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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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Abstract

Introduction: Leptospirosis is a health problem in tropical countries where rats serve as the reservoir of Leptospira contamination. Previous investigations implementing the Bi-index to assess rat density in Leptospirosis endemic areas are highly limited. This study aimed 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 were selected as the study sites based on Leptospirosis data in Puskesmas Gayamsari. Live traps were positioned in one case house and 39-49 neighboring houses in a 100m radius, on three consecutive days. Trapped rats were collected for species identification, morphometrics evaluation, and calculation of Bi-index and rat indices, while environmental parameters were obtained through observation. Results and Discussion: 67.1% of participants were women, private employees, and aged 17-55, while trap success ranged from 2.5-26.5% with the Bi, diversity, dominance, and evenness indices of 0.02-0.32, 0.94-1.09, 0.36-0.44, and 0.79-0.96, respectively. Trapped species included Rattus norvegicus, Rattus tanezumi, and Mus musculus with proportions of 61.3%, 34.1%, and 4.7%, respectively. The presence of rats was associated with closeness to the river containing stagnant water, frequent flooding, water entering houses during floods, open trash bins, and rubbish bins around the houses. 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 was correlated with water drainage and garbage management, hence further investigation was recommended to determine Leptospira bacterial infection in rodents.

INTRODUCTION

Leptospirosis is still a global health problem in humans with a prevalence of 5 and 14 per 100,000 inhabitants in endemic and epidemic areas, respectively (1). A high prevalence (33.6%) of this infection has been found in Asian countries such as India, Malaysia, Iran (2), and Bangladesh (3). Based on the characteristics of sufferers, the prevalence ratio in women is higher than in men (1.4:1) where those of productive age are more susceptible to being infected with *Leptospira* (4). Additionally, reports showed that the prevalence in Indonesia was 39.2% comprising cases spread across nine provinces (5). The incidence of Leptospirosis was greater in urban compared to rural areas (6) including Semarang City 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 the feces and urine become a reservoir and source of contamination with *Leptospira* bacteria in the floor, soil, and water around the settlements. The most dominant genus of rats infected with this bacteria is *Rattus* (8), while the wider spread of bacteria contaminations, as well as infections, is influenced by factors comprising elevation, landscape fragmentation, urbanized areas, precipitation, forest closeness, and rodent density (9). The dominance of rat species in settlements varies according to geographic conditions such as *R. norvegicus* and *R. argentiventer* being the most abundant in urban and rural areas, respectively (10-11). Several studies reported rat species including *R. norvegicus, R. tanezumi, B. indica*, and *B. bengalensis* as reservoirs of Leptospirosis (12-13).

Data on the presence, distribution, variation, and dominance of rat species in endemic areas is useful for supporting mitigation and control programs in residential environments (14). Rodent surveys conducted 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%, among which 15.6% were confirmed positive for *Leptospira* infection (15). Other results from Tanzania showed three genera with proportions of *Mus* 51.8%, *Rattus* 44.7%, and *Suncus* 3.5%, with the identification of the highest *Leptospira* infection in *R. norvegicus* (13). Additionally, several investigations performed in Indonesia reported the diversity of *Rodentia* species in Leptospirosis endemic areas. Two genera with respective species proportions of *R. rattus* 74.1%, *R. norvegicus* 14.8%, and *Suncus murinus* 11.1% were found in West Jakarta (16). Meanwhile, three species including *R. tanezumi* 53.3%, *R. norvegicus* 33.3%, and *M. musculus* 13.3% were identified in Semarang City, with trap success of 11.8% signifying a high rat population (17).

An effective method for predicting the probability of rats appearing in certain areas is Bi-index (18), which accounts for the proportion trapped, the areas considered, and land use. Previous investigations on rat density in Leptospirosis endemic areas did not implement Bi-index, specifically in Indonesia. Studies in Malaysia reported that residential environmental conditions influenced the incidence of Leptospirosis, particularly the altitude of the location, house walls, distance to plantations/agriculture, and history of exposure to flooding (19). A similar phenomenon was reported in Saint Lucia, United States, where poor management and the condition of trash cans easily accessible to rats had an impact on morbidity (20).

A study in Klaten district, Central Java Province reported an association between environmental conditions and the risk of Leptospirosis, including settlements in flood areas, the presence of rats, poor residential conditions, as well as the distance between houses and nearby rivers (21). Semarang City is included among Leptospirosis endemic urban areas, triggering the need to monitor environmental conditions and the presence of rats to support disease control efforts. Therefore, this study aimed to use the Bi-index in monitoring the presence and density of rats, as well as the environmental characteristics in Leptospirosis endemic areas in Semarang City.

METHODS

Study Sites

This study was part of the investigation on the genomic as well as epidemiological characteristics of the Plague, Leptospirosis, Rickettsiosis, and Hantavirus (PESTORITA) in East and Central Java Province conducted by the National Research and Innovation Agency in 2023. Additionally, the Leptospirosis data reported from the Sub-district Office of *Puskesmas* in 2022 covered four endemic areas, including Muktiharjo Kidul I, Muktiharjo Kidul II, Sambirejo, and Siwalan villages, which were selected as study sites. The number of participants was 50 each in the first three sites comprising 100 traps respectively, while 40 were assessed in Siwalan comprising 80 traps positioned in a 100-m radius around the houses presenting Leptospirosis cases (18).

Study Variables and Data Collections

A total of 14 variables observed included participant characteristics (age, sex, and occupation), study sites, trap placement, number of trapped rats, trap success, Bi-index, rat sex, species, and indices (diversity, dominance, and evenness). Some of the remaining variables were species distribution and rat morphometry (total, tail, hind leg, and ear length, as well as body weight). Additionally, environmental parameters such as ditch existence, river existence, puddles of ditch water, house distance to the river, puddles around houses, flooded houses, flood water entering the house, trash bins outside, opened trash bins, and rubbish piles were examined alongside the distribution of Leptospirosis cases.

Rat Trapping and Identification

Rats were caught using a single live trap installed in each house of the participants in all study sites, with a trap distribution of 100 in Muktiharjo Kidul I, 100 in Muktiharjo Kidul II, and 100 in Sambirejo villages, while 80 were positioned in Siwalan village. The method of rat trapping and species identification was in accordance with the PESTORITA standard operating procedures (SOP) (22).

Household Interview and Environmental Observations

Characteristics of participants were collected through interviews performed during preparation for the installation of live traps. Meanwhile, environmental

observation included parameters such as the existence and conditions of ditches, rivers, and trash bins, stagnant water presence, flooding history, water entering the house during floods, and rats living around the house. Interviews with households were conducted to complete and confirm the observation results, followed by recording the data in an observation sheet and determining the location coordinates using the GPS waypoint application on a cellphone.

Data Analysis

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

Ethical Clearance

Data collection was conducted after obtaining permission and an ethical clearance certificate from the Semarang City Government. Additionally, the study protocol was reviewed and ethical approval was received 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 performed when permission had been obtained from the neighborhood head.

RESULTS

Characteristics of Participants

A total of 190 households were engaged in this study, where 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 belonged to private employment groups such as labor, entrepreneurs, and households, while the least were government officers. In general, the distribution of cases and non-cases of Leptospirosis based on gender showed nearly equal conditions. The proportion of women in the case and non-case groups was slightly different, leading to insignificant statistical test results. Related conditions were found in the distribution of cases based on age groups, where only the 17-25 age group had a higher proportion. However, ages 56-65 as well as >65 years old were found in the

non-case group, and the distribution by occupation was identical between the case and non-case groups. The results of the Chi-square analysis of the sex, age, and occupation variables did not show significant differences (p>0.05).

Trap Success and Bi-Index

A total of 380 traps were installed in 190 houses with varying results which included trap success ranging from 2.5 to 26.5%. Two locations, namely Muktiharjo Kidul I and Siwalan village showed the highest trap success and high Bi-index values. However, Sambirejo and Muktiharjo Kidul II subdistricts were classified with medium and low rat density, as presented in Table 1.

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

Trapping Sites	Number of Traps	Number of Trapped Rats	Trap Success (%)	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

Rat Species, Diversity and Dominance Indices, and Morphometrics

A total of 129 rats caught in this study were grouped into three species, including *R. norvegicus*, *R. tanezumi*, and *M. musculus*. The highest number of trapped rats was obtained in the Muktiharjo Kidul I and Siwalan villages, and the least was in Muktiharjo Kidul II. *R. norvegicus* and *R. tanezumi* were trapped in all sites, while *M. musculus* was only found in Siwalan village, and female rat percentages were higher than male in nearly all sites (Table 2).

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

Rat Species	Study Sites							
	Sambirejo		Muktiharjo Kidul I		Siwalan		Muktiharjo kidul II	
	n	%	n	%	n	%	n	%
R. 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
R. 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
M. musculus								
Male	0	0	0	0	1	16.7	0	0
Female	0	0	0	0	5	83.3	0	0

Siwalan and Muktiharjo Kidul I villages had a diversity index in the medium category, while the other two were categorized as low-density, and, all trapping sites showed a low dominance along with a stable evenness index (Table 3).

**Table 3. Shanon-Wiener Diversity, Dominance, and Evenness Indeces of Rats**

Study Sites	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

Morphometric data showed that the majority of trapped rats had a total length (body and tail) range similar to adult rat size, namely between 300-500 for *R. norvergicus*, 245-445 for *R. tanezumi*, and 160-200 mm for *M. musculus*. These also signified the majority in the adult age category, although a small portion of the *R. norvegicus* was identified as juveniles based on the total body size which was still below standard. Based on the body size, the species *R. norvegicus* and *R. tanezumi* trapped in the Muktiharjo Kidul I and Siwalan were bigger compared to the Sambirejo and Muktiharjo Kidul II (Table 4).

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

Species	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 (g)	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 (g)	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 (g)	0	0	0	0	0	0	20	41	30.00	0	0	0
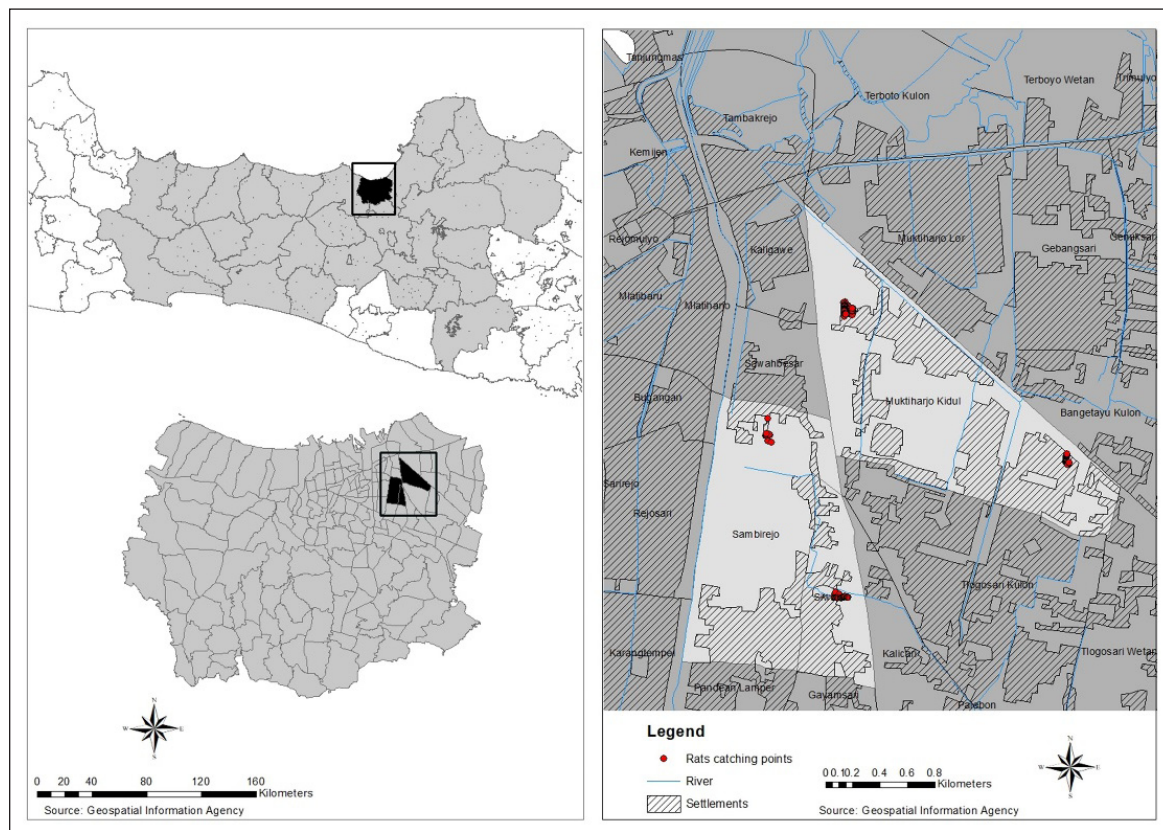
Figure 1. Study Sites in Semarang City, Central Java Province, Indonesia
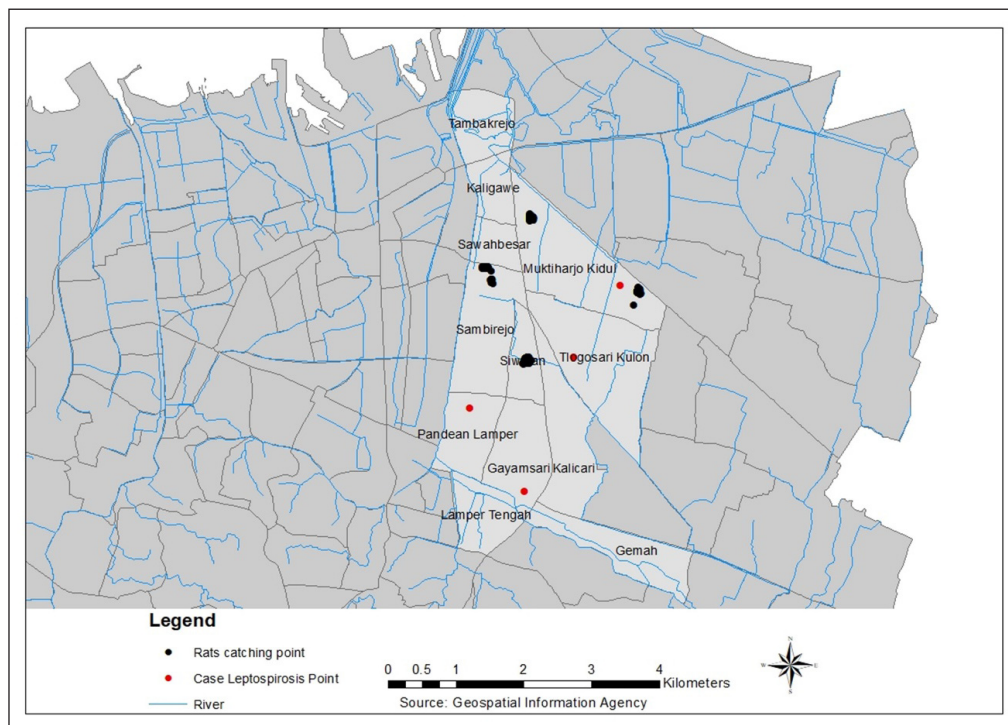
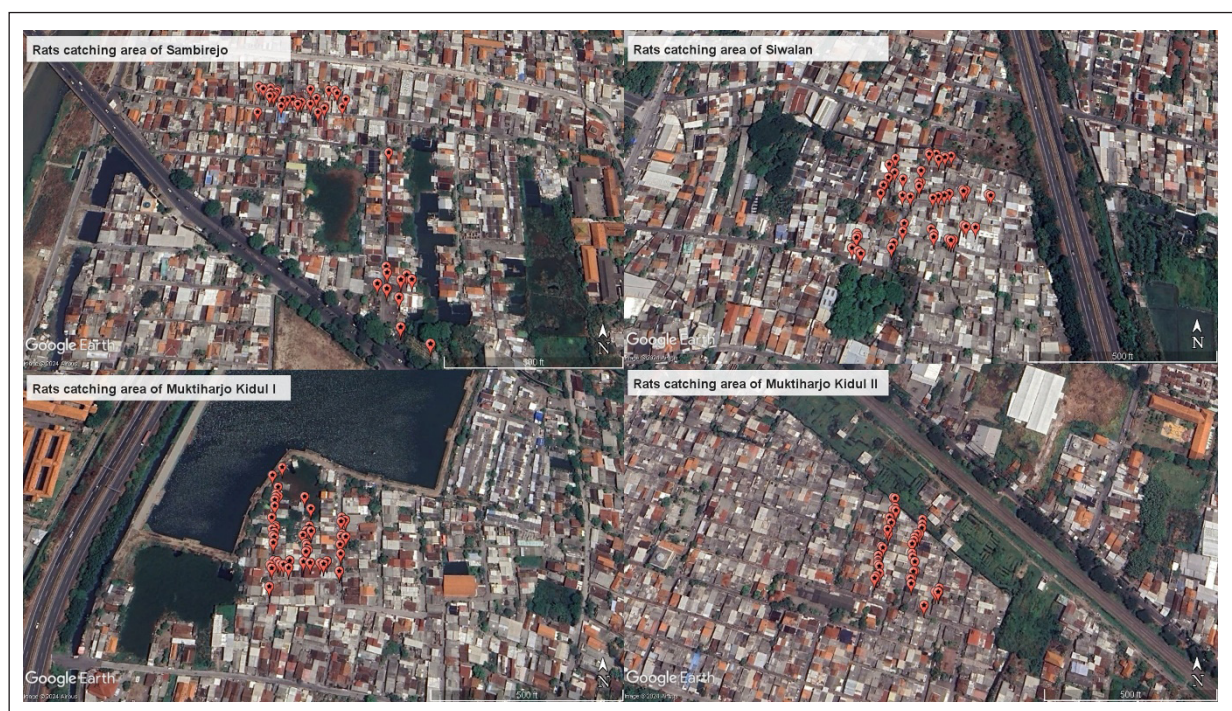**Figure 2. Distribution of Leptospirosis Cases in Study Sites**

Figure 3. The Rats Trapping Sites

Environmental Conditions and the Rat Presence in Settlements

Five of the 10 environmental parameters that showed a correlation with the presence of rats in the house (Table 5) included proximity to the river with stagnant water (p=0.042), frequent flooding (p=0.000), water entering the house during floods (p=0.000), the presence of open trash bins (p=0.023), and the existence of rubbish piles around the house (p=0.009). According to Figure 1 and 2, a total of four Leptospirosis cases in 2023 were spread across four sub-districts in the Gayamsari sub-district, Semarang City. During this study, 380 rat traps were installed in four fishing sites found in the villages of Siwalan, Sambirejo, and Muktiharjo Kidul. There was one site each for trapping rats in Siwalan and Sambirejo, while two were targeted in Muktiharjo Kidul Village (Figure 3).

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

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

DISCUSSION

Characteristics of Participants

The results showed that female participants were more dominant in the study sites, a condition suggesting the great engagement of this gender in promoting healthy homes and residential environments, and this was similar to the majority of female reported from Vietnam (4). However, several studies stated that men were more at risk of being infected with *Leptospira* (20-21), a phenomenon signifying the need to investigate whether women truly care more about their health and self-protection than men. The average age of participants assessed was 35.46 years, regarded as a productive stage predisposed to a greater opportunity of obtaining health-beneficial information, and this result was similar to a previous report of participants living in urban areas with a mean age of 33.4 years (6). In general, socio-demographic factors including gender, age, and occupation were identical based on participant status, cases, and non-cases. The gender and age variables

showed balanced conditions based on participant status, where these results were similar to reports from studies conducted in Sabah, Malaysia (23) and Ponorogo, Indonesia (24). However, other investigations stated that the 25-39 age group had a higher risk for significant incidence of Leptospirosis (6). The employment variable showed no differences which might be due to the participants not engaging in risky jobs, such as farming, livestock breeding (25), meat preparation in abattoir, working in rice fields, hunting, veterinary, and caring for wild animals (26).

Trap Success and Bi-index

The trap success value showed that Semarang City contained a high density of Leptospirosis reservoirs, suggesting an early warning sign of the vulnerability of the region to potential Leptospirosis risk. 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 value of ≥7% in residential areas and ≥2% in gardens (27). These data presented an extremely high rat population in urban endemic areas with a high potential for disease transmission, while the 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 rats between houses, leading to high exposure to feces and urine, which impact disease transmission over short distances (28). Similarly, several investigations reported that the domestic rat population correlated with population density in a settlement due to abundant food sources such as rubbish and food scraps (12). This situation was identical to the results of an exploration performed on rodentia density in urban and rural areas of Nicaragua over two years where trap success was 20.2% (28). Even though trapping in this study was only carried out for two months, success values obtained were equivalent to those found in Nicaragua and higher for some locations. The result was also consistent with another study from Makassar City which reported trap success of 25% in flood-prone areas (29).

The trap success observed in this study correlated with the Bi-Index value, hence a high success value would influence the potential for certain rat species to be caught. Even though the Bi-index was originally used to evaluate *Bandicota indica* (18), the method could be applied to other species. The four trapping sites showed high to low Bi-index categories, which were similar to the results of investigations in Thailand reporting medium

Bi-index value in urban land, where several rat species appeared at fishing sites because of the attraction of human activities providing food opportunities. However, the phenomenon of areas with high Bi-Index values does not frequently signify the presence of Leptospirosis cases. The two main factors influencing this condition are the phenomenon of underdiagnosis of Leptospirosis due to insufficient understanding of the disease signs and symptoms by health workers as well as misdiagnosis occurrence since the detection tool available at *Puskesmas* is an antibody-based rapid diagnostic test (RDT). Supposing patients access health services in a period of 5-7 days from the fever onset, RDT device will show a false negative result (30).

Rats Species, Diversity and Dominance Indices, and Morphometrics

Among the three species of rats identified from urban residential areas in this study, *R. norvegicus* was dominant, correlating with various reports where this species was cosmopolitan both as a pest and disease carrier in urban areas worldwide (14). Furthermore, the observation is supported by several factors influencing the activity of *R. norvegicus* in urban areas, such as 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 prepare nests (32). Slightly different results were reported from Los Angeles, United States, where more *M. musculus* species were trapped in green open spaces in urban areas than *R. Norvegicus* (33). However, a smaller number of rat species were found in this study compared to the report of *Bandicota indica*, *R. norvegicus*, and *R. tanezumi*, as well as *Suncus murinus* from other areas in Semarang City (34). The observations signify urban settlements as areas that allow the breeding and mobility of various species of rats, leading to great potential for the transmission of rodent-borne diseases, hence urban settlements should be targeted for rat control focusing mainly on the *R. Norvegicus* species (35). The proportion of female rats trapped was higher than male in all sites, a phenomenon associated with reasons including the narrower roaming area of female rats and the tendency of being in a heat period or pregnant, which led to searching for food outside. Despite the high mobility of rats, the distances covered were similar and the areas traversed individually were relatively the same. This high activity has a propensity to 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 trapped, hence higher species variation leads to an increase in the diversity index value. The high diversity index at the Siwalan and Muktiharjo Kidul II sites showed the existence of ideal habitats and food types for rat breeding (37). In this study, the Simpson's dominance index showed no competition between rat species in all locations, which was supported by the presence of different habitats. Generally, *R. norvegicus* is a peridomestic species that prepares nests in ditches, while *R. tanezumi* and *M. musculus* are domestic rats (32) active in different places. The species *R. tanezumi* is often found on house roofs, while *M. musculus* is identified in cupboards and warehouses (36). Furthermore, the evenness index value showed stable results in all locations because the four fishing points had a nearly balanced environment.

Morphometric data on *R. norvegicus* and *R. norvegicus javanus* showed a body weight of 38-535 g and a total length of 249-510 mm, where body weight <200 g in this species was included in the juvenile category. The results were consistent with an investigation in America reporting that several *R. norvegicus* species were still classified as juveniles (38). Other morphometric data including body weight, total length, and tail length of *R. norvegicus* were 268.2 g, 400.5 mm, and 192.7 mm respectively, which generally showed that trapped rats belonged to adult categories (39). A similar observation was found in *R. tanezumi* where a small number of rats were identified to be juveniles based on body weight measurements, and the species was classified as an adult provided the weights ranged between 65-300g (40). *M. musculus* with a maximum body weight of 41g was identified in this study, while the four examined species trapped in Semarang City settlements were larger than those in Qatar (39).

Environmental Conditions and the Rat Presence in Settlements

A total of 5 among the 10 aspects of environments observed around the houses of participants were significantly related to the rat presence. Furthermore, the existence of rubbish piles near the house and open bins was significantly related to the presence of rats in the residential areas. Observation results showed that every house had rubbish bins produced from plastic barrels or used tires, but only one-third of all trash bins had lids, serving as ideal conditions supporting the availability of food for rats (20,41). These results are similar to other studies where rubbish accumulation is directly proportional to Leptospirosis incidence (42), and the presence of rubbish around the house environments increases the number of rat population (43). Consequently, the public needs to be informed about this phenomenon to improve

cleanliness in the house environments 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 study was conducted during the dry season, but there were still many flooded rivers originating from residential wastewater. Poor sanitary conditions were a good growing environment for the rats, specifically the *R. norvegicus* species, thereby increasing exposure to the risk of *Leptospira* bacterial contamination in standing water (6), as these bacteria could survive in normal to slightly alkaline water pH ranging from 7.3-7.7 (45). Another aspect was the history of flooding and water inflow, where flood waters submerged gutters and rat nests, causing migration to safe places, including inside houses. Other places identified as safe from waterlogging included mounds of land with bushes and the spaces around residential areas (36). These results are useful to public health workers in determining priority efforts and strategic targets for controlling Leptospirosis, with community empowerment in drainage systems and residential waste management being a key factor.

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CONCLUSION

In conclusion, the results showed that *R. norvegicus* was dominant among the three species of rats found with high density in the Leptospirosis endemic areas of Semarang City. Additionally, the presence of rats in settlements at the sites assessed was related to the proximity of stagnant rivers, flooding history, frequent flooding of houses, the existence of open rubbish bins, and the availability of rubbish piles near the houses. Morphometric data showed that the majority of rats were adults, hence further investigation was recommended to determine *Leptospira* infection in feces and urine, along with the level of community participation in efforts to control rodents in residential areas.

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