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## Risk Factors for the Spread of *Leptospira sp.* in Rats Using Success Trap and iiPCR Methods in Probolinggo Harbor

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### ABSTRACT

This study aimed to identify the distribution of rats and environmental risk factors associated with the spread of *Leptospira sp.* in Probolinggo Harbor using success trap and iiPCR methods. A total of 150 traps were deployed across 66 strategic locations around the harbor, capturing 19 rats, with a rat capture rate of 4.2%. Among the captured rats, 21.05% tested positive for *Leptospira sp.*, with *Rattus tanezumi* constituting 75% of the infections and *Rattus norvegicus* accounting for the remaining 25%. Path analysis (SmartPLS 4.0) revealed significant associations between gutter conditions, vegetation, humidity, and signs of rat activity with the presence of *Leptospira sp.* Environmental factors such as gutter conditions, temperature, humidity, and vegetation were found to significantly influence the prevalence of *Leptospira sp.*, with *Rattus tanezumi* identified as the primary vector, particularly in locations with open waste systems or abundant vegetation. These findings highlight the critical role of environmental factors in the transmission of leptospirosis, emphasizing the need for targeted environmental management strategies, including rodent control programs and improvements in sanitation, to mitigate risks in harbor areas.

**Keywords:** Leptospirosis, *Leptospira sp.*, Success trap, iiPCR, Environmental health

### PENDAHULUAN

Leptospirosis is an infectious disease caused by *Leptospira sp.* bacteria and is recognized as a significant global health concern, particularly in tropical and subtropical regions. The disease is associated with high morbidity and mortality rates, with an estimated 1 million cases and nearly 60,000 deaths reported annually worldwide (Costa et al., 2015). Transmission occurs through direct contact with infected animals or indirect exposure to environments contaminated by their urine, such as water or soil (Costa et al., 2015; Mwachui et al., 2015; Terpstra, 2003; Zhang et al., 2012). Environmental factors, including high humidity and the presence of rats as primary reservoirs, exacerbate the risk of disease spread.

There has been an observed increase in the incidence of leptospirosis in tropical regions, including Southeast Asia. In Malaysia, the state of Perak has recorded the highest incidence and mortality rates since 2004 (Garba et al., 2017; Thayaparan et al., 2013). Despite a decline in cases in Northeast Thailand since 2001, mortality from severe leptospirosis increased between 2001 and 2012, with pulmonary hemorrhage identified as the leading cause of death. Studies conducted in Vietnam during the 1990s and 2000s demonstrated a notable prevalence of leptospirosis, with seroprevalence rates varying between

10 and 80 percent, contingent on geographical location and population group (Dung et al., 2022; Thai et al., 2006; Thipmontree et al., 2014). In Indonesia, between 2011 and 2019, cases were documented in 12 provinces, with 920 cases and 122 deaths in 2019, resulting in a case fatality rate (CFR) of 13.3% (Ariani & Wahyono, 2021).

In Indonesia, leptospirosis remains a critical public health issue. Tropical regions like East Java, especially during the rainy season, are particularly vulnerable to the disease. Probolinggo Harbor is among the areas with a high risk of leptospirosis due to its role as a port hub. Harbor environments often have conditions conducive to the presence of rats, such as poor drainage systems, vegetation, and high humidity, making them potential hotspots for *Leptospira sp.* transmission. Previous studies reported an average leptospirosis infection rate of 22.23% among rats in Indonesian harbors.

The potential for the spread of leptospirosis is high in East Java, particularly during the rainy season. From January to March 2023, the East Java Health Office documented 249 cases of leptospirosis, representing nearly 40% of the total 602 cases identified in 2022. (Dinas Kominfo Jatim, 2023; Subiyantoro, 2024).

This study aims to address this gap by identifying environmental risk factors associated with the spread of *Leptospira sp.* in rats within the Probolinggo Harbor area.

Using success trap methods for rat capture and iiPCR molecular analysis, this research seeks to provide deeper insights into the mechanisms of leptospirosis transmission and serve as a foundation for targeted environmental risk management strategies in harbor regions.

## RESEARCH METHODS

Probolinggo Harbor was selected due to its status as a high-risk area for leptospirosis, especially during the rainy season. The harbor's environmental characteristics, including poor drainage systems, dense vegetation, and humid conditions, support the proliferation of rats as reservoirs of *Leptospira* sp. The study was conducted in two zones within the harbor area: buffer zone: Kampung DOK, a residential area close to the harbor with significant rat activity, and perimeter zone: DABN Pier, Pelindo Pier, and Fishery Pier, where high human and cargo activity occur. These zones were chosen to represent diverse environmental and activity profiles.

### Rat capture procedures.

Rats were captured using 150 traps set over three consecutive days, following a systematic process outlined by (Arasy et al., 2023). The traps were strategically placed in areas showing clear signs of rat activity, such as near drainage systems, vegetation, and waste disposal sites. Peanut butter, a well-documented attractant for rodents, was used as bait to enhance trapping efficiency. Each day, the traps were deployed between 16:00 and 17:00 WIB and subsequently checked the following morning between 06:00 and 09:00 WIB.

The trapping success rate was calculated as the proportion of rats captured relative to the total number of traps deployed. This study achieved a success rate of 4.2%, notably exceeding the 1% benchmark recommended by the Directorate of P2P (2015). Upon capture, the rats were humanely euthanized using atropine at a dosage of 0.02–0.05 mg/kg body weight, followed by ketamine HCl at 50–100 mg/kg body weight. This method ensured ethical handling while allowing for the collection of kidney samples for further analysis.

### Rat identification

Captured rats were identified based on morphological characteristics such as body weight, total length, tail length, and skull measurements. Species identification was cross-referenced with a rodent identification key. Documentation included physical traits such as fur color and texture.

### Rat dissection

The mice were then placed on an enamel tray. An incision was made on the abdomen using tweezers and scissors, extending laterally to the right and left until reaching the base of the hind paw. The same procedure was repeated until the base of the front legs was reached. The colon and duodenum are elevated until the placenta is visible, allowing for the determination of its status and the retrieval of the kidneys. The number of embryo candidates on the left and right sides of the placenta should be counted and recorded. The kidneys are then removed using tweezers and scissors and washed with

70% alcohol before being placed in a vial or pot containing 70% alcohol. They are then labeled with coded paper and stored at room temperature prior to undergoing laboratory examination.

### Rat kidney examination

The process comprises four distinct stages: sample preparation, sample extraction, mixing of reagents, and test execution.

### Data analysis

The analysis aimed to evaluate the influence of environmental factors on the presence of *Leptospira* sp. bacteria and validate the hypothesized relationships. Using Partial Least Squares (PLS) with licensed SmartPLS 4.0 software, the study ensured construct validity through outer loading values ( $>0.7$ ) and average variance extracted (AVE  $>0.5$ ). Reliability was confirmed with Cronbach's alpha and composite reliability ( $>0.7$ ). Hypotheses were tested using path coefficients, t-statistics ( $>1.96$ ), and p-values ( $<0.05$ ). Parameters analyzed included gutter conditions, vegetation, humidity, and rat activity, revealing their roles in supporting the presence of *Leptospira* sp..

## RESULTS AND DISCUSSION

### Identify the distribution of rats using the success trap method

The trap sites in the buffer and perimeter areas of Probolinggo Port (see Figure 1).



**Figure 1.** A Map Illustrating The Location Of Trap Installations encompass a number of locations, including DABN Pier, Pelindo Pier, PPI Mayangan Pier, and Kampung DOK

A total of 150 traps were deployed over a three-day period in a variety of locations. Twenty-five traps were placed at DABN Pier, 46 at Pelindo Pier, 25 at PPP Pier, and 54 at DOK Village. In accordance with the guidelines set forth by the Directorate of P2P (Direktorat P2P, 2015), the installation of traps in residential areas is typically divided into two sections: 40% of the traps are placed inside the house, and 60% are placed in the garden or field. The results of rat capture based on the location and time of observation are presented in Table 1.

**Table 1**  
 The Results Of The Rat Trapping Exercise, Presented By Trap Location And Observation Date

No	Trap Installation Location	Number of Mice Caught		
		Observation Date		
		May 18, 2024	May 19, 2024	May 20, 2024
1	DABN Pier	1	-	1
2	Pelindo Pier	3	1	1
3	PPP Pier	2	2	-
4	DOK Village	5	2	1
	Total	11	5	3

Source: Primary Data

In this study, the rat species captured included sewer rats (*R. norvegicus*), house mice (*R. tanezumi*), and house mice (*M. musculus*), as listed in Table 2. The

success rate of trapping in the buffer and perimeter areas of Probolinggo Harbor was 4.2%.

**Table 2**  
 The Identification Results Of The Captured Rat Species Based On The Trap Setting Location

No	Installation Location	Species			Total
		<i>M. musculus</i>	<i>R. norvegicus</i>	<i>R. tanezumi</i>	
1	DABN Pier	-	2	-	2
2	Pelindo Pier	1	3	1	5
3	PPP Pier	1	-	3	4
4	DOK Village	1	1	6	8
	Total	3 15.79%	6 31.58%	10 52.63%	19 100.00%

Source: Primary Data

The prevalence of *Leptospira* sp. in captured rats is presented in Table 3. Of the 19 rats tested, 21.05% were positive for *Leptospira* sp. The highest prevalence was found in Kampung DOK, emphasizing the risk posed by residential areas near the harbor.

#### Identify the Presence of *Leptospira* sp. Bacteria in Rat Kidneys Using the iiPCR Examination Method

An examination utilizing the iiPCR method demonstrated the presence of *Leptospira* bacteria in the kidneys of rats captured in the Probolinggo Harbour region. A total of 19 rats were obtained, four of which were infected with *Leptospira* bacteria. Table 3 illustrates that the prevalence of rats infected with *Leptospira* bacteria in the harbor area reached 21.05%.

**Table 3**  
 Rats Infected With the Pathogenic Bacterium *Leptospira* Has Been Observed In Probolinggo Harbor

Location	Number of Mice Caught	iiPCR Results			
		Positive	%	Negative	%
Probolinggo Harbor	19	4	21.05	15	78.95

Source: Primary Data

The results of the iiPCR tests indicated that the majority of rats infected with *Leptospira* bacteria in Probolinggo Harbor belonged to the species *R. tanezumi*, as illustrated in Table 4.

#### Identify the environmental risk factors associated with the distribution of rats in probolinggo harbor

This research model was analyzed using the Partial Least Square (PLS) method and assisted by SmartPLS 4.0 software. Convergent Validity is carried out by looking at reliability items (validity indicators) indicated by the value of the loading factor. Loading factor is a number that shows the correlation between the score of a question item and the score of the indicator construct indicator that measures the construct. In this study, the loading factor limit used was 0.7. After data processing using SmartPLS 4.0, the results of the loading factor can be shown as shown in Table 5.

**Table 4**  
*Leptospira*-Infected Rats In Probolinggo Harbor, Based On The Captured Species Of Rats

Rat Species	n	%
<i>R. norvegicus</i>	1	25%
<i>R. tanezumi</i>	3	75%
Total	4	100%

Source: Primary Data

**Table 5**  
Convergent Validity

Outer Loadings - matrix	Agent	Environment Factors	Sanitation
Humidity		0.923	
Leptospira	0.976		
Gutter			0.960
Lighting		0.925	
Predators		0.935	
Temperature		0.907	
Landfill			0.921
Signs of existence	0.975		
Vegetation			0.966

Source: Primary Data

Table 5 demonstrates significant loadings for environmental factors such as gutter conditions (0.960), vegetation (0.966), and humidity (0.923). These factors were strongly correlated with the presence of *Leptospira*

*sp.* The variable in this study has an AVE value of > 0.5, as shown in the table below, which can be shown in Table 6.

**Table 6**  
Construct Reliability And Validity

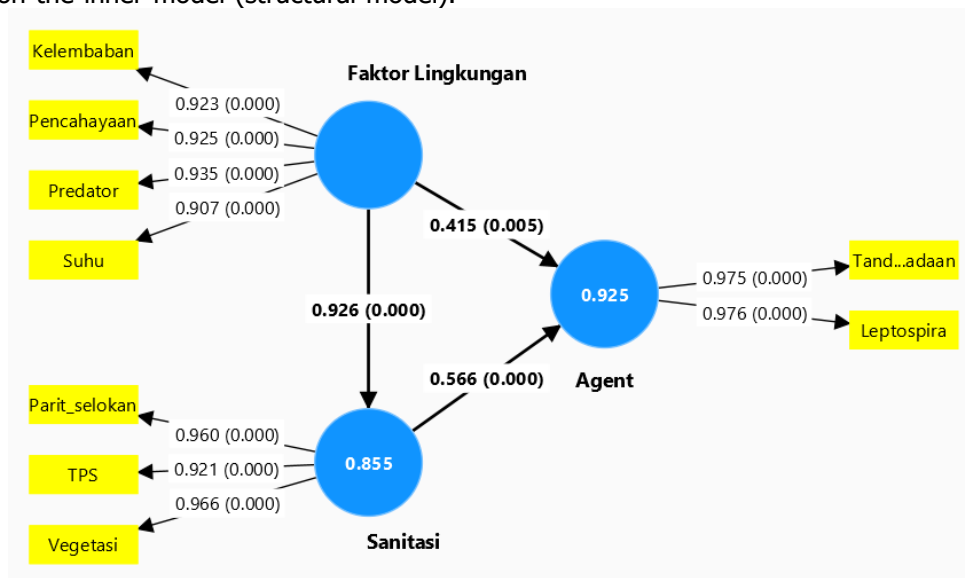
Construct Reliability and Validity - overview	Cronbach's alpha	Composite reliability (rho_a)	Composite reliability (rho_b)	Average variance extracted (AVE)
Agent	0.949	0.949	0.975	0.951
Environment Factors	0.942	0.942	0.958	0.851
Sanitation	0.945	0.945	0.965	0.901

Source: Primary Data

Based on the outer loading value and the AVE value, it can be concluded that the data of this study meets convergent validity. From the output results of SmartPLS in **Table 6**, the composite reliability value for all constructs is above 0.70. With the resulting value, all constructs have good reliability in accordance with the minimum value limit that has been required.

The inner model can be evaluated by looking at the r-square (reliability of the indicator) for the dependent construct and the t-statistical value of the path coefficient test. The higher the r-square value, the better the prediction model of the proposed research model. The value of path coefficients indicates the level of significance in hypothesis testing.

After testing the outer model that has met, the next test is carried out on the inner model (structural model).



**Figure 2.** Analysis Model With Partial Least Square Method



Provides the path coefficients (Figure 2), t-statistics, and p-values for the relationships between environmental factors and *Leptospira* sp. prevalence. Gutter conditions,

vegetation, and humidity were identified as significant predictors ( $p < 0.05$ ), while lighting and the presence of other animals showed no significant impact.

**Table 7** P-Values Of Path Analysis With The Partial Least Square Method

<b>Path coefficients – Mean, STDEV, T-values, P-values</b>	<b>Original sample (O)</b>	<b>Sampe mean (M)</b>	<b>Standard deviation (STDEV)</b>	<b>T-statistics ( O/STDEV )</b>	<b>P-value</b>
Environment Factors → Agent	0.415	0.412	0.148	2.801	0.005
Environment Factors → Sanitation	0.926	0.924	0.028	33.086	0.000
Sanitation → Agent	0.566	0.567	0.148	3.832	0.000

Source: Primary Data

Hypothesis testing is carried out based on the results of the Inner Model (structural model) test which includes r-square output, parameter coefficient and t-statistics. To see whether a hypothesis can be accepted or rejected by paying attention to the significance values between constructs, t-statistics, and p-values. The testing of this research hypothesis was carried out with the help of SmartPLS 4.0 software. These values can be seen from the bootstrapping results. The rules of thumb used in this study were t-statistics  $> 1.96$  with a significance level of p-value 0.05 (5%) and a positive beta coefficient. The value of testing the hypothesis of this research can be shown in Table 7 and the results of this research model can be described as shown in Figure 2.

#### **Identify the Distribution of Rats Using the Success Trap Method**

The rodent species captured in Probolinggo Harbor included the house mouse (*Mus musculus*), the sewer rat (*Rattus norvegicus*), and the house mouse (*Rattus tanezumi*). The *M. musculus* specimens were captured as a result of the deployment of traps in a variety of locations, including outdoor and indoor settings, as well as in proximity to human settlements and at docks. In contrast to other species within the *Mus* genus, house mice are inclined to reside in close proximity to human habitation. Although wild populations do exist, house mice are typically found in residential areas, on farms, and in commercial buildings (Phifer-Rixey et al., 2020). One of its defining characteristics is its commensalism with humans. The global dissemination of this species has predominantly occurred inadvertently via diverse modes of transportation, including cargo containers, luggage, and maritime and aerial conveyance, even encompassing vessels that reach distant islands (Broome et al., 2019; Wilson & Reeder, 2005).

The *R. norvegicus* mice were captured using traps positioned outside buildings with surrounding water gutters, including both open and closed configurations. The study site is situated in an area with high human mobility, which provides a conducive environment for the presence of rats. As Priyambodo (Priyambodo, 2006) notes, *R. norvegicus* is frequently encountered in drainage systems, including sewers and rivers, in residential areas,

markets, and warehouses in port cities and coastal regions. This rat is peridomestic, which indicates that the majority of its activities, including foraging, sheltering, and nesting, are conducted in outdoor environments (Astuti, 2013; Ristiyanto et al., 2014). *R. norvegicus* is one of the most prevalent species of rat in densely populated urban environments, particularly in slums (Sholichah et al., 2021; Sunaryo & Priyanto, 2022; Susanna et al., 2021; Udechukwu et al., 2021).

*R. tanezumi* mice were captured using traps that were placed outside and inside buildings, in both village areas and docks. As stated by Htwe et al. (Htwe et al., 2012), *R. tanezumi* can be found both indoors and outdoors. These rats frequently inhabit roof spaces, wall voids and ceilings, which can cause damage to the structure of the building (Barnett, 2001). As omnivores, their diet consists primarily of plant matter and they are active primarily at night, particularly at dusk and dawn. Additionally, these rats exhibit seasonal migration patterns, moving into fields as spring and fall crops mature and causing damage to crops before harvest (Miller et al., 2008; Singleton et al., 2004).

The majority of the rats captured in the Probolinggo harbour area were male, with a greater number of males than females. The absence of precipitation during the study period enabled male rats to more actively leave their nests to search for food or explore new territories. It is generally observed that male rats spend a greater proportion of their time outside their nests than female rats, due to their capacity to move farther from their homes. The presence of other rats in the vicinity of their habitat, particularly males, prompts them to seek alternative living quarters. Additionally, the limited food resources and the need to find a mate contribute to the high mobility of male rats (Astuti, 2013). These factors, when considered collectively, explain why male rats were captured more frequently than females during the study.

The results demonstrated that the success rate of capturing rats at the port of Probolinggo was 4.2%. This rate is notably higher than the standard set by the Directorate of P2P (Direktorat P2P, 2015), which is 1%. This elevated success rate is believed to be attributable to a number of factors, including the presence of environmental conditions that support the natural habitat

of rats, the strategic placement of traps in areas traversed by rats or in proximity to exit holes, and the utilization of bait that aligns with the preferences of rats. Furthermore, the traps were meticulously cleaned prior to utilization, and the rodent population in the vicinity was considerable. As posited by Astuti (Astuti, 2013), the efficacy of rat capture is contingent upon a multitude of variables, encompassing the nature of the bait, the manner of bait placement, the type of trap employed, the trap's location, and the rodents' capacity to rapidly discern the perils in their environment.

#### **Identify the Presence of *Leptospira* sp. Bacteria in Rat Kidneys Using the iiPCR Examination Method**

The results of the iiPCR test conducted at the port of Probolinggo indicated the presence of *Leptospira* bacteria in one *R. norvegicus* and three *R. tanezumi* mice, representing a relatively high prevalence. The elevated prevalence of infection is hypothesized to be associated with a number of factors, including the body weight of rats exceeding 200 grams, the number of adult rats, and the prevalence of female rats infected with *Leptospira* bacteria. As posited by Himsworth et al. (2013) and Desvars-Larrive et al. (Desvars-Larrive et al., 2020), the probability of *Leptospira interrogans* infection is heightened by a number of factors, including the body weight of the mouse, its age, its sex, the volume of adipose tissue it possesses, and the number of bites it receives. The body weight of the mouse was found to be positively associated with the status of infection with *Leptospira*. Mice of greater maturity were observed to have a greater chance of exposure and infection, which may be attributed to their longer environmental exposure. It is noteworthy that an elevated risk of leptospirosis in female rats has seldom been documented. However, findings from studies conducted by Easterbrook et al. (Easterbrook et al., 2007), Costa et al. (Costa, Porter, et al., 2014), and Minter et al. (Minter et al., 2017) indicate that female mice exhibit a heightened susceptibility to infection compared to males. This phenomenon has been observed to manifest primarily during early life stages, potentially due to the influence of behavioral differences or underlying physiological factors that render female mice more vulnerable to infection.

Two of the four rats that tested positive for *Leptospira* bacteria by iiPCR were found in DOK village. The presence of rats in the vicinity of a residence has been demonstrated to increase the risk of leptospirosis transmission by up to 4.5 times (Sarkar et al., 2002). The prevalence of *Leptospira* in *R. norvegicus* in urban areas was reported by De Faria et al. (De Faria et al., 2008) and Costa et al. (Costa, Ribeiro, et al., 2014) to range from 7% to 82%, while in *R. tanezumi* it ranged from 7% to 34% (Carter & Cordes, 1980; Hathaway & Blackmore, 1981). In Indonesia, studies on the prevalence of *Leptospira* in commensal rodents also demonstrated notable discrepancies. The prevalence of *Leptospira* in *R. norvegicus* was recorded between 3.36% and 72.72%, while in *R. tanezumi* it ranged from 2.94% to 25%

(Handayani & Ristiyanto, 2008; Mulyono & Handayani, 2013; Yuniyanto et al., 2012). As Himsworth et al. (2013) observe, the factors influencing *Leptospira* prevalence rates in mice remain incompletely understood.

#### **Identify the Environmental Risk Factors Associated with the Distribution of Rats in Probolinggo Harbor**

This study identified gutter conditions, vegetation, and humidity as significant environmental factors influencing the presence of *Leptospira* sp. in rats. Poorly maintained gutters, often clogged and damp, provide ideal breeding grounds for rats and act as reservoirs for *Leptospira* sp. bacteria. Similar findings were reported by Rahmawati (2012), who noted a higher prevalence of rats in areas with poor drainage systems. Rats utilize these areas for shelter and as pathways, increasing their interaction with contaminated environments.

Vegetation was also found to significantly contribute to rat activity and, consequently, *Leptospira* sp. transmission. Dense vegetation offers protection, food sources, and access points to human habitation, as supported by Traweger et al. (2006). Rats, particularly *R. tanezumi*, thrive in areas with abundant vegetation, where their activities may spread bacteria through urine contamination of the surrounding environment.

Humidity plays a critical role in sustaining *Leptospira* sp., as these bacteria require moist conditions to survive and proliferate (Evangelista & Coburn, 2010). High humidity levels, typical in tropical harbors, create favorable conditions for both rats and bacteria, thereby amplifying transmission risks.

The prevalence of *Leptospira* sp. among rats in this study (21.05%) aligns with findings from other harbor environments. For instance, Costa et al. (2014) reported prevalence rates ranging from 7% to 34% in urban areas of Brazil, while studies in New Zealand observed rates between 6.5% and 50% depending on environmental conditions (Carter & Cordes, 1980). Regionally, *Leptospira* sp. prevalence among rats in Indonesian ports has been reported to range from 22.23% to as high as 50% in specific areas (Manyullei et al., 2019). Compared to these figures, the prevalence observed in Probolinggo Harbor is moderate but highlights the need for intervention.

Globally, variations in prevalence rates can be attributed to differences in environmental conditions, species composition, and human activity patterns. For example, Himsworth et al. (2013) documented higher prevalence rates in urban slums of Canada, emphasizing the influence of poor sanitation and waste management. The study findings underscore the interplay between environmental factors and rat activity in driving *Leptospira* sp. transmission. Rats serve as primary reservoirs, and their urine contaminates soil, water, and other surfaces. Human exposure occurs indirectly through these contaminated mediums or directly via contact with infected rats.

The significant presence of *Rattus tanezumi* in this study, accounting for 75% of *Leptospira*-positive cases, aligns with its known behavioral traits. As an omnivorous

and highly adaptive species, *R. tanezumi* readily occupies both residential and harbor environments, increasing human-rat interactions and the risk of leptospirosis transmission.

## CONCLUSIONS

This study successfully identified the environmental factors influencing the presence of *Leptospira* sp. around Probolinggo Harbor. The analysis revealed that environmental factors such as gutter conditions, temperature, humidity, and vegetation significantly affect the presence of *Leptospira* bacteria. Out of 150 traps set at 66 strategic locations, the captured rat species included *R. norvegicus*, *R. tanezumi*, and *M. musculus*, with *R. tanezumi* being the most dominant species (52.63%).

The examination showed that 21.05% of the captured rats were positive for *Leptospira* sp., with *R. tanezumi* being the most infected species (75%). Environmental factors such as gutter conditions and abundant vegetation significantly influence the presence of *Leptospira*, making *R. tanezumi* the main vector for the transmission risk, particularly in areas with open waste systems or dense vegetation.

The findings of this study assume that the rat population around Probolinggo Harbor has a relatively stable ecological distribution and that environmental conditions such as temperature, humidity, and vegetation have remained consistent during the study period. Additionally, it is assumed that the prevalence of *Leptospira* sp. is primarily influenced by the environmental conditions assessed, with other potential risk factors remaining constant or negligible.

## SUGGESTION

Based on the findings of this study, it is recommended that the local authorities implement a targeted rodent control program around Probolinggo Harbor. This program should focus on regular monitoring and control of *R. tanezumi*, the primary rat species identified as a vector for *Leptospira* sp., particularly in areas with open waste systems and dense vegetation. The program could include the installation of additional traps and rodenticides and the management of vegetation around the harbor to prevent the spread of rats.

It is also recommended that portable diagnostic tools for the early detection of *Leptospira* sp. be developed and implemented. These tools could be used by local health authorities to quickly assess the risk of leptospirosis in areas with a high rat population and environmental factors conducive to the spread of the bacteria. Early detection would enable prompt interventions and reduce the risk of disease transmission to humans.

Additionally, efforts to improve environmental sanitation around the harbor, such as regular cleaning of gutters, reducing waste accumulation, and controlling vegetation, should be prioritized. Such measures will not only reduce the rat population but also mitigate the

environmental conditions that promote the survival and spread of *Leptospira* sp.

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