Infestation of Leptospira in Rodents at the Perimeter Area in "Pelabuhan Ratu" Port, Sukabumi District

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Abstract. Leptospirosis is a neglected zoonotic disease with global health implications. A significant impact of Leptospirosis happens in tropical regions, including Indonesia. Rodents play an essential role in the spread of Leptospirosis due to their rapid population growth and proximity to humans. This study aims to determine the rodent population density and the presence of leptospira bacterial infestations in the proximity of the "Pelabuhan Ratu" Port, Sukabumi district. This study's survey was conducted monthly for six months (January, February, March, May, June, and August) in 2021 and for three months (January, March, and April) in 2022. The study's survey was held for 15–16 h; a single trap was used to catch rodents. One hundred traps are placed on the port's perimeter inside and outside the building. Leptospira bacteria were detected using PCR in rodents captured between January and March 2022. According to the survey results, 120 rodents were captured, and the sex proportions of rodents collected in the survey were nearly equal. *Rattus norvegicus*, *Rattus tanezumi*, and *Bandicota indica* were all captured. The average population of dominant rodents in the 2021 survey was *R. tanezumi* (4.3), while *R. norvegicus* (17.3) was mainly found in the 2022 survey. The average success rate of traps in 2021–2022 was 7.83 and 24.3, respectively. Rodents were found to have Leptospira bacteria (29.09%). The density of rodents in the proximity of the "Pelabuhan Ratu" port is considered high (> 1). Leptospira is only found in *R. norvegicus* caught at the fish market in this study site. This information is critical for early detection in the effort to raise public awareness of the occurrence of Leptospirosis transmission. It is necessary to improve communication, information, and education to traders and visitors in maintaining personal and environmental hygiene and cross-sectoral collaboration to improve environmental sanitation, particularly in public spaces.

Keywords: Leptospira · Rodent · *Rattus norvegicus* · Market

1 Introduction

Leptospirosis is a neglected zoonotic disease (neglected disease) that can have a global impact on public health, particularly in economically vulnerable populations, such as

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those living in urban slums or rural areas. This disease has a high mortality rate and is widespread, but the burden of morbidity and mortality is not widely known throughout the world, so it is not always a public health priority [1, 2]. According to the findings of Torgerson et al. (2015), this disease is a significant health burden worldwide, exceeding and approaching the burden of other zoonotic diseases, particularly in low-income countries [2].

Indonesia is another tropical country that has reported Leptospirosis cases. These cases are frequently reported as outbreaks, particularly after a flood. Leptospirosis cases increased over the last decade (2011–2020), particularly in the last three years; in 2020, there were 906 cases spread across eight provinces, with the three highest provinces being Central Java, East Java, and Jakarta. West Java Province is also one of the provinces that contribute significantly and is ranked fifth for Leptospirosis cases in 2020, with 16.4% of deaths occurring in this province. The mortality rate for Leptospirosis cases had decreased since 2019 when it reached more than 30% [3].

Spirochaete bacteria are the cause of Leptospirosis (Genus: Leptospira). In nature, leptospires are found in the kidneys of reservoir animals and are transmitted to humans (directly or indirectly) through their urine [4]. Contaminated water or the environment, transmission through rodents' urine, and the presence of livestock as reservoirs are all critical sources of disease transmission. Leptospires are found in mammals [5] such as cattle, buffalo, horses, goats, dogs, cats, [6] and other rodents [7]. Rodents were first discovered as reservoirs and natural hosts capable of sustaining the presence of Leptospira without exhibiting symptoms. Several research findings revealed that several types of rodents tested positive for Leptospira bacteria in various areas [7–10].

Climate, environment, poor sanitation, the presence of rodents, type of occupation, and risky activities are all risk factors for Leptospirosis transmission [11–13]. Because of the rapid growth of the rodent population and its proximity to humans in both urban and rural areas, these animals play a significant role in the transmission of Leptospirosis. Poor sanitation conditions in densely populated residential areas in urban areas or agricultural areas in rural areas put people at risk of Leptospira bacteria transmission [12].

The type of occupation is also a risk factor for infection with the Leptospirosis bacteria (i.e. farmers, livestock workers, traditional market workers). According to research conducted in Malaysia [13, 14] and Indonesia [15], fresh meat and fish traders in traditional/wet markets had a history of Leptospirosis exposure. Wet floors, uncovered garbage piles, insufficient water supply, insufficient drainage, and human activities at the market can provide rodents with an abundant source of food, increasing the risk of Leptospirosis transmission [13].

Pelabuhan Ratu is a popular tourist destination and fishery centre; a fish market at the port sells a variety of fish species in this area. Like other traditional markets, the fish market frequently has poor sanitation, making it a risk factor for disease transmission, especially as a source of Leptospira bacteria transmission. The presence of Leptospira bacterial infestations, as well as the density of rodent populations in an area, are critical factors in preventing transmission to the community. Surveys must be conducted as an initial step toward controlling the spread of Leptospirosis. This survey aimed to determine the level of rodent density in the port perimeter area, including the fish market, and the presence of Leptospira-positive rodents.

2 Materials and Methods

2.1 Study Design

This study uses an observational design with a cross-sectional approach where the collection of rats is carried out once a month for six months (January, February, March, May, June, and August) in 2021 and for three months (January, March, and April) in 2022. The study was conducted in the Pelabuhan Ratu perimeter area, which is part of the territory of the Bandung Health Quarantine Office, Ministry of Health. The radius of the port's perimeter, including the fish market.

2.2 Sample Collection and Identification

Rodents were captured once per month for a total of nine samples. A single trap is used in the rodent catching method. The traps used are 34 × 21 × 16 cm and 28 × 16 × 13 cm in size. The bait is roasted coconut, placed in a trap with 100 traps. The traps are placed purposively around office buildings and the fish auction market within the radius of the port perimeter. Traps are mostly placed inside buildings or kiosks, especially in fish markets; some are outside buildings/kiosks.

The traps are set in the evening, and samples are collected the following day for 15–16 h (04.00 pm–7.00 am). Traps are placed around the port's perimeter, from the offices to the fish market. Traps were placed inside and outside the building, with most of them inside. Traps placed outside the building are frequently lost, so the following catch is mainly placed inside.

All types of rats caught will be further identified through several stages; the rats are anaesthetized according to the procedure [16] using ketamine (1 ml) and xylazine (0.75 ml) injected in one part of the hind leg/thigh; identified using the key "Mammals of the Indomalayan Region" [17, 18]; surgery to remove the rat kidney was conducted according to the procedure [16] (Fig. 1).

Kidney samples were stored in tubes with 70% alcohol. Examination of the identification of leptospira bacteria through the PCR method ("Pockit": nucleic acid analyzer – combining advanced insulated isothermal/iiPCR technology) with the following steps: the sample was inserted in a vial tube, 500 μ PBS was added, the sample pureed with a plastic mortar then centrifuged, 200 μ of the clear liquid transferred to the first hole of the extraction machine, checked for 45 min. Then the sixth hole is added with 50-μ buffer and centrifuged. The liquid was transferred to a 5-μ ertube, coded, and then centrifuged again. The ertube was set in the pockit test for 42 min; then, the results were checked on the screen to see whether the samples were positive or negative for leptospira bacteria. The identification of Leptospira bacteria on captured rodents was limited to rodents caught with a higher population (sample caught in January and March 2022) and adjusted for reagent availability in 2022.

2.3 Data Analysis

The success trap formula was used to analyze the data to determine the density of rodents at the study site, which is the percentage of rodents caught divided by the number

Fig. 1. Identification And Dissection Of Rodent Samples

of traps. Rodent density is classified into two categories: high and low, according to Minister of Health Regulation No. 50 of 2017, based on a standard quality value of one. In addition to rat density data, other supporting data are also presented, including rainfall data in Pelabuhan Ratu District obtained from BMKG Class I Bandung. Data were presented descriptively based on the density of rats caught and analyzed using the Pearson Correlation test (95% CI) to determine the relationship between rainfall and rat density when the survey was carried out using IBM SPSS Statistic 20. Data (mm) was converted first to cm to facilitate the visualization of rainfall. The data was analyzed descriptively to describe the level of rodent density and Leptospirosis bacterial infestation in rodents caught in the study area.

2.4 Study Limitation

There are two limitations of this study: 1) the samples examined for identifying Leptospira bacteria were only concluded from rat samples collected in January and March 2022 due to the limited reagents available. Therefore, the 2021 samples were still stored, and Leptospira bacteria could not be identified; [2]. The collection is not done thoroughly every month, so the density level by season cannot be seen clearly. Furthermore, this study did not assess the state of environmental sanitation using observation tools but instead made direct observations of environmental sanitation in the fish market at the port. Only rodent samples were examined for Leptospira bacteria, and no blood tests were performed to detect a history of Leptospirosis exposure in the community (especially market workers).

3 Results

The total number of rodents captured during this survey was 120, and the sex proportions of rodents captured were nearly the same, albeit quite variable per month catch per species. The number of male rodents caught in the 2022 survey was higher (56.2%), though it was nearly the same as the number of females. Only three rodent species were identified: *Rattus norvegicus*, *R. tanezumi*, and *Bandicota indica.* According to the average rodent density in 2021, *R. tanezumi* appears to be more dominantly caught per month than the other two species (Mean = 4.3). In contrast to the 2022 catch, *R. norvegicus* (Mean = 17.33) has a larger population than *R. tanezumi* (Table 1).

The study's nine-month results show a high rodent density because the success trap exceeds the standard quality value of > 1 per month. The rodent population density on the port's perimeter, particularly in the fish market, is high, especially for rodent catching in January and March 2022. (Fig. 2). Based on rainfall data, fluctuations in rat density are almost the same but look different in January 2022, where rainfall decreases but rat density increases (Fig. 1). The results of the Pearson correlation test show a positive correlation, but the results are not significant (p-value 0.186; r 0.339). Based on observations, the Fish Market in the port perimeter area has open sewerage, garbage dumps, wet floors, and inadequate sanitation. Furthermore, most fish market workers and customers are seen not wearing closed footwear, leaving them vulnerable to wet floors.

The PCR test on rodent kidney samples revealed that nearly all types of *R. norvegicus* (41.03%) had Leptospira serovar bacteria in their kidneys, while the other samples did

Table 1. Rodent Population Density Per Species Catch Per Month In The "Pelabuhan Ratu" Port Perimeter Area, 2021–2022

Year	Month	N (%) rodents catch per species			All rodents	
		Bandicota indica	*Rattus norvegicus*	*Rattus tanezumi*	*N male (%)*	N rodents
2021	January	1 (9.1)	4 (36.4)	6 (54.5)	5 (45.5)	11
	February	0	1 (14.3)	6 (85.7)	1 (14.29)	7
	March	2 (40)	0	3 (60)	3 (60)	5
	May	0	4 (57.1)	3 (42.9)	4 (57.14)	7
	June	0	1 (16.7)	5 (83.3)	5 (83.33)	6
	August	2 (18.2)	3 (27.3)	6 (54.5)	8 (72.72)	11
Mean		**0.83**	**2.17**	**4.83**	**3.83 (48.9)**	**7.83**
2022	January	6 (19.4)	17 (54.8)	8 (25.8)	13 (41.9)	31
	March	2 (8.3)	22 (91.7)	0	18 (75)	24
	April	2 (11.1)	13 (72.2)	3 (16.7)	10 (55.5)	18
Mean		**3.33**	**17.33**	**3.67**	**13.67 (56.2)**	**24.33**

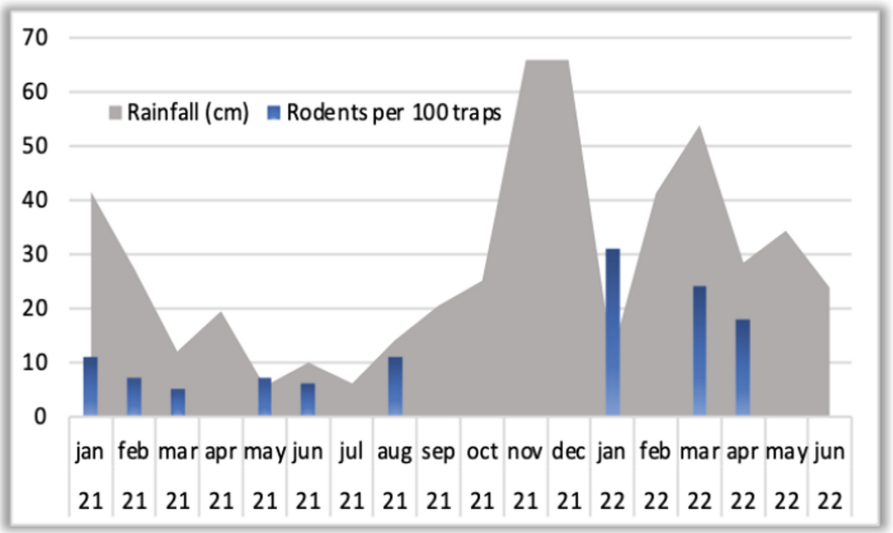

Fig. 2. Rodent Success Traps Vs Rainfall Per Months In Pelabuhan Ratu "Port" 2021–2022

Table 2. Infestation Of Leptospira Bacteria In Rodents Caught Between January And March 2022, In The "Pelabuhan Ratu" Port Perimeter Area

Rodent species	**N**	**Positive (%)**	**Negative (%)**
Rattus norvegicus	39	16 (41.03)	23 (58.97)
Rattus tanezumi	8	0	8 (100)
Bandicota indica	8	0	8 (100)
Infection rate	**55**	**16 (29,09%)**	**39 (70.91)**

not. Positive rodent samples were collected from a fish market. The overall positivity rate for the sample is 29.09% (Table 2).

4 Discussion

The findings of a 9-month rodent caught study in the port perimeter area show that the rodent population density is high. Three rodent species were caught in the study area, with *R. norvegicus* and *R. tanezumi* catching the most commonly caught. Only *R. norvegicus* caught in the fish market area tested positive for Leptospira serovars, with a 29.09% Leptospira positivity rate. Rodents are a significant reservoir and source of leptospira infection. Pathogenic leptospires in urine will be excreted continuously throughout its life. Leptospira can survive for weeks in dirty environments, mud, lakes, ponds, and rivers with a neutral or slightly alkaline pH. The presence of rodents is linked to human infection [19].

The rodent catching success rate describes the relative density of a rodent population in a given location. This is due to various factors, including bait selection, trap quality, and rodent density, in addition to trap placement [20]. The bait selection must be suited to the location where it is placed. Because it acts as a barrier when rodents enter, the trap's quality determines the catch's success. If the trap is not strong enough, rodents can escape more easily. Traps must also be placed in the correct location, along the path that rodents usually take, because rodents will cross the same path to find food, return to the nest, or another location [21].

The study discovered *R. norvegicus*, *R. tanezumi*, and *B.indica* rodents, with *R. novergicus* being the most abundant. According to research findings, *R. novergicus* has a high population in areas with abundant food sources, such as restaurants or markets [22]. Only R. novergicus was found positive for serovar bacteria after a kidney examination. *Rattus novergicus* prefers wet and humid environments; these conditions allow Leptospira to survive longer, increasing the risk of infection in rodents. This species carries several dangerous and commonly found serovars, such as icterohamorragie, ballum, and autumnalis. [4] Because R. novergicus is the most infected with the Leptospira bacteria that cause leptospirosis in humans, it is the primary source of transmission [23]. This rodent is nearly 78 times more likely than other rodents to become infected with Leptospira sp. [24]. The older the age of the rodent, the more the number of Leptospira in the body [25].

The sex proportions of rodents caught were nearly the same. Although the numbers were not all that different, more male rats were caught. This is consistent with research findings from the Port of Manokwari [26]. Male rodents may exhibit aggressive behaviour. On the other hand, several previous studies reported that more female rodents were captured [27, 28]. Female rodents are easier to catch because they enter and exit the nest frequently in search of food, whereas male rodents serve as nest guards [21].

Several studies concluded that environmental conditions, climatic, rodent density, and leptospirosis transmission were related [29–31]. Although this study did not show a significant correlation between monthly rainfall and rat density, other studies reported an effect of climate variables, such as an increase in the rat population during the rainy season [31]. Other regions also reported that the late summer (late summer) and an early rainy season (early fall) between Jan and March have the peak of Leptospira cases [30]., This study indicates a similar situation where the density of rats is seen to be high in January and March (2022). There is an increase in rainfall this year compared to 2021. In addition, the variables ecology, vegetation, food availability, and predator presence all influence the presence of rodents in a given area [32]. Poor waste management will make it easier for rodents to find food, increasing rodent density [33]. On the other hand, predators reduce rodent populations; an area with cats will have a lower density than one without cats [32].

The market, according to the study's findings, is a risk area for disease transmission, including Leptospira [34]. This is because the fish market is relatively wet and moist, with plenty of food available, making it ideal for rodent survival. Wet and humid conditions are also beneficial to leptospira. These bacteria can survive in standing water and soil for several weeks [35]. Market environmental hygiene is closely related to the presence

of rodents and the risk of contracting leptospirosis [36]. The market is closely related to waste and unsanitary conditions, such as poor drainage and open sewers. This condition will further support a rodent's breeding ground [9, 13].

Leptospirosis was found to be common among Malaysian market workers in a study [13]. Aside from rodent survival, hygiene and personal protection factors are essential in the risk of leptospirosis transmission. Market workers are more vulnerable to infection because they are exposed more frequently and do not wear any protective equipment [37]. High seroprevalence is caused by worker activity and the duration of exposure in a contaminated environment.

Longitudinal rodent density surveys and leptospira detection are urgently needed. This study could be beneficial for controlling and preventing leptospirosis in the Sukabumi, including the port area. The port serves as a point of entry for people and goods traffic, and it is located in a densely populated area; the discovery of serovars in rodents will impact the local community's health. Positive serovar results from rodents living near settlements suggest unfavourable environmental conditions that will aid in the spread of leptospira bacteria. Rodents easily move between houses to survive, causing leptospirosis to spread more widely.

5 Conclusion

The rodent population density in the port perimeter area, Pelabuhan Ratu, Sukabumi, is high. *Rattus norvegicus* and *R. tanezumi* are the most commonly caught species. Only *R. norvegicus* caught in the fish market area tested positive for Leptospira serovars, with a Leptospira positivity rate of 29.09 percent.

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