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Indonesian Journal of
Tropical and Infectious Disease

Vol. 11 No. 2 May – August 2023

Original Article

The Existence of *Leptospira interrogans* on Rats and The Transmission Potency in Public Areas: School, Traditional Market, and Settlement in Yogyakarta

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Received: June 23rd, 2022; Revised: August 2nd, 2023; Accepted: August 11st, 2023

ABSTRACT

Leptospirosis is a zoonotic disease caused by bacterial infection, Leptospira interrogans. Indonesia is known for being an endemic country of this disease and Yogyakarta Special Province has become one of the regions with high cases of leptospirosis. There was lack of information on the L. interrogans prevalence on rats at the public areas, such as school and traditional market. This research was conducted to determine and predict the potential leptospirosis transmission in public areas, especially in schools, traditional markets, and the settlement of Yogyakarta. Wild rats were collected from several public places (elementary schools, traditional markets, and Settlement areas) by using single live traps. The rat's blood was centrifuged to obtain the serum. The serum was tested by using immunochromatography of Leptotek Lateral Flow. The collected rats and shrews were euthanized and then identified for the species and the morphological features. Total of 27 rats (67.5%) and 13 (32.5%) shrews were collected. There were six species of collected rats, namely Rattus argentiventer, Rattus norvegicus, Rattus tanezumi, Rattus tiomanicus, and Bandicota bengalensis, while the collected shrew species was Suncus murinus. The rats and shrews from traditional market were negative with L. interrogans, however the positive result was in elementary schools (14.28%), that were from R. norvegicus and S. murinus, moreover the positive infection also showed in the settlements (57.14%), that were from R. argentiventer, R. norvegicus, and R. tiomanicus. These findings indicated that school and settlement must be a concern for the leptospirosis transmission.

Keywords: Leptospira interrogans; rats; school, settlement, traditional market, Yogyakarta.

Highlights: The novelty in this research was the potency of *Leptospira interrogans* transmission in public areas: school, traditional market, and settlement, as there was limited information on prevalence of infected rats in public areas.

How to Cite: Yuangga, S. A., Fahrunniam, Astuti, R. R. U. N. W. Astuti. The Existence of *Leptospira interrogans* on Rats and The Transmission Potency in Public Areas: School, Traditional Market, and Settlement in Yogyakarta. Indonesian Journal of Tropical and Infectious Disease. 11(2). 73–84. Aug. 2023.

DOI: 10.20473/ijtid.v11i2.46918

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INTRODUCTION

Leptospirosis or Weil's disease is a zoonotic disease caused by bacterial infection from *Leptospira interrogans*, known as pathologic bacteria with spiral morphological features. Leptospirosis mostly can be found in tropic and subtropic areas, and Indonesia is one of the endemic countries of the disease. This disease can be transmitted to humans and animals through reservoirs such as rats, mice, and shrews.[1,2]

Leptospirosis can be a serious problem but is still treatable with the right treatments. The symptoms of leptospirosis in humans are quite similar to other common diseases such as influenza and hepatitis. The similarity of the symptoms caused this disease to be overlooked by many people.[1] Mild symptoms of leptospirosis are fever, severe headache, sore muscles, diarrhea, and mild jaundice or icteric. Severe symptoms of leptospirosis are severe icteric or jaundice, kidney failure, bleeding manifestation, and anuria.[2]

The main transmission of this disease in humans mostly involves direct and indirect contact between *L. interrogans* infected urine and human skin. Direct transmission of *L. interrogans* can occur when human skin contact directly with infected urine or blood. Meanwhile, indirect transmission of this bacteria can occur when human skin contact with water, soil, or another medium that is contaminated by infected urine or blood.[2,3] Reservoirs of leptospirosis commonly known are animals like mammals, especially rodents. Leptospirosis in Indonesia is mostly caused or transmitted by rodents as reservoirs.[4] Rodents in Indonesia that usually become reservoirs of leptospirosis are *B. indica, R. norvegicus, R. exulans, Mus musculus,* and *Suncus murinus*.[2,4]

The Health Profile of Indonesia in 2016 from The Indonesian Ministry of Health shows that Indonesia is an endemic country of leptospirosis shows that Special Region of Yogyakarta is one of the regions in Indonesia with a high number of leptospirosis cases.[5] The Special Region of Yogyakarta had the highest number of leptospirosis cases in 2010 with 230 cases with 23 death cases.[6] Bantul Regency had the highest number of cases with 154 cases than other areas, yet Yogyakarta City had the highest Case Fatality Rate of leptospirosis.[7]

Diagnosis for leptospirosis can be conducted through IgG and IgM detection in the blood with Rapid Detection Test (RDT) using Lepto Tek Lateral Air Flow.[8] Diagnosis for leptospirosis also can be detected through IgG and IgM detection in Reservoir's blood. In previous research by Romadhona (2022), rats were collected from Settlement areas in four districts of Yogyakarta City (Wirobrajan, Tegalrejo, Kotagede, and Umbulharjo), and the blood's serum was analyzed using Lepto Tek Lateral Air Flow. Positive results were shown in 3 of the 29 rats collected from four districts.[9]

Information about the potential transmission of *L. interrogans* in public areas in Yogyakarta other than Settlement areas is still limited compared to the probability of people in Yogyakarta doing activities there and can be direct or indirect contact with the infected rat's urine. In this research, the public areas that are chosen as the focus of studying *L. interrogans* transmission in elementary schools, settlement, and traditional markets of Yogyakarta.

Elementary schools are chosen as representatives of potential studies about *L. interrogans* transmission in the school environment, considering children in elementary schools age are prone to disease and have crucial growth and development.[10,11] Considering the importance of children's health in schools, the study about the potential transmission of threatening diseases such as leptospirosis should be considered to be needed.

Traditional markets were also chosen as representatives of public areas, for this place become the center of people to do daily transactions for daily goods, such as fresh

foods. Traditional markets need some criteria for safety in health issues, such as good sanitation, to prevent some infectious diseases from emerging there.[12] Some traditional markets such as Demangan and Giwangan in Yogyakarta are still categorized as not healthy management.[13] Those issues can lead to environmental health problems such as transmission and infectious disease emergence in traditional markets. Furthermore, an unclean environment is liked by the rats, the reservoirs of *L. interrogans*.[14]

Therefore, this research was conducted to determine and predict the potential leptospirosis transmission in public areas, especially in schools, settlement, and traditional markets of Yogyakarta.

MATERIALS AND METHODS

Materials and Tools

Materials used in this research were collected from rats and shrews from public areas in Yogyakarta such as state elementary schools (Serayu, Sinduadi Timur, Karangwuni 1, Pogung Kidul), traditional markets (Demangan and Kranggan), and Settlement area (Sharehouse at Kocoran and Wirobrajan), labeling stickers, rats bait (dried fish, fried tofu, cheese, bread, food waste, etc.), Ketamine HCl, alcohol 70% and 96%, Rapid Test Kit Lepto Tek Lateral Air Flow (*Leptospira* IgG/IgM) from SD BIOSENSOR (Korea Selatan), assay diluent.

Tools that were used in this research were individual or single live traps, cloth sacks, digital scales, rulers, identification keys, sectioning kit, 1 ml and 3 ml syringe, EDTA venoject, microtube, mikropipet, and refrigerated centrifuge.

Methods

This research was using experimental analysis of wild rat's collection and detection of *L. interrogans* from the collected rats and shrew bloods, and descriptive analysis for morphometrical analysis, identification, and potential study of leptospirosis transmission.

Wild Rats and Shrews Collection

Rat's collection methods in this research were modified from basic methods for collection from instruction in The Indonesian Ministry of Health and collection methods from Ristiyanto's.[15,16] Rats were collected from four state elementary schools (Serayu, Sinduadi Timur, Pogung Kidul, Karangwuni 1), two traditional markets (Demangan and Kranggan), and two settlements (Kocoran and Wirobrajan). The traps were prepared using dried fish, fried tofu, cheese, or food waste as bait. The traps were installed in the afternoon and evening of each location with a minimum distance of 5 meters between traps, and then collected on the next day in the morning.

Blood Collection and Serum Extraction

Blood collection were conducted by using the modified method from the Indonesian Ministry of Health (2015) and collection methods from Ristiyanto's.[15,16] The rats in the live traps were put into cloth sack then the rats is released in the cloth sack, and it were anesthetized by using Ketamine HCl 50-100 mg/kg through intra muscular. The anesthetized rats were taken out from the sack and the blood collection conducted through cardio with using 3 ml syringe then transferred into EDTA Venoject.

The serum extraction is carried out with using a refrigerated centrifuge. The blood is transferred into a 1 ml microtube and centrifuged for 15 minutes at 3000 rpm. The collected serum was used in Rapid Detection Test in Lepto Tek Lateral Air Flow.

Rapid Detection Test with Lepto Tek Lateral Air Flow

Widiastuti and Jati[8] conducted whole blood detection of Leptospira IgG/IgM by using Lepto Tek Lateral Air Flow, while in this research was used the serum and whole blood as the materials for detecting *Leptospira* test. The 10 µl serum was inserted

into the sample well of the kit and then 3 drops of assay diluent were inserted into the buffer well. The result came out after 15 minutes and not longer than 30 minutes. The indicators in this kit consist of three categories; C (Control), G (IgG), and M (IgM). The negative result was when the stripe showed in the control indicator. Meanwhile, the positive result was when the stripe showed in either G or M, or both indicators. The stripe or smear in the G indicator showed the blood or serum sample was positive with *Leptospira* IgG, meanwhile, the stripe or smear in the M indicator showed the sample was positive with *Leptospira* IgM.

Identification of Collected Rats and Shrews

The Identification of wild rats and shrews were based on the identification keys book by The Indonesian Ministry of Health and Pinardi's.[15,17] Identification was carried out by using scales to weigh the rats and shrews. The quantitative morphology measurement was carried out by using a ruler to measure total body length (TL), head-body length (HB), tail length (T), head length (H), hind foot length (HF), and ear length (E). The qualitative morphological observation was carried out by describing the dorsal and ventral fur features, the shape of snout and body, and dorsal and ventral features of the tail.

Trap Success

The success of catching rats in an area was expressed as a successful trap. The trap success was calculated by using the formula[15]:

$$\text{Trap success} = \frac{\text{Number of rats caught}}{\text{Number of rat traps}} \text{ X } 100\%$$

Prevalence of *Leptospira interrogans* in Collected Rats

Prevalence of *L. interrogans* was calculated from the positive results of Lepto Tek Lateral Air Flow compared to the total sample of Lepto Tek Lateral Air Flow test.

RESULTS AND DISCUSSION

Identification, Distribution, and Trap Success of Collected Rats and Shrews

Table 1 showed that there were six kinds of species collected from elementary schools, traditional markets, and settlement areas in Yogyakarta. Those species were *R. argentiventer, R. norvegicus, R. tanezumi, R. tiomanicus, Bandicota bengalensis,* and *S. murinus*, which *R. norvegicus, R. tanezumi*, and *S. murinus* were found at three locations, while *R. argentiventer* and *R. tiomanicus* was only found at settlement. *B. bengalensis* was found at both elementary schools and traditional markets.

R. argentiventer, known as ricefield rat, was identified for having yellowish brown dorsal pelage and broken white ventral pelage.[18,19] *R. norvegicus,* known as Norway Rat or Brown Rat, has a long cylindrical body with total length of more than 350 mm and a blunt conus snout. *R. norvegicus* has a rough texture and greyish-brown colored dorsal and ventral pelage.[18,20] *R. tanezumi,* known as House Rat, was identified for having a rougher and glisten pelage than *R. norvegicus,* and having a yellowish brown color for the dorsal and ventral pelage.[18,21] *R. tiomanicus,* known as Shrub Rat or Tree Rat, was identified for having a greyish-brown dorsal pelage with a broken white or cream-colored ventral pelage.[18] *B. bengalensis* was identified for having typical black-colored dorsal and ventral pelage with rough texture pelage.[18] *S. murinus,* known as House Shrew, was identified as having distinctive quantitative and qualitative morphology differences. *S. murinus* typically has sharp snout with very short tail compared to other species collected from three locations. This species has pungent and distinctive body odor.[21] *S. murinus* has a

smooth and short pelage compared to other collected species.[22]

Morphometric measurement of the species collected from two or three locations was compared, especially based on total length which can be a typical analysis for size comparison. The variance in the total length of each species showed a particular trend that the rats and shrews species caught from traditional markets were bigger and longer than the species caught from elementary schools. The distinctive difference in size was shown in *R. norvegicus*, *B. bengalensis*, and *S. murinus* (Table 1). Morphometric differences between some species collected from traditional markets and elementary schools, especially the total length and the weight, can be affected by adaptation to the environment, which was related to the activities of the rats and shrews in eating patterns and habits.[23]

Table 1. Range of Body Weight and Morphometry of Collected Rats and Shrews from Elementary Schools, Traditional Markets, and Settlement in Yogyakarta

Morphometry	Elementary Schools	Traditional Markets	Settlement
Rattus argentiventer			
W (g)	-	-	64 - 137
TL (mm)	-	-	264 - 322
HB (mm)			139 - 151
T (mm)	-	-	125 - 171
HF (mm)	-	-	34 - 40
E (mm)	-	-	18 - 15
Rattus norvegicus			
W (g)	207 - 287	292 - 314	237- 298
TL (mm)	365 - 400	405 - 420	396 - 401
HB (mm)	188 - 213	229 - 226	204 - 216
T (mm)	176 - 187	175 - 194	180 - 197
HF (mm)	41 - 43	40 - 44	42 - 45
E (mm)	20 - 21	19 - 22	19 - 21
Rattus tanezumi			
W (g)	24 - 169	87	116
TL (mm)	222 - 359	284	324
HB (mm)	97 - 183	138	156
T (mm)	125 - 190	146	168
HF (mm)	25 - 35	36	38
E (mm)	15 - 22	20	18
Rattus tiomanicus			
W (g)	-	-	167
TL (mm)	-	-	358
HB (mm)	-	-	188
T (mm)	-	-	17
HF (mm)	-	-	37
E (mm)	-	-	19
Bandicota bengalensis			
W (g)	86 - 270	283	-
TL (mm)	285 - 395	405	-
HB (mm)	145 - 205	222	-
T (mm)	140 - 190	183	-
HF (mm)	35 - 40	42	-
E (mm)	18 - 20	21	-
Suncus murinus			
W (g)	27 - 50	40 – 63	44
TL (mm)	185 - 210	188 – 213	188 - 202
HB (mm)	109 - 119	116 – 140	117 - 126
T (mm)	68 - 91	63 – 83	71 - 76
HF (mm)	18 - 21	18 – 30	20 - 30
E (mm)	9 - 10	6 – 10	8 - 9

Abbreviation: W: Weight; g: gram; TL: Total body length; mm: millimeters; HB: Head-body length; T: Tail length; HF: Hindfoot length; E: Ear length

Table 2 showed the distribution and trap success in elementary schools, traditional markets, and settlement areas. The trap success of the rats was ideal to analyze the density of the rats in a certain area.[24] It was showed that rats can be found in all locations,

which indicates that there was a rat population at that location. The highest trap success was found in the traditional market at 23.3%. The trap success of rodents such as rats is categorized into high density if the number is higher than 7%.[24]

Table 2. The Distribution and Trap Success of Collected Rats and Shrews

Species	Location			Total (%)
	ES	TM	S	
R. argentiventer	0	0	4	4 (10%)
R. norvegicus	3	4	2	9 (22.5%)
R. tanezumi	7	1	2	10 (25%)
R. tiomanicus	0	0	1	1 (2.5%)
B. bengalensis	2	1	0	3 (7.5%)
S. murinus	3	8	2	13 (32.5%)
Total	15 (37.5%)	14 (35%)	11 (27.5%)	40 (100%)
Trap success	**8.82%**	**14.0%**	**13.75%**	**11.43%**

Abbreviation: ES = Elementary Schools; TM = Traditional Markets; S = Settlement

Traditional markets were a public space known for their unclean condition and for producing food waste. As Saragih et al also Wijayanti and Marbawati said that those conditions were suitable for rats to live in, because rats mostly live in an unclean location and near the food source.[14,25] The number of trap success at traditional markets in this research was higher than the trap success in other research conducted at several traditional markets in Semarang, such as Simongan, Jatingaleh, and Kedung Mundu Traditional market. The trap success at Simongan Traditional Market was 7.0%, the trap success at Kedung Mundu Traditional Market was 4.66%, and the trap success at Jatingaleh Traditional Market was 8.67%.[26,27]

The trap success in settlement in this research was 13.75%, and it was categorized as the high density of rats because it was higher than 7%.[24] Settlement areas that were chosen as the sampling location was sharehouse in Kocoran and Wirobrajan. The trap success at settlement in this research was slightly lower than the trap success in previous research at settlement in urban areas in four District of Yogyakarta. The trap success in four Districts, such as Wirobrajan, Umbulharjo, Kotagede, and Tegalrejo, was about 14.5%.[9] The difference in successful traps was probably due to the number of traps installed, the presence of rats in the area, as well as the density of the human population.

The lowest number of trap success was shown in Elementary schools, it was 8.82%. This number was higher than 7% and categorized as high density of rats.[24] This finding showed higher rat's population than in the previous research that conducted in Krapyak Islamic Boarding School of Yogyakarta. The trap success at Krapyak Islamic Boarding School was 5.9%[28], in contrast the trap success of this research in school was almost one and a half times. The difference in trap success between boarding schools and elementary schools in this research was the existence of residences within the school area may be the biggest factor of increasing the trap success in boarding school more than elementary schools as the sampling location in this research. Residence that close to the school could increase the food source of the rats in the school area. This condition was also supported by the rats caught from elementary schools, that were mostly collected from the school guard's home within the school area, which it can be correlated with the fact about residence for increasing the food source of the rats.[29]

The trap success of rats in each location can be affected by some factors, such as the quality of the live traps, the bait used, the rat's habit, and the location of the trap.[30] The right position of the installed rat trap would made a higher probability of trap success, because of the rat's thigmotaxis behavior which was explaining the behavior

of the rats to always go through the same track.[31]

R. tanezumi was the most caught species in this research. The distribution of the *R. tanezumi* in this research was 7 individuals caught from elementary schools, an individual caught from traditional markets, and 2 individuals caught from settlement. *Rattus tanezumi* was mostly found in elementary schools because *R. tanezumi* is a commensal Rat that is found in a house or buildings in Settlement.[32] *Rattus norvegicus* was the second most caught species in this research with a total number was 9 individuals. *Rattus norvegicus* was mostly caught from traditional markets for 4 individuals. This number was corresponding with the other research results about *R. norvegicus* is the most caught species in Simongan Traditional Market of Semarang.[26]

The factors that affecting the trap success of *R. norvegicus* were the habitat of *R. norvegicus* was at in a bad sanitation locations or in waterways, which was considered as the characteristic of some traditional markets in Yogyakarta.[13,18]

A number of 6 rats out of 33 rats that were caught showed positive for *L. interrogans.* and the prevalence of *L. interrogans* on the each species samples were showed in Table 3.

Table 3. Prevalence of *Leptospira interrogans*

Species	Positive of *L. interrogans* infection (n/N)*			Prevalence in Each Species (%)
	ES	TM	S	
R. argentiventer	(0/0)	(0/0)	(2/3)	66.67%
R. norvegicus	(1/3)	(0/4)	(1/2)	22.22%
R. tanezumi	(0/7)	(0/2)	(0/1)	0%
R. tiomanicus	(0/0)	(0/0)	(1/1)	100%
B. bengalensis	(0/2)	(0/1)	(0/0)	0%
S. murinus	(1/2)	(0/5)	(0/0)	14.28%
Total	(2/14)	(0/12)	(4/7)	**18.18%**
Prevalence in Each Location (%)	14.28%	0%	57.14%	

Abbreviation: ES = Elementary Schools; TM = Traditional Markets; S = Settlement*(n/N) = the number of positive samples/number of samples examined

Table 3 showed the prevalence of *L. interrogans* based on the species and the locations. The total prevalence of *L. interrogans* from all collected blood samples was 18.18%. The previous research by Astuti showed that the prevalence of *L. interrogans* from four District of Yogyakarta City was 10%.[9[in review]] Meanwhile, the previous research by Joharina et al (2019) showed the prevalence of *L. interrogans* in Bantul Regency was 20.4%.[33] The prevalence of *L. interrogans* in this research was higher than Romadhona's research but a little lower than Joharina's.[9,33] This research was conducted at several areas of Yogyakarta City and Sleman, thus can be said that prevalence of *L. interrogans* in several areas of Sleman and Yogyakarta City was higher than in four District of Yogyakarta City, but lower than in Bantul Region. The high result in prevalence of *L. interrogans* from this research can be considered as the presence of more *L. interrogans* in the collected rats and lead to higher potential transmission of leptospirosis.[33]

The prevalence of *L. interrogans* based on the location was showed that positive result was shown in the rats and shrews from elementary school and settlement, but there were negative results in all samples from traditional markets. The prevalence of *L. interrogans* in the rats was heterogenous based on the microhabitat of sampling location.[34] The positive results of *L. interrogans* from settlement and elementary schools was affected by the environment of the locations.

Based in Table 3, the prevalence of *L. interrogans* in settlement was 57.14% and 14.28% in elementary school. The prevalence in the settlement was shown four times higher than the prevalence in school. This can be assumed that the *L. interrogans* transmission in settlement is much easier to occur than the elementary schools. The transmission of *L. interrogans* can occur by direct contact between human skin and the *L. interrogans* infected urine or by indirect contact between

human skin and the soil or water contaminated by infected urine from the reservoirs.[2,3]

Thibeaux[35] stated about the suitable environment for *L. interrogans* was water and soil near the banks of rivers or water bodies, and this statement was correlated with the locations which was having the humid environment affected by the distance between water ways or water bodies.[35] The location of *L. interrogans* infected rats in the elementary school was found at Sinduadi Timur State Elementary School and Serayu State Elementary School. Sinduadi Timur State Elementary School is located 220 m from Selokan Mataram waterways and is also located around a residential area. Meanwhile, Serayu State Elementary School is located 550 m away from the Code River and located away from the settlement. The location of *L. interrogans* infected rats in the Wirobrajan District which is located 100 m from Winongo river.

The transmission of *L. interrogans* is considered to be correlated with the species of the rats and shrews. Cosson stated that the main host of the *L. interrogans* is the *Rattus* genus.[36] Ikawati's research stated that *R. norvegicus* and *R. tanezumi* have a higher probability of infection than *Suncus* species. *Rattus norvegicus* have almost 78-fold to be infected by *L. interrogans* compared to *Suncus.* Meanwhile, *R. tanezumi* is 8-fold to be infected by *L. interrogans* compared to *Suncus*.[37]

The results of this research showed that the *L. interrogans* infected rats and shrews species was *R. argentiventer, R. norvegicus, R. tiomanicus,* and *Suncus murinus.* The highest prevalence was 100% from *R. tiomanicus* (Table 3). It was shown that this prevalence was higher than the prevalence showed in Joharina's research at Bantul Region with 31%.[33] The prevalence of *L. interrogans* from *R. tiomancus* showed as the highest prevalence because the higher rate of infection in certain area will affect the transmission of *L. interrogans* easier to occur between or within the rat species.[38] *Rattus argentiventer* and *R. norvegicus* is living in the humid area, which had high potential of *L. interrogans* transmission.[39] The prevalence of *R. norvegicus* in this research was 22.22%. It was lower than the prevalence in Joharina's research in Bantul Region with 43%, but it was higher than the prevalence in Sunaryo and Priyanto's with 12.5%.[33,38] Meanwhile, the prevalence of *L. interrogans* from *R. argentiventer* was 66.67%, and it was higher than the prevalence in Ramadhani and Widiastuti's reasearch with 7.69%.[40] The prevalence of *L. interrogans* from *S. murinus* in this research was 14.28%. It showed that the prevalence in this research was higher than the prevalence in Ikawati's research with 1.6% and Ramadhani's research with 6.7%.[36,41]

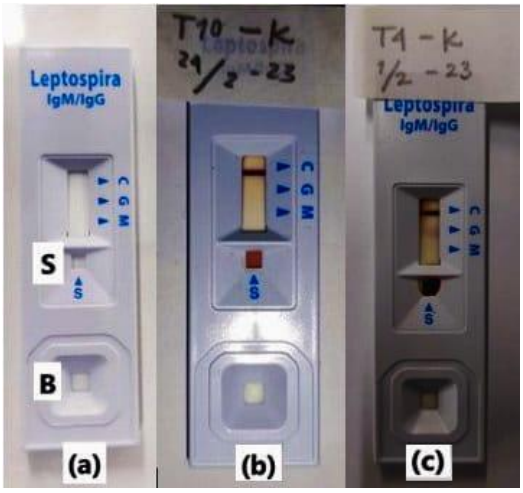

Figure 1. Lepto Tek Lateral Air Flow Kit with C, G, and M indicators (a), Sample well (S), and Diluent Assay Well (B). The Negative result showed in (b) and the positive result showed in (c).

Test results with leptotek showed that in samples rat's that were positively infected with *L. interrogans*, they appeared a line on the IgG and/or IgM line (Figure 1). Table 4 showed the IgG and IgM detection in Lepto Tek Lateral Air Flow from the blood samples. In total of 66.67% and 33.33% of rat's serum were positif with IgG and IgM respectively. The positive results from elementary schools were shown by IgG detection in *R. norvegicus* (50%) and IgM detection in *S.*

murinus. The positive results from settlements were shown by IgG detection in *R. norvegicus*, *R. argentiventer* , and *R. tiomanicus*, and also IgM detection in *R. argentiventer*.

Table 4. IgG and IgM Detection in Lepto Tek Air Flow from *L. interrogans* infected Rats

Location	Species	Indicator		
		Control	IgG	IgM
Elementary Schools	*R. norvegicus*	+	+	-
	S. murinus	+	-	+
Settlement	*R.argentiventer* I	+	+	-
	R.argentiventer II	+	-	+
	R. norvegicus	+	+	-
	R. tiomanicus	+	+	-
			66.67%	33.33%

The IgM detection in the samples indicates the acute phase of early-stage infection in the reservoir. The IgM detection can be detected in the first two months of infection. The IgM level was appearing earlier than IgG and will quickly be followed by IgG. The IgG detection from the samples indicated the secondary immune response after early detection and can be categorized as the chronic stage of *L. interrogans*.[1,42]

The presence of rats is important, because apart from carrying *L interrogans* bacteria that cause leptospirosis, they can also transmit plague, that it caused by bacterial infection, *Yersinia pestis*, and both of the diseases are zoonotic.[43] A healthy lifestyle, with a clean environment is one of the factors to avoid the presence of rats in our environment and prevent leptospirosis transmission

STRENGTH AND LIMITATION

The strength of this study was that the information regarding the prevalence of bacteria *L. interrogans* in wild rats in public areas, especially schools and traditional markets, is still very limited, and this research can be a starting point and a reference for other researchers. The limitation of this study was the need for exploration and integration of data from leptospirosis patients to provide more description and evaluation of risk factors for leptospirosis in humans.

CONCLUSIONS

Six blood samples from collected rats and shrews from settlement and schools were confirmed positive of *L. interrogans.*, while there was negative results of rats from traditional markets.The prevalence of *L. interrogans* in settlement was 57.14% and at the school was 14.28%. These findings revealed that school and settlement must be a concern for the leptospirosis transmission.

ACKNOWLEDGEMENT

Authors thanks to the Faculty of Biology UGM through the KDM (Kolaborasi Dosen Mahasiswa) grand for funding, head and staff Parasitology Division Laboratory of Animal Systematic UGM, head and staff of Elementary Schools (Serayu, Sinduadi Timur, Karangwuni 1, Pogung Kidul), head and staff of the Traditional Market (Demangan and Kranggan), and the communites in Wirobrajan and Kocoran Sleman Yogyakarta.

ETHICAL CLEARANCE

The research protocol was approved by the Research Ethics Committee, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia (Reference number 092/EC-FKH/Eks./2022 and 094/EC-FKH/Eks./2022).

FUNDING

This research was supported by Faculty of Biology UGM through KDM (Kolaborasi Dosen Mahasiswa), grant no. 1147/UN1/FBI.1/KSA/PT.01.03/2022.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest in this research.

AUTHOR CONTRIBUTION

Raden Roro Upiek Ngesti Wibawaning Astuti (RRUNW), Salsabila Rifda Yuangga (SRY), Fahrunniam (FN). Conceptualization: RRUNW. Funding acquisition: RRUN. Methodology: RRUNW, SRY, FN. Original draft preparation: SRY and FN. Writing review, editing and validation: RRUNW.

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