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Analysis of Environmental Factors that Influence the Existence of Rats Positive for *Leptospira sp.* in Kendari City

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Abstract: Rats have the potential to transmit Leptospirosis to humans. The incidence of leptospirosis in Sulawesi Province in 2017 there were 2 suspected cases of leptospirosis in Silea Village, Buke District, Kab. South Konawe, which is directly adjacent to the city of Kendari, and the leptospirosis suspect finally died. In 2022, suspected cases of leptospirosis will increase again to 4 cases, this data is based on laboratory examinations which show positive leptospirosis in Kendari City. The aim of the research is to determine environmental factors that influence the presence of mice, including the presence of rubbish, temperature, air humidity, standing water, sewer conditions and identification of Leptospira sp bacteria in caught mice. The design of this research is cross sectional. Collecting the results of observations and examination of leptospira bacteria in rat kidneys was carried out using Portable PCR, while determining the type of leptospira serotype was carried out using the MAT (Microscopic Agglutination Test). The results of this study found that the variable temperature (ρ = 0.014), the variable humidity (ρ = 0.035), the variable the presence of rubbish and the condition of the sewers (ρ = 0.000) which can be interpreted as that there is a relationship between several environmental factors and the presence of mice positive for Leptospira sp bacteria. . Meanwhile, for the waterlogging variable (ρ = 0.281), it was concluded that there was no relationship between waterlogging and the presence of mice and positive Leptospira sp bacteria. There is a relationship between temperature, humidity, the presence of rubbish, and the condition of the sewers and the presence of mice positive for leptospira sp bacteria. in Kendari City in 2023. People in Kendari City should pay more attention to the cleanliness of their living environment.

Keywords: Environmental Factors; Leptospira sp; Rats Positive

Introduction

Rat species that have been found to be found as reservoirs in Indonesia include Rattus exulans, Rattus hoffmani, Rattus norvegicus, Rattus argentiventer, Rattus tanezumi, and Maxomys bartelsi. Rats have an important role as disease reservoirs, which greatly influence the life, welfare, safety and socio-economics of society (Supranelfy et al., 2019). Transmission of diseases carried by mice can occur either directly or indirectly through ectoparasites. Rat ectoparasites have an important role in the transmission of disease to humans, where ectoparasites act as biological factors (Setyaningrum et al., 2020).

Diseases that can be transmitted by mice include Yersiniosis, leptospirosis, plague and salmonella (Menona & Jolly, 2020). Pathogens that mice can transmit to humans are Entamoebahistolytica, Lymphochytis choriomeningitis, and Giardia muris (Yulianto & Candra, 2019). Leptospirosis is a zoonotic disease and is an emerging infectious disease caused by bacteria of the genus Leptospira (Adamu & Neela, 2023). Leptospirosis is transmitted directly or indirectly from animals to humans through the urine or body organs of mice containing Leptospira bacteria (Paleri, 2020).

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Based on data from Med Crave into the world of Research, the incidence of leptospirosis worldwide in 2018 was around 1.03 million cases with 58,900 deaths. The countries experiencing leptospirosis outbreaks are Thailand and Sri Lanka. The cause of leptospirosis comes from around 19 species, one of the most commonly known is Spirochete, this bacteria is corkscrew shaped with a hook tip (Jamal & A., 2018). The number of leptospirosis cases in Indonesia in 2018 reached 894 cases with 148 deaths. The increase in the number of leptospirosis sufferers occurs during the rainy season and becomes a pandemic when floods occur which are also accompanied by extreme weather. Flood water causes Leptospira bacteria to spread outside and very easily infect humans either through flood water, soil or mud as well as plants that have been infected with the urine of animals that have previously been infected with Leptospira (Haryono et al., 2020).

The results of Rikhus Vektora in 2016 showed that there were reports of reservoirs of leptospirosis in mice from ecosystems far from residential areas. In mice from remote ecosystems positive for leptospirosis were: Bunomyschrysocomus, Bunomyspenitus, Bunomyscoelestis, leopoldamys Edwarsi, Leopoldamyssabanus, Maxomysrajah, Maxomyssurifer, Maxomyswattsii, Maxomyswhiteheadi, Melomysbennisteri, Sundamysmaxi and Sundamysmuelleri. The incidence of leptospirosis in Southeast Sulawesi Province has never been specifically reported, but in 2017 there were 2 suspected cases of leptospirosis in Silea Village, Buke District, Kab. South Konawe, which is directly adjacent to the city of Kendari, and the leptospirosis suspect finally died. In 2022, suspected cases of leptospirosis will increase again to 4 cases, this data is based on laboratory examinations which showed positive leptospirosis in Kendari City.

Transmission of leptospirosis is related to environmental factors, both abiotic and biotic. Abiotic environmental factors include rainfall index, air temperature, water temperature, air humidity, light intensity, water pH and soil pH. Biotic environmental factors include vegetation, trap success and the prevalence of Leptospira in mice (Supranelfy et al., 2019). Apart from that, mice infected with Leptospira bacteria that are near residential areas have the potential to transmit this bacteria to humans around them if environmental conditions support it, in the form of poor sanitation, careless waste disposal and food storage to ensure their survival. This analysis aims to determine environmental factors related to the presence of mice that are positive for Leptospira sp bacteria in Kendari City.

Method

The type of research is a cross-sectional analytical observational study to determine the relationship between the distribution of the presence of rats that are positive for leptospira bacteria and environmental factors that influence the presence of rats, including the presence of rubbish, temperature, air humidity, standing water, and gutter conditions. Descriptive research is research carried out without providing any intervention to the research subject, so that the researcher only observes and analyzes existing phenomena with the environmental components being studied. Collection of observation and measurement results at the research location and examination of leptospira bacteria in rat kidneys were carried out in the field using Portable PCR while determining the type of leptospira serotype using the MAT method.

Rat Sampling

Rat sampling includes: Tools and materials needed as follows: live trap/single lifetrap, cutting board, knife, zinc sheet measuring 20 x 20 cm, long tweezers/cake tongs, cloth bag (blacu), field label, pencil, label thread , Japanese ribbon, raffia rope, wire, cutting pliers, permanent marker, medium thick gloves for bait used in the daily food of the people at the survey location.

Determining the location of trap installation

Rats are cosmopolitan animals, meaning they occupy almost all habitats. To get the expected catching results, when installing traps you need to pay attention to the following things: finding traces of rats such as food remains or litter (fallen fruit), scraps, traces of feces and run-ways. Traps can also be placed around plants and trees. Public information about the presence of rats is also very useful in determining trap success.

Identification of Mice with Portable PCR

POCKITTM Reagent Set uses insulated isothermal polymerase chain reaction (iiPCR) technology to detect specific nucleic acid solutions in bacteria (Wilkes et al., 2014). The test is based on iiPCR for qualitative detection of bacteria where hydrolysis bonds with fluorogenic markers used on viruses and bacteria can produce a fluorescent signal when a specific DNA sequence is amplified.

MAT Method

Many methods are used to diagnose leptospirosis, one of which is the serological method with the MAT (Microscopic Agglutination Test) test for serological diagnosis of leptospirosis, as a powerful and cheap method, which is still widely used in many laboratories

throughout the world (Guedes et al., 2021). The working principle of the MAT test is to incubate samples from patients with various serovars of Leptospira. The sample commonly used for the MAT test is diluted patient blood serum mixed with serotype-determined Leptospira, and the presence or absence of aggregation is determined under a dark field microscope to calculate the antibody titer (Oyamada et al., 2021). A positive result from the MAT test is the presence of clots in the sample to which certain Leptospira serovar antigens have been added, which shows a reaction between serum and Leptospira antigens. A serovar that reacts with the patient's serum is indicated as the infecting serovar. Information on the infecting serovar obtained by the MAT test is used for epidemiological studies. The MAT method starts from preparing the leptospira antigen culture, examining the growth of the leptospira culture, taking the culture, to the MAT test (Jayasundara et al., 2021).

Result and Discussion

In the research that has been carried out, the results obtained are descriptions of the distribution of catches, identification of the presence of leptospira sp. bacteria, types of serotypes of leptospira sp bacteria and analysis of environmental factors that influence the presence of leptospirosis in mice. The research results can be seen in the following graph and table.

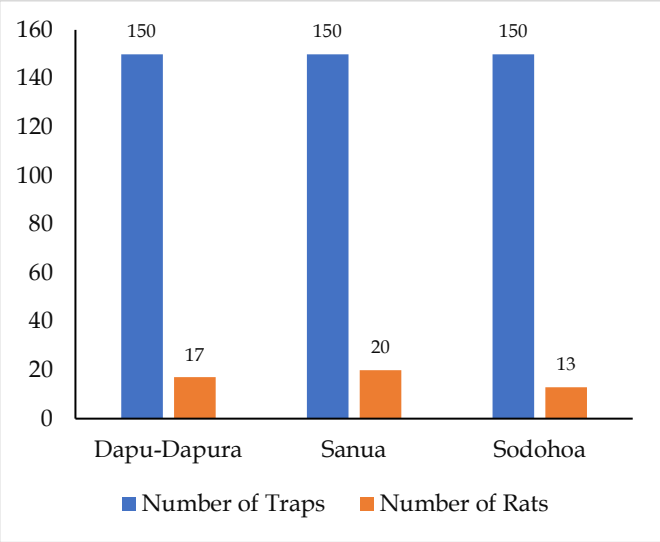

Figure 1. Distribution of Caught Rats based on Number of Traps in Kendari City

Based on Figure 1, it can be seen that the number of traps installed for 3 consecutive days was 450 traps. On day 1 there were 19 traps caught, on day 2 there were 12 traps and on day 3 there were 19 traps. The number of mice caught in 50 traps in Dapu-Dapura, Sanua and Sodoha Villages, West Kendari District, Kendari City.

Rat Density (Success Trap) in Kendari City

The results of the study showed that 49 mice were caught for 3 consecutive days. Every day 150 traps are installed. Based on the calculations above, the results obtained were 10.9% trap success in Kendari City. Caught mice are identified using a mouse identification table to determine their type. The captured mice were anesthetized using chloroform until they fainted and then identified (Chrismayanti, 2023). Identification of the type of rat is seen by paying attention to external morphological characteristics consisting of hair color and type, tail color and length, skull size and shape. Apart from that, other determinations are made by measuring body weight, measuring the total length of the body and tail, length of the tail measured from the base of the anus to the tip of the tail, length of the hind feet measured from the heel to the tip of the toes without nails, length of the ears measured from the base of the leaf. ear to tip of earlobe, body weight, and number of nipples in female mice and number of testicles in male mice. The results of measurements and observations of mouse types are adjusted to the mouse identification key.

The most numerous rat identification results obtained in West Kendari District were 40 Rattus norvegicus rats, 7 Rattus tanezumi rats, 3 Rattus diardii rats. The total number of mice caught was 50. This research is in line with research conducted by Syamsuar, M et al in 2019 which stated that the research results showed that several types of mice were obtained, namely, Rattus Tanezumi (75.00%) and Rattus Norvegicus (25.00%) species (Manyullei et al., 2019).

Rattus norvegicus is a rodent whose habitat in settlements and residences is very dependent on human life. This rat has a total length of 300 – 400 m, tail length 170 – 230 mm, hind leg length 42 – 47 mm, ear size 18 – 22 mm, hair on the back is blackish brown and hair on the chest and stomach is gray (Yuliadi et al., 2016).

Rattus tanezumi is a type of rat that is often found in home areas (roofs, rooms, kitchens), offices, hospitals, warehouses or schools. This type of mouse is known as a house mouse which has the characteristics of a medium sized body, a total length of 220-380 mm, a tail length of 101-190 mm, hind legs measuring 20-39 mm and ears measuring 13-23 mm. R. tanezumi has dark yellowish brown back hair with longer guide hairs and reddish brown to dark gray belly hair (Yuliadi et al., 2016).

Rattus diardii is the scientific name of the house rat, this type of rat's life is very dependent on human life. When food is not available, it really disrupts the continuity of the reproductive process (Ivakdalam, 2016). This type of rat has a body length of 100 – 190 mm, and has a tail length that is longer or equal to body

length. The length of the hind legs is 35 mm and the ears are 20 mm. The nose has a cone shape, the body shape is cylindrical, the tail does not grow hair, and has a body weight ranging from 70 – 300 g. It has slightly coarse textured hair that is blackish brown on the dorsal part and the color on the ventral part is almost the same as the color of the hair on the dorsal part. Female rats have pairs of nipples on the chest and 3 pairs on the stomach (10 pieces) (Sapriyadi et al., 2016).

There are several factors that have been proven to influence the incidence of leptospirosis, namely the presence of mice in and around the house, Leptospira sp bacterial infection occurs due to residential environmental conditions where mice are often found (Samekto et al., 2019). All types of mice have the potential to have parasites in their bodies. Rat ectoparasites can be found in their hair, while endoparasites can be found in the rat's internal organs, including the intestines, liver, heart and lungs. Rats as carriers of ectoparasites and endoparasites have the potential to cause several diseases such as plague, plague and leptospirosis (Manyullei et al., 2019).

Identify the presence of leptospira sp bacteria

Table 1. Results of Identification of Leptospira sp. on Rats in West Kendari District, Kendari City

Types of Rats	Total Pinjal		Total Positif *Leptospira sp.*	
	n	%	n	%
Rattus tanezumi	3	20.0	5	19.2
Rattus norvegicus	12	80.0	21	80.8
Rattus diardii	0	0.0	0	00.0
Total	15	100	26	100

The results of identification of Leptospira sp bacteria through PCR examination in table 1 show that the majority of mice were positive for Leptospira sp bacteria. found in the kidneys of Rattus norvegicus rats with a percentage of 80.8%. This figure is followed by Rattus tanezumi mice with a positive percentage of 19.2%, and Rattus diardii which were not identified as positive for Leptospira sp bacteria. Leptospira bacteria, especially the species L. Icterrohaemorrhagiae, often attack sewer rats (Ratus norvegicus) and house mice (Ratus tanezumi) (Mursyafah, 2018).

The results of this research are different from Tolistiawaty's research in that the results of rat catching were carried out in three locations, namely Lalombi Village, Wani Village and Labuan Panimba Village, 51 rats were obtained, with 44 of the species R. tanezumi (86.3%) and R. norvegicus as many as 7 individuals (13.7%). The results of the PCR examination showed that only 1 rat was positive for the Ratus norvegicus type (Tolistiawaty, 2021).

The presence of a reservoir of mice that are detected positive for Leptospira is one of the risk factors for leptospirosis. The Leptospira bacteria detected in this study nested in the kidney tubules of mice and were excreted in the urine. This bacteria is transmitted directly through the urine and body fluids of infected animals or indirect contact through water or soil contaminated by Leptospira bacteria (Karpagam & Ganesh, 2020). Rats can excrete Leptospira bacteria continuously through urine throughout their lives so that they can be a source of transmission of leptospirosis, especially in humans (Putz & Nally, 2020). Leptospira can contaminate water and food with the risk of causing infection and disease in humans (Ratnaningsih et al., 2023).

Leptospirosis is caused by pathogenic leptospira bacteria which are classified into several species based on DNA-DNA hybridization and also classified into several serovars based on the MAT test (Piredda et al., 2023). The bacteria L. Icterohaemorrhagiae javanica and L. cynopteri are serogroups of the Leptospira group of pathogens found in mice and known to be virulent for humans (Delgado et al., 2022). Research to differentiate between pathogenic and non-pathogenic leptospira is very important and useful for epidemiological data in controlling leptospirosis in the community. Certain serovars will develop into commensals or have a mild pathogenic relationship with their reservoir animal species, for example serovar harjo in cattle, serovar canicola in dogs, and mice by icterohaemorrhagiae and copenhageni (Mursyafah, 2018).

Identify the presence and type of serotype of Leptospira sp bacteria

Based on the MAT test results in table 2, it is known that the types of leptospira serovars found were Grippothyphosa (Gri 20), Icterohaemorhagic (Ict 20), and Bataviae (Bat 80). The MAT test uses 2 live antigens and is the most popular serological test used as a reference test for all other serological tests. The working principle of the MAT test is to incubate samples from patients with various serovars. The leptospira samples commonly used for the MAT test are blood serum. The MAT test results are declared positive if there are clots in the sample to which certain Leptospira serovar antigens have been added, which shows a reaction between serum and Leptospira antigens. A serovar that reacts with the patient's serum is indicated as the infecting serovar (Riyadi & Sunarno, 2019).

Table 2. Results of Identification of Serotypes of Leptospira sp Bacteria in West Kendari District, Kendari City

Species	Types of Leptospira Serotypes			
	Gri 20	Ict 20	Dja 20	Bat 80
Rattus norvegicus	-	1	1	-
Rattus tanezumi	3	2	1	1
Rattus diardii	-	-	-	-
Total	3	3	2	1

Another study showed that from a total of 51 samples, 13 blood samples were exposed to Leptospira sp bacteria. At 10x dilution, the serovars detected were Bangkinang, Icterohaemorrhagie, Djasiman, Hebdomanis, Manhao, Mini, and Batavia. Meanwhile, for the 20x dilution, the serovar types are Icterohaemorrhagie and Djasiman (Tolistiawaty, 2021). Meanwhile, the results of research conducted by Ardanto et al. (2018) showed that the results of the MAT test on rat serum in Bulukumba Regency showed that R. marmosurus was infected with Leptospira serovars Icterohaemorragiae and Djasiman in one individual in HDP, B. chrysocomus and R. hoffmanni were infected with serovar Grippotyphosa in NHJP , and R. tanezumi infected with serovar Icterohaemorragiae.

Examinations using the MAT method are less effective or sensitive than examinations that use samples taken at the start of an acute infection. The serovars of Leptospira bacteria in Indonesia are not completely known for certain, so that negative tests carried out on mouse and human blood samples do not mean they are not infected with Leptospira, but it could be that the infecting serovar is not the type of serovar used for testing (Tolistiawaty, 2021; Widjajantin et al., 2018).

Analysis of Environmental Factors with Leptospira sp. in Kendari City

Based on table 3, it shows that the temperature variable obtained a ρ value of 0.014, which means that there is a relationship between temperature and the presence of mice and positive bacteria Lep tospira sp. In the humidity variable, a ρ value of 0.035 was obtained, which means that there is a positive relationship between humidity and the presence of rats. Meanwhile, the results of research conducted by Ardanto et al. (2018) showed that the results of the MAT test on rat serum in Bulukumba Regency showed that R. marmosurus was infected with Leptospira serovars Icterohaemorragiae and Djasiman in one individual in HDP, B. chrysocomus and R. hoffmanni were infected with serovar Grippotyphosa in NHJP , and R. tanezumi was infected with serovar Icterohaemorragiae Leptospira sp. In the variables of the presence of rubbish and the condition of the gutters, a ρ value of 0.000 was obtained, which means that there is a relationship between the presence of rubbish and the condition of the gutters and the presence of rats positive for Leptospira sp bacteria. In the standing water variable, the ρ value was 0.281, which means that there is no relationship between standing water and the presence of mice and positive Leptospira sp bacteria.

Table 3. Analysis of Environmental Factors with Leptospira sp. in Kendari City

Variable		The presence of Leptospira sp.				Total		*p* Value
		Negative		Positive				
		n	%	n	%	n	%	
Temperature	Optimal	21	60	14	40	35	100	0.014
	Not optimal	3	20	12	80	15	100	
Humidity	Optimal	21	60	14	40	35	100	0.035
	Not optimal	4	25	12	75	16	100	
Existence of Garbage	Fulfil	14	88	2	12	16	100	0.000
	Not Fulfil	10	29	24	71	34	100	
Sewer Conditions	Good	15	88	2	12	17	100	0.000
	Not Good	9	27	24	73	33	100	
Puddle	Not yet	6	67	3	33	9	100	0.281
	Yet	18	44	23	56	41	100	

The number of mice identified as positive for Leptospira sp bacteria. namely 25 animals and 80.8% of them were rats of the Rattus norvegicus type. Environmental factors greatly influence the incidence of leptospirosis which is also related to geographical conditions. Research conducted by Janah, et al (2021) shows that the results of spatial environmental risk analysis show that the potential risk factors for leptospirosis are residential land use, population density of 500-1,249 people/km², altitude of 0-199 meters above

sea level, low rainfall. , no history of flooding, river buffer (Musbir, 2022).

Direct transmission of leptospirosis between mice occurs through various transmission routes. Mice are exposed to Leptospira bacteria through vertical transmission from their mothers or exposure to the bacteria in the nest before weaning (Sari, 2023). Rats will leave the nest after weaning, then be exposed to a polluted environment and direct contact with other rats. Once sexually mature, mice have an additional risk of contracting leptospirosis through sexual contact (Ardanto et al., 2018).

The relationship between temperature and mice that are positive for leptospira sp bacteria

The temperature is measured using a thermometer at the place where the trap is placed. Temperature is one of the abiotic factors in the existence of mice, mice are mammals that have a limited temperature range, namely the upper limit temperature is more deadly for mice than the lower limit temperature (Husni et al., 2023). Temperatures in the range of 28.1-32.7°C have the potential to become a breeding ground for Leptospira (Putri et al., 2019). The results of this study indicate that there is a relationship between temperature variables and the presence of mice positive for leptospira bacteria (ρ = 0.014). At the research location, it is known that the average temperature is in the range of 29 – 31°C.

The relationship between humidity and mice that are positive for Leptospira sp bacteria

Air humidity influences the survival of Leptospira in water and soil environments. The growth rate of bacteria, fungi or microorganisms in the room will be faster if the room has humidity above 60%. So leptospirosis bacteria can easily grow in damp places (Ilma et al., 2023). The results of this study indicate that there is a relationship between humidity variables and the presence of mice positive for leptospira bacteria (ρ = 0.035). At the research location it is known that the average humidity is above 60%. Research in Sri Lanka also stated that the average air humidity during 2006-2015 had an influence on the peak incidence of leptospirosis. (Ehelepola et al., 2019).

The relationship between the presence of rubbish and mice that are positive for Leptospira sp bacteria

The condition of open rubbish dumps is a potential place that rats like most because of the presence of piles of rubbish, namely leftover food, fish, bread, etc. The results of this study show that there is a relationship between the variable presence of trash and the presence of mice positive for leptospira bacteria (ρ = 0.000). A study in Boyolali Regency conducted by Setyaningsih et al. (2022) also stated that there is a significant relationship between the presence of waste and the incidence of Leptospirosis. This means that the presence of rubbish has a 2.13 times higher risk of transmitting leptospirosis (Setyaningsih et al., 2022).

The relationship between sewer conditions and mice that are positive for Leptospira sp bacteria

Sewers or waste water drainage channels are places that are most often used as residences or routes for mice to enter and exit the house. Sewers are one of the places where mice can live. The sewer will become a place of transmission of leptospirosis when the sewer is contaminated by rat urine which contains Leptospira bacteria (Kemenkes, 2017).

Poor sewer conditions can be a route for rats. These conditions can increase direct or indirect contact with mice and their droppings. The condition of gutters that overflow when it rains can also cause puddles around the house. The overflow of water is caused by the dam being made of concrete so it cannot absorb water (Ilma et al., 2023). The results of this study indicate that there is a relationship between the variable condition of the sewers and the presence of mice positive for leptospira bacteria (ρ = 0.000). Research by Husni et al (2022) supports this by finding that there is a relationship between the variable condition of the gutter (ρ = 0.016) and the presence of rats (Husni et al., 2023). Research by Ginting et al (2022) also states that the condition of sewers is related to the transmission of leptospirosis. Where the results of statistical analysis show an OR value of 4.29, which means the possibility of transmitting leptospirosis in poor sewer conditions is 4.29 times higher than in good sewer conditions. (Ginting & Indarjo, 2022).

The relationship between the presence of standing water and mice that are positive for Leptospira sp bacteria

With leptospirosis, there is a lot of possibility of water pooling in the rainy season and not paying attention to the cleanliness of the environment inside and outside the house. Leptospirosis is closely related to environmental conditions which cause bacteria to multiply rapidly in poor environmental conditions (Ilma et al., 2023). Leptospira bacteria can survive for several months in standing water, whereas in damp soil these bacteria can survive for at least three weeks (Ardanto et al., 2018). The results of this study showed that there was no relationship between the waterlogging variable and the presence of mice positive for leptospira bacteria (ρ = 0.281). This research is in line with research in Pati Regency which also found no relationship between the incidence of leptospirosis and the presence of standing water (Samekto et al., 2019).

Conclusion

Rat identification results obtained in West Kendari District showed several types of rats, of the 50 rats caught, 40 of them were Rattus norvegicus (sewer rats) then 7 were Rattus tanezumi (house rats) and 3 were Rattus diardii. The number of mice identified as positive for Leptospira sp bacteria. namely 25 animals and 80.8% of them were rats of the Rattus norvegicus type. The test results based on the MAT test results showed that the types of leptospira serovars found were Grippothyphosa (Gri 20), Icterohaemorhagic (Ict 20), and Bataviae (Bat 80). The results of the analysis of environmental factors found that the variable temperature (ρ = 0.014), the variable humidity (ρ = 0.035), the variable the presence of rubbish and the condition of the sewers (ρ = 0.000) which can be interpreted as that there is a relationship between several environmental factors and the presence of mice positive for Leptospira bacteria. sp. Meanwhile, for the standing water variable, the ρ value was 0.281, which means that there is no relationship between standing water and the presence of mice and positive Leptospira sp bacteria.

Author Contribution
This article was prepared by three authors, namely A.A.A., N, and M.S.A. All writing members worked together at every stage of preparing this article.

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The authors declare no conflict of interest.

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