Detection of Leptospira Bacteria in Rats in Ciomas Sub-district, Bogor District, West Jawa Using Real-Time PCR Method

Gunawan Gunawan[1(✉)], Hanny Zamsiar[2], Agus A. Wibowo[2], Yahidin Selian[3], and Dessy L. Utami[4]

[1] National Research and Innovation Agency (BRIN), Research Organization for Health, Bogor, Indonesia
r.gunawand@gmail.com
[2] Jakarta Institute of Environmental Health Engineering and Disease Control, Jakarta, Indonesia
[3] Vector Contorl, Ministry of Health Indonesia, Jakarta, Indonesia
[4] Respati University, Yogyakarta, Indonesia

Abstract. Leptospirosis is a zoonotic disease caused by bacteria from the genus *Leptospira*. *Leptospira* bacteria can infect rats, dogs, cats, raccoons, cattle, horses, and other mammals. The main reservoir of leptospirosis in Indonesia is rats. Indonesia is a country with a high risk of developing leptospirosis, considering the occurrence of flooding and the presence of stagnant water, and poor drainage and sanitation conditions in several residential areas. This study aims to detect *Leptospira* spp. bacteria in kidney tissues of rats in the Ciomas Sub-district, Bogor District, West Java. This study was a descriptive-analytic study with a cross-sectional design. Its survey was conducted in Batu City Village, Mekarjaya, and Parakan, Ciomas Sub-district. The rats caught were counted for density, identified the species, and taken kidney samples to be examined for the presence of *Leptospira* using the Real-Time Polymerase Chain Reaction (RT-PCR) method. The density of rats in this study, which was based on the success of catching rats in Parakan Village, was 26%, Mekarjaya 17.3%, and Kota Batu 15.3%. The total number of rats caught was 88. The rat species caught were *Rattus tanezumi* (54.5%), *Bandicota indica* (21.6%), *Rattus norvegicus* (20.5%) and *Mus musculus* (3.4%). The results of the PCR test found that 22 out of 88 rat samples were positive for *Leptospira.* All species caught were positive for Leptospira with the following percentages, *Bandicota indica* (50%), *Rattus norvegicus* (27.3%), *Rattus tanezumi* (18.2%), and *Mus musculus* (4.5%). *Leptospira* bacteria were successfully detected in rat kidneys using the RT- PCR method. The public can increase their awareness by keeping the environment clean in an effort to prevent leptospirosis transmission.

Keywords: Leptospira · Rats · PCR

1 Introduction

Leptospirosis is a bacterial disease that affects both humans and animals [1]. The sources of infection in humans are usually due to direct or direct contact through water, plants,

I. Nurlaila et al. (Eds.): ICHR 2022, AHSR 56, pp. 949–956, 2023.
https://doi.org/10.2991/978-94-6463-112-8_86

or soil contaminated with the urine of infected animals or consuming food contaminated with Leptospira bacteria. It occurs worldwide but is especially present in tropical and subtropical areas that experience heavy rainfall [2, 3].

Leptospira bacteria can infect approximately 160 species of mammals, including rats, pigs, cats, dogs, raccoons, cattle, horses, and other mammals [4]. The main reservoirs of Leptospirosis, rodents or rats, are most commonly found [5]. The species of rat reported as carriers of leptospira bacteria are *Rattus norvegicus*, *Rattus diardii, R. bartelsi, R. argentiventer* and *R. tanezumi* [3, 6].

Indonesia is a country with a high risk of developing leptospirosis, considering the occurrence of flooding and the presence of stagnant water, and poor drainage and sanitation conditions in several residential areas. In 2019, 920 leptospirosis cases were reported in Indonesia, with 122 deaths caused by the disease. The cases have been reported in nine provinces (DKI Jakarta, Banten, West Java, Central Java, DI Yogyakarta, East Java, Maluku, South Sulawesi, and North Kalimantan). In 2020, leptospirosis cases were reported from eight provinces: East Java, Central Java, West Java, DKI Jakarta, DI Yogyakarta, Banten, North Kalimantan, and South Sulawesi. In 2021, leptospirosis cases were reported from eight provinces: Central Java, East Java, Yogyakarta, Banten, DKI Jakarta, North Kalimantan, West Java, and East Kalimantan [7].

Nationally, leptospirosis cases in Indonesia in 2020 were 906 cases. During the last ten years since 2011, there has been a tendency to increase cases of Leptospirosis, especially in the last three years. In 2020, the highest Case Fatality Rate (CFR) occurred in West Java Province (16.4%). West Java Province experienced a significant increase in cases from 2 cases to 32 cases in 2019 and increased again to 55 cases in 2020 [7].

Bogor Regency is one of the areas in West Java with the most cases of Leptospirosis. Based on data from the Bogor District Health Office, it was recorded that in 2018 there were 2 cases in Jonggol District, 2019, there were 3 cases with 1 case died in Klapa Nunggal District, 2020, there was 1 case in Bojong Kulur District, and in February 2021 there was the latest case in the village Batu City, Ciomas District [8]. The existence of a new case in a sub-district is an extraordinary event (KLB), so it is necessary to investigate risk factors from human, environmental, and reservoir factors. A faster molecular-based identification method for Leptospira bacteria with high sensitivity and specificity has been successfully developed. One of these methods is the Polymerase Chain Reaction (PCR) using the 16S rRNA gene target (16S ribosomal Ribonucleic acid/Ribonucleic acid encoding the 16S ribosome and the LipL32 gene). Pathogen-specific Leptospira can be detected using genes such as *LipL32, Liga, or ligB* [9]. Our objective was to detect *Leptospira spp.* Bacteria in kidney tissues of rats Batu City, Mekarjaya and Parakan, Ciomas Sub-district, Bogor District, East Java.

2 Materials and Methods

2.1 Study Design

This research is a survey activity conducted by the Jakarta Institute of Environmental Health Engineering and Disease Control – Jakarta, Indonesia, with a cross-sectional design study to detect *Leptospira* spp. Bacteria in kidney tissues of rats in Kota Batu Village, Mekarjaya and Parakan, Ciomas Sub-district, Bogor District in September 2021.

The trap was installed in 75 houses. The rats caught were counted for density, identified the species, and taken kidney samples to be examined for the presence of *Leptospira* using the real-time polymerase chain reaction method.

Study Sites
The study sites were in Batu City, Mekarjaya and Parakan, Ciomas Sub-district, Bogor District, East Java.

Population and Sample
The populations of the study were all rats who lived at Kota Batu Village, Mekarjaya and Parakan, Ciomas Sub-district, Bogor District in East Java. We installed 150 traps in 75 houses on location. Every house is installed with two traps, inside and outside of the house. The caught rats were into a cloth bag and then brought into field laboratories for identification, and kidney samples were taken to be examined for the presence of *Leptospira* using the real-time polymerase chain reaction.

2.2 Laboratory Testing

The Rats caught were anesthetized with Xylazine and Ketamine HCL. The kidney sample from 88 rats was collected from Kota Batu Village, Mekarjaya, and Parakan, Ciomas. The DNA isolation procedures followed the manual procedure from QIAamp DNA Mini and Blood Mini Handbook [10]. The protocol was applied; it consists of ATL buffer, proteinase K+, AL buffer, and absolute ethanol to ensure complete tissue lysis. Hereafter, DNA extraction took place according to the manufacturer's protocol. The extracted DNA samples were stored at − 20 °C. And then tested by real-time quantitative polymerase chain reaction (RT-qPCR).

This protocol is for use with the QuantiNova Probe RT-PCR Kit. Procedure: Thaw 2x QuantiNova Probe RT-PCR Master Mix, QuantiNova Yellow Template Dilution Buffer, template DNA, primers, probes, and RNase-Free Water. Mix the reaction thoroughly and dispense appropriate volumes into PCR tubes or wells of a PCR plate. Add template DNA to the individual PCR tubes or wells containing the reaction mix. Program the real-time cycler according to the program outlined in Table 1. Place the PCR tubes or plates in the real-time cycler and start the cycling program [11]. Using this approach the forward primer, LipL32F: 5′-AGA GGT CTT TAC AGA ATT TCT TTC ACT ACC T-3′, reverse primer, LipL32R: 5′-TGG GAA AAG CAG ACC AAC AGA-3′ [12].

The real-time quantitative polymerase chain reaction (RT-qPCR) procedures followed the manual procedures from Quanti Nova Probe RT-PCR [11]. Program the real-time cycler according to the program outlined in Table 1.

3 Results

3.1 The Density of Rats

Based on research conducted in Batu City Village, Mekarjaya, and Parakan, the results of success trap calculations in the three villages were more than 1%. The highest success trap calculation in Parakan Village (26%) was followed by Mekarjaya Village (17%) and Batu City Village (15%). The least number of rats obtained in Batu City Village was 23 (15%) (Table 2).

Table 1. Program the real-time cycler

Step	Time	Temperature
Reverse transcription	10 min	45 °C
PCR initial activation step	2 min	95 °C
Two-step cycling:		
Denaturation	5 s	95 °C
Combined annealing/extension	5 s	60 °C
Number of Cycle	40	

Table 2. Success trap based on the location of the study

Village	The number of traps installed	Number of days of arrest	Number of rats caught	*Success trap* (%)
Batu City	50	3	23	15
Mekarjaya	50	3	26	17
Parakan	50	3	39	26

Table 3. Species of the rats captured in Kota Batu Village, Mekarjaya and Parakan, Ciomas Sub-district, Bogor District.

Village	**Species of the rats**								**Total**	
	R. tanezumi		***R. norvegicus***		***B. indica***		***M. musculus***			
	∑	*%*	∑	*%*	∑	*%*	∑	*%*	∑	*%*
Kota Batu	16	69,6	6	26,1	1	4,3	0	0	23	100
Mekarjaya	15	57,7	6	23,1	5	19,2	0	0	26	100
Parakan	17	43,6	6	15,4	13	33,3	3	7,7	39	100
Total	48	54,5	18	20,5	19	21,6	3	3,4	88	100

3.2 Identification the Species

To determine the species with the measurement morphology of rat body, questionnaire, and identification using the rat identification key. The meaning scale is nominal. The species rats were *R. tanezumi, R. norvegicus, Bandicota indica* and *Mus musculus*. The rat species caught were *R. tanezumi* as many as 48 (54.5%), *R. norvegicus* as many as 18 (20.5%), *B. indica* as many as 19 (21.6%), and *Mus musculus* as many as 3 (3.4%) (Table 3).

Table 4. Prevalence of Leptospira infection

Species of the rats	*Leptospira infection*				Total	
	Positive		Negative			
	∑	%	∑	%	∑	%
Rattus tanezumi	4	8,3	44	91,7	48	100
Rattus norvegicus	6	33,3	12	66,7	18	100
Bandicota indica	11	57,9	8	42,1	19	100
Mus musculus	1	33,3	2	66,7	3	100
Total	22	25,0	66	75,0	88	100

3.3 Detection of Leptospira spp.

All rats caught in this study were taken by their kidneys to identify *Leptospira* bacteria using the *Real-Time Polymerase Chain Reaction* (RT-PCR) method. The number of rat kidney samples examined was 88 samples. A total of the 88 kidney samples examined 22 positive samples of *Leptospira* bacteria (25%). In this study, all species examined were confirmed to contain *Leptospira* bacteria (Table 4).

4 Discussion

The main reservoirs of Leptospirosis are rodents or rats. In Indonesia are most commonly found. The success of rat capture is seen from the results of trap success carried out inside and outside the house [13]. Rat capture success rates describe the relative density of rats at the survey site [14]. In this study, based on the results of rat capture carried out for three days with a total of 150 traps, in general, the calculation of trap success in each region was different.

The highest success trap calculation in Parakan Village (26%) was followed by Mekarjaya Village (17%) and Batu City Village (15%). This success trap is used as an estimate of the relative density of rats in an area. An area is said to have a high density of rats if the success of the capture is more than 7%; this means that the density of rats in Batu City, Mekarjaya and Parakan, Ciomas Sub-district, Bogor District, East Java is very high. According to Manyulei *et al*. 2019, trap success under normal conditions is 7% in the home habitat and 2% outside the house/garden. This can likely be caused by several factors, including the availability of feed and drinking rats, both inside and outside the home environment. The large availability of feed and drinks in the surrounding environment affects a large number of rat populations [15]. The high success trap is related to environmental conditions in locations that are not kept clean. The presence of non-flowing got is a factor that supports the breeding of rats and the nesting place of rats [20].

The results of the rat capture survey showed that the most commonly obtained rat species was *R. tanezumi*. According to research conducted by Priyanto *et al*., 2020 that this is reasonable considering that the habitat of *R. tanezumi* is a settlement and has been

known to be a type of domestic rat whose distribution follows the existence of residential areas. It is related to the food source of these rats are foodstuffs commonly consumed by humans [16].

Based on the species of rats, the results in the present study were similar to Mauron et al., 2011 investigated the dynamics of the carrier density of rats' *leptospira* in highly endemic areas in New Caledonia. The rats caught near settlements, and the species were *R. tanezumi, R. norvegicus, R. exulans, and M. musculus* [17]. In line with research conducted by Gunawan et al. l, 2019, the caught species of rats were *R. tanezumi, R. argentiventer, R. norvegicus, M. Musculus, Paruromys dominator, Maxomys sp*, and *Rattus sp* [3].

The examination of Leptospira bacteria in this study was carried out using the real-time PCR method with the primer used *Lip32*. Molecular Leptospirosis (RT-PCR) examination provides several advantages compared to conventional PCR, including that it is easier to do and can save time, reduce the level of contamination, and does not require post-reaction analysis [8]. The use of PCR tests in the detection of Leptospirosis is more accurate. Then the use of the *Lip32* gene as a primer is able to detect pathogenic *leptospira* bacteria. But this method has not been able to detect serovar-level *leptospira* [18].

In this study, as many as 99 rat kidney samples were successfully carried out with RT-PCR examination, with the results of 22 out of 88 positive samples of *leptospira* bacteria. Based on the results of the PCR examination, more *B. indica* rat species were found to show positive leptospira, followed by *R. norvegicus, R. tanezumi*, and *M. musculus.* This is in line with research conducted by Loan et al. l 2015, and all 275 rats were investigated by RT-PCR for leptospira. A total of 16 rats tested positive. *Bandicota indica* and *R. norvegicus* rats had the highest prevalence of infection (10.8% and 6.9%, respectively)) [19]. Transmission of Leptospirosis can occur easily in densely populated areas, especially if the rat population is dense and accompanied by poor sanitation of the environment. The success of the arrests is closely related to the dietary habits/household waste in the area. The presence of scattered litter will become a place where rats forage and nest [20].

The existence of rats as reservoirs is very important to know in efforts to control and break the chain of transmission of Leptospirosis [21]. The highest density of rats is in Parakan Village (26%) due to the existence of a factory/shoe craftsman in the village. A large number of piles of goods that are not well organized and scattered garbage make the optimal environment like a shelter for rats. Supported by non-routine waste transportation facilities that cause piles of waste that become a source of food for rats that support the survival of rats. The presence of waste in the home environment has a risk of 8.46 times getting Leptospirosis, while the presence of garbage around the house has a risk of 10.9 times greater getting Leptospirosis compared to the condition where there is no waste. Open litter conditions have a 16.3 times greater risk of spreading Leptospirosis [22]. *Leptospira* bacteria were successfully detected in rat kidneys using the RT- PCR method. The public can increase their awareness by keeping the environment clean in an effort to prevent leptospirosis transmission.

Acknowledgement. The author is grateful to the Jakarta Institute of Environmental Health Engineering and Disease Control - Jakarta, Indonesia, and the Research Organization for Health, National Research and Innovation Agency, Indonesia.

References

1. Paixão S, Alves-martin MF, Michely S, Starke-buzetti WA, Luana M, Tiago D, et al. Serology, isolation, and molecular detection of Leptospira spp. from the tissues and blood of rats captured in a wild animal preservation centre in Brazil. Prev Vet Med. 2014;115:69–73.
2. Vitale M, Agnello S, Chetta M, Amato B, Vitale G, Di C, et al. Human leptospirosis cases in Palermo Italy. The role of rodents and climate. J Infect Public Health [Internet]. 2017; https://doi.org/10.1016/j.jiph.2017.07.024
3. Gunawan, Wibawa T, Wijayanti MA, Anastasia H. Detection of Leptospira spp . in kidney tissues isolated from rats in the Napu and Bada Highlands of Poso District , Central Sulawesi. J Vektor Penyakit. 2020;14 No 1:17–26.
4. Ko AI, Goarant C, Picardeau M. Leptospira: The Dawn of the Molecular Genetics Era for an Emerging Zoonotic Pathogen. Nat Rev Microbiol. 2009;7(10):736–47.
5. Lorenzo-Morales DJ. Epidemiology, Surveillance and Laboratory Diagnosis of Leptospirosis in the WHO South-East Asia Region. World ' s largest Science , Technology & Medicine Open Access book publisher; 2012. 214–226 p.
6. Ristiyanto, Wibawa T, Budiharta S, Supargiono. Prevalensi Tikus Terinfeksi Leptospira interogans. Vektora. 2015;7 nomer 2:85–92.
7. Kementerian Kesehatan RI. Profil Kesehatan Indonesia 2020. 2021. 205–207 p.
8. Dinas Kesehatan Kabupaten Bogor. Laporan Dinas Kesehatan Kabupaten Bogor Tahun 2021. 2021.
9. Podgorsek D, Sabljic ER, Logar M, PavLovic A, Remec T, Baklan Z, et al. Evaluation of real-time PCR targeting the lipL32 gene for diagnosis of Leptospira infection. BMC Microbiol. 2020;20;59:1–9.
10. Qiagen. QIAamp DNA Mini and Blood Mini Handbook. 2016. p. 32–6.
11. Qiagen. RT-PCR Handbook. 2015. 14–20 p.
12. Villumsen S, Pedersen R, Borre MB, Ahrens P, Jensen JS, Krogfelt KA. Novel TaqMan ® PCR for detection of Leptospira species in urine and blood : Pit-falls of in silico validation. J Microbiol Methods [Internet]. 2012;91(1):184–90. https://doi.org/10.1016/j.mimet.2012.06.009
13. Manyullei S, Birawida AB, Suleman IF. Studi Kepadatan Tikus Dan Ektoparasit Di Pelabuhan Laut Soekarno Hatta Tahun 2019. J Nas Ilmu Kesehat. 2019;2.
14. Sholichah Z, Ikawati B, Marbawati D, Majid M, Ningsih P. Peran Tikus Got (Rattus norvegicus) dari Kelompok Tikus dan Suncus sebagai Penular Utama Leptospirosis di Semarang. J Vektor Penyakit. 2021;15 No 1:53–62.
15. Priyotomo YC. Studi Kepadatan Tikus dan Ektoparasit di Daerah Perimeter dan Buffer Pelabuhan Laut. J Kesehat Masy. 2015;3(April):86–96.
16. Priyanto D, Raharjo J. Domestikasi Tikus : Kajian Perilaku Tikus dalam Mencari Sumber Pangan dan Membuat Sarang Rat Domestication : Study on Foraging and Nesting Behavior. Balaba. 2020;Vol 16 No(Juni 2020):67–78.
17. Mauron C, Goarant C, Perez J, Brescia F. Rodent Abundance Dynamics and Leptospirosis Carriage in an Area of Hyper-Endemicity in New Caledonia. PLoS Negl Trop Dis. 2011;5(10).
18. Ardanto A, Yuliadi B, Martiningsih I, Bagus D, Putro W, Sih A. Leptospirosis pada Tikus Endemis Sulawesi (Rodentia : Muridae) dan Potensi Penularannya Antar Tikus dari Provinsi Sulawesi Selatan. Balaba. 2018;14(No 2):135–46.

19. Loan HK, Cuong N Van, Takhampunya R, Kiet BT, Campbell J, Bryant JE, et al. How Important Are Rats As Vectors of Leptospirosis in the Mekong Delta of Vietnam ? Vector-Borne Zoonotic Dis. 2015;15(1):56–64.
20. Supranelfy Y, S NH, Oktarina R. Analisis Faktor Lingkungan Terhadap Distribusi Jenis Tikus Yang Terkonfirmasi Sebagai Reservoir Leptospirosis Di Tiga Kabupaten Di Provinsi Sumatera Selatan. Vektora J Vektor dan Reserv Penyakit. 2019;11(1):31–8.
21. Ramadhani T, Yunianto B. Reservoir dan Kasus Leptospirosis di Wilayah Kejadian Luar Biasa. Kesmas Natl Public Heal J. 2012;7(4):162.
22. Nugroho A, Trapsilowati W, Yuliadi B, Indriyani S. Faktor Lingkungan Biotik Dalam Kejadian Luar Biasa Leptospirosis Di Kabupaten Tangerang, Banten, Indonesia. Vektora J Vektor dan Reserv Penyakit. 2018;10(2):91–6.