kit's prescribed procedure.

PCR (Polymerase Chain Reaction) process

The steps of PCR examination was carried out on DNA samples using Go Taq Green Master Mix (Promega) with the pair of primers as follows: rpoB-F-CCTCATGGGTTCCAACATGCA and rpoB-R-CGCATCCTCRAAGTTGTAWCCTT. The details of the PCR reaction are as follows: pre-denaturation of 94 ° C for 2 minutes, followed by 40 amplification cycles consisting of denaturation at 94 ° C for 30 seconds, annealing step at 55°C for 1 minute. Then extension step at 72°C for 1 minute followed by a final extension for 20 minutes at 72°C. The identification of the PCR results was carried out by electrophoresis using agarose 1.5% at 100 volts for 15 minutes. Visualization of specific DNA is carried out using a UV transilluminator.

Nucleotide Sequencing

The rpoB gene PCR product was purified using a DNA Fragments Extraction Kit (Product of Geneaid) Gel/ PCR according to the procedure recommended by the kit. The sequencing reaction uses primers used in the previous PCR process. The product of PCR and primer are then sent to the sequencing service provider (1st base) to determine the nucleotide base sequence. Phylogenetic trees are arranged based on DNA sequences of the rpoB gene by including reference sequences based on neighbor-joining using the Phylogeny.fr program.

RESULTS

The catching rat findings showed that the rat population in Bakaran Kulon Village is of medium density. It is based on the village trap's 4.36 percent success rate. Figure 1 shows the distribution of rats and shrew species present in the village of Bakaran Kulon

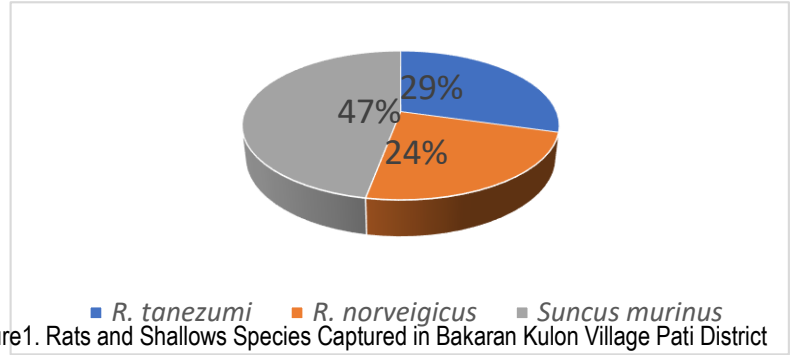

Figure1. Rats and Shallows Species Captured in Bakaran Kulon Village Pati District

Figure 1 shows that two rat species were found in Bakaran Kulon Village, namely *R.tanezumi* and *R.norvegicus* and one insectivorous species, namely *Suncus murinus*. The rat species caught in the village showed a relatively balanced composition of *R tanezumi* and *R. norvegicus*. PCR analysis examined a total of 17 rat kidney samples to detect the presence of Leptospira DNA and 2 of these showed positive results for the rpoB gene. Positive PCR electrophoresis findings are shown in Figure 2.

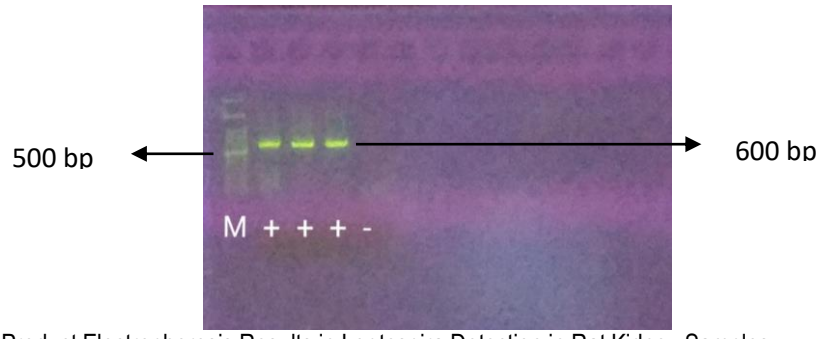

Figure 2. PCR Product Electrophoresis Results in Leptospira Detection in Rat Kidney Samples

The electrophoresis of positive PCR samples is shown in Figure 2. Lane 1 contains sample 21 PCR products, lane 2 contains PCR products for 37, lane 3 contains positive control of *Leptospira interrogans* serovar Djasiman culture, and lane 4 contains negative control of *Eschericia coli* bacterial culture. Two pools of kidney samples were obtained from the *R norvegicus* and S murinus each of 1 tub sample which provided

positive results for the rpoB gene. It suggests that these rats and insectivores have leptospires in their bodies. The full results are presented in Table 1.

Table 1. Leptospira positive samples based on Leptospira rpoB gene detection

No.	Number of isolat	Species	PCR results
1.	21	*R. norveigicus*	Positive
2.	37	*Suncus murinus*	Positive

Phylogenetic trees based on partial sequences of the rpoB gene nucleotide reveal clustering built by bootstrapping using the neighbor-joining process in MEGA6 (Figure 3)

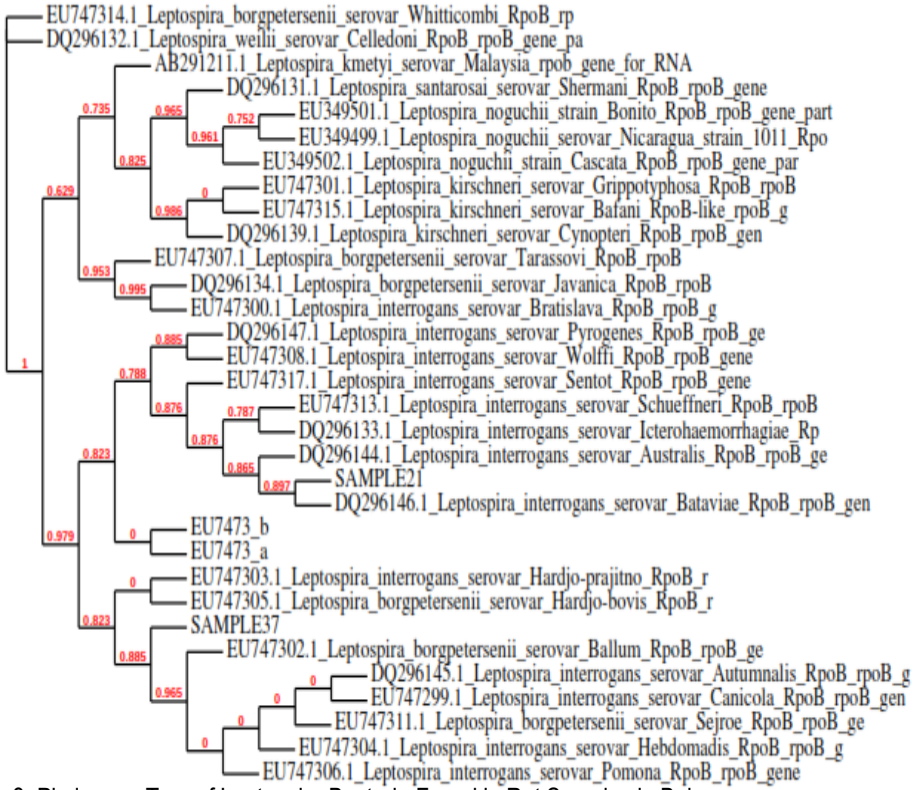

Figure 3. Phylogeny Tree of Leptospira Bacteria Found in Rat Samples in Bakaran Kulon Village Pati District

Phylogenetic work showed that 1 strain of *Leptospira borgpetersenii* serovar ballum and 1 strain of *Leptospira interrogans* serovar bataviae were of the 2 strains of Leptospira bacteria found in the rat population.

The mapping results (Figure 3) show that the distance between the location of the detection of positive Leptospira-containing rat in Bakaran Kulon Village was relatively close to the location of patients with leptospirosis.

Legend :

- *R. norvegicus* dan *S. murinus* with rpoB positive
- negative rats
- rat roaming buffer 30 meters and 60 meters

Figure 4. Spatial Distribution of Positive Leptospires in Bakaran Kulon Village

DISCUSSION

In this study, the detection of Leptospira was performed only in rats and shallows, while the detection of Leptospira in the environment (samples of soil and water) was not carried out. This is based on the fact that *L. Interrogans* can not replicate in the environment after being excreted from an animal source, meaning that, from an epidemiological viewpoint, the environment can not function as a Leptospira source. Therefore, while the environment plays an important factor in the dissemination of pathogenic leptospires, it can not function alone in preserving the continuity of the transmission process of leptospirosis (6). The results of the rat capture showed that the Trap Performance Index in the Bakaran Kulon Village showed a not too high figure (4.36%) consisting of *S. Murinus* (47%), *R. Tanezumi* (29%) and *R. Norveigicus* (24% of the population). This shows that the incidence of cases of leptospirosis is not only linked to the high population density of rats in the region. The rats caught in the area of study are dominated by the *Rattus tanezumi*. This is in line with several studies carried out in the Central Java Province, Demak, and Semarang City. Those studies reported that the dominant rat species found in the capture of rats around the settlement was *R. tanezumi* (9, 10). *Rattus tanezumi* is a commensal rat in Southeast Asia and East Asia. *Rattus tanezumi* is often found in indoor and outdoor habitats. In outdoor habitats, *R. tanezumi* is the dominant pest for rice fields, which often resulting in crop damage. Once the harvest season is over, *R. tanezumi* will migrate to the neighborhood to look for food sources. Because of its vast habitat, *Rattus tanezumi* has the opportunity to carry a variety of pathogens, including zoonotic agents including Leptospira (11).

Based on the PCR analysis by the detection of the rpoB gene, it has been shown that the organisms

with positive results containing pathogenic Leptospira are *R.norveigicus* and *S murinus*. Previous research stated that Leptospira infection in rats was influenced by species and age of rat. The species most commonly infected with Leptospira were R. *tanezumi* and *R. Norvegicus*. It is indicating the *R. Norveigicus* may serve as a source of Leptospira infection and spread leptospires to humans and the surrounding environment. The possibility of humans being exposed to Leptospira from *R. norveigicus* is even greater because of the habitat of these rats that are close to the human environment. The analysis using the rpoB segment could be used to conduct screening in identifying new isolates of the Leptospira bacteria using similarities to identified species. This technique can be applied to various types of samples both from clinical and environmental samples (12).

Based on phylogenetic trees, it is seen that from the two positive samples, one of them has a genetic relationship that is quite close genetically to *Leptospira borgpetersenii* serovar Ballum and the other has a genetically close relative to *Leptospira interrogans* serovar bataviae. The results of this study differ from those conducted in Boyolali, where the pathogenic Leptospira bacteria found in rats mostly show a close relationship with *Leptospira borgpetersenii* serogrup Sejroe. Epidemiologically, *Leptospira borgpetersenii* serogroup Sejroe is frequently correlated with the study of the bovine population (13) and Boyolali is a district that is a center for dairy farming. The *Leptospira borgpetersenii* serovar Ballum was not considered to have a special relationship with certain ecosystems or other contagious animal organisms. Whereas *Leptospira interrogans* serviaar Bataviae have been reported to be found frequently in domestic rat populations (14).

The spatial distribution shows that the location of rats that positively contain Leptospira bacteria in Bakaran Kulon Village is near the most recent cases of leptospirosis in the area. The results of the spatial analysis showed that there was one positive shear within a distance of fewer than 60 m from leptospirosis cases. Also, leptospira-positive rats (*R. norveigicus*) were detected in 30 m cases of leptospirosis. The location of cases of leptospirosis in both villages is within the range of the region of normal daily activity of the rat. The average distance of daily operation of rats with a lot of feed is 30 m and not more than 200 m, while the average distance that can be traveled by *Rattus norveigicus* was 33.7 m (15). The findings of this research suggest the likelihood of a role for rats with leptopsira in the transmission of leptospirosis. Evidence on positive rats by the molecular method should also be considered a significant finding because the presence of rodents does not directly mean the risk of disease or the transmission of bacteria to the environment. Recent research suggests that rat detection can be used as an early indicator of human exposure risk to Leptospirosis, especially in urban areas with low incomes (16). Many studies have also done the use of GIS to imagine leptospirosis-related epidemiology. GIS should have a detailed and practical way of clearly visualizing trends of communicable disease transmission and identifying the spatial distribution and early warning of epidemic outbreaks (17, 18).

The drawback of this work lies in the time of catching a mouse whose laq is quite long since the case was detected. Catching rats in this study was conducted approximately 4 weeks after leptospirosis was observed. A long duration of mouse capture from the time of case finding increases the risk of bias in the assessment of leptospirosis in infected animals. It is possible because the contagious rat can migrate to other locations. Catching rat in this study was conducted approximately 4 weeks after leptospirosis cases were reported. While it can not be established that the source of infection of dead leptospirosis cases arose from positive rat found in

this study, these results could be an awareness about the potency of the rat as a source of transmission.

The advantage of this study is that it uses a valid method that is reliable in detecting Leptospira bacteria that are using PCR. The PCR method can be performed at different locations of the Leptospira bacterial genome to make this process a standard tool. Postponement in leptospirosis diagnosis can lead to more severity which is likely to lead to death. Therefore traditional approaches such as cultivation or microscopic agglutination test (MAT) need a live culture of Leptospira bacteria with high biohazard rates. The PCR method has the benefit that it can detect leptospira bacteria from various samples (19). The strength of this research is that the use of genes specific to identify pathogenic leptospires and has a high variation between serovars. Besides, the results of PCR detection could also determine the detected serovar in this study. When treating this disease, the rapid diagnosis of leptospirosis is very critical. This is because the late diagnosis can cause several complications, such as pancreatic inflammation, cerebral hemorrhage, pulmonary bleeding, and other complications requiring intensive therapy. Another advantage of this study is that it uses GIS and conducts phylogenetic studies to complement research results related to Leptospira detection.

The findings of this study indicate the importance of performing epidemiological surveillance in the region to assess the level of risk in the region. Doing a rat density survey on its own is not enough to see the extent of risk of leptospirosis. Since the number of rats without having information about positive leptospira bacteria can not provide data on the actual level of risk in the field. Rat control measures are also successful by reducing the rat population in a variety of ways, such as the use of rodenticides, and also by reducing the existence of an ecosystem that may increase the risk of leptospirosis transmission. Avoid standing water, and water from agricultural runoff, and minimize animal contamination in food or garbage.

CONCLUSION

Two positive samples of the rpoB gene from *R norvegicus* and *S murinus* were identified from the rat samples teste. Of the two positive samples, one showed the closest relationship with *Leptospira borgpetersenii* serovar Ballum and the other showed the closest relationship with *Leptospira interrogans* serovar bataviae. Leptospira-positive rats were in the home range of rats (30 m and 60 m). The location of positive rat containing Leptospira bacteria collects around leptospirosis sufferers, and clustered leptospirosis sufferers. Detection of leptospires using the molecular PCR method is useful in determining the extent of risk of leptospirosis. The use of this method is very good for routine surveillance of the prevention of leptospirosis, particularly in endemic areas.

ACKNOWLEDGMENT

We acknowledge Banjarnegara Health Research and Development for funding this research. We thank the laboratory staff who help during data collection in the area of study

REFERENCES

1. Karpagam KB, Ganesh B. Leptospirosis: a Neglected Tropical Zoonotic Infection of Public Health Importance—an Updated Review. European Journal of Clinical Microbiology & Infectious Diseases. 2020;39(5):835-46. https://doi.org/10.1007/s10096-019-03797-4
2. Gasem MH, Hadi U, Alisjahbana B, Tjitra E, Hapsari MMDEAH, Lestari ES, et al. Leptospirosis in Indonesia: Diagnostic Challenges Associated with Atypical Clinical Manifestations and Limited Laboratory Capacity. BMC Infect Dis. 2020;20(1):179-. https://doi.org/10.1186/s12879-020-4903-5
3. De Brito T, Silva AMGd, Abreu PAE. Pathology and Pathogenesis of Human Leptospirosis: a Commented Review. Rev Inst Med Trop Sao Paulo. 2018;60:e23-e.

https://doi.org/10.1590/s1678-9946201860023

4. Naing C, Reid SA, Aye SN, Htet NH, Ambu S. Risk Factors for Human Leptospirosis Following Flooding: A Meta-Analysis of Observational Studies. PloS one. 2019;14(5):e0217643-e.https://doi.org/10.1371/journal.pone.0217643

5. Boey K, Shiokawa K, Rajeev S. Leptospira Infection in Rats: A Literature Review of Global Prevalence and Distribution. PLoS Negl Trop Dis. 2019;13(8):e0007499-e. https://doi.org/10.1371/journal.pntd.0007499

6. Bierque E, Thibeaux R, Girault D, Soupé-Gilbert M-E, Goarant C. A Systematic Review of Leptospira in Water and Soil Environments. PLOS ONE. 2020;15(1):e0227055. https://doi.org/10.1371/journal.pone.0227055

7. Mohamad Ikbal N, Bhassu S, Simarani K, Uni S, Chan C, Omar H. Prevalence and Genetic Divergence of Leptospira interrogans and Leptospira borgpetersenii in House Rats (Rattus rattus)from Peninsular Malaysia. Asian Pacific Journal of Tropical Medicine. 2019;12(10):463-71. https://doi/org/10.4103/1995-7645.269906

8. Bevans AI, Fitzpatrick DM, Stone DM, Butler BP, Smith MP, Cheetham S. Phylogenetic Relationships and Diversity of Bat-Associated Leptospira and the Histopathological Evaluation of These Infections in Bats from Grenada, West Indies. PLoS Negl Trop Dis. 2020;14(1):e0007940. https://doi.org/10.1371/journal.pntd.0007940

9. Widiastuti D, Sholichah Z, Agustiningsih A, Wijayanti N. Identification of Pathogenic Leptospira in Rat and Shrew Populations Using rpoB Gene and Its Spatial Distribution in Boyolali District. 2016. 2016:7. http://dx.doi.org/10.21109/kesmas.v11i1.798

10. Arief M, Ristiyanto R, Farida Dwi H, Dimas Bagus WP, Esti R. Seroprevalensi Leptospira pada Rattus Norvegicus dan Rattus Tanezumi Berdasarkan Jenis Kelamin dan Umur. Vektora: Jurnal Vektor dan Reservoir Penyakit. 2015;7(1):7-14. https://www.neliti.com/publications/126742/seroprevalensi-leptospira-pada-rattus-norvegicus-dan-rattus-tanezumi-berdasarkan#cite

11. Loan HK, Van Cuong N, Takhampunya R, Kiet BT, Campbell J, Them LN, et al. How Important are Rats as Vectors of Leptospirosis in the Mekong Delta of Vietnam? Vector Borne Zoonotic Dis. 2015;15(1):56-64. https://doi.org/10.1089/vbz.2014.1613

12. Philip N, Garba B, Neela VK. Long-Term Preservation of Leptospira spp.: Challenges and Prospects. Applied Microbiology and Biotechnology. 2018;102(13):5427-35. https://doi.org/10.1007/s00253-018-9047-9

13. Zarantonelli L, Suanes A, Meny P, Buroni F, Nieves C, Salaberry X, et al. Isolation of Pathogenic Leptospira Strains from Naturally Infected Cattle in Uruguay Reveals High Serovar Diversity, and Uncovers a Relevant Risk for Human Leptospirosis. PLoS Negl Trop Dis. 2018;12(9):e0006694. https://doi.org/10.1371/journal.pntd.0006694

14. Benacer D, Thong KL, Verasahib KB, Galloway RL, Hartskeerl RA, Lewis JW, et al. Human Leptospirosis in Malaysia: Reviewing the Challenges After 8 Decades (1925-2012). Asia-Pacific journal of public health. 2016;28(4):290-302. https://doi.org/10.1177/1010539516640350

15. Minter A, Costa F, Khalil H, Childs J, Diggle P, Ko AI, et al. Optimal Control of Rat-Borne Leptospirosis in an Urban Environment. Frontiers in Ecology and Evolution. 2019;7(209). https://doi.org/10.3389/fevo.2019.00209

16. Pellizzaro M, Martins CM, Yamakawa AC, Ferraz DdC, Morikawa VM, Ferreira F, et al. Molecular Detection of Leptospira spp. in Rats as Early Spatial Predictor for Human Disease in an Endemic Urban Area. PloS one. 2019;14(5):e0216830-e. https://doi.org/10.1371/journal.pone.0216830

17. Zhao J, Liao J, Huang X, Zhao J, Wang Y, Ren J, et al. Mapping Risk of Leptospirosis in China Using Environmental and Socioeconomic Data. BMC Infect Dis. 2016;16:343-. https://doi.org/10.1186/s12879-016-1653-5

18. Mohammadinia A, Saeidian B, Pradhan B, Ghaemi Z. Prediction Mapping of Human Leptospirosis Using ANN, GWR, SVM and GLM Approaches. BMC Infect Dis. 2019;19(1):971. https://doi.org/10.1186/s12879-019-4580-4

19. Sumanta H, Wibawa T, Hadisusanto S, Nuryati A, Kusnanto H. Genetic Variation of Leptospira Isolated from Rats Catched in Yogyakarta Indonesia. Asian Pacific Journal of Tropical Medicine. 2015;8(9):710-3. https://doi.org/10.1016/j.apjtm.2015.07.029