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Potential transmission of leptospirosis from rats and ectoparasites in the Health Office of Port of Surabaya Class I

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Abstract

Purpose: This study attempted to confirm leptospirosis in rats and ectoparasites in the working area of Tanjung Perak Port and the working region of Gresik Port Health Office of Port of Surabaya Class I. **Methods:** This study is a descriptive study using a cross-sectional approach. The samples were rats. The rats are identified to determine the type, then shaved to take ectoparasites and dissected to take the kidneys. Testing of pathogenic *Leptospira* bacteria using the ii-PCR method. The study lasted from October 2022 to February 2023. **Results:** The rat species were *Rattus norvegicus*. Based on PCR examination, pathogenic *Leptospira* bacteria were found in the kidneys of rats *Rattus norvegicus*. *Rattus norvegicus* was infected with the pathogenic *Leptospira* bacteria in the Port of Tanjung Perak working area at 56,67% and in the Port of Gresik working area at 43,75%. The ectoparasites found are fleas, lice, and mites. Both study sites did not find Pathogenic *Leptospira* bacteria in ectoparasites (fleas and mites). Fleas infestation of *Rattus norvegicus* in the working area of Tanjung Perak Port increased by 80% and in the work area of Gresik Port by 68,75%. **Conclusion:** The pathogenic *Leptospira* bacteria was found in both research sites in *Rattus norvegicus's kidneys* but not in ectoparasites (fleas and mites). Flea infestation is also high in both research locations.

Keywords: fleas; leptospirosis; mites; pathogenic *Leptospira*; rats

INTRODUCTION

Leptospira bacteria can cause leptospirosis [1]. The *Leptospira* genus is divided into 20 species. It has been categorized into three main subgroups: nine species are pathogenic, five species are intermediate or have less clear pathogenicity, and six species are saprophytic, which are free-living and do not infect animals [2]. Leptospirosis in humans is caused by various serovars that infect domestic or wild animals [3]. Animals that act as intermediaries for the *Leptospira* bacteria in humans are wild rats, cats, pigs, cows, sheep, horses, buffaloes, squirrels, hedgehogs, civets, and squirrels.

Rats are the main reservoir of *Leptospira* and play an essential role in the occurrence of leptospirosis in humans [1]. Pathogenic *Leptospira* are present in the kidney tubules of certain animals [4]. Various pathogenic *Leptospira* species have been detected in rat kidneys and urine [5]. In addition, it is suspected that the ectoparasites in the rats can be infected with pathogenic *Leptospira* bacteria. European researchers state the possibility of transmission of leptospirosis by *Ixodes ricinus*, the most common tick species in Europe [6]. In flooded areas, ticks can function as a reservoir, allowing the persistence of *Leptospira* in the interval between subsequent floods. Research on the transmission of leptospirosis through ectoparasites still needs to be done [6], even though the ectoparasites in the rat body are very diverse in number and type [7].

This disease can cause multi-organ failure that affects humans, especially in tropical countries. The number of leptospirosis cases in the world is estimated at 1,000,000 cases, with 60,000 deaths each year [8,9]. The incidence of leptospirosis is also estimated to increase by more than 10 per 100,000 people each year in tropical countries [1].

In Indonesia, 734 cases of leptospirosis were reported in 2021, spread across eight provinces, one of which is East Java. Leptospirosis cases in East Java increased from 272 in 2020 to 312 in 2021 [10]. The East Java Provincial Health Office said 2021 data on leptospirosis cases in Gresik Regency was 19.

Leptospirosis can be prevented and controlled by conducting rat sentinel surveillance and detecting *Leptospira* spp. in rats. The aim is to determine the density of rats' detection of *Leptospira* spp. in rats, perform data analysis, and prepare recommendations.

In 2021, the Surabaya Class I Port Health Office (KKP) stated that trap success in one of its working areas, the Port of Tanjung Perak Surabaya and the Port of Gresik is more than 7%. No standard for measuring rat population density exists, but trap success can indicate rat density [11]. Rat density in an area is considered high if the trap success value is above 7% [12].

Based on the results of examination of rat kidney specimens from KKP Class I Surabaya conducted by the Vector Control Working Team at the Directorate of Health Surveillance and Quarantine on January 31, 2022, 4 (21%) of 19 rat kidney specimens were positive for *Leptospira* with details of 1 positive specimen from the working area of Tanjung Perak Port and three positive specimens from the Gresik Port working area.

Tanjung Perak Port and Gresik Port have several cargo and passenger ports. Tanjung Perak Port is the second largest port in Indonesia and connects Surabaya with other ports inside and outside the country. Meanwhile, the port of Gresik only serves domestic crossings [13]. The port is also the entry point for rats from various regions, countries, and goods and people [12].

For this reason, this research also detected *Leptospira* bacteria in rat kidneys and ectoparasites. In Indonesia, no research has detected *Leptospira* bacteria in ectoparasites. The PCR method used is iiPCR. This method requires a short time to detect nucleic acids and can be done anywhere and at any time [14].

As an effort to detect early risk factors for leptospirosis, a fast and precise molecular examination is needed to detect the presence of pathogenic *Leptospira* bacteria in the kidneys and rat ectoparasites in the work area of Tanjung Perak Port and Gresik Port of KKP Class I Surabaya.

METHODS

This research is a descriptive study with a cross-sectional approach and has received approval from the Ethics Commission of the Faculty of Medicine, Public Health and Nursing, Gadjah Mada University, Number: KE/FK/1274/EC/2022, dated 6 October 2022 with a validity period of one year. This research was carried out in the working areas of Tanjung Perak Port and Gresik Port of Class I KKP Surabaya from October 2022 to February 2023. PCR examination was conducted at the Vector and Disease Carrier Laboratory of the Directorate General of Disease Prevention and Control, Ministry of Health, Republic of Indonesia.

The population of this study consisted of rats in the work area of Tanjung Perak Port and Gresik Port

of KKP Class I Surabaya, with the research subjects being rats. The inclusion criteria of this study were adult rats, which were measured by body weight and size according to the type of rat. At the same time, the exclusion criteria from this study were pre-adult rats and rats that died in traps and shrews.

Rats were caught using a single live trap. The number of traps installed every day is 100 pieces [1]. Traps were set in the afternoon at 16.00-17.00 WIB for five consecutive days. The next day, at 06.00-09.00, the trapped rats were checked. Traps containing rats and traps with missing bait are replaced with new traps daily. Rats that were found alive were put in cloth bags. Then, the rats were euthanized by injecting the rats with atropine at a dose of 0.02-0.05 mg/kg body weight of the rats and continued with ketamine HCl at a dose of 50-100 mg/kg body weight for the rats in the thick muscles of the rat's thighs. After the rats were unconscious/fainted, they were identified. After that, the rats were combed to find ectoparasites in the hair on all the outer limbs of the rats, such as the folds of the earlobe, the folds of the thighs, and the body and upper body, and were taken from the rat's pouch. The ectoparasites obtained were put in a tube containing 70% alcohol and labeled and identified. The next step is dissecting the rats to take the kidneys. Examination/detection of Leptospira bacteria in rat kidneys and ectoparasites using the Insulated Isothermal Polymerase Chain Reaction (iiPCR) method with *lipL32* reagent primer.

Examination of the presence of pathogenic *Leptospira* in the kidneys and rat ectoparasites begins with DNA isolation. DNA isolation using Taco Preloaded DNA Extraction Set. The DNA extraction and PCR examination steps begin by grinding a sample of 40 mg of kidney or ectoparasites using a grinder. Then add 500 µL PBS. After that, centrifuge in the cube for 5 minutes. Gently open the cover film of the Preloaded 48 well Extraction Plate. Transfer 200 µL of sample into well one on the preloaded plate. Then, open the Tacomini machine, insert the mixing comb and preloaded extraction plate filled with samples, close the machine, and start the extraction program by pressing the on button. After completing the extraction program, remove the preloaded 48-well extraction plate and mixing comb. The next step is to move the nucleic acid from well six into a new microtube and label the R-tube in the label area. After that, prepare one premix for each sample. Add 50 µL Premix Buffer B to each premix tube, then add 5 µL of DNA extraction or P(+) control to each premix tube. Rotate the premix tube for 10 seconds in the mini centrifuge. Put 50 µL of premix or sample in the R-tube. Close the top of each R-tube. Make sure the R-tube is appropriately closed. Place the R-tube on the Pockit Micro Series machine. Wait for the result in 45 minutes [15–17].

The data obtained is entered into Microsoft Excel and calculates trap success, percentage of rats infected with pathogenic *Leptospira* bacteria, percentage of ectoparasites infected with pathogenic *Leptospira* bacteria, and infestation of fleas.

RESULTS

All rat species caught were brown rats (*Rattus norvegicus*, Berkenhout, 1769), with success traps in the Tanjung Perak Port working area of 9.75% and the Gresik Port working area of 6.75%, as presented in **Table 1.**

The traps in the Tanjung Perak Port working area's perimeter area, as shown in Figure 1, are inside the yellow line. They are located in the outer building of the Gapura Surya Nusantara passenger terminal. The environment around the terminal contains bushes, gutters, piles of used goods, and standing water in the parking area (Figure 1).

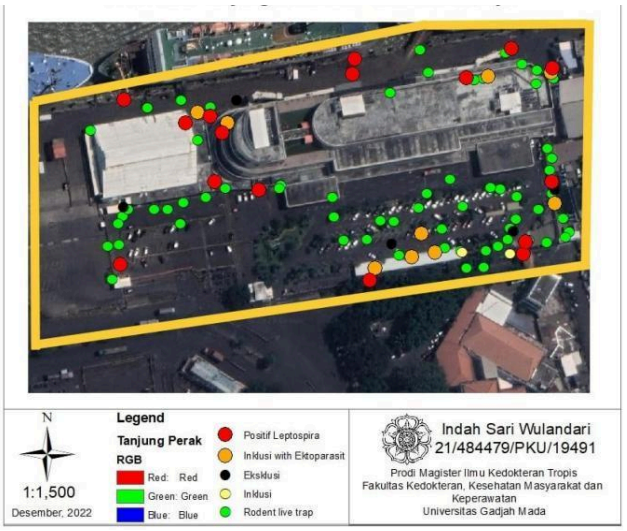

Figure 1. Map of the Distribution of Rats Infected with Pathogenic *Leptospira* Bacteria in the Work Area of Tanjung Perak Port of Class I KKP Surabaya

The traps in the perimeter area of the Gresik Port working area are in the outer building of the passenger terminal, the outer and inner structures of the Food Processing Site (TPP), and the outer office buildings. The environment around the port has bushes, gutters, and piles of goods (Figure 2).

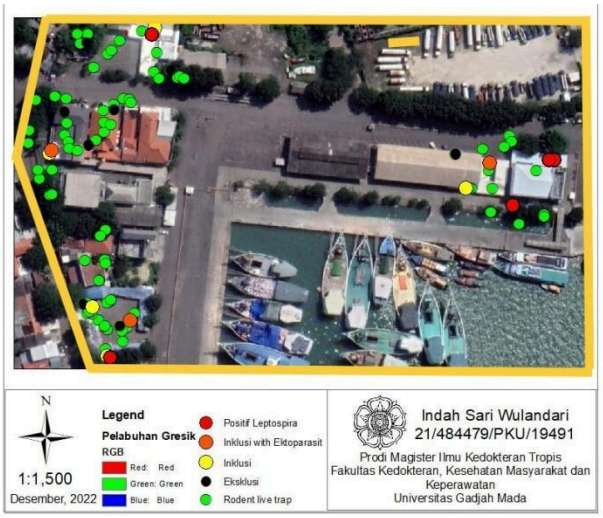

Figure 2. Map of Distribution of Rats Infected with Pathogenic *Leptospira* Bacteria in the Working Area of Gresik Port Class I KKP Surabaya

The results of the PCR examination showed the presence of pathogenic *Leptospira* bacteria in the kidneys of rats obtained from the working area of Tanjung Perak Port, Surabaya, and the working area of Gresik Port. The number of rats containing pathogenic *Leptospira* bacteria in the Tanjung Perak Port working area was 17 out of 30, and in the Gresik Port area, there were 7 out of 16 rats.

Table 2 shows that rats were infected with pathogenic *Leptospira* bacteria in the Tanjung Perak Port working area, 56,67%, and in the Gresik Port working area, 43,75%. The percentage of male rats positive for pathogenic *Leptospira* bacteria in the working area of Tanjung Perak Port, Surabaya, 26,7%, and female rats, 30,0%, is relatively higher than the percentage of male rats positive for pathogenic *Leptospira* bacteria in the working area of Gresik Port, 31,25 % and female rats, 12,50%.

Examination of ectoparasites found a variety of types, fleas (Siphonaptera), mites (Acarina), and ticks (Anoplura). The identification of ectoparasites is shown in Table 3. Mites were the most common ectoparasites found in the working area of the Port of Tanjung Perak, with 118 mites, but fleas were the most common type of ectoparasites found in the working area of the Port of Gresik, with 57 total.

Not all of the ectoparasites found in rats could be tested for PCR because they did not reach the minimum weight of 40 mg. PCR examination was carried out only on fleas and mites with the results that none contained pathogenic *Leptospira* bacteria in the Tanjung Perak Port and Gresik Port working areas.

Flea infestations in the Tanjung Perak Port working area are higher than in the Gresik Port working area. Flea infestation in the Tanjung Perak Port working area is 80%, while the Gresik Port working area is 68,75%.

Table 1. Identification of rat species

Rat species/sex		Research sites			
		Port of Tanjung Perak		Port of Gresik	
		n	%	n	%
Rattus norvegicus	Male	20	51,28	9	33,33
	Female	19	48,72	18	66,67
	Total	39[a]	100,00	27[a]	100,00
Trap success (%)*		39/400 = 9,75		27/400 = 6,75	

Nore : *) Trap success of Tanjung Perak Port : 39 rats per 400 traps; Port of Gresik : 27 rats per 400 traps

Table 2. Rats infected with pathogenic *Leptospira* bacteria

Rat species/sex		Research site/PCR result							
		Port of Tanjung Perak				Port of Gresik			
		Positive		Negative		Positive		Negative	
		n	%	n	%	n	%	n	%
Rattus norvegicus	Male	8	26,67	6	20,00	5	31,25	4	25,00
	Female	9	30,00	7	23,33	2	12,50	5	31,25
	Total	17	56,67	13	43,44	7	43,75	9	56,25

Table 3. Identification of ectoparasites

Rats species / Ectoparasites		Research site			
		Port of Tanjung Perak		Port of Gresik	
		n	%	n	%
Rattus norvegicus	Fleas (Siphonaptera)	77	39,29	57	50,00
	Mites (Acarina)	118	60,20	54	47,37
	Ticks (Anoplura)	1	0,51	3	2,63
	Total	196	100,00	114	100,00

DISCUSSION

The prevalence of leptospirosis in *Rattus norvegicus* is higher than in other rat species [18,19]. It is caused by its habitat in a humid and wet environment so it can support the survival of *Leptospira* [20].

Rattus norvegicus is the main reservoir of leptospirosis. This might be caused by *Leptospira* spp. having a relatively high affinity for specific receptors (PRR) in the kidneys. This is thought to be due to the body weight of the rats caught being more than 300 grams, the number of adult rats, and the number of positive female rats for pathogenic *Leptospira*. Factors that increase the risk of infection are body weight, age, sex, and fat volume, number of bite wounds [21,22]. Body weight has a positive relationship with *Leptospira interrogans* infection status. The more mature the rats, the more they were exposed and infected with it. This can occur due to environmental exposure [21].

Rats can be infected with pathogenic *Leptospira* for a long period of time and do not show any clinical symptoms. Pathogenic *Leptospira* will be stored in the kidney tubules and excreted in rat urine [23]. High rainfall and many puddles are factors for leptospirosis transmission [24].

Rattus norvegicus is often found in waterways, sewers, and gutters in residential areas and markets, warehouses in port cities, and human settlements on the coast [25]. It is periodical, meaning that most of its life activities are carried out outside the home [7,26]. Several previous studies have shown that it is a dominant rat species in densely populated urban environments, especially in slum urban areas [27].

They will not come out of the nest when it rains, so after the rain subsides, the male rats will come out to find food or find a new home range. They will stop its movement when it rains [28]. In addition, male rats are active outside longer. Male rats can move outside the range of their homes. If there are other rat species around the house, *Rattus rattus*, the male rat will look for another place to live. Other factors that cause male rats to often move outside the home, including less available food and looking for a partner [26]. In contrast, female rats have a role in finding food for their children. In addition, they will come out of the nest to get food during pregnancy and lactation [20].

Mites are the most common ectoparasites found in rats. This can happen because the rat species found is *Rattus norvegicus*, a peridomestic rat. Peridomestic rats contain more mites than domestic rats, both in terms of number and type. The development of mites will be optimal at a temperature of 25°C-30°C with humidity close to 100% [7].

Fleas are ectoparasites found infesting rats in both harbors. Fleas like dry places and are protected from sunlight. The effect of temperature and humidity on the development and survival of fleas is still the subject of research to date, but there are studies stating that unfavorable conditions for the development of fleas are temperatures above 28,5°C with humidity ranging from 60%-67% and optimal conditions at a temperature of 18°C-27°C with humidity of 70%-90% [7]. With low rainfall, the optimum temperature for flea life is above 27°C with low rainfall [29]. The evolution of flea and host interactions is related to host environmental factors, host factors such as flea habitat, physiological adaptation and ability to spread, isolation, and specifications [7].

Lice are the fewest ectoparasites found in rats. Lice do not like wet conditions like *Rattus norvegicus* whose habitat is in waterways. The optimum temperature for lice development is the host's body temperature range, which is 23°C – 38°C with 60%-80% humidity [7].

Ticks were not found in rats caught. This can happen because ticks' life cycle requires plants while waiting for their host [7]. In this study, no ticks were found, while research stated that *Ixodes* sp., a tick species, contains pathogenic *Leptospira* bacteria [6].

Flea infestation differences can be influenced by weather factors, such as warm temperatures and humidity, that support the growth of ectoparasites, especially fleas and mites [30]. Male rats have a negative relationship between testosterone levels and their immune system. Male rats are also more aggressive (increased testosterone) and experience

stress more often, so they are easily infested with ectoparasites. In addition, the mobilization of male rats is wider and allows contact with other animals so that they can be infected with more ectoparasites [31].

The trap success is relatively high. It can caused by the environmental conditions at the study sites, the rat's habitat, the placement of the appropriate mouse traps on the paths of the rats or near the exit holes for the rats, the type of bait according to the preferences of the rats, washing the traps before use and the rat population in the area is indeed high, the type of bait, bait installation, type of trap, laying traps, and the behavior of rats, they can learn quickly anything harmful to them [26]. The trapping success above 7% is included in the high category so that it can increase the risk of transmitting leptospirosis [12].

The strength of this research is that it has detected *Leptospira* bacteria in ectoparasites. The limitations of this research are that the number of samples is small, and only one species of rat was caught, so there was no information regarding the presence of pathogenic *Leptospira* bacteria in other rats.

This research is very useful for policyholders to avoid transmission of leptospirosis at the Port of Tanjung Perak and Gresik Port, considering that the symptoms of this disease are not specific.

CONCLUSION

Pathogenic *Leptospira* bacteria were found in the kidneys of *Rattus norvegicus*, so it is necessary to watch out for its transmission, especially to workers and residents around the Ports of Tanjung Perak and Ports of Gresik. Ectoparasites (fleas and mites) were not detected to be infected with pathogenic *Leptospira* bacteria in both study locations, but further detection of arthropod borne diseases is necessary.

Flea infestation was also high in the two study locations, and it is feared that this could be a risk factor for rodent borne diseases. So it is necessary to educate and socialize the community around the port through communication media such as posters and pamphlets regarding the dangers of diseases transmitted by rats.

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