VARIATION OF RAT SPECIES AND IDENTIFICATION OF LEPTOSPIROSIS IN SEVERAL TYPES OF ECOSYSTEMS IN SOUTHEAST SULAWESI PROVINCE

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Abstract. Rat is one of the mammals of which species are studied related to their role as disease reservoirs of various tropical diseases. One of the diseases that is transmitted from rats to humans or other mammals is leptospirosis. The purpose of this study was to provide an overview of the variation of field-caught rat species in various ecosystems and the results of the leptospirosis examination in Southeast Sulawesi Province. The rats were caught using 100 live traps for three consecutive days. The locations of trapping were in six ecosystems: forests near and far from settlements, non-forest locations near and far from settlements, and coastal areas near and far from settlements. The results showed that the total number of rats caught was 339, consisting of 18 species. Several species were found, *ie, Bunomys andrewsi, B. chrysocomus, B. coelestis, B. penitus, Maxomys musschenbroekii, M. watsi, Mus sp, Paruromys dominator, Rattus exulans, R. hoffmanni, R. marmosurus, R. nitidus, R. argentiventer, R. tanezumi, R. norvegicus, R. xanthurus, Rattus* sp, and *Taeromys celebensis*. The most dominant species found was *R. tanezumi*. The sylvatic rat species such as *Paruromys dominator* were mostly found in forest and coastal ecosystems far from settlements. The results of laboratory tests using the microscopic agglutinin test (MAT) technique showed that 32 rats were positive for leptospirosis. The positive rat species were found near settlements in forest and coastal ecosystems. There were eight *Leptospira* serovars: Icterohaemorrhagiae, Hebdomadis, Djasiman, Bataviae, Bangkinang, Grippotyphosa, Pomona and Robinsoni. The study concludes that the variety of rat species in the research location was relatively wide. In addition, the serovars found were also varied. Moreover, one rat could contain more than one serovar. This study revealed that 32 rats positive

containing *Leptospira* sp have the potential to be a source of transmission of leptospirosis to humans. Information on leptospirosis-positive rats is essential for the surveillance of leptospirosis in humans.

Keywords: leptospirosis, rat, South East Sulawesi, reservoir

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INTRODUCTION

Zoonoses are diseases that can be transmitted from vertebrate animals to humans and vice versa through food (foodborne), air (airborne), and direct contact with sick animals. Significant changes in the world that are currently happening have triggered the occurrence of emerging and re-emerging zoonoses, 60.3% originating from animals and 71.8% originating from wild animals (World Organisation for Animal Health, 2021). Indonesia is a country that is biogeographically located between two regions with different biogeography, ie, the Oriental and Australian regions; this condition causes the presence of wild animals in Indonesia to be very diverse and spread in various habitats and ecosystems (Kirnowardoyo, 1991). It also affects the spread of disease vectors and reservoirs (Simpson, 1977).

Rats have been known as reservoirs of the disease since 1320 BC. One of the diseases that have the potential to be transmitted from rats to humans or other pets is leptospirosis (Ristiyanto *et al*, 2015). Leptospirosis is caused by *Leptospira* bacteria, which are spread through the urine or blood of infected animals. Rats are of all the that can act as a reservoir for leptospirosis. Leptospirosis from domestic, peridomestic, and sylvatic rats is very likely to be transmitted to humans (Khariri, 2019). Rats are the primary source of *Leptospira* transmission to humans and livestock (Boey *et*

al, 2019; Kusmiyati *et al*, 2005; Ristiyanto *et al*, 2015). According to Maryanto *et al* (2020), 186 species of rats are widely distributed in various habitats in Indonesia. Transmission of leptospirosis to humans is usually through direct or indirect contact with the urine of infected animals. Many cases of leptospirosis are not reported because of the difficulty of clinical diagnosis and the high cost of laboratory tests.

Indonesia is one of the endemic countries for leptospirosis (Gasem *et al*, 2020). In 2020, eight provinces, namely, DKI Jakarta, West Java, Central Java, DI Yogyakarta, East Java, Banten, North Kalimantan, and South Sulawesi, reported cases of leptospirosis (MOH RI, 2021). As many as 906 cases of leptospirosis were reported in 2020 (MOH RI, 2021). During the last ten years, there has been a trend of increasing cases of leptospirosis, especially in the previous three years. However, there was a decrease in deaths over the three years. The case fatality rate (CFR) from leptospirosis in 2020 at the provincial level was much lower than it was in 2019. In 2020, there were three provinces with a more than 30% CFR. However, in 2020, there was no single province with a CFR >30%, and even the highest CFR 16.4%, occurred in West Java (MOH RI, 2021).

The purpose of this paper was to provide an overview of the variation of rat species and *Leptospira* serovars based on the results of the special research on disease vector reservoir in Southeast Sulawesi. This information is essential for the surveillance of leptospirosis transmission from rats to humans.

MATERIALS AND METHODS

This study is a cross-sectional study. Data collection was carried out during special research on disease vector reservoir 2016. The research has obtained research ethics clearance from the Health Research Ethics Committee of the Health Research and Development Agency, No. LB. 02.01/5.2/KE.355/2014.

Research sites

The field-caught rats were carried out in six ecosystems, ie, the coastal ecosystem near the settlement (PDP), coastal ecosystem far from the settlement (PJP), non-forest ecosystem near the settlement (NHDP), non-forest ecosystem far from the settlement (NHJP), forest ecosystem the near settlement (HDP), and forest ecosystem far from the settlement (HJP), which are located in three regencies, namely, Bombana, Konawe, and Muna Regencies (Fig 1).

Rat trap's installation

Rats were caught in six types of ecosystems. In each ecosystem, rats were caught for three days. The type of trap used is a live trap with roasted coconut bait. Traps were set every evening at 16.00 hr inside and outside the house. The traps were taken the following day. Catching rats in each ecosystem used 100 single live traps measuring 12x12x28 cm. Traps were installed from 14.00-17.00 hr local time.

The rats were taken from the traps starting at 06.00 hr local time the next day. The captured rats were put in a calico bag and then taken to the field laboratory for blood collection and identification of the rat species.

Rat species identification

The rats were identified by observing their morphological and morphometric characteristics. Morphological characters included hair color and type, tail color, scales, and hair on the tail. The morphometric characteristics comprised body weight, total length, tail length, hind paw length, ear length, skull length, and the number of nipples in female rats. The measurements and observation results matched with the rat identification key being used (Health Research and Development Agency, 2015). Furthermore, the results of rat identification in the field were re-confirmed. The species of the rats were re-identified at the Reservoir Reference Laboratory, Institute for

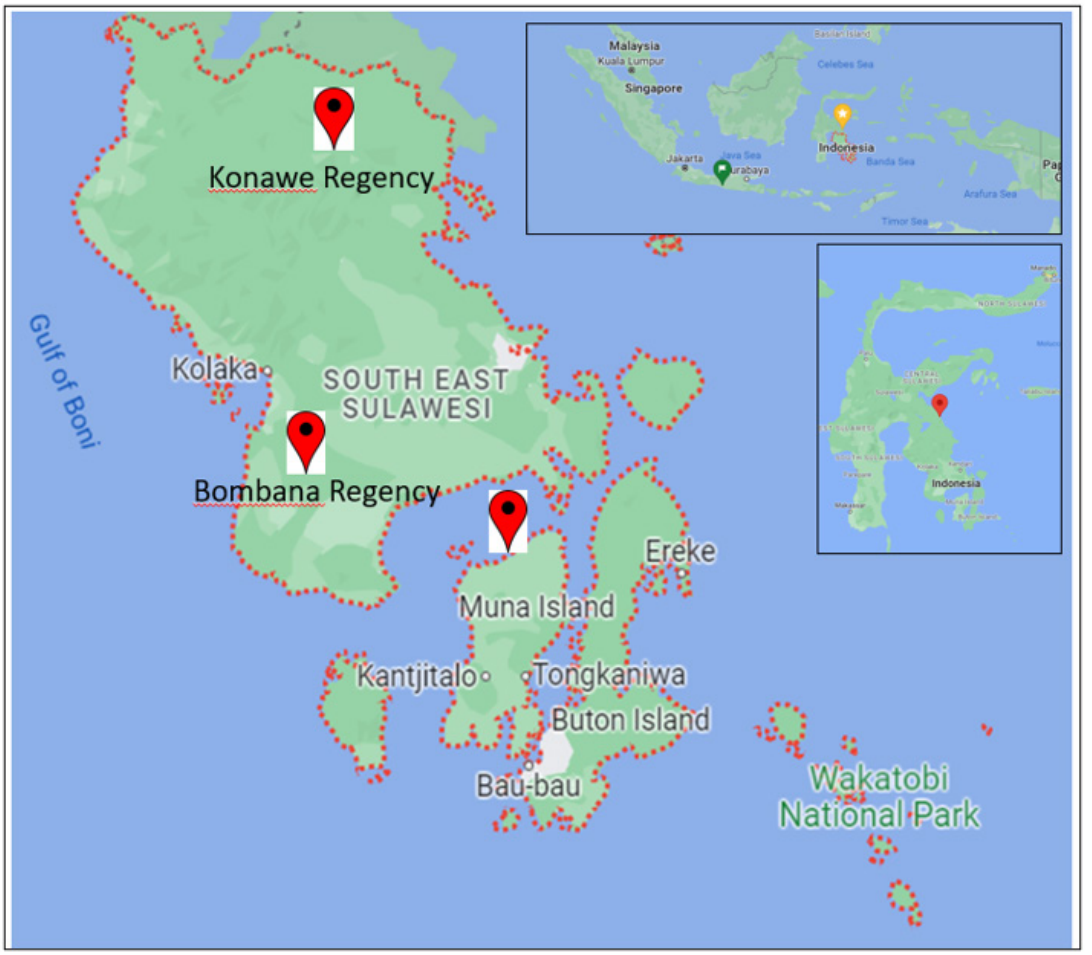

Fig 1 - Map of the location of Special Vektora Research in Muna, Bombana, and Konawe Regencies Southeast Sulawesi Province

Source: https://www.google.com/maps/place/South+East+Sulawesi/@-4.4920258,120.4960414,7z/data=!3m1!4b1!4m5!3m4!1s0x2d98ecde0b6b7183:0x621d7c439f04a4ed!8m2!3d-4.14491!4d122.174605

Vector and Reservoir Control Research and Development (IVRCRD) Salatiga using identification keys from various sources (Suyanto, 2006; Musser, 2014; Corbet and Hill, 1992).

Rat blood sampling

During the field, samples were processed by following the applicable ethical rules, including taking blood from the heart and collecting rats' kidneys. The blood collection of rats was carried out intracardially with a syringe of 3 ml and a hypodermic needle 22G. Blood samples was put in a vacutainer tube and centrifuged at a speed of 3000 rpm for 5 minutes to separate the serum. The formed serum is taken using a Pasteur pipette and put in a sterile cryotube. The serum was stored at temperatures 4-8°C until the MAT test was performed. (Health Research and Development Agency, 2015).

Serological and biomolecular examinations were conducted at the Bacteriology Laboratory, IVRCRD Salatiga. The serological study used the microscopic agglutination test (MAT) method, with 15 serovars (Bangkinang, Canicola, Grippotyphosa, Icterohaemorrhagiae, Pyrogenes, Hardjo, Hebdomadis, Pomona, Djasiman, Robinsoni, Bataviae, Mini, Sarmin, Manhao, and Rama). The results of the rat serum examination are considered positive if the titer is 1:20 (B2P2VRP, 2016).

RESULTS

Rat species identification

The number of field-caught rats during the study in Southeast Sulawesi Province in 2016 was 339. A total of five genera (*Rattus, Bunomys, Maxonys, Paruromys, Taeromys* and *Mus*) and 18 species of rats were identified (Fig 2). The most common rat species identified during the study were *Rattus tanezumi* followed by *R. exulans, R. hoffmanni, R. argentiventer* and *R. nitidus.* Several species of forest rats are only found endemic in Sulawesi. They are *Paruromys dominator*, *Bunomys chrysocomus, B. coelestis* and *Taeromys celebensis*.

The location for catching rats in this study was divided into six systems (Table 1). This study showed that the coastal ecosystem near the settlement was the location where the most rats were found. *R. tanezumi*, a domestic rat

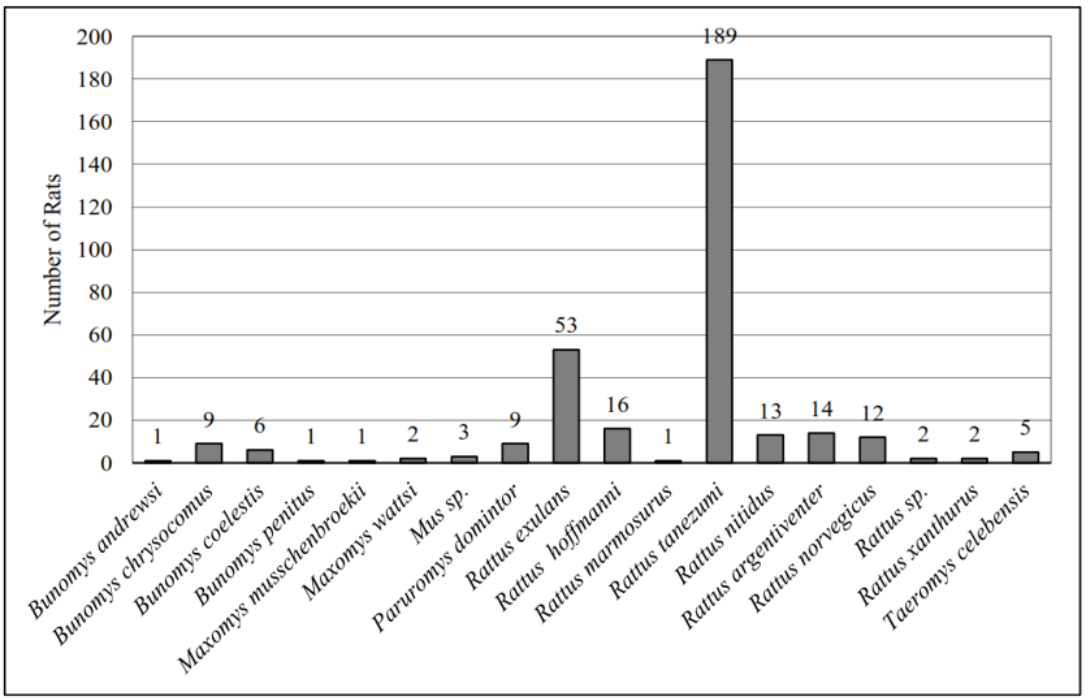

Fig 2 - Species of rats and their numbers in Southeast Sulawesi Province

or house rat, was found at this location. Several species of forest or sylvatic rats, including endemic species of Sulawesi Island, *ie, B. chrysocomus*, *B. penitus*, *B.andrewsi*, *B. coelestis*, *Paruromys dominator* and *Taeromys celebensis*, were found in forest ecosystems far from settlements.

Species of rats and *Leptospira* bacterial serovars in Southeast Sulawesi Province

Rat serum samples were examined by the microscopic agglutination test (MAT) method for *Leptospira* serovar identification. MAT method was applied using 15 serovars (Bangkinang, Canicola, Grippotyphosa, Icterohaemorrhagiae, Pyrogenes, Hardjo, Hebdomadis, Pomona, Djasiman, Robinsoni, Bataviae, Mini, Sarmin, Manhao, and Rama).

Table 1
Species of rats caught in each ecosystem in Southeast Sulawesi Province in 2016

Rat species	Number of rats in various ecosystem types						
	HDP	HJP	NHDP	NHJP	PDP	PJP	Total
Bunomys andrewsi	-	1	-	-	-	-	1
Bunomys chrysocomus	-	8	-	-	1	-	9
Bunomys coelestis	-	6	-	-	-	-	6
Bunomys penitus	-	-	-	1	-	-	1
Maxomys muschenbroekii	-	-	-	-	1	-	1
Maxomys wattsi	-	1	-	-	-	1	2
Mus sp	-	-	-	-	1	2	3
Paruromys domintor	-	-	-	-		9	9
Rattus exulans	5	3	2	24	11	8	53
Rattus hoffmanni	3	1	-	-	5	7	16
Rattus marmosurus	-	-	-	-	-	1	1
Rattus tanezumi	26	3	40	18	70	32	189
Rattus nitidus	-	-	11	-	2	-	13
Rattus argentiventer	-	-	3	2		9	14
Rattus norvegicus	-	-	6	-	6	-	12
Rattus sp	-	-	-	1	1	-	2
Rattus xanthurus	-	-	-	-	-	2	2
Taeromys celebensis	-	-	-	1	-	4	5
Total number of rat	34	23	62	47	98	75	339

HDP: Forest ecosystem near settlements; HJP: Forest ecosystem far from settlements; NHDP: Non-forest ecosystem near settlements; NHJP: Non-forest ecosystem far from settlements; PDP: Coastal ecosystem near settlements; PJP: Coastal ecosystem far from settlements

Note: - means no rat of the respective species was found in ecosystem

The examination results show that each rat positive for *Leptospira* bacteria contained different serovars. The serovars found were Robinsoni, Icterohaemorrhagiae, Grippotyphosa, Bangkinang, Bataviae, Djasiman, Hebdomadis, and Pomona. Various species of rats contain more than one serovar. Seven species of rats were found to have two serovars, and even one species of rat (*B. chrysocomus*) had three serovars at once. Among the eight serovars, Ictiohaemorrhagie was found in 11 of all positive rats. This number was the highest compared to the number of other serovars. Serovar Pomona was only found in one rat.

Based on the location for catching rats, the forest ecosystem far from settlements (HJP) was where the most rats positive for *Leptospira* bacteria were found, followed by the coastal ecosystem far from settlements (PDP) and forest ecosystem near settlements (HDP). The location of the non-forest ecosystem near settlements (NHDP) had the least number of *Leptospira*-positive rats (only three rats), namely *R. nitidus* (two rats) and *R. tanezumi*. The results of the examination can be seen in Table 2.

DISCUSSION

Several species of forest or sylvatic rats, namely *B. chrysocomus*, *B. penitus*, *B. andrewsi, B. coelestis, Paruromys dominator* and *Taeromys celebensis*, are endemic species in Sulawesi. These rats are often found in forest ecosystems far from settlements, which are their natural habitat. It indicates that the condition of the forest ecosystem in Southeast Sulawesi is still quite good in supporting the life of those rat species.

An important finding from this study is that the rats that are most commonly hunted, traded, and consumed by some people in Sulawesi were seropositive for *Leptospira* bacteria; they were *Bunomys coelestis, B. chyssocomus, B. fratotum, B. prolatus* and *R. argentiventer*. Most of the rats are hunted and captured in areas around forests, oil palm plantations, and rice fields. In the last few decades, rat hunting in Sulawesi not only has been common in North Sulawesi Province but also in Central Sulawesi and

Table 2

Species of rats and serovars of *Leptospira* bacteria in Southeast Sulawesi Province in 2016

Rat species	Serovar	Ecosystem
Maxomys wattsi	Rob 20	HJP
Rattus tanezumi	ICT 20	PDP
Rattus tanezumi	Gri 160	PDP
Maxomys muschenbroekii	Gri 20	PDP
Rattus tanezumi	Ict 320, Dja 320	PDP
Rattus tanezumi	40 . Tires	HDP
Rattus tanezumi	Bat 40	HDP
Rattus tanezumi	Dja 80, Heb 20	HDP
Rattus tanezumi	dja 40	HDP
Bunomys chrysocomus	Ict 20, Rob 40	HJP
Bunomys chrysocomus	Gri 320	HJP
Bunomys chrysocomus	Ict 40, Dja 20, Rob 20	HJP
Bunomys chrysocomus	dja 20	HJP
Bunomys chrysocomus	ICT 20	HJP
Rattus exulans	Heb 40, Rob 20	NHJP
Bunomys penitus	ICT 20	NHJP
Rattus tanezumi	ICT 20	PDP
Rattus tanezumi	Gri 20	PJP
Rattus hoffmanni	Ict 80 , Dja 20	HDP
Rattus tanezumi	dja 20	HDP
Rattus tanezumi	Bat 80	HJP
Rattus nitidus	Gri 20	NHDP
Rattus nitidus	dja 20	NHDP
Rattus tanezumi	Ict 40, Dja 20	NHDP
Rattus tanezumi	heb 20	NHJP
Rattus tanezumi	ICT 20, Dja 20	NHJP

Table 2 (cont)

Rat species	Serovar	Ecosystem
Rattus tanezumi	dja 20	NHJP
Rattus tanezumi	Pom 40	PDP
Rattus tanezumi	ICT 20	PJP
Rattus tanezumi	dja 20	PJP
Rattus tanezumi	dja 40	PJP
Rattus tanezumi	dja 40	PJP

HDP: Forest ecosystem near settlements; HJP: Forest ecosystem far from settlements; NHDP: Non-forest ecosystem near settlements; NHJP: Non-forest ecosystem far from settlements; PDP: Coastal ecosystem near settlements; PJP: Coastal ecosystem far from settlements

Gorontalo Provinces (Latinne *et al*, 2020). The captured rats are sold in traditional markets in North Sulawesi Province and several restaurants in Poso and Parigi Moutong, Central Sulawesi Province. Ninety-one percent of the rats are sold as roasted rats, and the rest are sold alive. The wildlife trade is an anthropogenic factor that enables the transmission of pathogens from wildlife to humans. The consumption of wild animal meat in the last 20 years has triggered the emergence of zoonotic diseases with known causes and effects on humans and new emerging zoonotic diseases (Friant *et al*, 2015; Saylors *et al*, 2021).

Bushmeat trading, such as rats in Sulawesi, increases the frequency with which humans are exposed to the pathogens they carry and spreads pathogens from one area to another. The body fluids (urine, feces, and saliva) secreted by the small number of rats traded alive (9%) during the transport process from hunting grounds to markets pose a risk of spreading pathogens, including leptospirosis (Jobbins and Alexander, 2015).

Identification of serovars in rats and humans is very important as a surveillance component to prevent and control leptospirosis transmission.

If the presence of pathogenic *Leptospira* bacteria serovars in an area is established, the information can be conveyed to the public so that they maintain the cleanliness of their surrounding environment, especially from reservoir animals, namely rats. *Leptospira* serovar could be identified by using the microscopic agglutination test (MAT) method. This method is the gold standard in the study of leptospirosis (Riyadi and Sunarno, 2019).

One of the advantages of MAT is that it has better sensitivity, specificity, and accuracy compared to other methods. It is also a diagnostic method for detecting serovars infecting animals and humans. A positive reaction from MAT shows the presence of clots or agglutination due to anti-*leptospira* antibodies in blood serum bound to *Leptospira* bacteria. Examination using this method is less effective or sensitive in examinations that use samples in early acute infection (Rahardianingtyas, 2012). Examination using this method requires antigens of *Leptospira* serovars that are widely circulating in the area to detect the serovars present in the area. *Leptospira* serovars in Indonesia are not fully known with certainty (Setiawan, 2008). Therefore, in the test conducted on the blood samples of rats and humans that gave negative results, it does not mean that they are not infected with *Leptospira* bacteria, but it could be that the infecting serovars are not of the serovars used for testing.

Leptospira bacteria are spirochetes that multiply in the body reservoir (in animal kidneys) and can be transmitted to humans directly from the urine of infected animals. The main reservoir is rats which typically do not show any symptoms of illness (Joharina *et al*, 2018). Serovars from pathogenic *Leptospira* bacteria have specific reservoir hosts, for example, serovar Hardjo has cattle as the specific host; serovar Canicola serovar has dogs as its specific hosts; and serovar Icterohaemoorhagiae is specifically found in rats (Rahardianingtyas, 2012). *Leptospira* infection may be asymptomatic, mild, severe, acute, or chronic. The disease tends to be milder in the reservoir host and more painful when *Leptospira* serovars infect unsuitable host. Clinical signs are often associated with kidney disease, liver disease, or reproductive dysfunction, but other organs can be infected as well (Picardeau, 2017).

Leptospira interogans serovar icterohaemorrhagiae is common in house rats and rarely found in other reservoir hosts. This serovar is very virulent in humans. A study conducted by Sumanta *et al* (2015) discovered that from 99 rats caught in the Yogyakarta region that were successfully amplified using the 16S rRNA gene target; 6 rats were positive for *Leptospira* bacteria, including *Leptospira interrogans* and *Leptospira borgpetersenii*. Based on the results of the MAT examination in this study, *Leptospira* bacteria were detected in both domestic and sylvatic rats. The most commonly found serovar was Icterohaemorrhagie. Thus, the potential for transmission of pathogenic *Leptospira* to humans is high.

This study found that the forest ecosystem far from settlements (HJP) had the most samples of rats that were positive for *Leptospira* bacteria, followed by the coastal ecosystem near settlements (PDP) and forest ecosystem near settlements (HDP). The risk of transmission of leptospirosis to humans is potentially high, considering the presence of these rats in areas near settlements. This study concludes that the variety of species in the research location is quite high. In addition, the serovars found were also varied, even one rat could contain more than one serovars. This is a potential source of transmission of leptospirosis to humans. The results of the study on species of rats and serovars can become information important information in disease surveillance, especially by community health clinics and the health office, to prevent transmission of leptospirosis in the Southeast Sulawesi region.

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CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflicts of interest.

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