Original Article

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Detection of Leptospira sp. Bacteria and Factors Related to The Incidence of Leptospirosis in Semarang City

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Abstract

Objective: The Case Fatality Rate (CFR) of Leptospirosis in Semarang City has shown a tendency to increase from 2019 to 2022. In 2022, there were 30 cases reported, with 8 deaths (CFR 27%). The purpose of this study was to detect *Leptospira* sp. bacteria in rats and analyze the risk factors for leptospirosis in Semarang City.

Methods: This study employed an observational approach with a Case-Control Study design. The research was conducted in Semarang City from November to December 2023. Non-random sampling techniques were used, specifically the total sampling method, resulting in a sample size of 45 case groups and 45 control groups. Bivariate analysis was performed using the Chi-square test, with a significance level set at <0.05.

Results: Based on the Chi-Square test results, the following variables were found to be associated with the incidence of leptospirosis in Semarang City: the presence of rats (p<0.0001), the presence of *Leptospira* sp. bacteria (p<0.0001), house conditions (p=0.260), sewer condition (p<0.0001), presence of pets (p=0.001), condition of landfills (p=0.001), presence of vegetation (p=0.005), temperature (p=0.299), humidity (p=0.495), lighting (p=0.023), history of wounds (p=0.001), and knowledge level (p=0.025).

Conclusion: The risk factors for leptospirosis in Semarang City include the presence of rats, the presence of *Leptospira* sp. bacteria, sewer conditions, the presence of pets, conditions of landfills, the presence of vegetation, lighting, history of injury, and level of knowledge.

Keywords: Detection, leptospirosis, microscopy, rats, risk factors

Introduction

Currently, zoonoses remain a global threat to public health, with zoonoses accounting for 60.3% of all emerging infectious diseases (EIDs).[1] Leptospirosis, caused by the bacteria *Leptospira* sp., is a zoonotic disease transmitted by animals carrying infective *Leptospira* bacteria.[1] Animals such as rats, dogs, and farm animals like cattle and pigs can act as reservoirs for the disease. The bacteria reside in the kidneys of the host and are excreted in urine.[2] Transmission of leptospirosis can occur through direct contact with environments contaminated with the urine of animals carrying *Leptospira* sp. bacteria, or through indirect contact with environments at risk of contamination. The bacteria can enter the human body through wounds, skin abrasions, or ingestion, such as consuming water contaminated with the bacteria. *Leptospira* bacteria can survive in water and soil for weeks to months. Transmission primarily occurs from animals to humans, with human-to-human transmission being very rare.[3]

Leptospirosis is a significant health issue in

Indonesia, particularly in flood-prone areas.[4] According to the 2021 Indonesian Health Profile, leptospirosis cases in Indonesia have been increasing in the past five years, with 734 cases and a Case Fatality Rate (CFR) of 11.4% reported in 2021.[2] Central Java Province has been declared an endemic area for leptospirosis, with 113 cases and a CFR of 22.12% recorded in 2021.[1] Data from the Semarang City Health Office indicates that the CFR of leptospirosis has been on the rise from 2019 to 2022. In 2022, there were 30 cases reported, resulting in 8 deaths (CFR 27%).[5]

Based on previous research conducted in Demak in 2020 and in Banten in 2021, the risk factors for the incidence of leptospirosis include: presence of stagnant water, sewer conditions, use of personal protective equipment, behavioral habits of washing feet and washing hands with soap, presence of pets, history of injury, environmental conditions, conditions of landfills, and the presence of vegetation.[3,6,7] The purpose of this study was to detect *Leptospira* sp. bacteria in rats and analyze the risk factors for leptospirosis in Semarang City.

Methods

This study is an observational study conducted in Semarang City from November to December 2023, using a Case-Control Study approach. The study population consisted of all leptospirosis patients (cases) recorded in the Semarang City Health Office working area from January 2022 to July 2023, as well as non-leptospirosis patients (controls) who were the closest neighbors of the cases. The sampling technique used in this study was non-random sampling, specifically the total sampling method, taking into account the exclusion and inclusion criteria. The inclusion criteria included recovered leptospirosis patients or families of leptospirosis patients who had recovered or died, as well as neighbors of leptospirosis patients. Exclusion criteria consisted of individuals who were unwilling to participate as respondents or had moved from their recorded address. Therefore, the case sample for this study consisted of 45 people, as did the control sample.

Prior to conducting the study, the researchers explained the purpose and objectives to the respondents, who were then asked to sign an Informed Consent Form if they agreed to participate. The tools and materials used in the field included rat traps, sacks, bait, masks, and gloves for catching rats, as well as pens, questionnaire sheets, thermohydrometers, cameras for environmental observation, interviews, and documentation. Rat catching took place over two days and two nights, with traps being checked every morning. Any captured rats were immediately taken to the Integrated Laboratory of the Faculty of Public Health, Diponegoro University, where they underwent the process of identifying rats and examining *leptospira* sp. bacteria found in their feces, urine, and blood using microscopic methods. The tools and materials used in the laboratory included ketamine, syringes, cloth sacks, surgical scissors, tweezers, stationery, scales, rulers, calipers, masks, and Chinese ink for examining the *leptospira* bacteria under a microscope.

The steps taken are the preparation of the object glass and negative painting. a. Clean the object glass using a cotton swab that has been soaked in 70% alcohol. b. Give the code to the bacterial culture on the left side of the object glass. c. Use an ode to take a small number of bacterial colonies. d. Add 1–2 drops of Chinese ink, then add 1-2 drops of Chinese ink again. Add 1–2 drops of Chinese ink, then mix thoroughly. e. Swap the mixture using another object glass or deck glass with a 45° tilt, so that a thin smear is formed. f. Let the smear dry for 15 minutes. g. After it has dried, observe using a microscope. The microscopic magnification used is 100x.[7]

The variables that were examined in this study include: the presence of rats, the presence of *Leptospira* sp. bacteria, house conditions, sewer conditions, the presence of pets, wound history, condition of landfills, the presence of vegetation, temperature, humidity, lighting, and knowledge level.

The data analysis involved using univariate analysis to determine the frequency distribution of each variable. Bivariate analysis was then performed to assess the relationship between the independent and dependent variables. The Chi-square test was used to calculate the p-value at a confidence level of <0.05 and a 95% Confidence Interval. Microsoft Excel software and SPSS v.24 were used for data analysis.

This study has been approved by the Health Research Ethics Commission under the reference number: 589/EA/KEPK-FKM/2023.

Results

The results of rat trapping in 90 houses over the course of 2 consecutive days are as follows: a total of 180 traps were set, and 43 houses

tested positive for rats. A total of 54 rats were caught, resulting in a relative density of 15%. Microscopic examination of these 54 rats revealed that 88% of them tested positive for *leptospira* bacteria.

Table 1 shows a comparison of different variables between the case and control groups. Out of the 45 case houses, 32 of which (71.1%) tested positive for caught rats, while 31 rats (68.9%) were positive for *Leptospira* sp. bacteria. Additionally, 22 houses (48.9%) had poor conditions, 36 house gutters (80%) were in poor condition, 33 houses (73.3%) had pets, 38 houses (84.4%) had inadequate garbage disposal conditions, 30 houses (66.7%) had vegetation, 26 houses (57.8%) had lighting levels below 60 lux, 31 cases (68.9%) had a history of injury, and 24 people (53.3%) had a lack of knowledge. In comparison, the 45 control houses had 12 houses (26.7%) that tested positive for caught rats, with 8 rats (17.8%) testing positive for *Leptospira* sp. bacteria. Furthermore, 19 houses (42.2%) had poor conditions, 16 house sewers (35.6%) were in poor condition, 18 houses (40%) had pets, 25 houses (55.6%) had inadequate landfill conditions, 23 houses (51.1%) had vegetation, 18 houses (40%) had lighting levels below 60 lux, 17 people (37.8%) had a history of injury, and 18 people (40%) had a lack of knowledge.

Based on the results of data analysis in Table 1 using the Chi-Square test (p-Value <0.05), it can be concluded that several independent

Table 1 Frequency Distribution and Chi-Square Test Data Analysis of Risk Factors for Leptospirosis in Semarang City

Variable		Incidence of Leptospirosis		p Value
		Case	Control	
		(n=45) (%)	(n=45)(%)	
Presence of rats	Yes	32 (71.1)	12 (26.7)	<0.0001
	No	13 (28.9)	33 (73.3)	
Presence of *Leptospira* sp.	Positive	31 (68.9)	8 (17.8)	<0.0001
	Negative	14 (31.1)	37 (82.2)	
House conditions	Bad	22 (48.9)	19 (42.2)	0.260
	Good	23 (51.1)	26 (57.8)	
Sewer condition	Bad	36 (80.0)	16 (35.6)	<0.0001
	Good	9 (20.0)	29 (34.4)	
Presence of pets	Yes	33 (73.3)	18 (40.0)	0.001
	No	12 (26.7)	27 (60.0)	
Condition of landfills	Bad	38 (84.4)	25 (55.6)	0.001
	Good	7 (15.6)	20 (44.4)	
Presence of vegetation	No	15 (33.3)	22 (48.9)	0.005
	Yes	30 (66.7)	23 (51.1)	
Temperature	Optimum	13 (28.9)	14 (31.1)	0.299
	Not optimum	32 (71.1)	31 (68.9)	
Humidity	>60%	15 (33.3)	16 (35.6)	0.495
	<60%	30 (66.7)	29 (64.4)	
Light	<60 lux	26 (57.8)	18 (40.0)	0.023
	>60 lux	19 (42.2)	27 (60.0)	
Injury history	Yes	31 (68.9)	17 (37.8)	0.001
	No	14 (31.1)	28 (62.2)	
Knowledge	Bad	24 (53.3)	18 (40.0)	0.025
	Good	21 (46.7)	27 (60.0)	

variables have a relationship with the incidence of leptospirosis in Semarang City. These variables include the presence of rats, the presence of *Leptospira* sp. bacteria, sewer conditions, the presence of pets, the condition of landfills, the presence of vegetation, lighting, history of injury, and level of knowledge.

Discussion

In this study, rats are identified as one of the animals that serve as reservoirs for the transmission of leptospirosis. The success of rat capture can be influenced by several factors: the quality of the trap, the accuracy of bait selection, and the placement of the trap. The quality of the trap plays a significant role in catching rats, as it acts as a barrier when rats enter the trap. If the trap is of poor quality, rats that have been caught may escape and damage the trap.[8] During the research in Semarang City, it was observed that many traps were closed, the bait was missing, but the rats were not caught and the traps were damaged. This could be due to the shape of the trap not matching the size of the rat, allowing rats that have entered to break out and potentially causing damage to the trap.

In addition to trap selection, the accuracy of bait selection also greatly influences the success of rat capture. The bait used in this study was a mixture of milkfish, tofu, meatballs, and roasted coconut. It was found that grilled coconut and milkfish mixed with tofu were the most preferred baits for rats in Semarang City. The placement of traps also plays a role in the success of catching rats. Traps are strategically placed in areas that are expected to be rat paths and frequently visited. Rats exhibit thigmotaxis, meaning they follow the same trajectory every time they search for food, return to the nest, and engage in other rat activities.[9]

There are three types of rodents that have been successfully found in the Semarang City area: Rattus norvegicus, Rattus tanezumi, and Mus munculus. The results showed that Rattus norvegicus was the most common rat found in Semarang City, with 27 rats. Meanwhile, there were 24 Rattus tanezumi and 3 Mus munculus. The presence of Rattus norvegicus in Semarang City may be due to the poor condition of the home environment, gutters, and garbage disposal. Rattus norvegicus is commonly found in waterways or sewers in urban settlements, which is why it is also known as the sewer rat. Rattus norvegicus is known as a reservoir of leptospirosis, a disease that can be transmitted to humans. These rats carry harmful serovars such as ballum and autumnal.[10] These results are consistent with the research conducted by Alfira *et al*, where Rattus norvegicus was found to be the most commonly caught rat in Tandang Village, Semarang City.[8]

Other species found in Semarang City in this study were Rattus tanezumi and Mus musculus. The native habitat of Rattus tanezumi and Mus musculus is inside the house, as these rats are domestic rats that forage, shelter, nest, and breed inside houses. Rats will migrate if they lack food.[10]

Leptospira sp. bacteria thrive in hot and humid soil, such as in tropical conditions. These bacteria can survive up to 43 days in suitable soil. *Leptospira* bacteria are very small and can only be seen under a microscope. Dark field microscopy is used to examine *Leptospira* sp. bacteria. The samples taken for examination include rat kidneys, rat blood, rat urine, and feces.

Unqualified house components and arrangements can increase the incidence of leptospirosis. Houses that are not rat-proof can increase the risk of rats entering the house. House components that do not meet health requirements can become a nesting place for various vectors and reservoirs that can cause diseases such as rats.[11] A rat-proof house or building is one whose foundation is made of strong materials such as concrete, the floor is made of concrete and is at least 10 cm thick, the walls are made of bricks or concrete, there are no cracks or gaps that rats can pass through, the gap between the door and the floor is ≤6mm, and any ventilation larger than 6mm is closed with a strong wire mesh. This study is consistent with research conducted in Banyumas in 2019, which showed no significant relationship between the condition of the home environment and the incidence of leptospirosis, with a p-value of 0.195.[12]

The sewer is the place that is most often used as a living space or a route in and out of the house for rats. Based on the research of Sukismanto *et al.*[13] in Sleman Yogyakarta in 2018, the success of catching rats in the house is higher in open sewage conditions than in houses with closed sewage channels. This is supported by Rahmawati's research in Boyolali in 2014, which found that 87% of respondents' houses with poor sewers had more rats caught.[14] The most important environmental factor for rat infestation was indicated by the presence of access from the sewage system. The peridomestic habits of rats cause them to be more strongly associated

with unused buildings and access sources, especially sewage systems.[14]

The pets found around the houses studied were cats and dogs. Based on the results of research by Sutikno *et al.*,[15] the animals that are most commonly kept at home are cats, accounting for 48.38% of households. This situation is thought to contribute to a relatively small presence of rat pests in residential houses in Pekanbaru City, around 23.64%.Based on the results of spatial analysis in Fajriyah *et al* research in Semarang in 2017, it is known that the presence of pets that have the potential to become reservoirs of *Leptospira* sp. is a risk factor of 13.6%.[16]

The condition of open rubbish dumps is a potential place favored by rats due to the presence of piles of garbage, such as food waste, fish, bread, etc. According to Lestari's research, the mapping results from the condition of open trash cans showed that there were more rats inside and outside the house.[17] The presence of garbage around the house can increase the rat population. Rats can also be attracted by improper waste management within the house. Nugroho's research showed that the presence of waste, especially food waste left in open bins, is associated with the spread of leptospirosis.[18]

The results of observations when catching rats in residential areas reveal that there are numerous shrubs, trees, and plants both inside and outside people's homes. Rats can easily enter houses that have branches or twigs creeping into them, and the presence of trees can also provide shelter for rats.[19] This finding is supported by Nordmeier's research on the Otago Peninsula in 2020, which indicates a significant relationship between the probability of rat presence and the amount of vegetation (p=0.009).[20]

Temperature is measured using a thermometer at the location where the trap is placed. Temperature is one of the abiotic factors affecting the existence of mice. Rats are mammals with a limited temperature range, with higher temperatures being more deadly for them compared to lower temperatures. The average temperature in respondents' homes is 27ºC – 31ºC, which is considered an environmental factor that can influence the high rat population. According to research by Supranelfy *et al*, rats that were caught and identified as *Leptospira* sp. were found at an optimal temperature range of 24.0°C–26.9°C.[21]

Humidity is measured using a multimeter around the mousetrap placement. High air humidity (>60%) is considered ideal for mouse activity. Poor lay out of buildings can exacerbate the humidity levels inside the house, turning it into a place for mice to live, hide, or pass through.[15] This aligns with the findings of Kusumajaya *et al*. research in the settlements of Ajibarang District, which showed a relatively high mouse density with an average air humidity in residential areas of 71%, surpassing the optimal humidity limit for mice.[22]

Lighting measurements were carried out at the location where the mouse trap was placed. Insufficient lighting increases the likelihood of presence of mice. Rats are primarily active at night and tend to search for food just before or at sunset. Yulianto *et al*.[23] research in Sukajadi Village, Pekanbaru, supports this, stating that there is a correlation between lighting and rat vectors in houses. Good lighting refers to artificial or natural illumination that thoroughly lights up a room with a minimum intensity of 60 lux.

Leptospira bacteria can enter the human body through wounds on the skin,[24] as well as through mucous membranes (nose, mouth, eyes) and from stagnant water. The transmission of leptospirosis is significantly influenced by a history of wounds, as *Leptospira* bacteria can enter the body through wounds. If a person with a wound comes into direct contact with an environment contaminated with *Leptospira* bacteria, they can contract leptospirosis. The most common type of wound is on the feet and hands, typically related to the person's work. Even the smallest wound or skin abrasion can become the gate for *Leptospira* bacteria to enter the human body.[25]

A person's health is greatly impacted by their knowledge. With sufficient knowledge, individuals will strive to live a clean and healthy lifestyle and undergo regular health checks. In Indonesia, despite leptospirosis causing significant health problems, it receives little attention. This lack of information contributes to the respondents' limited knowledge about leptospirosis.[8] There is still a lack of public awareness regarding the factors that contribute to the presence of rats, the transmission of leptospirosis from animals to humans, and how to prevent and control the disease. One limitation of this research is the suboptimal quality of mouse traps used in the field when catching mice.

In conclusion, this research identifies several variables related to the incidence of leptospirosis in Semarang City, including the presence of rats, the presence of *Leptospira*

sp. bacteria, the condition of sewers, the presence of pets, the condition of rubbish dumps, the presence of vegetation, lighting, history of injuries, and level of knowledge. It is recommended that health officers in the City of Semarang play an active role in increasing public knowledge about leptospirosis. Furthermore, the public should be encouraged to maintain a clean home environment and broaden their knowledge about preventing and controlling leptospirosis.

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