Inundation Sewers and Mapping the Prevalence Leptospirosis Throughout Rats in Central Java Province

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Abstract: Leptospirosis is a public health threat in countries with tropical climates, like Indonesia. Incidents in Central Java Province are still found every year and still threaten the local community. The goal of this study is to find out what connection there is between sewer puddles and mice that have found leptospira bacteria that can cause leptospirosis in the Regencies of Klaten, Sukoharjo, Karanganyar, and Boyolali in Central Java Province. This research uses descriptive analysis, a cross-sectional design study, and the Geographic Information System (GIS) to carry out mapping. From the research results, it was found that the rat species that were positive for leptospira bacteria were Rattus norvegicus (31%), Rattus tanezumi (44.8%), and Rattus argentiventer (24.1%). Apart from that, only the sampling area and the presence of puddles in the gutter had significant results with the mouse PCR results with $p<0.002$ and $p<0.03$. Due to this, there are still many mice infected with leptospira bacteria in Central Java Province. For this reason, it is hoped that the public can increase awareness to always maintain a clean environment so that mice cannot breed freely. Apart from that, related agencies can carry out more monitoring of areas where leptospirosis is still endemic.

Keywords: Inundation; Leptospirosis; Mapping; Rats

Introduction

Leptospirosis is a significant public health concern in Indonesia and other developing countries, with a high prevalence and substantial underreporting (Gasem et al., 2020). The diagnostic challenges associated with atypical clinical manifestations and limited laboratory capacity in Indonesia, leading to a severe underestimation of leptospirosis cases. The study reported an estimated 895 human cases in Indonesia during 2018, with a case fatality rate of 17.8%, indicating the gravity of the disease in the region. However, this case number is certainly a severe underestimate of leptospirosis in Indonesia given that the annual morbidity of leptospirosis in the population was recently estimated at 39.2 per 100,000 (Gasem et al., 2020). Furthermore, Indonesia has been established as a country with a high incidence of leptospirosis, signifying the substantial prevalence of the disease in the country (Noach & Noach, 2020).

The prevalence of leptospirosis in Indonesia is further supported by who conducted a study to investigate the type of reservoirs and estimate the prevalence of leptospirosis in rats and livestock animals in specific districts of Indonesia. This study provides direct evidence of the prevalence of leptospirosis in animals, which can serve as a reservoir for human infection (Sunaryo & Priyanto, 2022). The significant prevalence of leptospirosis in South-East Asian countries, including Indonesia, attributing it to occupational factors such as contact with intermediate Leptospira spp., further emphasizing the widespread nature of the disease in the region (Rahman et al., 2021).

The burden of leptospirosis in developing countries, including Indonesia, is exacerbated by underreporting and misdiagnosis (Hegazy et al., 2021).

The leptospirosis detection is not a routine test for febrile patients in Egypt and most developing countries, leading to underreporting and contributing to the neglected burden of leptospirosis worldwide (Hegazy et al., 2021). Similarly, the underestimation of leptospirosis in clinical and research settings, emphasizing the need for improved diagnostic methods to accurately assess the prevalence of the disease (Warnasekara et al., 2022).

Furthermore, the impact of leptospirosis in developing countries is evident, which reported that agricultural workers are the main occupational risk groups for leptospirosis in countries such as Thailand and Indonesia, highlighting the occupational risk factors associated with the disease (Dung et al., 2022). The lack of details from certain countries, including Malaysia, India, Bangladesh, and Vietnam, indicating the need for comprehensive research to understand the burden of leptospirosis in these regions (Sandhu et al., 2020).

The incidence of leptospirosis in developing countries, such as Indonesia, is closely linked to environmental factors, including the presence and condition of water ditches. The association between environmental risk factors and the incidence of leptospirosis, particularly in tropical and subtropical climates (Rafika et al., 2023). Several environmental risk factors for the incidence of leptospirosis, including the presence of standing water around the house, poorly maintained ditches, the existence of trash bins that did not meet requirements, the distance between the house and open drains, and the presence of rats inside and outside the house.

Similarly, several outbreaks of leptospirosis followed floods subsequent to heavy rainfall in various countries, highlighting flooding as a key driver of leptospirosis transmission (Ehelepola et al., 2019). The impact of environmental conditions, such as heavy rainfall and flooding, on the prevalence of leptospirosis. A significant percentage of homes of individuals with leptospirosis cases were located in flood zones, emphasizing the spatial association between flooding and the incidence of leptospirosis. A high percentage of patients with leptospirosis in Indonesia stated that there were puddles of water and many water ditches around their houses, indicating a potential link between the presence of water ditches and the risk of leptospirosis transmission (ILMA et al., 2022).

Moreover, a relationship between the presence of stagnant water, ditch conditions, and the incidence of leptospirosis, further emphasizing the role of water-related environmental factors in the transmission of the disease (Dewi & Yudhastuti, 2019).

The significance of environmental risk factors, including the absence of personal protective equipment and exposure to water sources, in contributing to the risk of leptospirosis (Hinjoy et al., 2019; Toemjai, 2023). This study aimed to examine the Inundation sewers and prevalence of leptospirosis according to water based contamination in District of Klaten, Sukoharjo, Karanganyar, dan Boyolali.

Method

Research design

This research is a descriptive epidemiological study using a cross-sectional research design. The target population is all rats found around the sampling area in Klaten, Karanganyar, Sukoharjo and Boyolali Regencies. Meanwhile, the study population in the research was mice that were around traps that had been installed in plantations, bushes and houses in the four districts in areas where there had been cases of leptospirosis. The samples in this study were mice that fell into traps that had been installed in several places in the area. To conduct this research, a minimum sample size must be met. The minimum sample size used is calculated using the following proportion estimation test Formula (Formula 1):

$$n = \frac{z_{1-\alpha/2}^2 P(1-P)}{d^2} \qquad (1)$$

Information:
P: Estimated Proportion
d: Absolute deviation
z: z value at the 1-α/2 confidence level
Known value:
P: 1.9% 45
d: 5%
CI: 95%
$n = \frac{(1,96)^2 0,019(1-0,019)}{0,05^2}$
n = 29

So, the minimum sample required for each region is 29 samples. The total sample required for the four districts (Regencies of Klaten, Sukoharjo, Karanganyar, and Boyolali) is 116 samples.

Research tools and methods

In this research, data was collected by taking samples of mice in several areas using the purposive sampling method. Apart from that, when collecting sample data, local environmental conditions are also identified. The following are the procedures used to take mouse samples:

Table 1. Procedures to collecting the data

First day	Second days
Determining the location for picking up mice can be done by paying attention to the following things, namely the presence of mouse traces such as food scraps, scraps, traces of feces, mouse tracks, proximity to plants/trees, and based on information from the surrounding community. The following are the procedures for collecting mice on the first day: Preparing mouse traps, determining whether the traps are still suitable for use, preparing bait using coconut measuring 3 x 3 cm which is burned and placed in the trap, assigning a number to the trap using Japanese tape, installing the trap, taking coordinates using GPS, taking documentation, and retrieve environmental parameters.	The next activity is taking mice which is done at 06.00 by placing a calico cloth in the mouth of the trap, after the mouse enters the cloth, the cloth is closed and given a field label, and traps that already contain mice can be washed using rice water.

Data processing and analysis

To prove that the mouse samples were positive for leptospirosis, a PCR test kit was used. The PCR kit used is Zymo Research Quick-DNA™ Miniprep Plus Kit D4068. Furthermore, in data processing, data management will be carried out, namely coding, editing, and cleaning data.

In the data editing section, the data will be checked for completeness and uniformity. After that, the data will be sorted again based on the inclusion and exclusion criteria. If there is data that does not match or falls within the exclusion criteria, the data will be excluded. In this section, data in the form of several variables will be recorded. Recoding is done by changing the numbers or letters contained in the initial data using the recode menu in SPSS based on the code that has been determined in the operational definition. So, the analysis process can be carried out easily. The data will be checked again to avoid data errors and check whether there is missing or odd data. The results of data analysis are presented in bivariate, univariate and map.

The research results were reanalyzed using Quantum Geographic Information System (QGIS) software to produce a mapping of the prevalence of leptospirosis at the research location.

Result and Discussion

Inundation in sewers result

From table 2 it can be seen that of the 29 samples that were positive for leptospirosis, the largest number of positive samples were in samples taken from Sukoharjo Regency with a total of 11 (37.9%). Meanwhile, in Karanganyar Regency, none of the samples taken were positive for leptospirosis. Of the 29 samples that were positive for leptospirosis, 18 (62.1%) were female. 29 samples were positive for leptospirosis, 25 samples were taken from outside the home. Of the 29 samples that were positive for leptospirosis, 13 samples belonged to the Rattus Tanezumi species, amounting to 44.8%. In addition, PCR results and inundation have a statistically significant relationship as proven by a p

value <0.03. If we look further, there were 15 samples (51.7%) at the sampling sites that had pools in the gutter and were identified as positive for leptospirosis.

Table 2. Sample distribution

Variable	Leptospirosis bacteria		p value
	Positif (%)	Negative (%)	
Districts			0.002
Sukoharjo	11 (37.9)	19 (19)	
Klaten	10 (34.5)	21 (21)	
Karanganyar	0	32 (32)	
Boyolali	8 (27.6)	28 (28)	
Sex of rats			0.471
Female	18 (62.1)	59 (59)	
Manly	11 (37.9)	41 (41)	
Samples Existence			0.130
Inside the house	25 (86.2)	74 (74)	
Outside	4 (13.8)	26 (26)	
Species of Rattus			0.420
Norvegicus	9 (31)	18 (18)	
Exulans	0	2 (2)	
Tanezumi	13 (44.8)	44 (44)	
Argentiventer	7 (24.1)	34 (34)	
Tyomanicus	0	2 (2)	
Inundation sewers			0.030
Yes	15 (51.7)	22 (22)	
No	14 (48.3)	78 (78)	

GIS results

The four districts that were the sampling locations consisted of Karanganyar Regency, Sukoharjo Regency, Klaten Regency and Boyolali Regency. Of the four districts, the districts where there were no positive leptospirosis samples were Karanganyar District. It can be seen on the map above that this region is colored green. Two other districts, namely Klaten District and Boyolali District, have 5-10 positive samples for leptospirosis (these two regions are colored yellow on the map). Meanwhile, the district with the most positive samples for leptospirosis was Sukoharjo District with 10-15 positive samples (marked in red on the map).
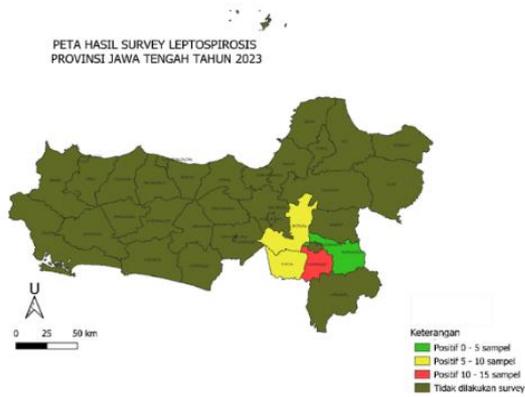
Figure 1. Map of Leptospirosis Survey Results

From the four areas where research has been conducted, it can be seen that Sukoharjo Regency has a greater number of leptospirosis positive mice than other areas. This can be seen on the map which shows red in Sukoharjo Regency, which indicates that this area has a positive count of leptospirosis with a number of 11-15. In line with the results of this research, a previous study in Yogyakarta, Indonesia, to estimate the prevalence of leptospirosis in rats and livestock animals. The study aimed to investigate the type of reservoirs and found evidence of leptospirosis in rats, highlighting their potential role as reservoirs for the disease (Sunaryo & Priyanto, 2022). To detected Leptospira bacteria in rats in Bogor District, West Java, indicating rats as the main reservoirs of leptospirosis in Indonesia. This finding further emphasizes the significant role of rats in the transmission of leptospirosis (Gunawan et al., 2023).

Furthermore, a study in Sri Lanka, which demonstrated the sero-prevalence of leptospirosis in peri-domestic rodents, including rats, indicating their potential role in the transmission of the disease. This study provides valuable insights into the prevalence of leptospirosis in different rodent species, including rats (Sunil-Chandra et al., 2022). In addition, The evidence of leptospirosis in wild infected rats, suggesting their potential role as reservoirs for the disease. This study provides important insights into the dynamics of leptospirosis in rat populations, highlighting their significance in the transmission of the disease (Ferrer et al., 2018).

Moreover, the presence of leptospirosis in Rattus tanezumi, indicating the potential of this rat species as a reservoir for leptospirosis in the Minahasa District of Sulawesi Utara, Indonesia. This finding underscores the importance of considering different rat species as potential reservoirs for the disease (Lobo et al., 2020).

From the results of research that has been carried out, the number of positive mice for leptospirosis which is female is greater than that of positive mice which are

male, amounting to 62.1%. Based on the provided references, several studies have investigated the prevalence and risk factors of Leptospira infection in rats. A study in Vienna, Austria, which found an overall prevalence of Leptospira interrogans in the kidney of rats to be 25%, with variations among different sites. While this study did not specifically focus on the sex of rats, it provides important information about the prevalence of Leptospira infection in urban rat populations (Desvars-Larrive et al., 2020). Similarly, a study in Vancouver, Canada, and Salvador, Brazil, to evaluate whether rat characteristics, including sex, were associated with the status of Leptospira interrogans. The study assessed various rat characteristics such as sex, weight, sexual maturity, pregnancy, and the presence of wounds, in relation to L. interrogans status. This study provides insights into the potential association between rat characteristics, including sex, and the carriage of Leptospira interrogans (Minter et al., 2019). Leptospira interrogans biofilm formation in Rattus norvegicus (Norway rats), which are known to be the main reservoir hosts of pathogenic Leptospira. While this study did not specifically focus on the sex of rats, it provides important information about the role of Norway rats as reservoir hosts for Leptospira interrogans (Santos et al., 2021).

The specific relationship between the sex of rats and leptospirosis may require further dedicated research to comprehensively understand the potential impact of sex on the prevalence and transmission of leptospirosis in rat populations. The presence of rats has been closely associated with the transmission and prevalence of leptospirosis; a zoonotic disease caused by pathogenic Leptospira spp. Several studies have investigated the role of rats in the epidemiology of leptospirosis, shedding light on their significance as potential reservoirs for the transmission of the disease.

The importance of understanding the distribution and abundance of pathogenic Leptospira spp. in the environment, including areas inhabited by rats (Schneider et al., 2018). The role of rats as potential reservoirs for the existence of Leptospira. This study aimed to provide insights into the association between the presence of rats and the prevalence of Leptospira in endemic areas (Ratnaningsih et al., 2023).

The characteristics of rats and shrews in endemic leptospirosis areas, emphasizing the presence of Leptospira interrogans and Leptospira borgpetersenni in rat populations, confirming their role as reservoirs for leptospirosis (Susanna et al., 2021). In addition, the molecular detection of Leptospira spp. in rats as an early spatial predictor for human disease in an endemic urban area. This study highlighted the importance of monitoring Leptospira in rat populations as a means of predicting and preventing human leptospirosis cases (Pellizzaro et al., 2019).

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From the results of the research conducted, PCR results and the presence of puddles in ditches have a statistically significant relationship. Sampling locations that had puddles in ditches and were identified as positive for leptospirosis were 51.7%. The inundation of sewers has been identified as a potential environmental factor contributing to the transmission of leptospirosis. The significance of environmental conditions in the residence of leptospirosis patients in the District of Probolinggo, shedding light on the potential role of inundated sewers in the transmission of the disease. The impact of environmental health factors on the prevalence of leptospirosis, providing valuable insights into the association between environmental conditions and the incidence of the disease. The aligns with the understanding that the urine of mice infected with Leptospira, contaminating sewer water, and the inundation or overflow of sewers can contribute to the transmission of leptospirosis (Damalia et al., 2021). This underscores the potential role of inundated sewers as a factor in the transmission of the disease, particularly in the context of environmental conditions that facilitate the presence of rats and the spread of the bacteria.

The dominant risk factors for leptospirosis in many tropical countries include activities that expose individuals to rodent urine, such as living in urban slums, proximity to sewers, and exposure to flood waters. This study provides direct evidence of the association between environmental factors, including water ditches, and the prevalence of leptospirosis in developing countries (Maze et al., 2018). The importance of rainfall and other meteorological factors as drivers of urban transmission of leptospirosis, particularly affecting vulnerable urban slum populations in developing country settings. This study underscores the significance of environmental conditions, including water-related factors, in contributing to the transmission of the disease (Cunha et al., 2022).

In humans mainly in developing countries and countries with poor housing and sanitation, due to animals (mainly rats) that are potential sources of contamination. The role of water ditches as potential sites for the transmission of leptospirosis, particularly in areas with poor housing and sanitation (Cardoso et al., 2022).

Three types of mice, namely Rattus norvegicus, Mus musculus, and Rattus tanezumi, that are widespread in the world and are associated with the transmission of leptospirosis. This study provides valuable insights into the diversity of mouse species that may serve as reservoirs for the disease (Anwar et al., 2020). A comparative in vivo study in mice, focusing on the role of TLR4 in persistent Leptospira interrogans infection. The study hypothesized that TLR4/MD-2 humanized transgenic mice (huTLR4) may be more susceptible to leptospirosis than wild-type mice, potentially constituting a model of acute human leptospirosis. This research provides important insights into the potential susceptibility of different mouse models to leptospirosis (Nair et al., 2021).

Conclusion

In sum, the research results showed that rats were positive for Leptospira and had areas with puddles in the gutter of 51.7% and the sampling area and the presence of puddles in the gutter had significant results with the PCR results of the rats.

Author Contributions
Conceptualization, S.Y., and D.S.; methodology, S.Y.; software, S.Y.; validation, S.Y. and M.K.A.; formal analysis, M.K.A.; investigation, S.Y.; resources, S.Y.; data curation, S.Y.; writing—original draft preparation, D.S.; writing—review and editing, D.P.; visualization, M.K.A.; supervision, S.Y.; project administration, S.Y.; funding acquisition. All authors have read and agreed to the published the last version of the manuscript.

Conflicts of Interest
The authors declare no conflict of interest.

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