

# The Potential of Rats and Bats as Reservoirs of Leptospirosis and Japanese Encephalitis (JE) in Muna Region, Southeast Sulawesi Province, Indonesia

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## Abstract

**Background:** Small mammals such as rats and bats are important animal reservoirs of various zoonotic diseases of public health importance. The potential of rats and bats as a reservoir of leptospirosis and Japanese Encephalitis (JE) in Muna Regency remains unknown. This study aimed to determine the presence of *Leptospira* spp and JE virus (JEV) in rats and bats in Muna Regency, Southeast Sulawesi Province.

**Methods:** A cross-sectional survey was carried out in three districts, namely Kabawo, Batalaiworu, and Katobu. Bats and rats were caught in six ecosystems spread across the three districts. Serological (Microscopic Agglutination Test, MAT) and molecular examinations (Polymerase Chain Reactions, PCR) were performed to detect *Leptospira* spp. and JEV in rats and bats, respectively.

**Results:** A total of 137 rats from seven species were successfully caught, namely *Mus* sp., *Rattus argentiventer*, *R. nitidus*, *R. exulans*, *R. hoffmanni*, *R. norvegicus*, and *R. tanezumi*. Of which, six species were confirmed positive for *Leptospira* spp by PCR, with the highest prevalence was found in *R. argentiventer* (50%) and *R. tanezumi* (36.4%). Meanwhile, a total of 86 bats were also successfully captured. The JEV antibody was detected in *D. viridis*, *R. celebensis* and *S. wallacei*.

**Conclusion:** Rats and bats are the potential reservoirs of leptospirosis and JE in Muna Regency. Most importantly, the study provides the first evidence of JE reservoirs (*R. celebensis* and *S. wallacei*) in Sulawesi. Surveillance of leptospirosis and JE are recommended.

**Keywords:** zoonotic, reservoir, rats, bats, leptospirosis, Japanese Encephalitis

## 1. Introduction

Zoonoses are diseases transmitted between vertebrate animals and humans through food (foodborne), air (airborne), and direct contact. The climate and environmental changes have triggered the emergence and re-emergence of zoonoses, of which  $\pm$  60.3% and 71.8% are transmitted by domestic animals and wild animals, respectively (World Health Organization, 2018).

Indonesia is an archipelago country biogeographically located between two animal distribution regions in the world, namely the Orientalis and Australia (Kirmowardoyo, 1991). It puts Indonesia possessing a high number and diversity of wildlife species distributed in various types of habitats and ecosystems, which in turn also affect the

distribution of vectors and reservoirs of diseases (Simpson, 1977).

Rats and bats are small mammals, which play an important role in many zoonotic infections. In Indonesia, a total of 153 species of genera identified belongs to the subfamily Murinae (rats). The previous studies reported that rats are believed as important zoonotic reservoirs for leptospirosis, hantavirus, typhus scrub, murine typhus, spotted fever group rickettsia, plague, schistosomiasis, rabies, and several other diseases in Indonesia (Ibrahim, 2005).

Since 1320 BC, rats are known as reservoirs of various diseases, 31 of which are caused by worms, 28 by viruses, 26 by bacteria, 14 by protozoa, and eight by rickettsias (Ristiyanto et al., 2014). One of the important rodent-borne diseases is leptospirosis caused by the pathogenic bacteria of the genus *Leptospira*. Rodents could carry and shed the *Leptospira* to the environment during urine excretion. Humans can be infected through direct or indirect contact with the urine of infected animals or *Leptospira*-contaminated water and soil. These bacteria could enter the human body through abraded skins or mucous membranes. Commonly, the disease is spread by domestic rats (Khariri, 2019).

Besides, Indonesia also has more than 200 species of bats (21% of the total species in the world). They belong to nine families, including Pteropodidae, Megadermatidae, Nycteridae, Vespertilionidae, Rhinolophidae, Hipposideridae, Emballonuridae, Rhinopomatidae, and Molossidae (Suyanto, 2001b). Some species are known as potential reservoirs for rabies, severe acute respiratory syndrome (SARS), Marburg virus infection, Nipah virus, Hendra virus, and Japanese Encephalitis (JE) (Suyanto, 2001b; Winoto, Graham, Nurisa, & Hartati, 1995). For instance, bats from the genus *Pteropus* have been reported as reservoirs of the Nipah virus in Sumatra (Sendow et al., 2013). Changes in the environment and deforestation trigger the transmission of the viruses from bats to humans (Kardena, I; Sukada, I; Abiyoga, P; Hartawan, D; Diamita, I; Robertson, 2014). A study in West Kalimantan demonstrated that bats also play a significant role in the transmission of the JE virus (JEV) in Kayanhilir and Kayanhulu Districts (Winoto et al., 1995). Further, a study in Tangerang, Banten Province showed that contact with bats or eating bat-leftover bites should be avoided to prevent zoonotic transmission from bats to humans (Wijayanti et al., 2016).

Even though some pieces of evidence have shown that rats and bats play an important role in carrying and transmitting the pathogens, however, the information regarding their potential as reservoirs of *Leptospira* and JEV in Southeast Sulawesi remains unknown. This study aimed to provide recent epidemiological data from a survey of the presence of *Leptospira* spp and JEV in rats and bats, particularly in Muna Regency.

## 2. Method

This study was part of the Rikhus Vektora Project (*Riset Khusus Vektora*) conducted in 2016, focusing on the survey on vectors and reservoirs. A cross-sectional survey was carried out in three districts located in Muna Regency, i.e. Kabawo, Batalaiworu, and Katobu Districts (Figure 1) in August 2016.

### 2.1 Study Sites

Muna Regency is located on Muna Island which is a separate land from Sulawesi Island (Wikipedia, 2020b). Muna is an island in the Southeast Sulawesi province of Indonesia with an area of 3,341.5 km<sup>2</sup>. It is located just southeast of the island of Sulawesi. Geographical conditions that are separated from the mainland of Sulawesi Island can cause a variety of fauna species that are interesting to study. Aside from that, this location still has a large forest area (Wikipedia, 2020a).

Bats and rats were caught in six ecosystems across the three districts. The six ecosystems were forest near the settlement (FNS, the site I), forest distant from the settlement (FDS, the site II), non-forest near to the settlement (NFNS, the site III), non-forest distant from the settlement (NFDS, the site IV), coast near the settlement (CNS, the site V) and coast distant from the settlement (CDS, the site VI).

Study sites were located in six ecosystems that were forest near the settlement (FNS: latitude -5.007" and longitude 122.430"; elevation 70 m above sea level), forest distant from the settlement (FDS: -5.007" and 122.446"; 79 m), non-forest near to the settlement (NFNS: -4.826" and 122.719" with elevation 93 m), non-forest distant from the settlement (NFDS: -4.833" and 122.709"; 113 m), coast near the settlement (CNS: -4.805" and 122.721"; 69 m) and coast distant from the settlement (CDS: -5.032" and 122.389"; 68 m. Muna regency has a 205.769 ha wide area. Muna Regency has a tropical climate like most regions in Indonesia, with an average temperature of around 26–30 ° C. Likewise with the seasons, Muna Regency has two seasons, namely the rainy season and the dry season.

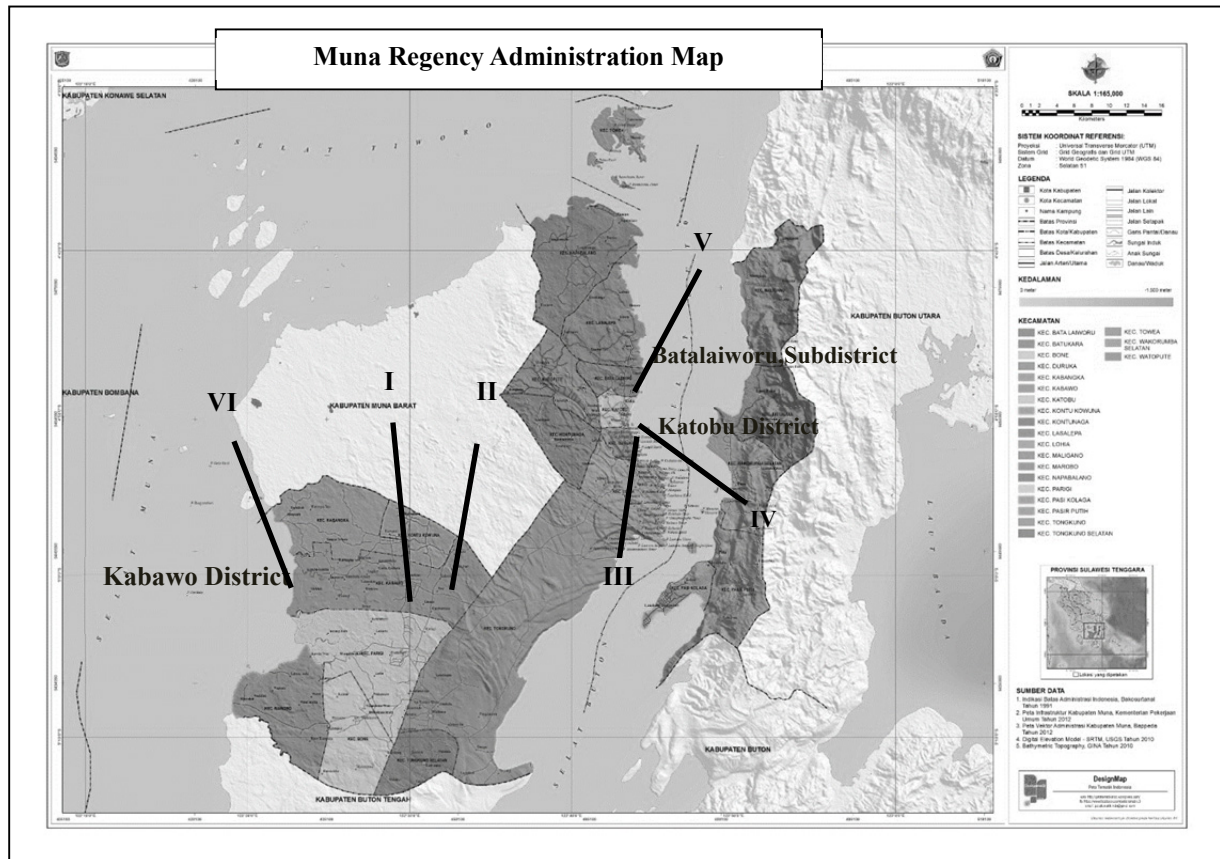


Figure 1. The map of trap in six location: I (FNS) = Forest Near Settlement; II (FDS) = Forest Distant Settlement; III (NFNS) = Non-Forest Near Settlement; IV (NFDS) = Non-Forest Distant Settlement; V (CNS) = Coast Near Settlement; VI (CDS) = Coast Distant Settlement

## 2.2 Data Collection

All steps of data collection followed the guideline of the survey on vectors and reservoirs collection in the field published by “*Riset Khusus Vektora*” (B2P2VRP, 2015). Traps were placed in the six predefined types of ecosystems. In each ecosystem, rats were collected during three consecutive days using 100 single live traps sized 12-cm x 12-cm x 28-cm. Roasted coconut was applied as baits. The traps were prepared at 4 PM, placed both inside and outside the house, and they were taken the next morning. The caught rats were put in calico bags and brought to the field laboratory for sample processing and identification. The sample processing involved blood taking through the heart. After that, blood was centrifuged to obtain serum for serological examination. All the rats' kidneys were also collected for molecular examination.

Meanwhile, bats were caught using mist nets and hand nets in one night in each ecosystem. Mist nets were prepared at 4 to 5 PM, then the caught bats were collected every hour from 7 to 10 PM. Besides, the bats were also collected at 6 AM the next morning.

## 2.3 Species Identification

The rats were identified based on morphological and morphometric characteristics (B2P2VRP, 2017), including the color and hair type, tail color, and scales and hair on the tail. While the latter, weight, total length, tail length, back foot length, ear length, skull length and the number of nipples in female rats were measured or counted. Rat species identification were validated by an expert in the Reservoir Reference Laboratory of the Center for Research and Development of Disease Vectors and Reservoirs (B2P2VRP) using Sulawesi rats key identification from various references (Aplin K.P et al., 2003; Corbet & Hill, 1992; G. Musser, 2014; G. G. Musser & Durden, 2014)

The bats identification was based on the morphometric characteristics. Body length was measured from the tip of the nose to the anal canal, the length forearm was measured from the base of the radius bone to the outer elbow. The length of hind foot was measured from the heel to the tip of the longest toe. Ear length was measured from the

base or base to the tip of the ear the furthest. The tragus length and antitragus length were measured from the base the base to the inner edge where the tragus / antitragus attaches to the head up to ends. Calf length (bet) was measured from knee to ankle. The tail length was measured from the base of the tailbone to the end of the tail. Identification activity refers in the manual (B2P2VRP Salatiga, 2016).

#### 2.4 Serological and Molecular Detection

Serological and molecular examinations were performed at the Bacteriology Laboratory, B2P2VRP, Salatiga, Central Java. The serological examination was carried out using the Microscopic Agglutination Test (MAT) 32 with 15 serovars (Bangkinang, Canicola, Grippotyphosa, Icterohaemorrhagiae, Pyrogenes, Hardjo, Hebdomadis, Pomona, Djasiman, Robinsoni, Bataviae, Mini, Sarmin, Manhao, and Rama). The results will be considered as positive if the titer is  $\geq 1: 20$  (Villanueva et al., 2010).

Molecular examination using the polymerase chain reaction (PCR) was performed on the kidneys. The DNA isolation was carried out according to the PureLink Genomic DNA Mini Kit protocol from Invitrogen (Cat. No. K182001) and the target of DNA fragments was amplified using reagent Go Taq® Green Master Mix (Promega, Cat. # M7122) with primer LipL32 (forward: 5'-ATCTCCGTTGCACTCTTTGC-3'; reverse: 5'-ACCATCATCATCATCGTCCA-3'). The thermal cycler machine was run with a denaturation temperature program of 95 °C for 5 minutes, amplification of 35 cycles at 94 °C for 30 seconds, 58 °C for 30 seconds (annealing), and 72 °C for 1 minute (extension), and then final extension at 72 °C for 7 minutes. The PCR products were visualized in agarose gel 2%. The LipL32 gene would form a DNA band-sized 474 bp (Ahmed et al., 2006).

Molecular examination of JE in bats using Reverse Transcription PCR (RT-PCR) was performed on blood sera. The RNA isolation was carried out according to the QIAamp Viral RNA Mini Kit protocol from Qiagen (Hilden, Germany). The results of the RNA isolation were tested using one-step RT-PCR on NS3 gene using the consensus primers FP (5'-AGA GCG GGG AAA AAG GTC AT-3') and RP (5'-TTT CAC GCT CTT TCT ACA GT-3') (Santhosh et al., 2007). The thermal cycler machine was run with a reverse transcription at 50 °C for 30 minutes, hot start at 94 °C for 2 minutes, amplification of 40 cycles at 94 °C for 15 seconds (denaturation), 55 °C for 30 seconds (annealing), and 68 °C for 1 minute (extension), and then final extension at 72 °C for 5 minutes. The primers corresponded to a PCR product of 162 bp (Garjito et al., 2019).

#### 2.5 Statistical Analysis

Descriptive analyses were carried out to describe the frequency and proportion of identified rats and bats species caught during the survey. Chi-square analysis was performed to determine the association between ecosystem types and the species of rats and bats found. All statistical analyses were carried out using SPSS 17 Programme (IBM Corp., Armonk, NY, USA).

### 3. Results

#### 3.1 Rodents Distribution

The species distribution of rats in each ecosystem spread in Muna Regency during the survey was significantly different ( $P < 0.001$ ) (Table 1). A total of 137 rats was successfully caught and classified into two genera consisting of seven species. The most abundant species was *R. tanezumi* (n=66; 48.2%), followed by *R. exulans* (n=18; 13.1%). In addition, an endemic species of Sulawesi Island, *R. hoffmanni* was also found in this study (n=12; 8.8%). The results showed a significant relationship between the species of the caught rats and the type of ecosystem ( $P$ -value  $< 0.001$ ).

Table 1. Distribution of rat species caught in six different ecosystems in Muna Regency, Southeast Sulawesi, 2016

Species	FNS		FDS		NFNS		NFDS		CNS		CDS		Total		p-value
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	
<i>Mus sp.</i>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	4.8	2	1.5	0.000
<i>Rattus exulans</i>	4	22.2	3	42.9	0	0.0	0	0.0	4	13.3	7	16.7	18	13.1	
<i>Rattus hoffmanni</i>	2	11.1	1	14.3	0	0.0	0	0.0	3	10.0	6	14.3	12	8.8	
<i>Rattus argentiventer</i>	0	0.0	0	0.0	3	12.5	2	12.5	0	0.0	9	21.4	14	10.2	
<i>Rattus nitidus</i>	0	0.0	0	0.0	11	45.8	0	0.0	2	6.7	0	0.0	13	9.5	
<i>Rattus norvegicus</i>	0	0.0	0	0.0	6	25.0	0	0.0	6	20.0	0	0.0	12	8.8	
<i>Rattus tanezumi</i>	12	66.7	3	42.9	4	16.7	14	87.5	15	50.0	18	42.9	66	48.2	
<b>Total</b>	<b>18</b>	<b>100.0</b>	<b>7</b>	<b>100.0</b>	<b>24</b>	<b>100.0</b>	<b>16</b>	<b>100.0</b>	<b>30</b>	<b>100.0</b>	<b>42</b>	<b>100.0</b>	<b>137</b>	<b>100.0</b>	

Comments: FNS, Forest Near Settlement; FDS, Forest Distant Settlement; NFNS, Non-Forest Near Settlement; NFDS, Non-Forest Distant Settlement; CNS, Coast Near Settlement; CDS, Coast Distant Settlement.

### 3.2 Detection of *Leptospira* in Rodents

Based on the PCR examination, the overall prevalence of *Leptospira* in rats was 29.2%. Six out of seven species were confirmed positive for leptospirosis using PCR. Of which, the highest prevalence of *Leptospira* spp was found in *R. argentiventer* (n=7/14; 50%) followed by *R. tanezumi* (n=24/66; 36.4%). Whereas based on MAT, only three species were found to be positive *Leptospira* and the overall prevalence was 9.5%. Based on the Chi-Square analysis, there was a correlation between species with the laboratory test results (using the PCR method) with a *P*-value < 0.05. The rats confirmed positive from *Leptospira* were detected in five ecosystems, i.e. FNS, FDS, NFNS, NFDS and CDS. None positive rat was found in the CNS ecosystem.

Table 2. Leptospirosis test results on rat species in Muna Regency, Southeast Sulawesi, 2016

Species	No. of rats	Method of Leptospirosis Tests				Comments
		PCR		MAT		
		Positive, (%)	p-value	Positive, (%)	p-value	Serovar
<i>Mus sp.</i>	2	0 (0.0)		0 (0.0)		
<i>Rattus exulans</i>	18	1 (5.6)		0 (0.0)		
<i>Rattus hoffmanni</i>	12	3 (25.0)		1 (8.3)		Ict 80, Dja 20
<i>Rattus argentiventer</i>	14	7 (50.0)		0 (0.0)		
<i>Rattus nitidus</i>	13	4 (30.8)	0.045	1 (7.7)	0.182	Gri 20
<i>Rattus norvegicus</i>	12	1 (8.3)		0 (0.0)		
<i>Rattus tanezumi</i>	66	24 (36.4)		11 (16.7)		Bat 80, Dja 20, Dja 40, Heb 20, Ict 20, Ict 40, Pom 40
<b>Total</b>	<b>137</b>	<b>40 (29.2)</b>		<b>13 (9.5)</b>		

Comments: PCR= Polymerase Chain Reaction, MAT= Microscopic Agglutination Test, Ict =Icterohaemorrhagiae, Dja = Djasiman, Gri = Grippotyphosa, Bat = Bataviae, Heb = Hebdomadis, Pom = Pomona.

Table 3. Rats that *Leptospirosis* confirmed using MAT in six different ecosystems in Muna Regency, Southeast Sulawesi, 2016

Ecosystem	Species	Type of serovar
Forest Near Settlement	<i>Rattus hoffmanni</i>	Ict 80 and Dja 20
Forest Distant Settlement	<i>R.tanezumi</i>	Bat 80
Non Forest Near Settlement	<i>R.nitidus</i>	Gri 20
	<i>R.tanezumi</i>	Ict 20 and Dja 20
Non Forest Distant Settlement	<i>R.tanezumi</i>	Gri 20, Heb 20, Ict 20 and Dja 20
Coastal Near Settlement	<i>R.tanezumi</i>	Pom 40
Coastal Distant Settlement	<i>R.tanezumi</i>	Ict 80 and Dja 20

Table 3 showed that *Rattus tanezumi* was the most confirmed *Leptospira* positive species, with various types of serovar. In term of ecosystem, based on MAT method, *R. hoffmanni* captured in the FNS was confirmed *Leptospira* positive with Ict 80 and Dja 20 serovars. In addition, *R. tanezumi* and *R. nitidus* caught in the FDS and NFNS ecosystems were also confirmed *Leptospira* positive with Bat 80 and Gri 20 serovars, respectively. The PCR results demonstrated that *R. tanezumi* caught in the FNS, FDS, and NFNS ecosystems were also confirmed positive from *Leptospira* infection. All rats trapped in the CNS ecosystem were free from *Leptospira* infection either based on MAT or PCR examinations.

### 3.3 Bats Distribution

The distribution of bat species caught in the present study can be seen in Table 4. There were 14 species of 10 genera rat trapped in Muna regency. The most captured bats were *Dobsonia viridis* (n=19) (Figure 2), followed by *Rousettus celebensis* (n=18) and *Styloctenium wallacei* (n=14, endemic species of Sulawesi). The endemic bat species trapped were *Dobsonia exoleta*, *D. viridis*, *Eonycteris spelaea*, *Nyctimene cephalotes*, *Pteropus alecto*, *Rousettus celebensis*, and *Styloctenium wallacei* (Figure 3). *Dobsonia viridis* and *Rousettus celebensis* were the most caught species (20.93%). *Dobsonia viridis* was most commonly found in the NFNS ecosystems and trapped in gardens and yards where papaya trees grew up. Whereas *Rousettus celebensis* was found in NFDS ecosystem and trapped in gardens and CNS ecosystem and trapped in the yard with papaya and guava vegetation. The second highest percentage was of *Styloctenium wallacei* (16.78%). Like *Dobsonia viridis*, this species was often found in the Non-Forest Near Settlement ecosystem.



Figure 2. *Dobsonia viridis* and *D.exoleta*

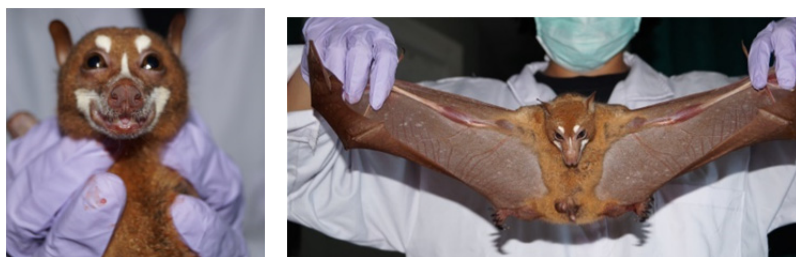


Figure 3. *Styloctenium wallacei* (Sulawesi stripe-faced fruit bat)

Table 4. Distribution of caught bats in six different ecosystems in Muna Regency, Southeast Sulawesi, 2016

Species	FNS		FDS		NFNS		NFDS		CNS		CDS		Total		P-value
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	
<i>Cynopterus brachyotis</i>	0	0.0	1	11.1	0	0.0	0	0.0	5	33.3	0	0	6	7.0	
<i>Cynopterus luzoniensis</i>	0	0.0	0	0.0	0	0.0	0	0.0	1	6.7	0	0.0	1	1.2	
<i>Cynopterus minutus</i>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	11.1	1	1.2	
<i>Dobsonia exoleta</i>	0	0.0	0	0.0	0	0.0	2	11.1	0	0.0	0	0.0	2	2.3	
<i>Dobsonia viridis</i>	1	9.1	1	11.1	9	37.5	5	27.8	3	20.0	0	0.0	19	22.1	
<i>Eonycteris spelaea</i>	0	0.0	0	0.0	2	8.3	1	5.6	0	0.0	0	0.0	3	3.5	
<i>Falsistrellus petersi</i>	0	0.0	1	11.1	0	0.0	0	0.0	0	0.0	0	0.0	1	1.2	
<i>Kerivoula hardwickii</i>	1	9.1	1	11.1	0	0.0	0	0.0	0	0.0	0	0.0	2	2.3	0.000
<i>Macroglossus minimus</i>	1	9.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.2	
<i>Nyctimene cephalotes</i>	4	36.4	1	11.1	0	0.0	0	0.0	0	0.0	0	0.0	5	5.8	
<i>Pteropus alecto</i>	0	0.0	0	0.0	0	0.0	2	11.1	0	0.0	0	0.0	2	2.3	
<i>Rousettus amplexicaudatus</i>	0	0.0	1	11.1	4	16.7	3	16.7	1	6.7	2	22.2	11	12.8	
<i>Rousettus celebensis</i>	1	9.1	1	11.1	3	12.5	5	27.8	5	33.3	3	33.3	18	20.9	
<i>Styloctenium wallacei</i>	3	27.3	2	22.2	6	25.0	0	0.0	0	0.0	3	33.3	14	16.3	
Total	11	100.0	9	100.0	24	100.0	18	100.0	15	100.0	9	100.0	86	100.0	

Comments: FNS = Forest Near Settlement, FDS = Forest Distant Settlement, NFNS = Non-Forest Near Settlement, NFDS = Non-Forest Distant Settlement, CNS = Coast Near Settlement CDS = Coast Distant Settlement.

The statistical analysis showed that there was a correlation between the difference in bat species and that in ecosystems. Twelve species collected from Muna Regency are commonly found in the island of Sulawesi. From this study in Muna Regency, Sulawesi Tenggara Province, 12 species from 10 genera were found. An endemic species in the island of Sulawesi, namely *Styloctenium wallacei*, was found. *Cynopterus brachyotis*, *Pteropus alecto*, *Rousettus amplexicaudatus*, *Eonycteris spelaea*, *Macroglossus minimus*, and *Kerivoula hardwickii* species were found in almost all regions of Indonesia. Based on the results of the study, there was a bat species commonly found on Sulawesi Island, but not found on Java and Sumatra, namely *Dobsonia viridis*.

*Dobsonia viridis* and *Rousettus celebensis* were the most caught species (20.93%). *Dobsonia viridis* was most commonly found in the NFNS ecosystems and trapped in gardens and yards on which papaya trees grew up. Whereas *R. celebensis* was found in NFDS ecosystem and trapped in gardens and the CNS ecosystem and trapped in the yard with papaya and guava vegetation. The second highest percentage was of *S. wallacei* (16.78%).

### 3.4 Detection of JEV in Bats

The result of confirmed positive rats from the JE can be seen in Table 5. It revealed that three species of rat was confirmed positive from JE, i.e. *D. viridis*, *R. celebensis*, and *S. wallacei*. They were known as the endemic species in Sulawesi Island.

Table 5. The species of bats caught in Muna Regency, Southeast Sulawesi confirmed positive for the JE virus.

Species	No. of bats	JE test results	
		No. of positive, (%)	No. of negative, (%)
<i>Cynopterus brachyotis</i>	2	0 (0.0)	2 (6.1)
<i>Dobsonia viridis</i>	7	1 (33.3)	6 (18.2)
<i>Falsistrellus petersi</i>	1	0 (0.0)	1 (3.0)
<i>Kerivoula hardwickii</i>	1	0 (0.0)	1 (3.0)
<i>Macroglossus minimus</i>	1	0 (0.0)	1 (3.0)
<i>Nyctimene cephalotes</i>	2	0 (0.0)	2 (6.1)
<i>Pteropus alecto</i>	2	0 (0.0)	2 (6.1)
<i>Rousettus amplexicaudatus</i>	6	0 (0.0)	6 (18.2)
<i>Rousettus celebensis</i>	9	1 (33.3)	8 (24.2)
<i>Styloctenium wallacei</i>	5	1 (33.3)	4 (12.1)
Total	36	3 (100)	33 (100)

#### 4. Discussion

In this study, the presence of *Leptospira* in rats and JEV in bats was successfully detected in some ecosystems during the survey in Muna Regency, Southeast Sulawesi. These findings highlighted the potential of such animals as reservoirs, which could play important role in maintaining disease transmission such as leptospirosis and JE in the region. The current study, to the best of our knowledge, is the first study that provides epidemiological evidence of the presence of *Leptospira* and JEV in rats and bats, respectively, in Southeast Sulawesi.

The dominance of *Rattus tanezumi*'s dominance is referred to several factors, one of which is the habitat of the rat. *R. tanezumi* has wide habitats, from primary forests, secondary forests, tropical rain forests, villages, plantations, office buildings, to residential areas, or at an altitude of 0-2000 meters above sea level (Maharadatunkamsi, 2011). Their acclimation ability to the various environment is one of the factors why the rats can spread widely and rapidly, leading it to be known as cosmopolitan animals (occupying almost all habitats). In this study, *R. tanezumi* was predominant in residential/home locations, for example in the Forest Near Settlement (FNS), Non-Forest Distant Settlement (NFDS), Coast Near Settlement (CNS), and Coast Distant Settlement (CDS) ecosystems. The results were supported by the theory and various studies about the habitat of *R. tanezumi*. In the Non-Forest Near Settlement ecosystem, the dominance of *R. tanezumi* was defeated by *R. nitidus*.

The second dominance was shown by *R. exulans*, with a percentage of 13.14%. Its habitat includes fields, gardens near settlements, plantations, and forests (Ristiyanto et al., 2014; Suyanto, 2001a). According to the activities, the rat prefers active outside houses (Ibrahim, 2005; Mulyono et al., 2016). This species can live at 0-2000 masl, like *R. tanezumi*. In addition, the rat is also categorized as commensal and a high destructive rodent (Farida et al., 2006). During the survey, *R. exulans* was found in the Forest Distant Settlement (FDS, primary forests), Forest Near settlement (FNS), Coast Near Settlement (CNS), and Coast Distant Settlement (CDS) ecosystems. The Forest Distant Settlement (FDS) ecosystem in Muna Regency was recently opened by the community to generate large gardens. This is in line with the theory that the existence of *R. exulans*, which is a commensal type in primary forest areas, is one indication of habitat destruction in an area. *R. exulans* activities are usually in a residential area, plantation, and rice fields. The existence of these rats in primary forests is possible, following humans' activities (Maharadatunkamsi, 2011).

In this research, *R. exulans* were found to dominate in the Coast Distant Settlement (CDS) ecosystem, around the ponds. *Rattus exulans* were also caught in the forest ecosystems, both near and far from settlements showing the characteristic for eastern Indonesia, by the presence of bright dark bicolor tails in the ventral-dorsal but not too contrast (G. G. Musser & Durden, 2014). This bicolor tail resembles the tail of *Maxomys mucchenbroeckii*, but the size is thin.

*Rattus argentiventer* was the third of the total rats collected. The characteristic of the species is yellowish-brown hair and gray belly hair with white edges. The habitat of *R. argentiventer* includes paddy fields, fields, and pastures,



where they get their favorite food in the form of rice, corn, or grass. They make nests in holes in the ground, under rocks, or in wood scraps (Suyanto, 2001a). In this study, *R. argentiventer* was the predominant species found in the Coast Distant Settlement (CDS) ecosystems, in the area of ponds with forest plants that make up the coastal ecosystem. The types of vegetations found were lontar (*Borassus flabellifer*), thistle (*Calotrophis gigantea*), ketapang (*Terminalia cattapa*), tallowwood, and some plants planted by farmers, such as chili, papaya, banana, and watermelon. The existence of watermelon in the field was believed as the source of feed for rats.

*Rattus nitidus*, *R. norvegicus*, *R. hoffmanni* dan *Mus* sp. were found in a few numbers indicating that their territory were not too broad. The characteristic of *R. nitidus* was similar to *R. norvegicus*, but *R. nitidus* has a ventral-dorsal bicolor tail although it is not too contrasting like the tail of the genus *Bunomys* (G. Musser, 2014). Even though rats found in Muna Regency were not endemic in Sulawesi and were commonly found in other areas, these results provided new information about the distribution of rats in that regency. The highest diversity of rat species was found in the Coast Distant Settlement (CDS) ecosystem. There were five of the seven species were collected at that location. The location of the CDS point in Muna Regency was an area with shrimp ponds with mangrove vegetation. The existence of these ponds was inferred as the source of food for rats from both natural areas and settlements.

The test for leptospirosis in the laboratory showed that the most vulnerable species to be exposed to leptospirosis was *R. tanezumi* or domestic rats. Several studies have also shown that cases of leptospirosis were found in rats near residential areas (Ardanto et al., 2018; Farida et al., 2006; Ikawati et al., 2013b). Human activities in the forest, such as collecting woods, hunting, and ecotourism activities could increase the risk of the transmission of leptospirosis to humans. Humans are infected through contact with water contaminated with the urine of infected animals or direct contact with reservoir animals. In the case of transmission in forests, humans might be infected by leptospirosis through standing water or polluted rivers in the forest or direct contact with infected animals, especially when hunting rats. Unfortunately, the endemic rats are slaughtered and sold in traditional markets in Sulawesi. Direct contact with rats, including their preparation before being cooked could be a route of transmission of the disease.

The rats detected positive for leptospirosis in Muna Regency were all from the genus *Rattus*, namely *R. tanezumi*, *R. argentiventer*, *R. nitidus*, *R. hoffmanni*, *R. norvegicus*, and *R. exulans*. The types of these rats belonged to the group of those having habitats in settlements or Non-Forest Near Settlement. The geographical environment of those sites could increase the potential of leptospirosis transmission associated with human activities near the rats' habitats. The domestic rats (*R. tanezumi*) caught at the house could be an indicator of the level of hygienic and sanitary house environment. The individual pattern of living of rats was influenced by the comparison of the differences observed in the histological and the quantitative histochemical activities in these mammalian species (Adeniyi et al., 2012).

The potential of rats as reservoirs of leptospirosis is generally dominated by commensal species. Based on the laboratory analyses that *R. tanezumi* or domestic rats were the most species with confirmed positive from leptospirosis (Ikawati et al., 2013; Ristiyanto et al., 2006; Ristiyanto et al., 2015; Sunaryo & Ningsih, 2014).. Rats trapped near houses potentially act as leptospirosis reservoirs. Rats living in natural habitats or being rarely in contact with human activities might have a low risk to be reservoirs of leptospirosis (Riyaningsih et al., 2012). Rats circulated in the habitats close to water tend to be potentially infected by *Leptospira*. For example, *R. norvegicus*, which primarily lived in sewers possess a high risk to be infected by *Leptospira* sp. Female rats can vertically transmit *Leptospira* spp. to the kids in the same location (Ramadhani & Yunianto, 2010).

The information about *Leptospira* positive rats in Muna Regency has never been reported. This current study is the first report of the potency of rats distributed in Muna Regency as a reservoir for leptospirosis. The previous studies conducted in South Sulawesi Province also showed new records of several endemic species of rats in Sulawesi with positive leptospirosis. The results indicated that various endemic rats such as *R. marmosurus*, *R. hoffmanni*, *B. chrysocomus*, *B. andrewsi* and *B. coelestis* were infected with leptospirosis (Ardanto et al., 2018).

These findings provide preliminary information for the management of the infectious disease program in the local health office to increase awareness of leptospirosis. Residents who are active in areas close to rat habitat are expected to be more aware by wearing Personal Protective Equipment (PPE) and maintaining personal hygiene. This information should also increase community self-awareness to keep their house clean, including the environment surrounding the house. People should be adequately informed that domestic rats are the important animal in transmitting *Leptospira*. It would be a threaten for their health if they do not keep the hygienic and sanitary surrounding the house.

Based on the statistical analysis, the confirmed positive rats from leptospirosis were related to the ecosystem and

species. This finding was in line with other researchers stating that the diversity of types of rats acted as a reservoir of *Leptospira* sp. in highland areas was different from lowland areas (Marbawati et al., 2016). Therefore, the efforts to tackle leptospirosis require a specific method (local specific) depending on the type of their habitat. Direct transmission of leptospirosis between rats occurs in various ways. Rats were vertically exposed to the bacteria *Leptospira* spp. from the parent or were exposed to bacteria in the nest before weaning. After the weaning period, the rat will come out from the nest and then be exposed to a polluted environment and direct contact with other rats. The other animal species are transmitted from *Leptospira* spp by drinking contaminated water (Narkkul et al., 2020). Rats also can be infected by *Leptospira* spp through sexual contact after reaching their adulthood (Minter, Diggle, Costa, Childs & M., 2017). In Thailand, people got the infection of *Leptospira* from bathing using contaminated water (Hinjoy et al., 2019).

To deal with various diseases transmitted through rats (especially leptospirosis) could be achieved from the knowledge of the bionomic approach. Preventing leptospirosis cannot be conducted individually. The point of controlling leptospirosis relies on environmental- and community-based control, including rat control. A study shows that disseminating knowledge on how to prevent leptospirosis to the community could increase their awareness (Farida et al., 2006). Control measures should be implemented including managing wastes properly, keeping the house clean, and maintaining the hygienic and sanitary of sewage. A study in Kedah proved that the predictive factors will help clinicians to identify severe leptospirosis cases earlier and develop their treatment plans so that the complications and mortality rate from severe leptospirosis can be reduced (Sandhu et al., 2020). Besides rats, in Brazil, *Leptospira* was found in bovine (65.69%), equine (20.32%), and canine (5.64%) (Polo et al., 2019).

Japanese encephalitis (JE) is a disease caused by a zoonotic virus that causes inflammation of the brain in young humans (5-9 years of age) and is transmitted through mosquitos as the vectors. The primary mosquito vector of JEV is *Culex tritaeniorhynchus*, although species such as *Cx. gelidus*, *Cx. fuscocephala*, and *Cx. annulirostris* are important secondary or regional vectors (van den Hurk et al., 2009). In Asia and Pacific vector JE is *Culex* mosquitoes, particularly, *Cx. tritaeniorhynchus* and *Cx. vishnui* which utilizes rice fields for larval development (van den Hurk et al., 2009; van den Hurk et al., 2019). The existence of JE-related viruses, vectors, and animals probable to be reservoirs in various regions of Indonesia requires vigilance against the possibility of outbreaks of this disease. The presence of JE in humans in Indonesia has been revealed based on clinical symptoms and serological examination. The latest cases of JE in Bali has become hyper-endemic, which was usually sporadic (Sendow & Bahri, 2005).

Previous data indicated that *Cynopterus brachyotis* was not found on Sulawesi Island (Suyanto, 2001b). *Rousettus celebensis* was found in NFDS ecosystem and trapped in gardens and CNS ecosystem and trapped in the yard with papaya and guava vegetation. This is following (Csorba et al., 2003) who stated that *R. celebensis* can be found in a variety of habitat types, such as secondary forests, agricultural areas, and disturbed habitats, while *D. viridis* is a species commonly found in disturbed forests, coconut gardens, and parks (Wiantoro, 2012). The second highest percentage was of *S. wallacei*. Like *D. viridis*, this species was often found in the Non-Forest Near Settlement ecosystem. It loves forest ecosystems with altitudes up to 1100 m asl, but it can also be found in disturbed forests (Patterson et al., 2017).

Besides mosquitoes, bats are also known to be reservoirs of JE causing fatal to human life. Fruit-eating bats reported as reservoirs of JE disease in Indonesia are *Rousettus leschenaulti*, *Cynopterus* sp., *Eonycteris* sp., *Myotis* sp (Winoto et al., 1995). In the present study, three species were confirmed positive as reservoirs of JE. The three species were *D. viridis* (one positive from one examined), *R. celebensis*, and *S. wallacei*. The three species were found in the Forest Distant Settlement (FDS), Non-Forest Distant Settlement (NFDS), and Coast Distant Settlement (CDS) ecosystem. Interestingly, the three species are only found in Sulawesi Island, but not exist in other islands in Indonesia. The flying ability of bats up to tens kilometers could be a pathway of viral transmission from one region to other regions. The finding of *R. celebensis* and *S. wallacei* as JE reservoirs in Sulawesi Island is a new evidence in Indonesia, so far. Meanwhile, *D. viridis* has been previously reported as a JE reservoir in Sulawesi Island.

Bats activities in humans' environment to find food is one of the vigilance risks of the transmission of the JE virus. When a bat eats fruit and leaves it, the part bitten by the bat can be a risk factor for the transmission of the JE virus. The presence of livestock such as pigs is also a risk factor for JE transmission. Even though bats distributed in Muna Regency were confirmed positive for JE, however no JE cases have been reported in humans. This finding should be an early warning system for controlling JE virus transmission. The discovery of rats detected positive for leptospirosis and bats confirmed positive for JE in Muna Regency showed that there is a potential for zoonotic disease transmission in the region.

## 5. Conclusion

Our study demonstrates that rats and bats are a potential reservoir of *Leptospira* and JEV in Muna Regency, respectively. These animals could be an important potential source of transmission for leptospirosis and JE infections in humans. This evidence highlights the importance of surveillance for leptospirosis and JE in the region. In addition, local health authorities need to manage and promote the strategy of the preventive program in order to mitigate the potential risk of zoonotic infections.

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## Shift Contributors

A.N.W, M.A.N, A.A, and R is the main contributors. “Conceptualization, A.N.W and M.A.N.; methodology, A.N.W.; software, M.A.N.; validation, A.A, R and P.W.D.; formal analysis, M.A.N.; investigation, A.A.; resources, R.; data curation, M.A.N.; writing—original draft preparation, A.N.W and M.A.N.; writing—review and editing, A.N.W, M.A.N, A.A, R, P.W.D and A.H.W. All authors have read and agreed to the published version of the manuscript.”

## Competing Interests Statement

The authors hereby declare that there is no potential conflict of interest in writing this article.

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