

SURVEY ON *LEPTOSPIRA* SPP INFECTION IN RAT SPECIES IN INDONESIA

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Abstract. Rats are the main reservoir of leptospirosis and play an important role in maintaining the *Leptospira* life cycle in nature. This study aimed to update the data on leptospirosis-confirmed rats in various ecosystems in Indonesia. The study was conducted from 2015 to 2018 in 29 provinces in Indonesia. The rat survey was carried out for three consecutive days using live traps with roasted-coconut as the bait. The locations of the rat survey were forests near settlements, forests far settlements, non-forests near and far settlements (rice fields, fields, gardens, and others), and coastal areas near and far settlements. *Leptospira* spp were detected in the kidneys of rats by the PCR method. The results of the study showed that of 9,161 rats, 2,310 from 35 species are confirmed to have leptospirosis. The dominant species of rat infected with leptospirosis in Indonesia consisted of eight types: *Rattus tanezumi* (n=1,623), *R. tiomanicus* (n=660), *R. norvegicus* (n=313), *R. exulans* (n=154), *R. argentiventer* (n=66), *Bandicota indica* (n=11), *Mus caroli* (n=15), and *M. musculus* (n=2). This study has also found that 27 other endemic rat species are confirmed positive for *Leptospira*: 14 species in western Indonesia, eight species in middle Indonesia, and five species in eastern Indonesia. Many rats were infected with *Leptospira* in ecosystems adjacent to the settlement (less than 3 kilometers) and the coastal areas.

Keywords: *Leptospira*, rats, reservoir, zoonosis

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INTRODUCTION

Indonesia is one of the endemic countries for leptospirosis, and rats are suspected of being the source of the transmission to humans and livestock (Gasem *et al*, 2020; Rajeev *et al*, 2020; Ristiyanto *et al*, 2015). It is known that 174 types of rats are widespread in various habitats in Indonesia (Suyanto *et al*, 2002). Eight of these rats have been known to carry pathogenic *Leptospira* bacteria (Sumanta *et al*, 2015). The interaction of *Leptospira* bacteria and rats causes pathogenic *Leptospira* bacteria to proliferate and become virulent to humans and other animals (Cilia *et al*, 2020)

Most species of mammals that inhabit all regions, except in polar regions, could be infected by *Leptospira*. However, only a few types of mammals act as hosts for *Leptospira* bacteria. Animals that carry chronic asymptomatic *Leptospira* are called reservoir hosts (Ellis, 2015). Rodents are, therefore, considered the major reservoir of infection. *Leptospira* are excreted in the urine of animals, and they infect human when they contact the urine of infected animals (Tilahun *et al*, 2013). The characteristics of the reservoir host are that *Leptospira* bacteria could colonize in animal kidneys in long term and release these bacteria in their urine, showing natural nidality; for example, each serovar tends to be retained in certain maintained or specific hosts (Ellis, 2015).

Indonesia's land area is 1,922,570 km², and its water area is 3,257,483 km². The land area is divided into agricultural land (33.2%), forest area (49.1%), arable land (14%), and permanent cropland (13.3%), with irrigated land covering an area of 6,722 thousand hectares (Knoema®,

2020). Despite making up only 1.3% of the planet's surface, Indonesia is home to many species. The 17,000 islands of the Indonesian archipelago are home to about 12% of the world's mammals, 16% of its reptiles and amphibians, 17% of its bird species, and 25% of its fish populations (Irawati and Widiatmoko, 2019). Indonesia has more mammal species than any other nation (Irawati and Widiatmoko, 2019; Suyanto *et al*, 2002).

Rats in urban and peri domestic environments are the most crucial source of leptospirosis transmission and that should be investigated (Boey *et al*, 2019). Rodents are considered the most significant reservoirs for human and animal infection (Shao *et al*, 2022). Identification of environmental factors and *Leptospira*-infected rodent are important to be monitored (Biscornet *et al*, 2021).

Indonesia has a relatively large diversity of rat fauna, and there are approximately 185 species spread across various types of ecosystems and habitats (Suyanto *et al*, 2002). Our study aimed to identify the type of rat that acts as a reservoir of leptospirosis. This study presents and discusses the results of the observation on types of rats, its distribution in ecosystem, and *Leptospira*-infected rats in various ecosystems in Indonesia. It is expected that this study will provide a reference for controlling leptospirosis risk factors related to the types of rats as a reservoir for leptospirosis and its habitat in Indonesia.

MATERIALS AND METHODS

Study area

The research was conducted in 29 provinces, 87 districts, and 270 survey points in Indonesia from 2015 to 2018. Even though this research was an animal survey and had nothing to do with human subjects, it has obtained ethical clearance from the Health Research Ethics Committee of the National Institute of Health Research and Development (NIHRD), No.

LB. 02.01/5.2/KE.355/2014 and No. LB. 02.01/2/KE.170/2018 because we had to precede the captured rats, so an approval from IRB in regard to animal ethics was needed. The location and rat-catching habitats were determined based on remote sensing satellite data and geographic information systems (Suryanta *et al*, 2019).

Survey and identification of rats

Rats were captured at each location by spreading 100 single live traps with roasted coconut as the bait (Permatawati, 2018). Trap installation was divided into two locations: 50 in the home/ and 50 outside the house/building. The installation distance between traps in outdoor habitat was at least 10 meters. In contrast, for habitats in the houses, traps were installed with two fruits in every house where rats were frequently seen. Global Positioning System (GPS) was recorded for each trap location. Trap setting was carried out in the afternoon (at 3-6 pm) and checked/collected in the morning (at 6-7 am). The duration of trapping at one location was two times during two consecutive nights. The captured-rats were put in labeled bags, brought to the field laboratory, and identified by species using an Indo-Malayan rat identification key (Krairojananan *et al*, 2020).

Confirmation of *Leptospira* infected rats

Before surgery for kidney retrieval, rats were anesthetized using ketamine-xylazine (4:3) with a dose of ketamine of 70-100 mg/kg body weight and dosage xylazine 2 mg/kg body weight. Both kidneys were taken and put into a glass bottle containing 70% alcohol to examine the pathogenic *Leptospira*. Rat carcasses were further burned and buried.

The DNA isolation of rat kidney specimen was extracted. The kidneys were rinsed with sterile distilled water, cut approximately 25 mg in the renal tubules, and inserted into a sterile 1.5 ml vial. The DNA from kidney tissue was isolated using an isolation kit Purelink Genomic DNA Mini Kit

(Invitrogen, Waltham, MA). Total 50 µl DNA products were obtained. The pathogenic *Leptospira* were detected by amplification of gene segments.

SecY the primer pairs: F-5' ATCCGATCATTTTTGCTTC 3' and R-5' CCGTCCCTTAATTTTAGCTTCTTC 3' were used. This primer amplified the DNA product by 549 base pairs (bps). The kidney tissue was amplified using Go Taq Green Master Mix (Promega, Madison, WI). The total reaction was 25 µl, consisting of 5 µl DNA, 12.5 µl of 2xMaster Mix, 2 µl (400 nM) primer, and 3.5 µl nuclease-free water. Amplification was run using Applied Biosystem Thermocycler SimpliAmp Thermal Cycler (Thermo Fisher Scientific, Madison, WI) with an initial denaturation temperature of 95°C for 5 minutes, 35 denaturation cycles at 94°C for 30 seconds, annealing 58°C for 30 seconds, and 72°C extensions for 1 minute. The final extension was run at 72°C for 7 minutes and incubated at 4°C. The amplicons were visualized with 2% agarose gel electrophoresis.

Data analysis

The data were analyzed descriptively for the number of rats caught, the average rats-caught per ecosystem for each province, and the percentage of rats with confirmed *Leptospira* bacteria. The types of rats per province were mapped to explore their distribution and geographical positions.

RESULTS

Characteristics of survey sites

Each district or city was determined by six survey points based on the ecosystems: forests near settlements, forests far from settlements, non-forests near settlements, non-forests far from settlements, coast near settlements, and coast far from settlements (Fig 1).

Coast ecosystems in this study were villages close proximity to the coastlines. Meanwhile, non-forest ecosystems referred to ecosystems that did

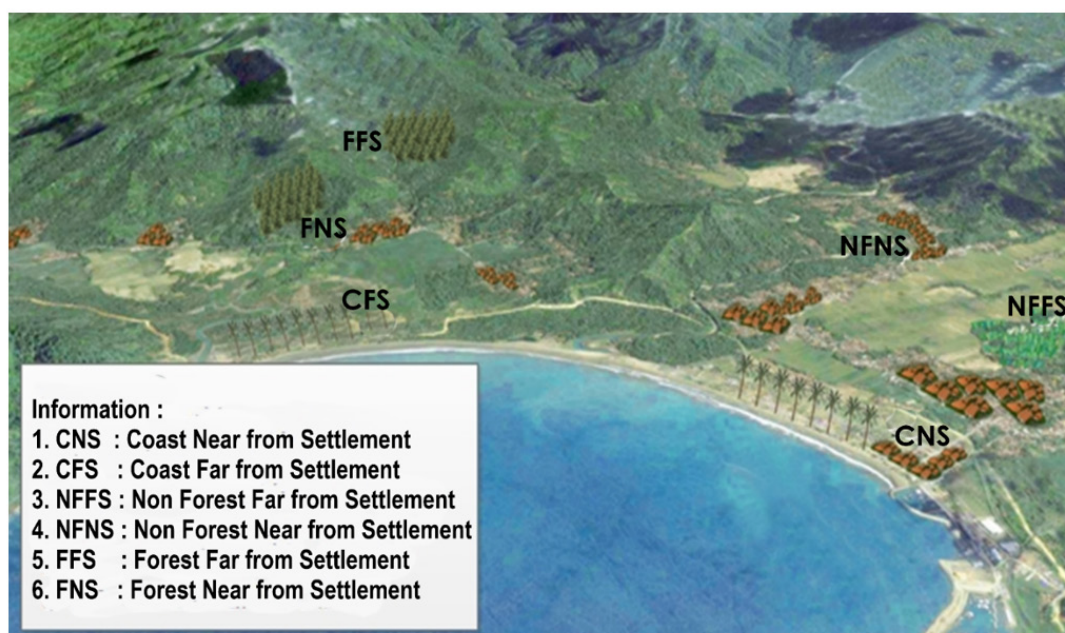


Fig 1 - Sketch of research site ecosystems

not include both coastal and forest, such as rice fields, gardens, and plantations. The operational definition was considered far from settlements if the ordinate point was within a radius of >3 kilometers from the nearest settlement.

Types of habitats where rats were caught in each ecosystem are presented in Table 1. Types of habitats in forest ecosystems included primary, secondary, homogeneous, mangrove, and coastal forests. Habitat gardens, fields, and plantations were found in forest, non-forest, and coastal ecosystems near settlements.

Types and number of *Leptospira* confirmed in rats by PCR method

This study caught 47 species of rats from 16 genera. The PCR result revealed 35 species of rats infected with *Leptospira* (Fig 3). This study revealed that the dominant types of rats infected with leptospirosis in Indonesia were

Table 1
Characteristic of habitats in each ecosystem surveyed

Survey habitat	Ecosystem					
	CNS	CFS	NFNS	NFFS	FNS	FFS
Primary forest	-	-	-	-	-	√
Secondary forest	-	-	-	-	-	√
Homogeneous forest	-	-	-	-	-	√
Mangrove forest	-	-	-	-	-	√
Beach forest	-	-	-	-	-	√
Plantation	-	-	√	√	√	-
Garden	√	-	√	√	√	-
Field	√	√	√	√	√	-
Settlement	√	-	√	-	√	-
Yard	√	-	√	-	√	-
Rice field	√	√	√	√	-	-
Beach	√	√	-	-	-	-
Pond	√	√	-	-	-	-

Notes: - means absent; √ means present

CNS: Coast near from settlements; CFS: Coast far from settlements; NFFS: Non-Forests far from settlement; NFNS: Non-forests near settlement; FFS: Forests far from settlement; FNS: Forests near settlement

Rattus tanezumi (n=1,623), *R. tiomanicus* (n=660), *R. norvegicus* (n=313), *R. exulans* (n=154), *R. argentiventer* (n=66), *Bandicota indica* (n=11), *Mus caroli* (n=15), and *M. musculus* (n=2). The eight types of rats become reservoir hosts for leptospirosis in Indonesia.

This study also found that other 27 types of endemic rats were confirmed to harbor *Leptospira* bacteria. Total 14 newly confirmed rat species are found in western Indonesia, namely Sumatra, Kalimantan, and Java.

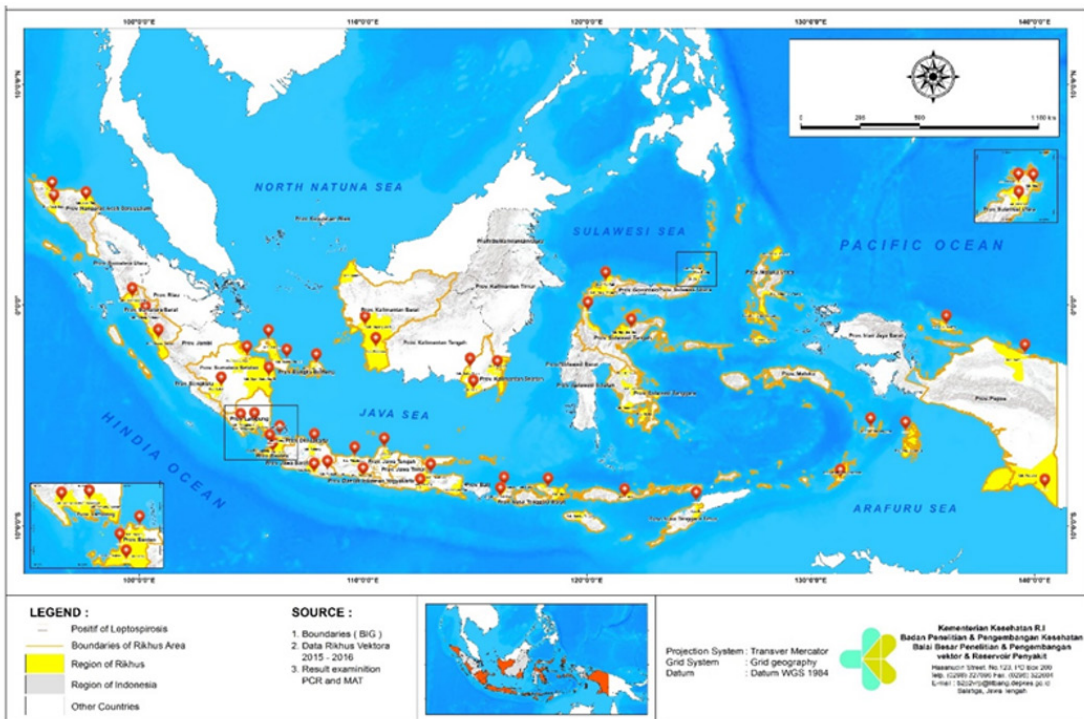


Fig 2 - Rat survey location points based on remote sensing satellite data and geographic information systems

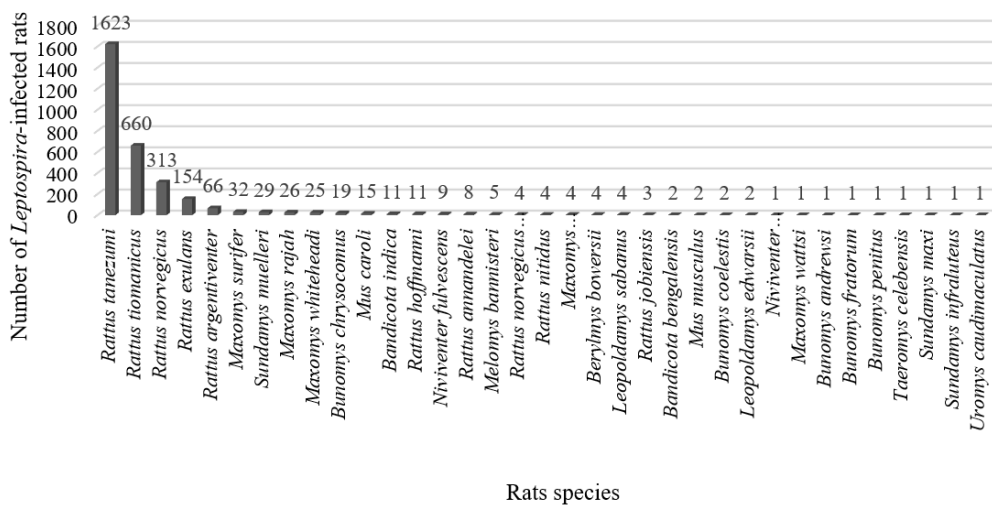


Fig 3 - Rat species and numbers of *Leptospira*-infected rats in Indonesia (2015-2018)

They were *Maxomys surifer*, *Sundamys muelleri*, *Maxomys rajah*, *Maxomys whiteheadi*, *Niviventer fulvescens*, *Rattus annandalei*, *Rattus norvegicus javanus*, *Bandicota bengalensis*, *Leopoldamys sabanus*, *Leopoldamys edwardsi*, *Berylmys bowersi*, *Sundamys maxi*, and *Niviventer cremoriventer*. Total eight types of rats positive for *Leptospira* in the middle or transitional regions of Indonesia, namely Bali, Sulawesi, West Nusa Tenggara, and East Nusa Tenggara were *Rattus hoffmanni*, *Rattus nitidus*, *Bunomys coelestis*, *Bunomys chrysocomus*, *Bunomys penitus*, *Taeromys celebensis*, *Bunomys andrewsi*, and *Bunomys fratorum*. And total five types of rats positive with *Leptospira* in eastern Indonesia, such as the Maluku Islands, North Maluku, Papua, and West Papua were *Melomys bannisteri*, *Melomys musschenbroeki*, *Rattus jobiensis*, *Melomys wattsi*, and *Uromys caudimaculatus*.

This study caught 2,310 rats in non-forest ecosystems near settlements, 2,297 in coast near settlements, and 1,744 in forests near settlements. More rats in the aforementioned areas were caught in ecosystems far from settlements, such as coast ($n=1,455$), non-forests ($n=847$), and forests ($n=710$). Of those, total 15.75%, 15.60%, 13.58%, 12.28%, 10.03%, and 8.73% of rats were confirmed with *Leptospira* bacteria in non-forests near settlement ecosystems, in coast far from settlement, in coast near the settlement, in non-forests far from the settlement, in forests near the settlement, and in forests far from the settlement, respectively (Fig 4).

Percentage of rats with infective *Leptospira* bacteria in 29 provinces

This study caught 9,616 rats from 29 provinces, 87 districts, and 270 research points. The percentage of rats confirmed with *Leptospira* using the PCR method in each province in Indonesia is presented in Fig 5.

Special Capital Region of Jakarta (DKI Jakarta) had the highest percentage of rats with *Leptospira*. On the other hand, East Nusa Tenggara Province and Riau Province had the lowest percentage (below 5%) of rats with *Leptospira*.

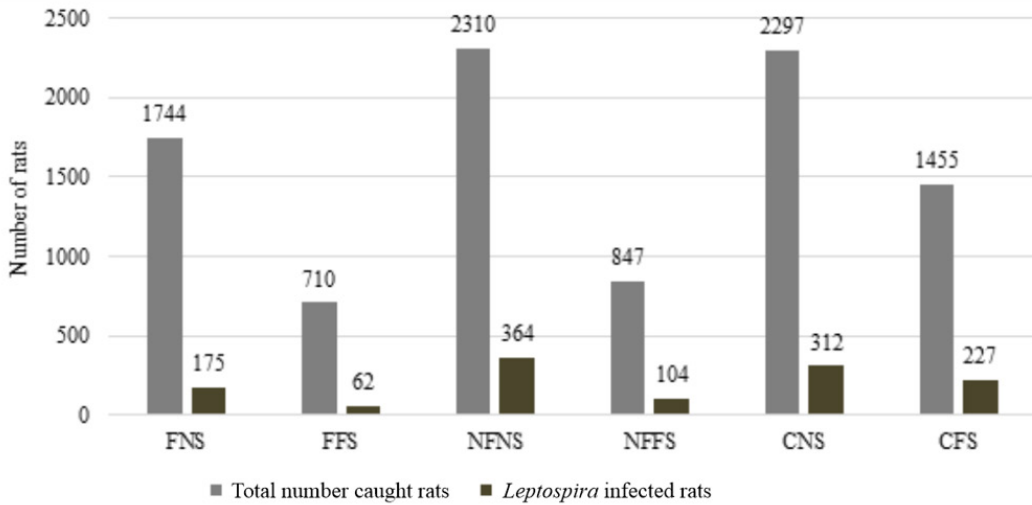


Fig 4 - Number of rats caught and confirmed positive *Leptospira* per ecosystem in Indonesia (2015-2018)

CNS: Coast near from settlements; CFS: Coast far from settlements; NFFS: Non-Forests far from settlement; NFNS: Non-forests near settlement; FFS: Forests far from settlement; FNS: Forests near settlement

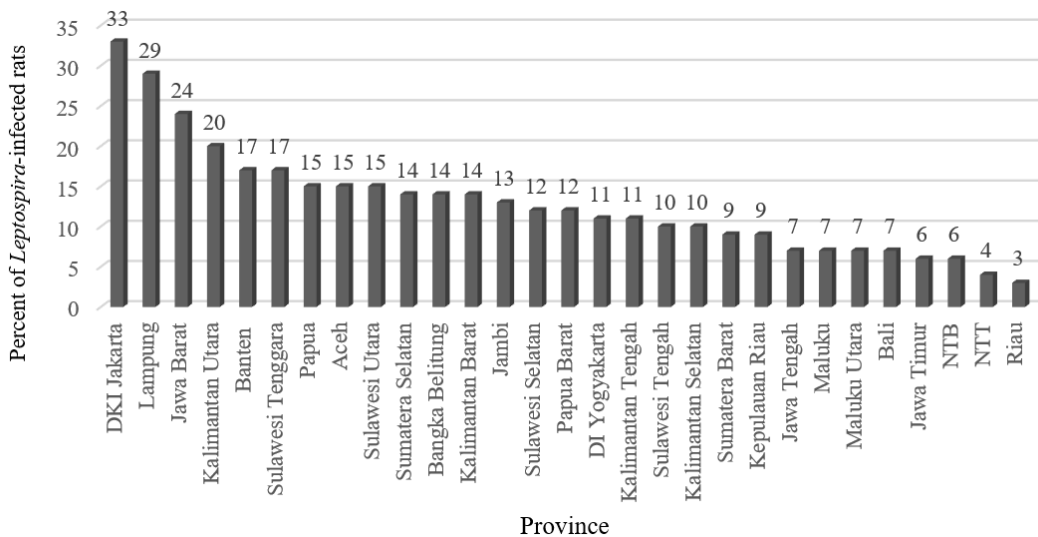


Fig 5 - Percentage of rats confirmed with *Leptospira* by polymerase chain reaction (PCR) method in Indonesia (2015-2018)

DISCUSSION

The results show that 35 of 47 species of rats are confirmed positive *Leptospira* bacteria: 14 species of rats found in Western Indonesia (Indo-Asian/Sahul Slab), eight species found in Central Indonesia (Transition), and five species found in Eastern Indonesia (Indonesia-Australia). Meanwhile, the remaining eight types of rats are confirmed with leptospirosis and had been found in all regions of Indonesia before this research was conducted (Sumanta *et al*, 2015).

To date, there are a few studies on the confirmation of rats infected with leptospirosis in all ecosystems in Indonesia. Consequently, leptospirosis becomes a neglected disease, the diagnosis and management of leptospirosis cases are relatively unknown to health workers, and epidemiological research, laboratory facilities, and infrastructure for leptospirosis detection are still very limited, especially outside of Java Island. Some studies revealed that the main inhibiting factors for leptospirosis monitoring are the availability of resources, awareness of good communication, lack of partner/researcher collaboration, incomplete data infrastructure, limited availability of the rapid diagnostic test (RDT), microscopic agglutination test (MAT), and polymerase chain reaction (PCR) in hospitals and health centers, unavailability of standard operating procedures (SOP) for leptospirosis surveillance systems, lack of evaluation standards, and insufficient activation of surveillance at community levels (Permatawati, 2018; Rachmawati and Khariri, 2020).

The diversity of rat species in Indonesia is relatively high for 174 species and spreads in various ecosystems. Thus, it is predicted that the types of rats as reservoir hosts for leptospirosis and places/habitats that transmit leptospirosis will be diverse and widespread. A study on leptospirosis in northeastern Thailand has revealed that *Leptospira* infection is prevalent in low-density *Bandicota indica* populations. In contrast, there is a low prevalence of *Leptospira* infection in high-density rodent populations of *R. exulans* and *R. rattus*, and their abundant rice fields refer to optimal rodent habitats (Krairojananan *et al*, 2020). A study conducted in Mahe Island has

revealed that *Leptospira* renal carriage could be predicted by identifying the species identification and describing landscape fragmentation and rainfall with infection prevalence that is positively correlated with these two environmental variables (Biscornet *et al*, 2021). Moreover, several environmental factors are associated with the carriage of *Leptospira* in *Rattus rattus* and *Rattus norvegicus*, such as low elevations, fragmented landscapes, the proximity of urbanized areas, increased distance from forests, and increased precipitation in the three-month preceding trapping (Biscornet *et al*, 2021).

Rattus tanezumi, house rats, and *R. norvegicus*, sewer rats, are most infected with *Leptospira* among other types of rats, especially in non-forest ecosystems near and far from settlements. *Leptospira* can survive, replicate, and can escape the phagocytic activity of macrophages in the reservoir host (Fraga *et al*, 2011). The survival ability of *Leptospira* in the reservoir host organ is stimulated by Toll-like receptor 4 (TLR4) and Toll-like receptor 2 (TLR2) in rats against lipopolysaccharides (LPS) of the bacteria (Santecchia *et al*, 2020; Santecchia *et al*, 2019).

Sewer rats and house rats are groups of domestic rats whose life activities are like foraging, breeding, and sheltering in human settlements (Ristiyanto *et al*, 2015). *R. rattus* and *R. norvegicus* play an important role in the transmission of leptospirosis in residential and urban areas (Pui *et al*, 2017). *R. exulans*, *R. argentiventer*, *Bandicota indica*, *Maxomys surifer*, *Sundamys muelleri*, *Maxomys rajah*, *Maxomys whiteheadi*, *Niviventer fulvescens*, *Rattus annandalei*, *Bandicota bengalensis*, *Leopoldamys sabanus*, *Leopoldamys cremori*, *Niviventer cremoriventer*, *Sundamys maxi*, *Sundamys infraluteus*, *Rattus hoffmani*, *Rattus nitidus*, *Bunomys coelestis*, *Bunomys chrysocomus*, *Bunomys penitus*, *Taeromys celebensis*, *Bunomys andrewsi*, *Bunomys fratorum*, *Melomys bannisteri*, *Melomys musschenbroeki*, *Rattus jobytus watts*, and *Rattus jobiensis* are frequently found outside the house. *R. jobiensis* are peri domestic rats found in gardens, paddy fields, fields, and plantations as well as sylvatic rats found in the forest (Himsworth *et al*, 2013; Maharadatunkamsi *et al*, 2020).

All ecosystems investigated in this study have shown that the number of rats infected with leptospirosis is more than 5%. The number of rats in settlements and beaches infected by leptospirosis is >10%, and that in the forest ecosystem far from the settlements is <10%. Although many rats infected with leptospirosis are found in ecosystems adjacent to settlements and coastal areas as well as forest ecosystems far from settlements, they could maintain the leptospirosis transmission cycle in nature. Ecosystem variables, location, altitudes, and temperatures have a significant relationship with the distribution of rats confirmed with positive *Leptospira* (Supranelfy *et al*, 2019). A Survey of rodent ecology in various environments or ecosystems in Southeast Asia has shown that the prevalence of rat species infected with *Leptospira* varies according to their habitat, for example, *L. interrogans* infection in rodents was restricted to humid habitats while *L. borgpetersenii* infection was equally frequent in both humid and dry habitats (Cosson *et al*, 2014). A study in Comilla, Bangladesh employed the sequencing of real-time polymerase chain reaction (qPCR) and has found that 13.1% (61/465) of the trapped rodents were infected with pathogenic *Leptospira*. Sequencing of the qPCR products identifies the presence of three species: *L. interrogans*, *L. borgpetersenii*, and *L. kirschneri*. Rodents of the *Bandicota* genus are more significantly positive than those of the *Rattus* and *Mus* genus (Krijger *et al*, 2019).

DKI Jakarta Province has the highest number of rats infected with Leptospirosis (33%). Jakarta is a lowland area and frequently suffers from floods, which become a risk factor for the occurrence of leptospirosis. Other risk factors include community behavior, health services, environment, socio economy, and demography. In the study of Widiyanti and Astuti (2016) on flood-vulnerable areas in Jakarta, 75% of the samples tested positive for *Leptospira*. Based on these results, the environment in Jakarta's flood-vulnerable areas supports *Leptospira's* survival). The pH environments are still suitable for *Leptospira* growth. Meanwhile, Okatini *et al* (2007) revealed that the significant risk factors for the incidence of leptospirosis in Jakarta are the condition and arrangement of houses with odds ratio (OR) = 3.96, wastewater disposal channel with OR = 1.98), socioeconomic level with OR

= 1.93, knowledge with OR= 17.6, and education with OR= 2.41.

Likewise, Suwannarong *et al* (2022) revealed that respondents who manage, hold, or consume rodents have a statistically significant relationship with the incidence of leptospirosis (adjusted OR = 3.137; 95% confidence intervals (CI): 1.914-5.139; $p < 0.001$). In addition, monthly household income, low level of knowledge, attitudes, and actions have a statistically significant relationship with the incidence of leptospirosis (OR = 0.57; 95% CI: 0.33-0.99; $p = 0.04$) (Suwannarong *et al*, 2022). Research in Northern Thailand has revealed that the prevalence of *Leptospira*-infected rats in endemic leptospirosis areas is higher than that in leptospirosis non-endemic areas (Della Rossa *et al*, 2016).

This study showed that rats with *Leptospira* (>5%) are found in every province and ecosystem, but cases of leptospirosis were relatively rare; leptospirosis cases are rarely reported in areas outside Java Island. These findings indicate that the iceberg phenomena of leptospirosis cases potentially occur in every province because no cases are reported, but in reality, there may be many infected people. Thus, the health service providers should improve their basic and advanced abilities to diagnose and detect cases of leptospirosis. Zoonotic diseases are closely related to animals and humans; thus, the planning and control efforts must involve many people-in-charge to deal with the health of animals, humans, and the environment. One health approach necessarily involves cross-sectors and cross-disciplinaries by integrating professional concepts in animal health and human health (Rachmawati and Khariri, 2020). The 'One Health' approach that integrates leptospirosis data across sectors is needed to determine the priority of leptospirosis prevention. Therefore, to more effectively conduct early diagnosis of leptospirosis cases, the capacity building of doctors and health workers should be improved through clinical lectures, cross-sector joint surveillance, and the development of laboratory networks (Handayani *et al*, 2019). These activities should be integrated with a one-health approach to control the transmission of leptospirosis to humans.

In summary, in Indonesia, there are 35 types of rats infected with leptospirosis: 22 rat species are found in western Indonesia (Sumatra, Kalimantan, and Java), eight rat species found in central Indonesia (Bali, Sulawesi, West Nusa Tenggara, and East Nusa Tenggara), and five rat species found in Eastern Indonesia (Maluku, North Maluku, Papua, and Papua West). The house rat (*Rattus tanezumi*), the tree rat (*R. tiomanicus*), the sewer mouse (*R. norvegicus*), and the field rat (*R. exulans*) were infected with leptospirosis and found in all provinces with the highest relative numbers (>10%) among other types. Ecosystems adjacent to settlements (distance less than 3 kilometers) and coastal areas were more commonly found infected-rats with leptospirosis. DKI Jakarta Province is the area with the highest number of *Leptospira*-infected rats in Indonesia. This number in this research is far more than that in previous reports.

ACKNOWLEDGMENTS

We thank the Director of the National Institute of Health Research and Development and Institute for Vector and Reservoir Control Research and Development Salatiga, who encouraged the research and article writing. We also thank the team involved in collecting data, all field investigators as well as coordinators during the Rikhus Vektora Project, and the local government who allowed us to conduct the research.

The Rikhus Vektora Project was funded by Vector and Reservoir Control Research and Development Salatiga, the National Institute of Health Research and Development, and the Ministry of Health.

CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflicts of interest.

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