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1. Cover Letter
2. Title Page
3. Whole Manuscript
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Cover Letter

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Semarang, April 27th, 2024

The Editor-in-Chief: Jurnal Kesehatan Lingkungan

Dear Sir,

Attached, please find our manuscript entitled: "***EVALUATION OF RATS DENSITY AND THE ASSOCIATED FACTORS IN LEPTOSPIROSIS ENDEMIC AREAS: THE FIRST REPORT ON THE USE OF BI-INDEX"*** which we would like to submit to the scientific journal that you run as an original research paper.

Information regarding the rat presence, density, and distribution in settlements of Leptospirosis endemic areas, and the associated factors must be updated and hardly accessible to the broad scientific community and the health policy planner. As a part of our attempts to evaluate the rat density and the associated factors in the settlement, we would like to share our data that might be important for the establishment of the Leptospirosis reservoir control program in the area and also provide the scientific information for control of the Leptospirosis reservoir in Indonesia.

We believe the manuscript would fill the data unavailability and be very relevant to your reader.

I am looking forward to hearing your favorable reply

Sincerely yours,

Sayono Sayono
On behalf of the authors

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Title page

EVALUATION OF RATS DENSITY AND THE ASSOCIATED FACTORS IN LEPTOSPIROSIS ENDEMIC AREAS: THE FIRST REPORT ON THE USE OF BI-INDEX

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Whole Manuscript

EVALUATION OF RATS DENSITY AND THE ASSOCIATED FACTORS IN LEPTOSPIROSIS ENDEMIC AREAS: THE FIRST REPORT ON THE USE OF BI-INDEX

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EVALUATION OF RATS DENSITY AND THE ASSOCIATED FACTORS IN LEPTOSPIROSIS ENDEMIC AREAS: THE FIRST REPORT ON THE USE OF BI-INDEX

ABSTRACT

Introduction: Leptospirosis is a health problem in tropical countries where rats are the reservoir of *Leptospira* contamination. The Bi-index is an effective formula to assess rat density in Leptospirosis endemic areas, but studies have not implemented this formula. This study aims to use the Bi-index in monitoring rat density and the associated factors in urban Leptospirosis-endemic areas. **Methods**: Four endemic areas in Semarang City (Muktiharjo-Kidul I and II, Sambirejo, and Siwalan) were selected as the study sites based on Leptospirosis data in Gayamsari Community Health Center. Live traps were placed at the case's house and 39-49 neighboring houses within a 100m radius on three consecutive days. Trapped rats were collected for species identification, morphometric measurements, and calculation of Bi, diversity, dominance, and evenness indices. Environmental parameters were collected through observation. **Result**: Two-thirds of participants were women, private employees aged 17-55. The success-trap ranged from 2.5-26.5% with the Bi, diversity, dominance, and evenness indices being 0.02-0.32, 0.94-1.09, 0.36-0.44, and 0.79-0.96. Three rat species were trapped, namely *Rattus norvegicus*, *Rattus tanezumi*, and *Mus musculus* with proportions of 61.3%, 34.1%, and 4.7%. Near the river with stagnant water, often being flooded, water entering the house during floods, open trash bins, and rubbish bins around the house were associated with rats. **Conclusion**: The rat density in Semarang City is high where *R. norvegicus* dominates. The presence of rats in residential areas is associated with water drainage and garbage management. Further investigation to determine Leptospira bacterial infection in rodents is needed.

Keywords: Rattus norvegicus, Rattus tanezumi, Rat density, Leptospirosis, Bi-index

INTRODUCTION

Leptospirosis is still a global health problem, mainly in developing countries with tropical climates, with a global incidence in the last decade of 1.3 million cases annually (1). Although the prevalence of Leptospirosis in the Asian region is relatively low (around 17%) compared to the American region, a high prevalence (33.6%) is still found in India, Malaysia, and Iran (2), especially in traditional market workers

(3). Based on the characteristics of sufferers, the prevalence ratio of Leptospirosis in women was higher than in men, amounting to 1.4:1, where those of productive age were more susceptible to being infected with Leptospira (4). Reports showed that the prevalence of Leptospirosis in Indonesia was 39.2%, where cases were spread across nine provinces (5). The incidence of Leptospirosis in urban was higher than in rural areas (6), where Semarang City is one of the urban areas with a total of 34 cases in 2021 (7).

The presence of rats in residential areas is an important factor in the transmission of Leptospirosis because their feces and urine become a reservoir and source of contamination of *Leptospira* bacteria in the floor, soil, and water around the settlements. The most dominant genus of rats infected with this bacteria is *Rattus* (8), and the wider spread of bacteria contaminations and infections is influenced by several factors, such as flooding, work, recreation areas, and rodent density (9). The dominance of rat species in settlement varies according to geographic conditions. Rat species in urban areas are dominated by *R. norvegicus* while in rural areas are *R. argentiventer* (10,11). Several studies have reported rat species that have been proven to be reservoirs of Leptospirosis, namely *R. norvegicus, R. tanezumi, B. indica*, and *B. bengalensis* (12,13).

Data on the presence, distribution, variation, and dominance of rat species in an endemic area is useful for supporting rat mitigation and control programs in residential environments (14). Surveys of rodents in urban settlements endemic to Leptospirosis from several countries show diverse species. A study in Selangor, Malaysia found three rodentia genera with respective proportions of *Rattus* 94.6%, *Tupaia* 4.9%, and *Suncus* 0.5%, and 15.6% of them were confirmed positive for *Leptospira* infection (15). Other findings from Tanzania reported three genera with proportions: *Mus* 51.8%, *Rattus* 44.7%, and *Suncus* 3.5%, and the highest *Leptospira* infection was found in *R. norvegicus* (13). Studies in Indonesia also report the diversity of *Rodentia* species in Leptospirosis endemic areas. Studies in West Jakarta, Indonesia found two genera with respective species proportions of *R. rattus* 74.1%, *R. norvegicus* 14.8%, and *Suncus murinus* 11.1% (16). Studies in Semarang City found three species of rats, namely *R. tanezumi* 53.3%, *R. norvegicus* 33.3%, and *M. musculus* 13.3%, with trap success of 11.8% which indicates a high rat population (17).

A formula for predicting the appearance of rats in an area is Bi-index (18). Apart from the aspect of the proportion of trapped rats, this formula also takes into account the area and land use so that the probability of a mouse species appearing in the location can be estimated. Even though studies on rat density in Leptospirosis endemic areas have been widely reported, the use of this formula has not been

implemented, especially in Indonesia. Studies in Malaysia reported that residential environmental conditions influenced the incidence of Leptospirosis, especially the altitude of the location, house walls, distance to plantations/agriculture, and history of exposure to flooding (19). A similar phenomenon was also reported in Saint Lucia, United States, where poor management of trash cans and the condition of trash cans that were easy for rats to access had an impact on morbidity (20). A study in Klaten district, Central Java Province, Indonesia also reported a link between environmental conditions and the risk of Leptospirosis, including settlements in flood areas, the presence of rats, poor residential conditions, and the distance between houses and nearby rivers (21). Semarang City is one of the urban areas where Leptospirosis is endemic in Indonesia. Environmental conditions and the presence of rats in the endemic areas need to be monitored to support the efforts to control this disease. This study aims to use the Bi-index in monitoring the presence, and density of rats, and the environmental characteristics in Leptospirosis-endemic areas of urban settlements in Semarang City.

MATERIAL AND METHODS

Study sites

This study was part of the research on the genomic and epidemiological characteristics of the Plague, Leptospirosis, Rickettsiosis, and Hantavirus (PESTORITA) in East and Central Java Province, Indonesia conducted by the National Research and Innovation Agency in 2023. This report uses partial data in four endemic areas in Semarang City, namely Muktiharjo Kidul I and II, Sambirejo, and Siwalan villages based on the Leptospirosis data in the Subdistrict Office of Public Health Center in 2022. Based on the location of the cases, four study sites were selected, namely Muktiharjo Kidul I, Sambirejo, Siwalan, and Muktiharjo Kidul II. The number of participants per location was 50 households within a 100-meter radius of the house of the case of Leptospirosis (18).

Rat trapping and identification

Rat trapping was carried out using a single live trap installed in each participant's house in each study site, with a trap distribution of 100 traps in Muktiharjo Kidul I, Muktiharjo Kidul II, and Sambirejo

villages, and 80 traps in Siwalan village. The methods of rat trapping and species identification refer to the PESTORITA standard operating procedures (SOP) (22).

Environmental observations

Observation of the environmental conditions of the house included the parameters of the existence and conditions of ditches and rivers, the existence of stagnant water, history of flooding, water entering the house during floods, the existence and conditions of indoor and outdoor rubbish bins, and the presence of rats around the house. Interviews with households were conducted to complete and confirm the observation results. The data was recorded in an observation sheet, while the location coordinates were determined using the GPS waypoint application using a Samsung A32 cellphone.

Data analysis

Descriptive data was analyzed and displayed in tables and maps. Map was produced using ArcGis 10.8, ESRI, and Google Earth software at the Epidemiology Laboratory, Faculty of Public Health, Muhammadiyah University, Semarang. Descriptive analysis included participant characteristic variables, success trap, Bi-index, rat morphometry, and environmental conditions, while the association of environmental parameters with the presence of rats was analyzed analytically with the Chi-Square test.

Ethical clearance

Data collection was carried out after research permission from the Semarang City Government and an ethical certificate was issued. This research protocol was reviewed and received ethical approval from the Health Research Ethics Commission (KEPK) of the National Research and Innovation Agency, with Referral Number: 049/KE.03/SK/05/2023. Live trap installation and interviews with households were carried out after obtaining permission from the head of the neighborhood.

RESULTS

A total of 190 households were involved in this study. The proportion of males was only one-third of all participants with a range and mean age of 17-69 and 35.46 years, the highest proportion in the young adult age group, and the lowest in the elderly. The majority of participants belong to private

employment groups (labor, entrepreneur, and household) and the least are government officers. In general, the distribution of cases and non-cases of Leptospirosis based on gender showed an almost equal condition. The proportion of women in the case and non-case groups was slightly different so the statistical test results were not significant. Almost similar conditions were found in the distribution of cases based on age groups, where only the 17-25 age group had a higher proportion of cases. However, the age groups 56-65 and >65 years old were only found in the non-case group. The distribution by occupation showed almost equal conditions between the case and non-case groups. The results of the Chi-square analysis of the three variables did not show significant differences ($p>0.05$).

A total of 380 traps were installed in 190 houses with varying results where trap success ranged from 2.5 to 26.5 percent. Two traping locations, namely Muktiharjo Kidul I and Siwalan village showed the highest success traps and high Bi-index values as well. In contrast, two study sites (Sambirejo and Muktiharjo Kidul II subdistricts) were classified as medium and low rat density (**Table 1**). A total of 129 rats were caught in this study which were grouped into three species, namely *R. norvegicus*, *R. tanezumi*, and *M. musculus*. The highest number of caught rats was obtained in the Muktiharjo Kidul I and Siwalan villages, and the least in the Muktiharjo Kidul II location. *R. norvegicus* and *R. tanezumi* were trapped in all study sites, while *M. musculus* was only found in Siwalan village. Female rat percentages were higher than male rats in almost all study sites (**Table 2**). The four trapping locations that have a diversity index in the medium category were Siwalan and Muktiharjo Kidul I villages. Meanwhile, the two other locations were categorized as low-density. All trapping locations have a low dominance index and a stable evenness index (**Table 3**).

Based on the range of morphometric data, it can be seen that the majority of rats caught were in the range of total length (body and tail) of adult mouse size, namely between 300-500 for *R. norvergicus*, 245-445 for *R. tanezumi*, and 160-200 mm for *M. musculus*. This data showed that the majority of the trapped rats were in the adult age category, although a small portion of the *R. norvegicus* was indicated as juveniles based on their total body size which was still below standard. Based on their body size, the species *R. norvegicus* and *R. tanezumi* trapped in the Muktiharjo Kidul I and Siwalan have larger bodies compared to the Sambirejo and Muktiharjo Kidul II trapping locations (**Table 4**).

Five of the ten environmental parameters showed a correlation with the presence of rats in the house (**Table 5**), namely the proximity of the house to the river with stagnant water ($p=0.042$), the house often being flooded ($p=0.000$), water entering the house during floods ($p=0.000$), the presence of open

trash cans (p=0.023), and the presence of piles of rubbish around the house (p=0.009). A total of 4 cases of Leptospirosis in 2023 were spread across four sub-districts in the Gayamsari sub-district, Semarang City (**Figure 1**). A total of 380 rat traps were installed in four fishing locations, namely Siwalan, Sambirejo, and Muktiharjo Kidul. There is one trapping location each for catching mice in Siwalan and Sambirejo villages, while in Muktiharjo Kidul Village there are 2 locations for trapping rats (**Figure 3**).

DISCUSSION

The characteristics of participants

The research results show that female participants dominate. This condition indicates that women participate greatly in promoting a healthy home and residential environment. This finding was similar to that reported in Vietnam, where most participants were female (4). However, ironically, men are more at risk of being infected with Leptospira as reported by several studies (20,21). This phenomenon raises the suspicion that needs to be researched that women care more about their health and self-protection than men. The average age of participants in this study was 35.46 years, which indicates they are of productive age so they have a greater opportunity to obtain information that is useful for their health. This finding is equivalent to a previous report where study participants were in urban areas with a mean of 33.4 years (6). In general, socio-demographic factors including gender, age, and occupation in this study show almost equal conditions based on participant status, cases, and non-cases. The gender and age variables show balanced conditions based on participant status, where these findings are similar to reports from studies conducted in Sabah, Malaysia (23) consistent with findings in Nepal (24), although other reports state that the 25-39 age group has a higher risk. higher incidence of Leptospirosis (6). The employment variable also shows no differences. This may be related to the types of work most at risk for Leptospirosis, namely farmers, livestock breeders (25), hunters, veterinarians, technicians, and livestock truck drivers (26), the types of work in this study do not include these groups.

Success Trap and Bi-index

The success trap value shows that Semarang City has a high density of Leptospirosis reservoirs. This finding can be an early warning sign of the vulnerability of the region to the potential risk of

Leptospirosis. The presence and density of rats in endemic areas need special attention because low density also creates opportunities for disease transmission by the rat reservoir (17). In general, trap success in this study reached 16.9%, higher than the normal standard trap success value of ≥7% in residential areas and ≥2% in garden areas (27). This data shows that the rat population in urban Leptospirosis endemic areas is very high and indicates a high potential for transmission as well. This study was conducted in densely populated residential areas with varying environmental sanitation conditions. The condition of dense and close housing structures can increase the mobility of mice between houses and result in high exposure to mouse feces and urine and impact disease transmission over short distances (28). This is by several findings that the domestic rat population is in line with population density in a settlement due to abundant food sources such as rubbish and food scraps (12). This situation is similar to the findings of a study on rodentia density in urban and rural areas of Nicaragua over two years where trap success was 20.2% (28). Even though the rat trapping in this study was only carried out for two months, success trap values were obtained which were equivalent to those found in Nicaragua, even higher for some locations. This finding is similar to another study from Makassar City which reported trap success of 25% in flood-prone areas (29).

The success traps in this study were in line with the Bi-Index value, where a high success trap value will influence the potential for certain rat species to be caught. Although the Bi-index was originally used to evaluate *Bandicota indica* (18), this formula can be applied to other species. The four rat-catching locations show high to low Bi-index categories. This finding is similar to the results of research in Thailand which reported that the Bi-index value in urban land was in the medium category, where several species of rats appeared at fishing locations because of the attraction of human activities which provided opportunities to find food. However, the phenomenon of areas with high Bi-Index values does not always indicate the presence of cases of Leptospirosis. The two main factors that influence this condition are the phenomenon of underdiagnosis of Leptospirosis because the signs and symptoms of the disease are not sufficiently understood by health workers and the occurrence of misdiagnosis because the detection tool available at public health centers is an antibody-based rapid diagnostic antibody (RDT). If the patient accesses health services within 5-7 days from the onset of fever, the RDT results will show a false negative result (30).

Rats species, the indices of diversity and dominance, and morphometrics

This study reports three species of rats in urban residential areas where *R. norvegicus* is the dominant species. This finding is in line with various other study reports where this species is cosmopolitan in urban areas worldwide, both as a pest and as a disease carrier (14). This is supported by several factors that influence the activity of *R. norvegicus* in urban areas, namely the presence of drainage channels, soil conditions, and dense settlements (31). The presence of open gutters and soil conditions are favorable for this rat species to dig and make nests (32). Slightly different findings were reported from Los Angeles, United States, where rat catching carried out in green open spaces resulted in more *M. musculus* species than *R. Norvegicus*, even though it was carried out in a similar area, namely urban areas (33) This study found a smaller number of rat species than research studies in other areas in Semarang City which found Bandicota indica, *R. norvegicus*, and *R. tanezumi*, as well as *Suncus murinus* (34). This fact shows that urban settlements are areas that have the potential for breeding and mobility of various species of rats, giving rise to great potential for the transmission of rodent-borne diseases. An important lesson that can be learned is to target urban settlements for rat control with the *R. Norvegicus* species as the main focus (35). The proportion of female mice caught was higher than male mice and occurred in all locations. This phenomenon is related to several reasons, namely the roaming area of female mice is narrower than that of male mice, the possibility of being in heat, or being pregnant so they have to go out to look for food. Even though the mobility of rats is quite high, the distances covered are the same and the areas traversed by individuals are relatively the same. This high activity can produce more urine, thereby increasing the chance of Leptospira bacterial contamination (36).

The Shannon-Wiener diversity index is highly dependent on the number of species caught. The higher the species variation, the diversity index value will also increase. The high diversity index at the Siwalan and Muktiharjo Kidul II locations indicates that these areas have ideal habitats and food types for rat breeding (37,38). Then the Simpson's dominance index showed that in all locations there was no competition between rat species. This is reinforced by the fact that the caught rat species have different habitats. *R. norvegicus* is a peridomestic type and makes nests in ditches and ditches, while *R. tanezumi* and *M. musculus* are domestic mice (32), but both of them are active in different places. The species *R. tanezumi* is often found on house roofs while *M. musculus* is often found in cupboards and warehouses (36). Furthermore, the evenness index value shows stable results in all locations. This is because the four fishing locations have an almost balanced environment.

Morphometric data on the species *R. norvegicus* and *R. norvegicus javanus* found a body weight in the range of 38-535 grams and a total length in the range of 249-510 mm, where body weight <200 grams in this species is still included in the juvenile category. The findings are similar to a study report from America that several types of *R. norvegicus* species are still classified as juveniles (39). Other morphometric data, namely body weight, total length, and tail length of *R. norvegicus* in this study were 268.2 gr, 400.5 mm, and 192.7 mm respectively, which overall shows that the mice caught were adult categories (40). A similar thing was also found in *R. tanezumi* where a small number of mice were indicated to be still juveniles based on body weight measurements. Other information reveals that the species is classified as an adult if it weighs between 65-300g (41). Likewise, this study found that the Mus musculus species had a maximum body weight of 41g. Overall, rats caught in Semarang City settlements showed that the four species studied were larger than those in Qatar (40).

Environmental conditions and the rat presence in settlements

This research observed ten aspects of the environment around the participants' homes, of which 5 (five) aspects were significantly related to the rat presence. The presence of piles of rubbish near the house and open rubbish bins is significantly related to the presence of rats in residential areas. Observation results show that every house has a rubbish bin, either made from plastic barrels or made from used tires. However, only a third of all trash cans have lids. These conditions are ideal for mice to get food (20,42). The findings are similar to other studies where the accumulation of rubbish is directly proportional to the incidence of Leptospirosis (43), and the presence of rubbish around the home environment will increase the number of rat populations (44). The public needs to know about this phenomenon to improve cleanliness in the home environment as an effort to prevent and control the rat population.

Reminiscent river condition parameters are part of the risk factors for Leptospirosis (45) where standing water becomes a medium for spreading bacteria to other places. This research was carried out during the dry season, but there were still many flooded rivers originating from residential wastewater. Poor sanitary conditions will be a good environment for mice, especially the *R. norvegicus* species, and increase exposure to the risk of Leptospira bacterial contamination in standing water (6). These bacteria can survive in normal to slightly alkaline water pH in the range of 7.3-7.7 (46). Another aspect is the history of flooding and water entering the house. Flood waters submerge gutters and rat nests, especially

R. novegicus so that they migrate to safe places, including inside houses. Other safe places from waterlogging include mounds of land with bushes and around residential areas (36).

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CONCLUSION

Three species of mice were found where *R. norvegicus* was the dominant species. The density of rats in the Leptospirosis endemic area of Semarang City is high, where the presence of rats in residential areas is related to the proximity of stagnant rivers, a history of flooding, houses being flooded, there are open rubbish bins, and there are piles of rubbish near the house. Morphometric data indicated that the majority of mice were adults. Further investigation is needed to determine Leptospira bacterial infection in rat feces and urine, and the level of community participation in efforts to control rodents in residential areas.

SOURCE OF FUNDING

National Research and Innovation Agency

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest regarding this research.

AUTHOR CONTRIBUTIONS

Conceptualization: ZA, SS, FDH. Data curation: ZA, FDH. Formal analysis: ZA, SS. Funding acquisition: National Research and Innovation Agency, Salatiga Co-Working Space. Methodology: ZA, RR, FDH, SS.

Project administration: ZA, FDH. Writing of original draft: ZA, SS. Internal review of manuscript: RR, FDH. Writing of review and editing: SS, FDH. All authors have read and approved the final manuscript.

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Table 1. Rat trap placement, number of trapped rats, success trap, and Bi-index in urban settlement of the endemic areas of Leptospirosis

Trapping Locations	Number of traps	Number of trapped rats	Success Trap (%)	Bi-index
Muktiharjo Kidul I	100	53	26.5	0.28
Siwalan	100	52	26.0	0.32
Sambirejo	80	19	11.9	0.11
Muktiharjo Kidul II	100	5	2.5	0.02

Table 2. Distribution of the species of tat-trapped species based on study sites

Rat Species	Study Sites							
	Sambirejo		Muktiharjo Kidul I		Siwalan		Muktiharjo kidul II	
	n	%	n	%	n	%	n	%
Rattus norvegicus								
Male	2	22.2	9	25.0	10	31.3	2	100.0
Female	7	77.8	27	75.0	22	68.8	0	0
Rattus tanezumi								
Male	4	40.0	6	35.3	4	28.6	0	0
Female	6	60.0	11	64.7	10	71.4	3	100.0
Mus musculus								
Male	0	0	0	0	1	16.7	0	0
Female	0	0	0	0	5	83.3	0	0

Table 3. The diversity index of Shanon-Wiener, dominance index, and evenness index of rats

Lokasi Penangkapan	Shanon-Wiener diversity index	Simpson's Dominance Index	Evenness Index
Sambirejo	0.94	0.42	0.86
Muktiharjo Kidul I	1.06	0.36	0.96
Siwalan	1.09	0.40	0.79
Muktiharjo Kidul II	0.95	0.44	0.86

Table 4. Rat morphometry based on the rat trapping location (study sites)

Species	Rat trapping location											
	Sambirejo			Muktiharjo Kidul I			Siwalan			Muktiharjo Kidul II		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Rattus norvegicus												
Total length (mm)	282	462	368.56	299	510	436.92	249	499	405.66	462	470	466.00
Tail length (mm)	130	207	171.89	143	239	204.44	130	239	186.16	221	234	227.50
Length of hind legs (mm)	32	45	41.33	38	48	43.97	24	48	40.47	41	46	43.50
Length of ears (mm)	16	24	20.33	17	24	20.53	16	23	20.00	21	22	21.50
Body weight (gr)	64	535	205.89	71	519	314.81	38	487	291.84	464	507	485.50
Rattus tanezumi												
Total length (mm)	232	397	348.90	202	416	347.65	245	415	344.86	355	408	389.67
Tail length (mm)	130	207	178.30	110	225	184.82	138	213	182.43	189	219	206.00
Length of hind legs (mm)	28	37	35.50	27	37	34.00	27	43	32.64	34	36	35.00
Length of ears (mm)	18	23	19.90	15	23	19.47	17	21	19.00	21	23	22.00
Body weight (gr)	27	197	123.90	19	227	121.06	39	454	148.86	117	207	175.67
Mus musculus												
Total length (mm)	0	0	0	0	0	0	165	256	219.00	0	0	0
Tail length (mm)	0	0	0	0	0	0	61	136	112.00	0	0	0
Length of hind legs (mm)	0	0	0	0	0	0	20	24	21.83	0	0	0
Length of ears (mm)	0	0	0	0	0	0	15	17	15.33	0	0	0
Body weight (gr)	0	0	0	0	0	0	20	41	30.00	0	0	0

Table 5. Environmental parameters associated with the presence of rats in the home environment

No	Environmental Parameters	Presence of rats				p
		Ya		Tidak		
		n	%	n	%	
1.	The existence of a ditch around the house					
	Yes	27	38.0	44	62.0	0.360
	No	3	21.4	11	78.6	
2.	The existence of a river around the house					
	Yes	6	31.6	13	68.4	0.911
	No	24	36.4	42	63.6	
3.	Puddles of water in the ditch					
	Yes	15	27.8	39	72.2	0.093
	No	15	48.8	16	51.6	
4.	The house is close to a puddle of river water					
	Yes	13	54.2	11	45.8	0.042
	No	17	27.9	44	72.1	
5.	There are puddles around the house					
	Yes	9	56.3	7	43.8	0.098
	No	21	30.4	48	69.6	
6.	The house is often flooded					
	Yes	16	72.7	6	27.3	0.000
	No	14	22.2	49	77.8	
7.	During a flood, water enters the house					
	Yes	14	87.5	2	12.5	0.000
	No	16	23.2	53	75.8	
8.	There is a trash can outside the house					
	Yes	27	37.0	46	63.0	0.527
	No	3	25.0	9	75.0	
9.	There are open trash cans around the house					
	Yes	25	44.6	31	55.4	0.023
	No	5	17.2	24	82.8	
10.	There are piles of rubbish around the house					
	Yes	12	63.2	7	36.8	0.009
	No	18	27.3	48	72.7	

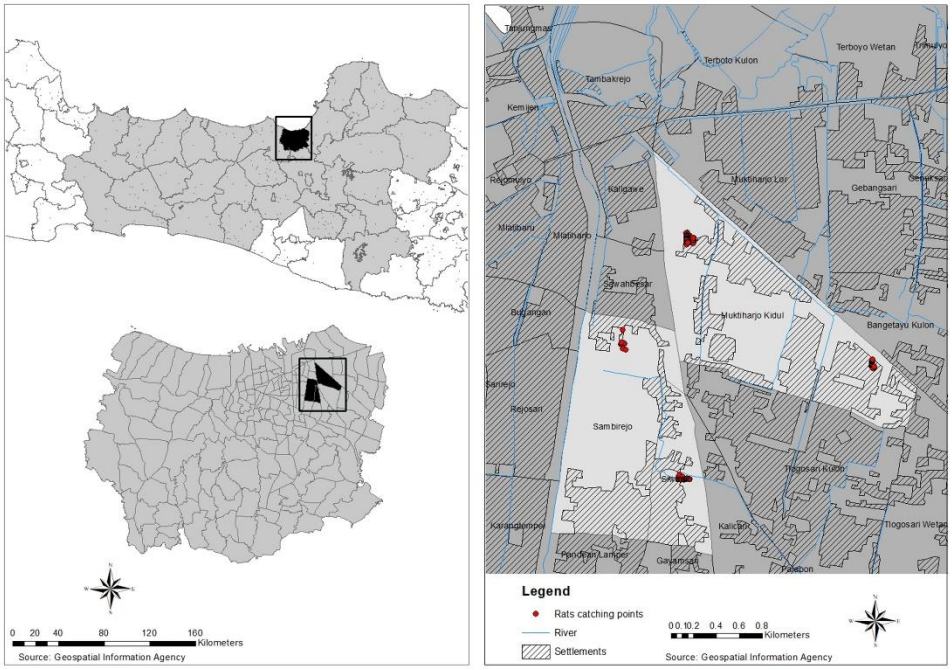

Figure 1. Study sites in Semarang City, Central Java Province, Indonesia

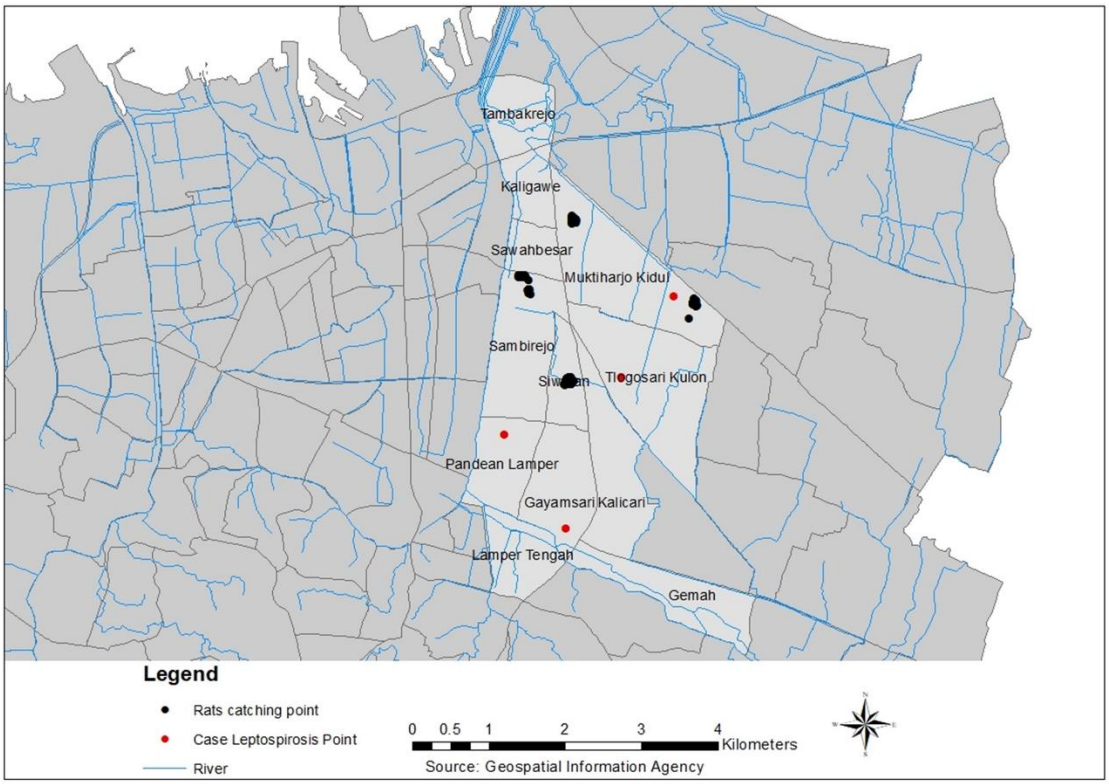

Figure 2. Distribution of Leptospirosis cases in study sites

Figure 3. The rats trapping locations

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Editorial Review

EVALUATION OF RATS DENSITY AND THE ASSOCIATED FACTORS IN LEPTOSPIROSIS ENDEMIC AREAS: THE FIRST REPORT ON THE USE OF BI-INDEX

Zainul Akbar[1], Ristiyanto Ristiyanto[2], Farida Dwi Handayani[2], Sayono Sayono[3,4]

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ABSTRACT

Introduction: Leptospirosis is a health problem in tropical countries where rats are the reservoir of *Leptospira* contamination. The Bi-index is an effective formula to assess rat density in Leptospirosis endemic areas, but studies have not implemented this formula. This study aims to use the Bi-index in monitoring rat density and the associated factors in urban Leptospirosis-endemic areas. **Methods**: Four endemic areas in Semarang City (Muktiharjo-Kidul I and II, Sambirejo, and Siwalan) were selected as the study sites based on Leptospirosis data in Gayamsari Community Health Center. Live traps were placed at the case's house and 39-49 neighboring houses within a 100m radius on three consecutive days. Trapped rats were collected for species identification, morphometric measurements, and calculation of Bi, diversity, dominance, and evenness indices. Environmental parameters were collected through observation. **Results dan Discussion**: Two-thirds of participants were women, private employees aged 17-55. The success-trap ranged from 2.5-26.5% with the Bi, diversity, dominance, and evenness indices being 0.02-0.32, 0.94-1.09, 0.36-0.44, and 0.79-0.96. Three rat species were trapped, namely *Rattus norvegicus*, *Rattus tanezumi*, and *Mus musculus* with proportions of 61.3%, 34.1%, and 4.7%. Near the river with stagnant water, often being flooded, water entering the house during floods, open trash bins, and rubbish bins around the house were associated with rats. **Conclusion**: The rat density in Semarang City is high where *R. norvegicus* dominates. The presence of rats in residential areas is associated with water drainage and garbage management. Further investigation to determine Leptospira bacterial infection in rodents is needed.

Keywords: Rattus norvegicus, Rattus tanezumi, Rat density, Leptospirosis, Bi-index

INTRODUCTION

Leptospirosis is still a global health problem in humans~~, mainly in developing countries with tropical climates,~~ with a global ~~incidence~~ prevalence ~~in the last decade of 1.3 million cases annually~~of 5 and 14 per 100,000 inhabitants in endemic and epidemic areas (1). Although the prevalence of Leptospirosis in the Asian region is relatively low (around 17%) compared to the American region, a high

prevalence (33.6%) is still found in India, Malaysia, and Iran (2), and Bangladesh, especially ~~in traditional market~~ among poultry farm and slaughterhouse workers (3). Based on the characteristics of sufferers, the prevalence ratio of Leptospirosis in women was higher than in men, amounting to 1.4:1, where those of productive age were more susceptible to being infected with Leptospira (4). Reports showed that the prevalence of Leptospirosis in Indonesia was 39.2%, where cases were spread across nine provinces (5). The incidence of Leptospirosis in urban was higher than in rural areas (6), where Semarang City is one of the urban areas with a total of 34 cases in 2021 (7).

The presence of rats in residential areas is an important factor in the transmission of Leptospirosis because their feces and urine become a reservoir and source of contamination of *Leptospira* bacteria in the floor, soil, and water around the settlements. The most dominant genus of rats infected with this bacteria is *Rattus* (8), and the wider spread of bacteria contaminations and infections is influenced by several factors, such as ~~flooding~~elevation, ~~work~~landscape fragmentation, ~~recreation~~ urbanized areas, precipitation, near from forest, and rodent density (9). The dominance of rat species in settlement varies according to geographic conditions. Rat species in urban areas are dominated by *R. norvegicus* while in rural areas are *R. argentiventer* (10,11). Several studies have reported rat species that have been proven to be reservoirs of Leptospirosis, namely *R. norvegicus, R. tanezumi, B. indica*, and *B. bengalensis* (12,13).

Data on the presence, distribution, variation, and dominance of rat species in an endemic area is useful for supporting rat mitigation and control programs in residential environments (14). Surveys of rodents in urban settlements endemic to Leptospirosis from several countries show diverse species. A study in Selangor, Malaysia found three rodentia genera with respective proportions of *Rattus* 94.6%, *Tupaia* 4.9%, and *Suncus* 0.5%, and 15.6% of them were confirmed positive for *Leptospira* infection (15). Other findings from Tanzania reported three genera with proportions: *Mus* 51.8%, *Rattus* 44.7%, and *Suncus* 3.5%, and the highest *Leptospira* infection was found in *R. norvegicus* (13). Studies in Indonesia also report the diversity of *Rodentia* species in Leptospirosis endemic areas. Studies in West Jakarta, Indonesia found two genera with respective species proportions of *R. rattus* 74.1%, *R. norvegicus* 14.8%, and *Suncus murinus* 11.1% (16). Studies in Semarang City found three species of rats, namely *R. tanezumi* 53.3%, *R. norvegicus* 33.3%, and *M. musculus* 13.3%, with trap success of 11.8% which indicates a high rat population (17).

A formula for predicting the appearance of rats in an area is Bi-index (18). Apart from the aspect of the proportion of trapped rats, this formula also takes into account the area and land use so that the probability of a mouse species appearing in the location can be estimated. Even though studies on rat density in Leptospirosis endemic areas have been widely reported, the use of this formula has not been implemented, especially in Indonesia. Studies in Malaysia reported that residential environmental conditions influenced the incidence of Leptospirosis, especially the altitude of the location, house walls, distance to plantations/agriculture, and history of exposure to flooding (19). A similar phenomenon was also reported in Saint Lucia, United States, where poor management of trash cans and the condition of trash cans that were easy for rats to access had an impact on morbidity (20). A study in Klaten district, Central Java Province, Indonesia also reported a link between environmental conditions and the risk of Leptospirosis, including settlements in flood areas, the presence of rats, poor residential conditions, and the distance between houses and nearby rivers (21). Semarang City is one of the urban areas where Leptospirosis is endemic in Indonesia. Environmental conditions and the presence of rats in the endemic areas need to be monitored to support the efforts to control this disease. This study aims to use the Bi-index in monitoring the presence, and density of rats, and the environmental characteristics in Leptospirosis-endemic areas of urban settlements in Semarang City.

MATERIAL AND METHODS

Study Sites

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This study was part of the research on the genomic and epidemiological characteristics of the Plague, Leptospirosis, Rickettsiosis, and Hantavirus (PESTORITA) in East and Central Java Province, Indonesia conducted by the National Research and Innovation Agency in 2023. This report uses partial data in four endemic areas in Semarang City, namely Muktiharjo Kidul I and II, Sambirejo, and Siwalan villages based on the Leptospirosis data in the Subdistrict Office of Public Health Center in 2022. Based on the location of the cases, four study sites were selected, namely Muktiharjo Kidul I, Sambirejo, Siwalan, and Muktiharjo Kidul II. The number of participants per location was 50 households within a 100-meter radius of the house of the case of Leptospirosis (18).

Rat Trapping a~~A~~nd Identification

Rat trapping was carried out using a single live trap installed in each participant's house in each study site, with a trap distribution of 100 traps in Muktiharjo Kidul I, Muktiharjo Kidul II, and Sambirejo villages, and 80 traps in Siwalan village. The methods of rat trapping and species identification refer to the PESTORITA standard operating procedures (SOP) (22).

Environmental Observations

Observation of the environmental conditions of the house included the parameters of the existence and conditions of ditches and rivers, the existence of stagnant water, history of flooding, water entering the house during floods, the existence and conditions of indoor and outdoor rubbish bins, and the presence of rats around the house. Interviews with households were conducted to complete and confirm the observation results. The data was recorded in an observation sheet, while the location coordinates were determined using the GPS waypoint application using a Samsung A32 cellphone.

Data Analysis

Descriptive data was analyzed and displayed in tables and maps. Map was produced using ArcGis 10.8, ESRI, and Google Earth software at the Epidemiology Laboratory, Faculty of Public Health, Muhammadiyah University, Semarang. Descriptive analysis included participant characteristic variables, success trap, Bi-index, rat morphometry, and environmental conditions, while the association of environmental parameters with the presence of rats was analyzed analytically with the Chi-Square test.

Ethical Clearance

Data collection was carried out after research permission from the Semarang City Government and an ethical certificate was issued. This research protocol was reviewed and received ethical approval from the Health Research Ethics Commission (KEPK) of the National Research and Innovation Agency, with Referral Number: 049/KE.03/SK/05/2023. Live trap installation and interviews with households were carried out after obtaining permission from the head of the neighborhood.

RESULTS

A total of 190 households were involved in this study. The proportion of males was only one-third of all participants with a range and mean age of 17-69 and 35.46 years, the highest proportion in the

young adult age group, and the lowest in the elderly. The majority of participants belong to private employment groups (labor, entrepreneur, and household) and the least are government officers. In general, the distribution of cases and non-cases of Leptospirosis based on gender showed an almost equal condition. The proportion of women in the case and non-case groups was slightly different so the statistical test results were not significant. Almost similar conditions were found in the distribution of cases based on age groups, where only the 17-25 age group had a higher proportion of cases. However, the age groups 56-65 and >65 years old were only found in the non-case group. The distribution by occupation showed almost equal conditions between the case and non-case groups. The results of the Chi-square analysis of the three variables did not show significant differences ($p>0.05$).

A total of 380 traps were installed in 190 houses with varying results where trap success ranged from 2.5 to 26.5 percent. Two traping locations, namely Muktiharjo Kidul I and Siwalan village showed the highest success traps and high Bi-index values as well. In contrast, two study sites (Sambirejo and Muktiharjo Kidul II subdistricts) were classified as medium and low rat density (**Table 1**). A total of 129 rats were caught in this study which were grouped into three species, namely *R. norvegicus*, *R. tanezumi*, and *M. musculus*. The highest number of caught rats was obtained in the Muktiharjo Kidul I and Siwalan villages, and the least in the Muktiharjo Kidul II location. *R. norvegicus* and *R. tanezumi* were trapped in all study sites, while *M. musculus* was only found in Siwalan village. Female rat percentages were higher than male rats in almost all study sites (**Table 2**). The four trapping locations that have a diversity index in the medium category were Siwalan and Muktiharjo Kidul I villages. Meanwhile, the two other locations were categorized as low-density. All trapping locations have a low dominance index and a stable evenness index (**Table 3**).

Based on the range of morphometric data, it can be seen that the majority of rats caught were in the range of total length (body and tail) of adult mouse size, namely between 300-500 for *R. norvergicus*, 245-445 for *R. tanezumi*, and 160-200 mm for *M. musculus*. This data showed that the majority of the trapped rats were in the adult age category, although a small portion of the *R. norvegicus* was indicated as juveniles based on their total body size which was still below standard. Based on their body size, the species *R. norvegicus* and *R. tanezumi* trapped in the Muktiharjo Kidul I and Siwalan have larger bodies compared to the Sambirejo and Muktiharjo Kidul II trapping locations (**Table 4**).

Five of the ten environmental parameters showed a correlation with the presence of rats in the house (**Table 5**), namely the proximity of the house to the river with stagnant water ($p=0.042$), the house

often being flooded (p=0.000), water entering the house during floods (p=0.000), the presence of open trash cans (p=0.023), and the presence of piles of rubbish around the house (p=0.009). A total of 4 cases of Leptospirosis in 2023 were spread across four sub-districts in the Gayamsari sub-district, Semarang City (**Figure 1**). A total of 380 rat traps were installed in four fishing locations, namely Siwalan, Sambirejo, and Muktiharjo Kidul. There is one trapping location each for catching mice in Siwalan and Sambirejo villages, while in Muktiharjo Kidul Village there are 2 locations for trapping rats (**Figure 3**).

DISCUSSION

The Ccharacteristics of Ppparticipants

The research results show that female participants dominate. This condition indicates that women participate greatly in promoting a healthy home and residential environment. This finding was similar to that reported in Vietnam, where most participants were female (4). However, ironically, men are more at risk of being infected with Leptospira as reported by several studies (20,21). This phenomenon raises the suspicion that needs to be researched that women care more about their health and self-protection than men. The average age of participants in this study was 35.46 years, which indicates they are of productive age so they have a greater opportunity to obtain information that is useful for their health. This finding is equivalent to a previous report where study participants were in urban areas with a mean of 33.4 years (6). In general, socio-demographic factors including gender, age, and occupation in this study show almost equal conditions based on participant status, cases, and non-cases. The gender and age variables show balanced conditions based on participant status, where these findings are similar to reports from studies conducted in Sabah, Malaysia (23) consistent with findings in ~~Nepal~~ Ponorogo, Indonesia (24), although other reports state that the 25-39 age group has a higher risk. higher incidence of Leptospirosis (6). The employment variable also shows no differences. This may be related to the types of work most at risk for Leptospirosis, namely farmers, livestock breeders (25), meat, abattoir, rice field workers, hunters, veterinarians, ~~technicians~~caretakers of wild animals,~~,~~ and ~~livestock truck drivers~~agricultural (26), the types of work in this study do not include these groups.

Success Trap and Bi-index

The success trap value shows that Semarang City has a high density of Leptospirosis reservoirs. This finding can be an early warning sign of the vulnerability of the region to the potential risk of

Leptospirosis. The presence and density of rats in endemic areas need special attention because low density also creates opportunities for disease transmission by the rat reservoir (17). In general, trap success in this study reached 16.9%, higher than the normal standard trap success value of ≥7% in residential areas and ≥2% in garden areas (27). This data shows that the rat population in urban Leptospirosis endemic areas is very high and indicates a high potential for transmission as well. This study was conducted in densely populated residential areas with varying environmental sanitation conditions. The condition of dense and close housing structures can increase the mobility of mice between houses and result in high exposure to mouse feces and urine and impact disease transmission over short distances (28). This is by several findings that the domestic rat population is in line with population density in a settlement due to abundant food sources such as rubbish and food scraps (12). This situation is similar to the findings of a study on rodentia density in urban and rural areas of Nicaragua over two years where trap success was 20.2% (28). Even though the rat trapping in this study was only carried out for two months, success trap values were obtained which were equivalent to those found in Nicaragua, even higher for some locations. This finding is similar to another study from Makassar City which reported trap success of 25% in flood-prone areas (29).

The success traps in this study were in line with the Bi-Index value, where a high success trap value will influence the potential for certain rat species to be caught. Although the Bi-index was originally used to evaluate *Bandicota indica* (18), this formula can be applied to other species. The four rat-catching locations show high to low Bi-index categories. This finding is similar to the results of research in Thailand which reported that the Bi-index value in urban land was in the medium category, where several species of rats appeared at fishing locations because of the attraction of human activities which provided opportunities to find food. However, the phenomenon of areas with high Bi-Index values does not always indicate the presence of cases of Leptospirosis. The two main factors that influence this condition are the phenomenon of underdiagnosis of Leptospirosis because the signs and symptoms of the disease are not sufficiently understood by health workers and the occurrence of misdiagnosis because the detection tool available at public health centers is an antibody-based rapid diagnostic antibody (RDT). If the patient accesses health services within 5-7 days from the onset of fever, the RDT results will show a false negative result (30).

Rats Sspecies, the indices of Ddiversity and Ddominance, and Mmorphometrics

This study reports three species of rats in urban residential areas where *R. norvegicus* is the dominant species. This finding is in line with various other study reports where this species is cosmopolitan in urban areas worldwide, both as a pest and as a disease carrier (14). This is supported by several factors that influence the activity of *R. norvegicus* in urban areas, namely the presence of drainage channels, soil conditions, and dense settlements (31). The presence of open gutters and soil conditions are favorable for this rat species to dig and make nests (32). Slightly different findings were reported from Los Angeles, United States, where rat catching carried out in green open spaces resulted in more *M. musculus* species than *R. Norvegicus*, even though it was carried out in a similar area, namely urban areas (33) This study found a smaller number of rat species than research studies in other areas in Semarang City which found Bandicota indica, *R. norvegicus*, and *R. tanezumi*, as well as *Suncus murinus* (34). This fact shows that urban settlements are areas that have the potential for breeding and mobility of various species of rats, giving rise to great potential for the transmission of rodent-borne diseases. An important lesson that can be learned is to target urban settlements for rat control with the *R. Norvegicus* species as the main focus (35). The proportion of female mice caught was higher than male mice and occurred in all locations. This phenomenon is related to several reasons, namely the roaming area of female mice is narrower than that of male mice, the possibility of being in heat, or being pregnant so they have to go out to look for food. Even though the mobility of rats is quite high, the distances covered are the same and the areas traversed by individuals are relatively the same. This high activity can produce more urine, thereby increasing the chance of Leptospira bacterial contamination (36).

The Shannon-Wiener diversity index is highly dependent on the number of species caught. The higher the species variation, the diversity index value will also increase. The high diversity index at the Siwalan and Muktiharjo Kidul II locations indicates that these areas have ideal habitats and food types for rat breeding (~~37,38~~37). Then the Simpson's dominance index showed that in all locations there was no competition between rat species. This is reinforced by the fact that the caught rat species have different habitats. *R. norvegicus* is a peridomestic type and makes nests in ditches and ditches, while *R. tanezumi* and *M. musculus* are domestic mice (32), but both of them are active in different places. The species *R. tanezumi* is often found on house roofs while *M. musculus* is often found in cupboards and warehouses (36). Furthermore, the evenness index value shows stable results in all locations. This is because the four fishing locations have an almost balanced environment.

Morphometric data on the species *R. norvegicus* and *R. norvegicus javanus* found a body weight in the range of 38-535 grams and a total length in the range of 249-510 mm, where body weight <200 grams in this species is still included in the juvenile category. The findings are similar to a study report from America that several types of *R. norvegicus* species are still classified as juveniles (~~39~~38). Other morphometric data, namely body weight, total length, and tail length of *R. norvegicus* in this study were 268.2 gr, 400.5 mm, and 192.7 mm respectively, which overall shows that the mice caught were adult categories (~~40~~39). A similar thing was also found in *R. tanezumi* where a small number of mice were indicated to be still juveniles based on body weight measurements. Other information reveals that the species is classified as an adult if it weighs between 65-300g (~~41~~40). Likewise, this study found that the Mus musculus species had a maximum body weight of 41g. Overall, rats caught in Semarang City settlements showed that the four species studied were larger than those in Qatar (~~40~~39).

Environmental C~~c~~onditions and the R~~r~~at P~~p~~resence in S~~s~~ettlements

This research observed ten aspects of the environment around the participants' homes, of which 5 (five) aspects were significantly related to the rat presence. The presence of piles of rubbish near the house and open rubbish bins is significantly related to the presence of rats in residential areas. Observation results show that every house has a rubbish bin, either made from plastic barrels or made from used tires. However, only a third of all trash cans have lids. These conditions are ideal for mice to get food (20,~~42~~41). The findings are similar to other studies where the accumulation of rubbish is directly proportional to the incidence of Leptospirosis (~~43~~42), and the presence of rubbish around the home environment will increase the number of rat populations (~~44~~43). The public needs to know about this phenomenon to improve cleanliness in the home environment as an effort to prevent and control the rat population.

Reminiscent river condition parameters are part of the risk factors for Leptospirosis (~~45~~44) where standing water becomes a medium for spreading bacteria to other places. This research was carried out during the dry season, but there were still many flooded rivers originating from residential wastewater. Poor sanitary conditions will be a good environment for mice, especially the *R. norvegicus* species, and increase exposure to the risk of Leptospira bacterial contamination in standing water (6). These bacteria can survive in normal to slightly alkaline water pH in the range of 7.3-7.7 (~~46~~45). Another aspect is the history of flooding and water entering the house. Flood waters submerge gutters and rat nests, especially

R. novegicus so that they migrate to safe places, including inside houses. Other safe places from waterlogging include mounds of land with bushes and around residential areas (36).

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CONCLUSION

Three species of mice were found where *R. norvegicus* was the dominant species. The density of rats in the Leptospirosis endemic area of Semarang City is high, where the presence of rats in residential areas is related to the proximity of stagnant rivers, a history of flooding, houses being flooded, there are open rubbish bins, and there are piles of rubbish near the house. Morphometric data indicated that the majority of mice were adults. Further investigation is needed to determine Leptospira bacterial infection in rat feces and urine, and the level of community participation in efforts to control rodents in residential areas.

SOURCE OF FUNDING

National Research and Innovation Agency

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest regarding this research.

AUTHOR CONTRIBUTIONS

Conceptualization: ZA, SS, FDH. Data curation: ZA, FDH. Formal analysis: ZA, SS. Funding acquisition: National Research and Innovation Agency, Salatiga Co-Working Space. Methodology: ZA, RR, FDH, SS. Project administration: ZA, FDH. Writing of original draft: ZA, SS. Internal review of manuscript: RR, FDH. Writing of review and editing: SS, FDH. All authors have read and approved the final manuscript.

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Table 1. Rat Ttrap pPlacement, Nnumber of tTrapped Rrats, sSuccess tTrap, and Bi-Iindex in Uurban Ssettlement of the eEndemic Aareas of Leptospirosis

Trapping Locations	Number of traps	Number of trapped rats	Success Trap (%)	Bi-index
Muktiharjo Kidul I	100	53	26.5	0.28
Siwalan	100	52	26.0	0.32
Sambirejo	80	19	11.9	0.11
Muktiharjo Kidul II	100	5	2.5	0.02

Table 2. Distribution of the Sspecies of Rtat-trapped Sspecies Bbased on sStudy Ssites

Rat Species	Study Sites							
	Sambirejo		Muktiharjo Kidul I		Siwalan		Muktiharjo kidul II	
	n	%	n	%	n	%	n	%
Rattus norvegicus								
Male	2	22.2	9	25.0	10	31.3	2	100.0
Female	7	77.8	27	75.0	22	68.8	0	0
Rattus tanezumi								
Male	4	40.0	6	35.3	4	28.6	0	0
Female	6	60.0	11	64.7	10	71.4	3	100.0
Mus musculus								
Male	0	0	0	0	1	16.7	0	0
Female	0	0	0	0	5	83.3	0	0

Table 3. The Ddiversity Iindex of Shanon-Wiener, Ddominance Iindex, and Eevenness Iindex of rats

Lokasi Penangkapan	Shanon-Wiener diversity index	Simpson's Dominance Index	Evenness Index
Sambirejo	0.94	0.42	0.86
Muktiharjo Kidul I	1.06	0.36	0.96
Siwalan	1.09	0.40	0.79
Muktiharjo Kidul II	0.95	0.44	0.86

Table 4. Rat Mmorphometry Bbased on the Rrat Ttrapping Llocation (sStudy sSites)

Species	Rat trapping location											
	Sambirejo			Muktiharjo Kidul I			Siwalan			Muktiharjo Kidul II		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Rattus norvegicus												
Total length (mm)	282	462	368.56	299	510	436.92	249	499	405.66	462	470	466.00
Tail length (mm)	130	207	171.89	143	239	204.44	130	239	186.16	221	234	227.50
Length of hind legs (mm)	32	45	41.33	38	48	43.97	24	48	40.47	41	46	43.50
Length of ears (mm)	16	24	20.33	17	24	20.53	16	23	20.00	21	22	21.50
Body weight (gr)	64	535	205.89	71	519	314.81	38	487	291.84	464	507	485.50
Rattus tanezumi												
Total length (mm)	232	397	348.90	202	416	347.65	245	415	344.86	355	408	389.67
Tail length (mm)	130	207	178.30	110	225	184.82	138	213	182.43	189	219	206.00
Length of hind legs (mm)	28	37	35.50	27	37	34.00	27	43	32.64	34	36	35.00
Length of ears (mm)	18	23	19.90	15	23	19.47	17	21	19.00	21	23	22.00
Body weight (gr)	27	197	123.90	19	227	121.06	39	454	148.86	117	207	175.67
Mus musculus												
Total length (mm)	0	0	0	0	0	0	165	256	219.00	0	0	0
Tail length (mm)	0	0	0	0	0	0	61	136	112.00	0	0	0
Length of hind legs (mm)	0	0	0	0	0	0	20	24	21.83	0	0	0
Length of ears (mm)	0	0	0	0	0	0	15	17	15.33	0	0	0
Body weight (gr)	0	0	0	0	0	0	20	41	30.00	0	0	0

Table 5. Environmental Pparameters Aassociated with the ppPresence of rats Rats in the hHome Eenvironment

No	Environmental Parameters	Presence of rats				p
		Ya		Tidak		
		n	%	n	%	
1.	The existence of a ditch around the house					
	Yes	27	38.0	44	62.0	0.360
	No	3	21.4	11	78.6	
2.	The existence of a river around the house					
	Yes	6	31.6	13	68.4	0.911
	No	24	36.4	42	63.6	
3.	Puddles of water in the ditch					
	Yes	15	27.8	39	72.2	0.093
	No	15	48.8	16	51.6	
4.	The house is close to a puddle of river water					
	Yes	13	54.2	11	45.8	0.042
	No	17	27.9	44	72.1	
5.	There are puddles around the house					
	Yes	9	56.3	7	43.8	0.098
	No	21	30.4	48	69.6	
6.	The house is often flooded					
	Yes	16	72.7	6	27.3	0.000
	No	14	22.2	49	77.8	
7.	During a flood, water enters the house					
	Yes	14	87.5	2	12.5	0.000
	No	16	23.2	53	75.8	
8.	There is a trash can outside the house					
	Yes	27	37.0	46	63.0	0.527
	No	3	25.0	9	75.0	
9.	There are open trash cans around the house					
	Yes	25	44.6	31	55.4	0.023
	No	5	17.2	24	82.8	
10.	There are piles of rubbish around the house					
	Yes	12	63.2	7	36.8	0.009
	No	18	27.3	48	72.7	

Figure 1. Study Ssites in Semarang City, Central Java Province, Indonesia

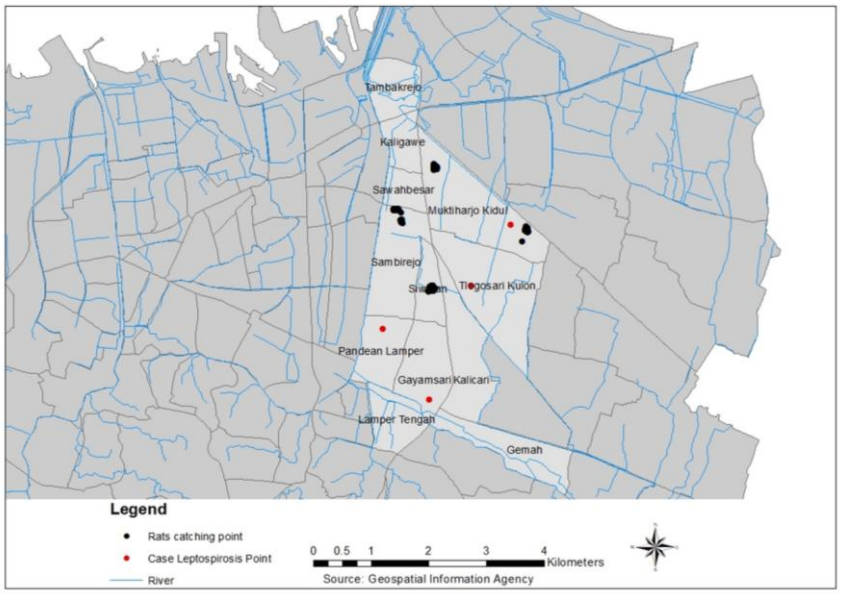

Figure 2. Distribution of Leptospirosis Ceases in Sstudy Ssites

Figure 3. The Rrats Ttrapping Llocations

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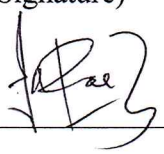
Farida Dwi Handayani		20-05-2024
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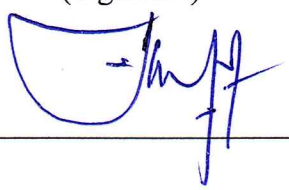
Sayono Sayono		20-05-2024

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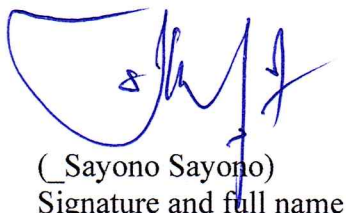

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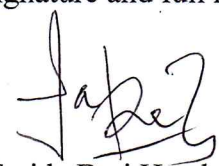

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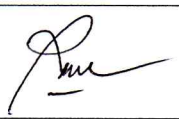
Ristiyanto Ristiyanto	ristiyanto.salatiga@gmail.com	

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EVALUATION OF RATS DENSITY AND THE ASSOCIATED FACTORS IN LEPTOSPIROSIS ENDEMIC AREAS: THE FIRST REPORT ON THE USE OF BI-INDEX

ABSTRACT

Introduction: Leptospirosis is a health problem in tropical countries where rats are the reservoir of *Leptospira* contamination. The Bi-index is an effective formula to assess rat density in Leptospirosis endemic areas, but studies have not implemented this formula. This study aims to use the Bi-index in monitoring rat density and the associated factors in urban Leptospirosis-endemic areas. **Methods**: Four endemic areas in Semarang City ~~(Muktiharjo-Kidul I and II, Sambirejo, and Siwalan)~~ were selected as the study sites based on Leptospirosis data in *Puskesmas* Gayamsari ~~Community Health Center~~. Live-traps were placed in the sample of houses (~~at the~~ one case~~'s~~ house and 39-49 neighboring houses within a 100m radius) on three consecutive days. Trapped ~~-~~rats were collected for species identification, morphometric ~~measurements~~, and calculation of Bi and~~, diversity, dominance, and evenness~~rat indices. Environmental parameters were collected through observation. **Results and Discussion**: Two-thirds (67.1%) of participants were women, private employees, and ~~-~~aged 17-55. The success-trap ~~ranged from~~ ~~-~~2.5-26.5% with the Bi, diversity, dominance, and evenness indices being 0.02-0.32, 0.94-1.09, 0.36-0.44, and 0.79-0.96. ~~Three rat species were trapped, namely~~ The rat-trap species were *Rattus norvegicus*, *Rattus tanezumi*, and *Mus musculus* with proportions of 61.3%, 34.1%, and 4.7%. Near the river with stagnant water, often being flooded, water entering the house during floods, open trash bins, and rubbish bins around the house were associated with rats. The high rat density, dominant species, and correlated environmental conditions are strategic targets in controlling Leptospirosis in Semarang City. **Conclusion**: The rat density (dominated by *R. norvegicus*) in Semarang City is high ~~where *R. norvegicus* dominates. The presence of rats in residential areas is~~and is ~~associated~~ correlated with water drainage and garbage management. Further investigation to determine Leptospira bacterial infection in rodents is needed.

Keywords: Rattus norvegicus, Rattus tanezumi, Rat density, Leptospirosis, Bi-index

INTRODUCTION

Leptospirosis is still a global health problem in humans with a global prevalence of 5 and 14 per 100,000 inhabitants in endemic and epidemic areas (1). ~~Although the prevalence of Leptospirosis in the Asian region is relatively low (around 17%) compared to the American region, a~~A high prevalence (33.6%) of Leptospirosis is still found in India, Malaysia, and Iran (2), and Bangladesh~~, especially among poultry farm and slaughterhouse workers~~ (3). Based on the characteristics of sufferers, the prevalence ratio of Leptospirosis in women was higher than in men, amounting to 1.4:1, where those of productive age were more susceptible to being infected with Leptospira (4). Reports showed that the prevalence of Leptospirosis in Indonesia was 39.2%, where cases were spread across nine provinces (5). The incidence of Leptospirosis in urban was higher than in rural areas (6), where Semarang City is one of the urban areas with a total of 34 cases in 2021 (7).

The presence of rats in residential areas is an important factor in the transmission of Leptospirosis because their feces and urine become a reservoir and source of contamination of *Leptospira* bacteria in the floor, soil, and water around the settlements. The most dominant genus of rats infected with this bacteria is *Rattus* (8), and the wider spread of bacteria contaminations and infections is influenced by several factors, such as elevation, landscape fragmentation, urbanized areas, precipitation, near from forest, and rodent density (9). The dominance of rat species in settlement varies according to geographic conditions. Rat species in urban areas are dominated by *R. norvegicus* while in rural areas are *R. argentiventer* (10,11). Several studies have reported rat species that have been proven to be reservoirs of Leptospirosis, namely *R. norvegicus, R. tanezumi, B. indica*, and *B. bengalensis* (12,13).

Data on the presence, distribution, variation, and dominance of rat species in an endemic area is useful for supporting rat mitigation and control programs in residential environments (14). Surveys of rodents in urban settlements endemic to Leptospirosis from several countries show diverse species. A study in Selangor, Malaysia found three rodentia genera with respective proportions of *Rattus* 94.6%, *Tupaia* 4.9%, and *Suncus* 0.5%, and 15.6% of them were confirmed positive for *Leptospira* infection (15). Other findings from Tanzania reported three genera with proportions: *Mus* 51.8%, *Rattus* 44.7%, and *Suncus* 3.5%, and the highest *Leptospira* infection was found in *R. norvegicus* (13). Studies in Indonesia also report the diversity of *Rodentia* species in Leptospirosis endemic areas. Studies in West Jakarta, Indonesia found two genera with respective species proportions of *R. rattus* 74.1%, *R. norvegicus* 14.8%, and *Suncus murinus* 11.1% (16). Studies in Semarang City found three species of

rats, namely *R. tanezumi* 53.3%, *R. norvegicus* 33.3%, and *M. musculus* 13.3%, with trap success of 11.8% which indicates a high rat population (17).

A formula for predicting the appearance of rats in an area is Bi-index (18). Apart from the aspect of the proportion of trapped rats, this formula also takes into account the area and land use so that the probability of a mouse species appearing in the location can be estimated. Even though studies on rat density in Leptospirosis endemic areas have been widely reported, the use of this formula has not been implemented, especially in Indonesia. Studies in Malaysia reported that residential environmental conditions influenced the incidence of Leptospirosis, especially the altitude of the location, house walls, distance to plantations/agriculture, and history of exposure to flooding (19). A similar phenomenon was also reported in Saint Lucia, United States, where poor management of trash cans and the condition of trash cans that were easy for rats to access had an impact on morbidity (20).

A study in Klaten district, Central Java Province, Indonesia also reported a link between environmental conditions and the risk of Leptospirosis, including settlements in flood areas, the presence of rats, poor residential conditions, and the distance between houses and nearby rivers (21). Semarang City is one of the urban areas where Leptospirosis is endemic ~~in Indonesia~~. Environmental conditions and the presence of rats in the endemic areas need to be monitored to support the efforts to control this disease. This study aims to use the Bi-index in monitoring the presence, and density of rats, and the environmental characteristics in Leptospirosis-endemic areas of urban settlements in Semarang City.

MATERIAL AND METHODS

Study Sites

This study was part of the research on the genomic and epidemiological characteristics of the Plague, Leptospirosis, Rickettsiosis, and Hantavirus (PESTORITA) in East and Central Java Province, Indonesia conducted by the National Research and Innovation Agency in 2023. This report uses partial data in four endemic areas in Semarang City, ~~namely~~ there were Muktiharjo Kidul I and II, Sambirejo, and Siwalan villages based on the Leptospirosis data in the Subdistrict Office of ~~Public Health Center~~ *Puskesmas* in 2022. Based on the location of the cases, four study sites were selected, ~~namely~~ there were Muktiharjo Kidul I, Sambirejo, Siwalan, and Muktiharjo Kidul II. The number of participants per location ~~was~~ 50 households in Muktiharjo Kidul I (100 traps), 50 households in Muktiharjo Kidul II (100

traps), 50 households in Sambirejo (100 traps), and 40 households in Siwalan (80 traps) within a 100-meter radius of the house of the case of Leptospirosis (18).

Research Variables and Data Collections

As many as fourteen variables were observed in this study, there were participant characteristics (age, sex, and occupation), study sites, trap placement, number of trapped rats, success trap, Bi-index, rat species, distribution of rat species, rat sex, rat indices (diversity index, dominance index, and evenness index), rat morphometry (total length, tail length, length of hind legs, length of ears, and body weight), environmental parameters (ditch existence, river existence, puddles of ditch water, the distance of house to the river, puddles around house, flooded house, flood water enter the house, trash can outside house, opened the trash can, and rubbish piles), and distribution of Leptospirosis cases.

Rat Trapping and Identification

Rat trapping was carried out using a single live trap installed in each of ~~each~~ the research participant~~'s~~' ~~house~~ houses in each study site, with a trap ~~trap~~ distribution of 100 ~~traps~~ in Muktiharjo Kidul I, 100 in Muktiharjo Kidul II, and 100 in Sambirejo villages, ~~and~~ while 80 traps in Siwalan village. The methods of rat trapping and species identification refer to the PESTORITA standard operating procedures (SOP) (22).

Household Interview and Environmental Observations

Participant characteristics were collected through interviews during preparation for live trap installation. Observation of the environmental conditions of the house included the parameters of the existence and conditions of ditches and rivers, the existence of stagnant water, history of flooding, water entering the house during floods, the existence and conditions of indoor and outdoor ~~rubbish~~ trash bins, and the presence of rats around the house. Interviews with households were also conducted to complete and confirm the observation results. The data was recorded in an observation sheet, while the location coordinates were determined using the GPS waypoint application using a ~~Samsung A32~~ cellphone.

Data Analysis

Descriptive data was analyzed and displayed in tables and maps. Map was produced using ArcGis 10.8, ESRI, and Google Earth software at the Epidemiology Laboratory, Faculty of Public Health, Muhammadiyah University, Semarang. Descriptive analysis included variables of participant characteristics ~~variables~~, success trap, Bi-index, rat morphometry, and environmental conditions, while the ~~association~~ correlation of environmental parameters with the presence of rats was analyzed analytically with the Chi-Square test.

Ethical Clearance

Data collection was carried out after research permission from the Semarang City Government and an ethical certificate was issued. This research protocol was reviewed and received ethical approval from the Health Research Ethics Commission (KEPK) of the National Research and Innovation Agency, with Referral Number: 049/KE.03/SK/05/2023. Live trap installation and interviews with households were carried out after obtaining permission from the head of the neighborhood.

RESULTS

The Characteristics of Participants

A total of 190 households were involved in this study. The proportion of males was only one-third of all participants with a range and mean age of 17-69 and 35.46 years, the highest proportion in the young adult age group, and the lowest in the elderly. The majority of participants belong to private employment groups (labor, entrepreneur, and household) and the least are government officers. In general, the distribution of cases and non-cases of Leptospirosis based on gender showed an almost equal condition. The proportion of women in the case and non-case groups was slightly different so the statistical test results were not significant. Almost similar conditions were found in the distribution of cases based on age groups, where only the 17-25 age group had a higher proportion of cases. However, the age groups 56-65 and >65 years old were only found in the non-case group. The distribution by occupation showed almost equal conditions between the case and non-case groups. The results of the Chi-square analysis of the three variables (sex, age, and occupation) did not show significant differences ($p>0.05$).

Success Trap and Bi-Index

A total of 380 traps were installed in 190 houses with varying results where trap success ranged from 2.5 to 26.5 percent. Two traping locations, namely Muktiharjo Kidul I and Siwalan village showed the highest success traps and high Bi-index values as well. In contrast, two study sites (Sambirejo and Muktiharjo Kidul II subdistricts) were classified as medium and low rat density (**Table 1**).

Rats Species, the Indices of Diversity and Dominance, and Morphometrics

A total of 129 rats were caught in this study which were grouped into three species, namely *R. norvegicus*, *R. tanezumi*, and *M. musculus*. The highest number of caught rats was obtained in the Muktiharjo Kidul I and Siwalan villages, and the least in the Muktiharjo Kidul II location. *R. norvegicus* and *R. tanezumi* were trapped in all study sites, while *M. musculus* was only found in Siwalan village. Female rat percentages were higher than male rats in almost all study sites (**Table 2**). The four trapping locations that have a diversity index in the medium category were Siwalan and Muktiharjo Kidul I villages. Meanwhile, the two other locations were categorized as low-density. All trapping locations have a low dominance index and a stable evenness index (**Table 3**).

Based on the range of morphometric data, it can be seen that the majority of rats caught were in the range of total length (body and tail) of adult mouse size, namely between 300-500 for *R. norvergicus*, 245-445 for *R. tanezumi*, and 160-200 mm for *M. musculus*. This data showed that the majority of the trapped rats were in the adult age category, although a small portion of the *R. norvegicus* was indicated as juveniles based on their total body size which was still below standard. Based on their body size, the species *R. norvegicus* and *R. tanezumi* trapped in the Muktiharjo Kidul I and Siwalan have larger bodies compared to the Sambirejo and Muktiharjo Kidul II trapping locations (**Table 4**).

Environmental Conditions and and the Rat Presence in Settlements

Five of the ten environmental parameters showed a correlation with the presence of rats in the house (**Table 5**), namely the proximity of the house to the river with stagnant water (p=0.042), the house often being flooded (p=0.000), water entering the house during floods (p=0.000), the presence of open trash cans (p=0.023), and the presence of piles of rubbish around the house (p=0.009). A total of 4 cases of Leptospirosis in 2023 were spread across four sub-districts in the Gayamsari sub-district, Semarang City (**Figure 1**). A total of 380 rat traps were installed in four fishing locations, namely Siwalan, Sambirejo, and Muktiharjo Kidul. There is one trapping location each for catching mice in

Siwalan and Sambirejo villages, while in Muktiharjo Kidul Village there are 2 locations for trapping rats (**Figure 3**).

DISCUSSION

The Characteristics of Participants

The research results show that female participants dominate. This condition indicates that women participate greatly in promoting a healthy home and residential environment. This finding was similar to that reported in Vietnam, where most participants were female (4). However, ironically, men are more at risk of being infected with Leptospira as reported by several studies (20,21). This phenomenon raises the suspicion that needs to be researched that women care more about their health and self-protection than men. The average age of participants in this study was 35.46 years, which indicates they are of productive age so they have a greater opportunity to obtain information that is useful for their health. This finding is equivalent to a previous report where study participants were in urban areas with a mean of 33.4 years (6). In general, socio-demographic factors including gender, age, and occupation in this study show almost equal conditions based on participant status, cases, and non-cases. The gender and age variables show balanced conditions based on participant status, where these findings are similar to reports from studies conducted in Sabah, Malaysia (23) consistent with findings in Ponorogo, Indonesia (24), although other reports state that the 25-39 age group has a higher risk. higher incidence of Leptospirosis (6). The employment variable also shows no differences. This may be related to the types of work most at risk for Leptospirosis, namely farmers, livestock breeders (25), meat, abattoir, rice field workers, hunters, veterinarians, caretakers of wild animals, and agricultural (26), the types of work in this study do not include these groups.

Success Trap and Bi-index

The success trap value shows that Semarang City has a high density of Leptospirosis reservoirs. This finding can be an early warning sign of the vulnerability of the region to the potential risk of Leptospirosis. The presence and density of rats in endemic areas need special attention because low density also creates opportunities for disease transmission by the rat reservoir (17). In general, trap success in this study reached 16.9%, higher than the normal standard trap success value of ≥7% in residential areas and ≥2% in garden areas (27). This data shows that the rat population in urban

Leptospirosis endemic areas is very high and indicates a high potential for transmission as well. This study was conducted in densely populated residential areas with varying environmental sanitation conditions. The condition of dense and close housing structures can increase the mobility of mice between houses and result in high exposure to mouse feces and urine and impact disease transmission over short distances (28). This is by several findings that the domestic rat population is in line with population density in a settlement due to abundant food sources such as rubbish and food scraps (12). This situation is similar to the findings of a study on rodentia density in urban and rural areas of Nicaragua over two years where trap success was 20.2% (28). Even though the rat trapping in this study was only carried out for two months, success trap values were obtained which were equivalent to those found in Nicaragua, even higher for some locations. This finding is similar to another study from Makassar City which reported trap success of 25% in flood-prone areas (29).

The success traps in this study were in line with the Bi-Index value, where a high success trap value will influence the potential for certain rat species to be caught. Although the Bi-index was originally used to evaluate *Bandicota indica* (18), this formula can be applied to other species. The four rat-catching locations show high to low Bi-index categories. This finding is similar to the results of research in Thailand which reported that the Bi-index value in urban land was in the medium category, where several species of rats appeared at fishing locations because of the attraction of human activities which provided opportunities to find food. However, the phenomenon of areas with high Bi-Index values does not always indicate the presence of cases of Leptospirosis. The two main factors that influence this condition are the phenomenon of underdiagnosis of Leptospirosis because the signs and symptoms of the disease are not sufficiently understood by health workers and the occurrence of misdiagnosis because the detection tool available at public health centers is an antibody-based rapid diagnostic antibody (RDT). If the patient accesses health services within 5-7 days from the onset of fever, the RDT results will show a false negative result (30).

Rats Species, the Indices of Diversity and Dominance, and Morphometrics

This study reports three species of rats in urban residential areas where *R. norvegicus* is the dominant species. This finding is in line with various other study reports where this species is cosmopolitan in urban areas worldwide, both as a pest and as a disease carrier (14). This is supported by several factors that influence the activity of *R. norvegicus* in urban areas, namely the presence of

drainage channels, soil conditions, and dense settlements (31). The presence of open gutters and soil conditions are favorable for this rat species to dig and make nests (32). Slightly different findings were reported from Los Angeles, United States, where rat catching carried out in green open spaces resulted in more *M. musculus* species than *R. Norvegicus*, even though it was carried out in a similar area, namely urban areas (33) This study found a smaller number of rat species than research studies in other areas in Semarang City which found Bandicota indica, *R. norvegicus*, and *R. tanezumi*, as well as *Suncus murinus* (34). This fact shows that urban settlements are areas that have the potential for breeding and mobility of various species of rats, giving rise to great potential for the transmission of rodent-borne diseases. An important lesson that can be learned is to target urban settlements for rat control with the *R. Norvegicus* species as the main focus (35). The proportion of female mice caught was higher than male mice and occurred in all locations. This phenomenon is related to several reasons, namely the roaming area of female mice is narrower than that of male mice, the possibility of being in heat, or being pregnant so they have to go out to look for food. Even though the mobility of rats is quite high, the distances covered are the same and the areas traversed by individuals are relatively the same. This high activity can produce more urine, thereby increasing the chance of Leptospira bacterial contamination (36).

The Shannon-Wiener diversity index is highly dependent on the number of species caught. The higher the species variation, the diversity index value will also increase. The high diversity index at the Siwalan and Muktiharjo Kidul II locations indicates that these areas have ideal habitats and food types for rat breeding (37). Then the Simpson's dominance index showed that in all locations there was no competition between rat species. This is reinforced by the fact that the caught rat species have different habitats. *R. norvegicus* is a peridomestic type and makes nests in ditches and ditches, while *R. tanezumi* and *M. musculus* are domestic mice (32), but both of them are active in different places. The species *R. tanezumi* is often found on house roofs while *M. musculus* is often found in cupboards and warehouses (36). Furthermore, the evenness index value shows stable results in all locations. This is because the four fishing locations have an almost balanced environment.

Morphometric data on the species *R. norvegicus* and *R. norvegicus javanus* found a body weight in the range of 38-535 grams and a total length in the range of 249-510 mm, where body weight <200 grams in this species is still included in the juvenile category. The findings are similar to a study report from America that several types of *R. norvegicus* species are still classified as juveniles (38).

Other morphometric data, namely body weight, total length, and tail length of *R. norvegicus* in this study were 268.2 gr, 400.5 mm, and 192.7 mm respectively, which overall shows that the mice caught were adult categories (39). A similar thing was also found in *R. tanezumi* where a small number of mice were indicated to be still juveniles based on body weight measurements. Other information reveals that the species is classified as an adult if it weighs between 65-300g (40). Likewise, this study found that the Mus musculus species had a maximum body weight of 41g. Overall, rats caught in Semarang City settlements showed that the four species studied were larger than those in Qatar (39).

Environmental Conditions and the Rat Presence in Settlements

This research observed ten aspects of the environment around the participants' homes, of which 5 (five) aspects were significantly related to the rat presence. The presence of piles of rubbish near the house and open rubbish bins is significantly related to the presence of rats in residential areas. Observation results show that every house has a rubbish bin, either made from plastic barrels or made from used tires. However, only a third of all trash cans have lids. These conditions are ideal for mice to get food (20,41). The findings are similar to other studies where the accumulation of rubbish is directly proportional to the incidence of Leptospirosis (42), and the presence of rubbish around the home environment will increase the number of rat populations (43). The public needs to know about this phenomenon to improve cleanliness in the home environment as an effort to prevent and control the rat population.

Reminiscent river condition parameters are part of the risk factors for Leptospirosis (44) where standing water becomes a medium for spreading bacteria to other places. This research was carried out during the dry season, but there were still many flooded rivers originating from residential wastewater. Poor sanitary conditions will be a good environment for mice, especially the *R. norvegicus* species, and increase exposure to the risk of Leptospira bacterial contamination in standing water (6). These bacteria can survive in normal to slightly alkaline water pH in the range of 7.3-7.7 (45). Another aspect is the history of flooding and water entering the house. Flood waters submerge gutters and rat nests, especially *R. novegicus* so that they migrate to safe places, including inside houses. Other safe places from waterlogging include mounds of land with bushes and around residential areas (36). These findings are an important input for public health workers in determining priority efforts and strategic

targets for controlling Leptospirosis. Community empowerment in drainage systems and waste management in residential areas is a key factor.

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CONCLUSION

Three species of mice were found where *R. norvegicus* was the dominant species. The density of rats in the Leptospirosis endemic area of Semarang City is high, where the presence of rats in residential areas is related to the proximity of stagnant rivers, a history of flooding, houses being flooded, there are open rubbish bins, and there are piles of rubbish near the house. Morphometric data indicated that the majority of mice were adults. Further investigation is needed to determine Leptospira bacterial infection in rat feces and urine, and the level of community participation in efforts to control rodents in residential areas.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest regarding this research.

AUTHOR CONTRIBUTIONS

Conceptualization: ZA, SS, FDH. Data curation: ZA, FDH. Formal analysis: ZA, SS. Funding acquisition: National Research and Innovation Agency, Salatiga Co-Working Space. Methodology: ZA, RR, FDH, SS. Project administration: ZA, FDH. Writing of original draft: ZA, SS. Internal review of manuscript: RR, FDH. Writing of review and editing: SS, FDH. All authors have read and approved the final manuscript.

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Table 1. Rat Trap Placement, Number of Trapped Rats, Success Trap, and Bi-Index in Urban Settlement of the Endemic Areas of Leptospirosis

Trapping Locations	Number of traps	Number of trapped rats	Success Trap (%)	Bi-index
Muktiharjo Kidul I	100	53	26.5	0.28
Siwalan	100	52	26.0	0.32
Sambirejo	80	19	11.9	0.11
Muktiharjo Kidul II	100	5	2.5	0.02

Table 2. Distribution of the Species of Rat-trapped Species Based on Study Sites

Rat Species	Study Sites							
	Sambirejo		Muktiharjo Kidul I		Siwalan		Muktiharjo kidul II	
	n	%	n	%	n	%	n	%
Rattus norvegicus								
Male	2	22.2	9	25.0	10	31.3	2	100.0
Female	7	77.8	27	75.0	22	68.8	0	0
Rattus tanezumi								
Male	4	40.0	6	35.3	4	28.6	0	0
Female	6	60.0	11	64.7	10	71.4	3	100.0
Mus musculus								
Male	0	0	0	0	1	16.7	0	0
Female	0	0	0	0	5	83.3	0	0

Table 3. The Diversity Index of Shanon-Wiener, Dominance Index, and Evenness Index of rats

Lokasi Penangkapan	Shanon-Wiener diversity index	Simpson's Dominance Index	Evenness Index
Sambirejo	0.94	0.42	0.86
Muktiharjo Kidul I	1.06	0.36	0.96
Siwalan	1.09	0.40	0.79
Muktiharjo Kidul II	0.95	0.44	0.86

Table 4. Rat Morphometry Based on the Rat Trapping Location (Study Sites)

Species	Rat trapping location											
	Sambirejo			Muktiharjo Kidul I			Siwalan			Muktiharjo Kidul II		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Rattus norvegicus												
Total length (mm)	282	462	368.56	299	510	436.92	249	499	405.66	462	470	466.00
Tail length (mm)	130	207	171.89	143	239	204.44	130	239	186.16	221	234	227.50
Length of hind legs (mm)	32	45	41.33	38	48	43.97	24	48	40.47	41	46	43.50
Length of ears (mm)	16	24	20.33	17	24	20.53	16	23	20.00	21	22	21.50
Body weight (gr)	64	535	205.89	71	519	314.81	38	487	291.84	464	507	485.50
Rattus tanezumi												
Total length (mm)	232	397	348.90	202	416	347.65	245	415	344.86	355	408	389.67
Tail length (mm)	130	207	178.30	110	225	184.82	138	213	182.43	189	219	206.00
Length of hind legs (mm)	28	37	35.50	27	37	34.00	27	43	32.64	34	36	35.00
Length of ears (mm)	18	23	19.90	15	23	19.47	17	21	19.00	21	23	22.00
Body weight (gr)	27	197	123.90	19	227	121.06	39	454	148.86	117	207	175.67
Mus musculus												
Total length (mm)	0	0	0	0	0	0	165	256	219.00	0	0	0
Tail length (mm)	0	0	0	0	0	0	61	136	112.00	0	0	0
Length of hind legs (mm)	0	0	0	0	0	0	20	24	21.83	0	0	0
Length of ears (mm)	0	0	0	0	0	0	15	17	15.33	0	0	0
Body weight (gr)	0	0	0	0	0	0	20	41	30.00	0	0	0

Table 5. Environmental Parameters Associated with the Presence of Rats in the Home Environment

No	Environmental Parameters	Presence of rats				p
		Ya		Tidak		
		n	%	n	%	
1.	The existence of a ditch around the house					
	Yes	27	38.0	44	62.0	0.360
	No	3	21.4	11	78.6	
2.	The existence of a river around the house					
	Yes	6	31.6	13	68.4	0.911
	No	24	36.4	42	63.6	
3.	Puddles of water in the ditch					
	Yes	15	27.8	39	72.2	0.093
	No	15	48.8	16	51.6	
4.	The house is close to a puddle of river water					
	Yes	13	54.2	11	45.8	0.042
	No	17	27.9	44	72.1	
5.	There are puddles around the house					
	Yes	9	56.3	7	43.8	0.098
	No	21	30.4	48	69.6	
6.	The house is often flooded					
	Yes	16	72.7	6	27.3	0.000
	No	14	22.2	49	77.8	
7.	During a flood, water enters the house					
	Yes	14	87.5	2	12.5	0.000
	No	16	23.2	53	75.8	
8.	There is a trash can outside the house					
	Yes	27	37.0	46	63.0	0.527
	No	3	25.0	9	75.0	
9.	There are open trash cans around the house					
	Yes	25	44.6	31	55.4	0.023
	No	5	17.2	24	82.8	
10.	There are piles of rubbish around the house					
	Yes	12	63.2	7	36.8	0.009
	No	18	27.3	48	72.7	

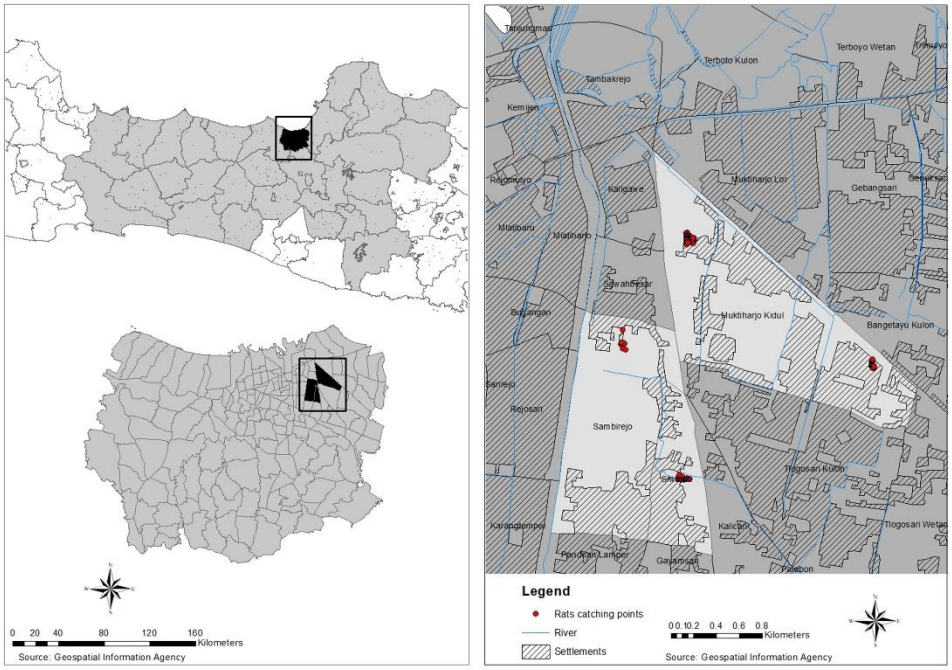

Figure 1. Study Sites in Semarang City, Central Java Province, Indonesia

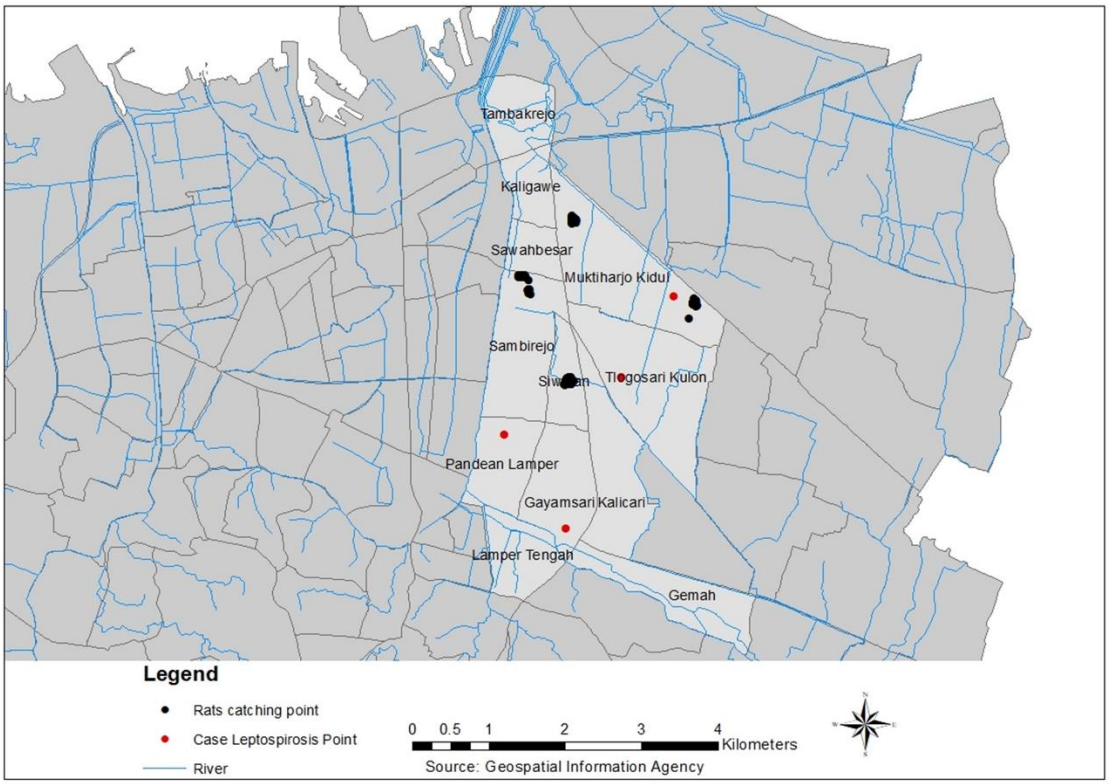

Figure 2. Distribution of Leptospirosis Cases in Study Sites

Figure 3. The Rats Trapping Locations

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[JKL] New notification from JURNAL KESEHATAN LINGKUNGAN

1 pesan

Zida Husnina, S.KM, M.PH <zidahusninajkl@gmail.com> 1 Juli 2024 pukul 09.21
Balas Ke: "Aditya Sukma Pawitra, S.KM, M.KL." <jkesling@fkm.unair.ac.id>
Kepada: Sayono <say.epid@gmail.com>

You have a new notification from JURNAL KESEHATAN LINGKUNGAN :

There is new activity in the discussion titled "Final Editorial Check and Request for Proofreading" regarding the submission "EVALUATION OF RATS DENSITY AND THE ASSOCIATED FACTORS IN LEPTOSPIROSIS ENDEMIC AREAS: THE FIRST REPORT ON THE USE OF BI-INDEX ".

Link: https://e-journal.unair.ac.id/JKL/authorDashboard/submission/57085

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[JKL] Editor Decision

1 pesan

Zida Husnina, S.KM, M.PH <zidahusninajkl@gmail.com> 10 Juli 2024 pukul 10.33
Kepada: Sayono Sayono <say.epid@gmail.com>, Zainul Akbar <zainul.akbar29@gmail.com>, Ristiyanto Ristiyanto <istiyanto.salatiga@gmail.com>, Farida Dwi-Handayani <fari018@brin.go.id>

Sayono Sayono, Zainul Akbar, Ristiyanto Ristiyanto, Farida Dwi-Handayani:

We have reached a decision regarding your submission to JURNAL KESEHATAN LINGKUNGAN , "EVALUATION OF RATS DENSITY AND THE ASSOCIATED FACTORS IN LEPTOSPIROSIS ENDEMIC AREAS: THE FIRST REPORT ON THE USE OF BI-INDEX : The First Report on the Use of Bi-index".

Our decision is to: Accept Submission

jkesling@fkm.unair.ac.id_____ Jurnal Kesehatan Lingkungan https://e-journal.unair.ac.id/JKL

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LETTER OF ACCEPTANCE (LoA)

Number : 21/UN3.1.10/PJ/JKL/2024

To
Sayono Sayono,
Faculty of Public Health, Universitas Muhammadiyah Semarang, Semarang 50273, Indonesia

Dear Author/s
The reviewers have completed their review of your paper submitted for the Jurnal Kesehatan Lingkungan (*Journal of Environmental Health*). The final decision is made based on the peer-review reports, the scientific metric, and the relevance. We are pleased to inform you that your paper has now been accepted and will be published in the forthcoming issue

Manuscript number	JKL-2024-57085
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Tittle	Evaluation of Rat Density and the Associated Factors in Leptospirosis Endemic Areas: The First Report on the Use of BI-Index
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It is further mentioned for your information that our journal is double-blind peer-reviewed. It is covered by the index SCOPUS, SINTA (National Accredited SINTA-2), DOAJ, and many other indexes.

Yours sincerely,

Aditya Sukma Pawitra, S.KM, M.KL
Editor in Chief