Detection of *Leptospira* spp. in kidney tissues isolated from rats in the Napu and Bada Highlands of Poso District, Central Sulawesi

Deteksi Leptospira spp. pada Ginjal Tikus di Dataran Tinggi Napu dan Bada, Kabupaten Poso, Provinsi Sulawesi Tengah

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INFO ARTIKEL

Article History:
Received: 09 Jul. 2019
Revised: 11 Mar. 2019
Accepted: 31 Jan. 2020

Contributorship:
Gunawan is the main contributor. Tri Wibawa, Mahardika Agus Wijayanti, and Hayani Anastasia are the member contributors in this aricle.

Kata kunci:
Leptospira,
16S rRNA,
LipL32,
PCR,
Ginjal Tikust

Keywords:
Leptospira,
16S rRNA,
LipL32,
PCR,
Kidney's Rat

A B S T R A C T / A B S T R A K

Leptospirosis masih merupakan masalah kesehatan global karena mempengaruhi kesehatan manusia di daerah pedesaan dan perkotaan, baik di negara industri maupun mnegara berkembang. Tujuan penelitian adalah untuk mendeteksi bakteri Leptospira spp di jaringan ginjal dari tikus di Dataran Tingi Napu dan Bada Kabupaten Poso, Provinsi Sulawesi Tengah. Ginjal tikus sebanyak 63 sampel dikoleksi dari Dataran Tinggi Napu dan Bada Kabupaten Poso, Provinsi Sulawesi Tengah pada bulan Mei – Juni 2018. PCR digunakan untuk mendeteksi Leptospira. Karakterisasi molekuler dilakukan berdasarkan gen 16SrRNA dan LipL32. Data dianalisis secara deskriptif untuk menggambarkan keberadaaN Leptospira yang patogenik. Analisis filogenetik dilakukan dengan menggunakan perangkat lunak Mega 6.2. Sebanyak 63 tikus berhasil ditangkap selama penelitian yang terdiri dari jantan dan betina, masing masing 36 ekor (75,1%) dan 27 ekor (42,9%). Spesies tikus adalah R. exulans, R. tanezumi, R. argentiventer, R. norvegicus, M. Musculus, Paruromys dominator, Maxomys sp, dan Rattus sp. DNA Leptospira patogenik terdeteksi pada tikus dengan spesies R. argentiventer dan Paruromys dominator menggunakan gen 16SrRNA dan LipL32 Sekuen sampel dengan target gen LipL32 menunjukkan kesamaan dengan L. interrogans serovar Hardjo, serovar Autumnalis, Lai, Icterohaemorrhagiae, Balico, Grippotyphosa, Mini, Canicola, Hebdomadis; L. noguchii serovar Pomona dan L. kirschneri. Sedangkan sekuen sampel dengan target gen 16S rRNA menunjukkan kesamaan dengan L. interrogans serovar Canicola,Copenhagen, Autumnalis, Pyrogenes, Javanica, Icterohaemorrhagiae, Manilae, Bratislava, Linhae, Hebdomadis, dan L. kirschneri serovar Grippotyphosa. Metode PCR dengan target gen 16SrRNA dan LipL32 mampu mendeteksi Leptospira spp. pada tikus dengan spesies R. argentiventer dan P. dominator.

Leptospirosis is still a global health problem because it affects human health in rural and urban areas, both in industrialized and developing countries. The aim of the study was to detect Leptospira spp. bacteria in kidney tissues isolated from rats in the Napu and Bada Highlands of Poso District, Central Sulawesi Province. Kidneys sample from 63 rats were collected from Napu and Bada Highlands of Poso District, Central Sulawesi Province in May-June 2018. Polymerase Chain Reaction (PCR) was used to detect Leptospira. The molecular characterizations were conducted based on the 16SrRNA and LipL32 genes. Data were analyzed descriptively to describe the presence of pathogenic Leptospira DNA. Analysis phylogenetic was performed using MEGA 6.2 software. A total of 63 rats was successfully caught during the study consisting of males and female for 36 (57.1%) and 27 (42.9%), respectively. The species of rats were *R. exulans, R. tanezumi, R. argentiventer, R. norvegicus, M. Musculus, Paruromys dominator, Maxomys* sp.*, and Rattus* sp. The pathogenic of Leptospira DNA was detected in rats with R. argentiventer and Paruromys dominator species using the 16S rRNA and LipL32 gene. Sample sequences using LipL32 target gene is a close similarity with L. interrogans serovar Hardjo, serovar Autumnalis, Lai, Icterohaemorrhagiae, Balico, Grippotyphosa, Mini, Canicola, Hebdomadis; L. noguchii serovar Pomona and L. kirschneri whereas the sample sequence using 16S rRNA target gene showed similarity with L. interrogans serovar Canicola, Copenhagen, Autumnalis, Pyrogenes, Javanica, Icterohaemorrhagiae, Manilae, Bratislava, Linhae, Hebdomadis, and L. kirschneri serovar Grippotyphosa. The PCR method with the target gene 16SrRNA and LipL32 are able to detect Leptospira spp. in rats R. argentiventer and P. dominator species.

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INTRODUCTION

Leptospirosis remains a global health problem because it affects human health in rural and urban areas, both in industrialized and developing countries. The disease is a zoonosis caused by bacteria of the genus *Leptospira* that spread all over the world, particularly in the tropical and subtropical with high rainfall.[1] Transmission of pathogenic *Leptospira* bacteria to humans can occur primarily through direct contact with water or soil contaminated with the urine of infected animals or consumption of contaminated food.[2]

Leptospira spp. Are divided into 21 species, consisting of 9 pathogenic species (L. *interrogans, L. kirschneri, L. noguchii, L. alexanderi, L. weilii, L. alstonii, L. borgpetersenii, L. santarosai,* and *L. kmetyi),* 5 intermediate species *(L. inadai, L. fainei*, *L. broomii*, *L. licerasiae* and *L. wolffii)* and 7 non-pathogenic species *(L. biflexa, L. meyeri, L. wolbachii, L. vanthielii, L. terpstrae, L. yanagawae* and *L. Idonii).*[3-5] Pathogenicity of *Leptospira* spp. is influenced by a protein from the outer surface of the membrane that plays a role in the interaction between the bacteria and the host tissue.[6] This outer surface protein has been shown to bind with various components of the extracellular matrix in vitro, including fibronectin, collagen, laminin, and elastin.[7]

Leptospira pathogenic species have different protein profiles and lipopolysaccharides on the outer surface of the membrane. *LipL32* protein *is* found on the outer surface of the *Leptospira* spp membrane.[7] Detection of *Leptospira* pathogenic species can be investigated using the PCR method with target gene 16S rRNA and *LipL32*. The target of the *LipL32* gene with highly conserved structures only found in pathogenic strains and important to detect virulence factors.[6,8]

Leptospira bacteria can infect approximately 160 species of mammals, including rats, pigs, dogs, cats, raccoons, cattle, horses, and other mammals.[4] The main reservoirs of leptospirosis, rodents or rats, are most commonly found. The essential leptospirosis reservoirs in America are dogs, livestock (cattle, horses, pigs, and sheep), rats, wild animals, and cats[9,10]. The species of rat reported as carriers of the bacteria *Leptospira* are *Rattus norvegicus, R. diardii, R. bartelsi, R. argentiventer,* and *R. tanezumi.*[10-12]

Extremely severe cases of leptospirosis in humans can lead to multi-organ failure leading to death.[12] According to data from WHO, cases of leptospirosis in humans in subtropical climates amount to 0.1-1 per 100,000 persons per year, whereas in the tropics there are more than 10 cases per 100,000 people annually.[13] The clinical diagnosis of leptospirosis is challenging because the manifestation of clinical symptoms is various. It has non-specific symptoms that are similar to other infectious diseases in the tropics, such as dengue fever or dengue hemorrhagic fever, scrubs typhus and malaria; laboratory facilities are inadequate for diagnosis of leptospirosis.[13,14]

In Indonesia, the incidence of leptospirosis in humans has been reported in the province of West Java, Central Java, Yogyakarta, Lampung, South Sumatra, Bengkulu, Riau Islands, West Sumatra, North Sumatra, Bali, West Nusa Tenggara, South Sulawesi, North Sulawesi, East Kalimantan, West Kalimantan, Jakarta, East Java and Banten.[13,15] The Ministry of Health of Indonesia reported, there were 857 cases and 82 deaths (CFR 9.57%) in 2001, 239 cases and 29 deaths (CFR 12.13%) in 2012, and 641 cases and 60 deaths (CFR 9.36%) in 2013 because of leptospirosis.[16]

Central Sulawesi consists of 12 districts and one municipality, namely the Banggai Islands, Banggai, Morowali, Poso, Donggala, Tolitoli, Buol, Parigi Moutong, Tojo Una Una, Sigi, Banggai Laut, Morowali North and Palu City.[17] Based on data from the Ministry of Health of the years 2009-2013, the incident of leptospirosis cases in Central Sulawesi has never been reported .[18]

The results of Research Special Vector and Reservoir Diseases (Vektora) conducted in 2014 and 2015 by the Center for Research and Development of Vector and Reservoir Diseases (B2P2VRP) reported that *Leptospira* spp. in rats was found in Donggala, Tolitoli, Parigi Moutong and Tojo Una-Una.[15,16] For these reasons, the present study is fundamentally needed to investigate in the Napu and Bada Highlands of Poso district, Central Sulawesi Province by detecting the

bacteria in the kidneys of rats captured in those areas. This study was aimed to detect *Leptospira* spp. bacteria in kidney tissues isolated from rats in the Napu and Bada Highlands of Poso District, Central Sulawesi Province.

METHODS

The study was approved by the animal ethics committee Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia, with number 0028/EC-FKH/Eks/2018. The study was an analytical study.

To determine the species with the measurement morphology of rat body, questionnaire and identified using the mouse identification key. The measuring scale is nominal. Kidneys sample from 63 rats were collected from Napu and Bada Highlands of Poso District, Central Sulawesi Province in May-June 2018. The DNA isolation procedures followed the manual from *Wizard Genomic DNA Purification Kit*. The materials needed were *isopropanol*, 70% *ethanol* and *Proteinase K*. Kidney was washed with distilled water and cut as many as 10-20 mg and put into a 1.5 ml tube. The next process was added 600 ml of *Nuclei Lysis Solution* into Eppendorf and putting it on gel ice packs. The kidneys were ground using a grinder or pellet pestle and then mixed homogeneously. After that, the mixture was incubated at 65°C for 30 minutes. When it was done, 20 ml *Proteinase K* was added. The mixture was incubated at 55°C for overnight.[19] After that, the samples were taken and placed at room temperature. After adding 200 ml of *Protein Precipitation Solution*, the samples were placed on gel ice packs for five minutes. The centrifuge was performed at the speed of 16,000 G for four minutes. Next, the supernatant or upper surface layer was carefully taken and transferred to a new 1.5 ml tube. And then, 600 ml of *isopropanol* was added to the sample and mixed using a vortex for 10 seconds .[19] Next, the samples were centrifuged at the speed of 16,000 G for one minute. Then, 600 ml of 70% *ethanol* was added and centrifuged at the speed of 16,000 G for one minute. The supernatant was carefully removed using a micropipette. The Eppendorf was opened, and the DNA pellets were left to dry. The final step was conducted by adding 100 ml of *DNA Rehydration Solution* and incubating it for 60 minutes at 65°C. It was distorted every 30 minutes. The DNA was stored at 2-8°C19. The DNA concentration was tested by spectrophotometer.

PCR technique using primers *LipL32* and 16S rRNA was used to detect pathogenic *Leptospira* in kidney tissues. The first step in the PCR process was preparing tools and materials, such as the DNA template, *Go Taq® Green Master Mix*, Primary *Lipl32*, *Nuclease-Free Water*, positive control, and negative control. PCR was run using PCR *Applied Biosystems 9700 machine*. The program used was denaturation temperature of 94 °C for 5 min; one cycle followed by 35 cycles for one minute at 94 °C, one minute at 58 °C, one minute at 72°C, and the final stage of extension at 72°C for five minutes.[5]

Table 1. Primers for PCR with the gene target Lipl32

Primer	Sequence Primer	Ribbon size
LipL32-F	5'-GCATCGAGAGGAATTAACATCA3'	474 bp
LipL32-R	5'-CATGCAAGTCAAGCGGAGTA3'	
16S rRNA-F	5'-CATGCAAGTCAAGCGGAGTA3'	571 bp
16S rRNA-R	5'-AGTTGAAGCCCGCAGTTTTC3'	

PCR products were electrophoresed using 1% agarose gel. The product of the PCR amplification is declared positive if DNA was obtained at position 571 and 474 bp [20].Pathogenic *Leptospira* DNA sequencing was carried out in several positive samples of *Leptospira*. Sequencing was carried out at PT 1st BASE Sequencing using the Sanger method. Data were analyzed descriptively to describe the presence of pathogenic *Leptospira* DNA.

RESULTS

A total of 63 rats was successfully caught during the study consisting of males and females for 36 (57,1%) and 27 (42,9%) respectively. The species of rats were *R. exulans, R. tanezumi, R. argentiventer, R. norvegicus, M. Musculus, Paruromys dominator, Maxomys sp,* and *Rattus sp (Table 2)*. The most caught species of rats were *R. tanezumi* for 38,1% (24/63). The species of rats captured in this study were distribution in gardens, fields, yards, swamps and others location.

Table 2. Species of the rats captured in the Napu and Bada Highlands of Poso District, Central Sulawesi Province.

No	Species	Sex				Total	
		Male		Female			
		n	%	n	%	n	%
1	*R. exulans*	10	62,5	6	37,5	16	25,4
2	*R. tanezumi*	14	58,3	10	41,7	24	38,1
3	*R. argentiventer*	6	75,0	2	25,0	8	12,7
4	*R. norvegicus*	1	100,0	0	0,0	1	1,6
5	*M. musculus*	1	100,0	0	0,0	1	1,6
6	*P. dominator*	0	0,0	3	100,0	3	4,8
7	*Maxomys* sp.	3	60,0	2	40,0	5	7,9
8	*Rattus* sp.	1	20,0	4	80,0	5	7,9
	Total	**36**	**57,1**	**27**	**42,9**	**63**	**100,0**

Detection of *Leptospira* spp. based on *LipL32* gene

The *LipL32* gene was able to successfully identify the presence of *Leptospira* pathogenic species found in one rat (1/63). The PCR product was shown by 474 bp (Figure 1). The phylogenetic tree of one DNA sequence of the lipl32 showed that *Leptospira* isolated from the rat in the Napu Highland has a close relationship with *L. interrogans* serovar Hardjo, serovar Autumnalis, Lai, Icterohaemorrhagiae, Balico, Grippotyphosa, Mini, Canicola, Hebdomadis; *L. noguchii* serovar Pomona and *L. kirschneri* (Figure 2).

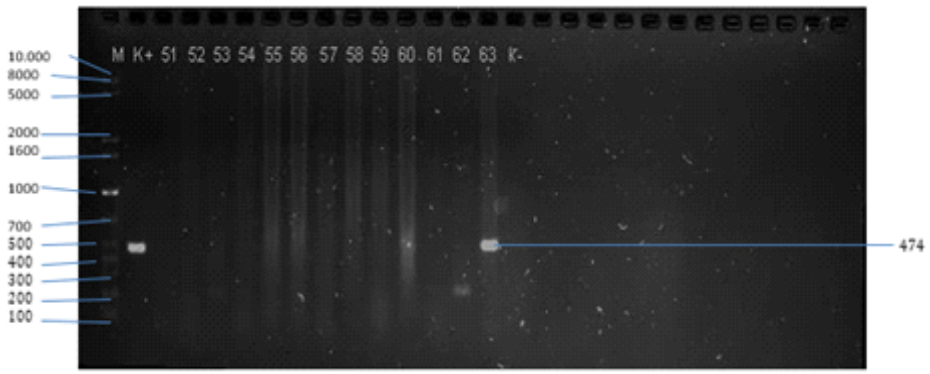

Figure 1. Electrophoresis of standard PCR product on 1% agarose gel. Marker (M) is a 1 kb ladder. Lane 63 (sample 63) is representative of the PCR product. The outcome of the PCR amplification produced 474 bp.

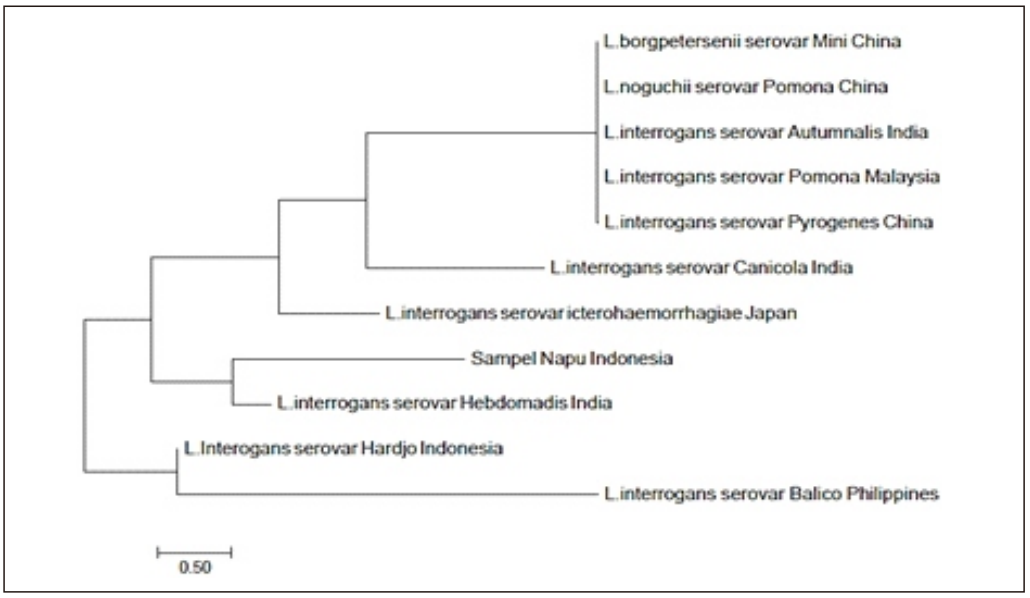

Figure 2. *Leptospira* Phylogenetic Tree based on Lipl32 gene sequences. The phylogenetic tree was constructed by using Neighbor-Joining Methods with 1000 bootstrap replication.

Detection of *Leptospira* spp. based on 16S rRNA gene

The results of PCR with 16S rRNA gene were positively detected from three of 63 samples (3/63) with 571 bp (Figure 3). The phylogenetic tree of 16S rRNA showed that *Leptospira* isolated from the rat in the Napu and Bada Highland has a close relationship with *Leptospira* including *L. interrogans* serovar Canicola, Copenhageni, Autumnalis, Pyrogenes, Javanica, Icterohaemorrhagiae, Manilae, Bratislava, Linhae, Hebdomadis, and *L. kirschneri* serovar Grippotyphosa (Figure 4).

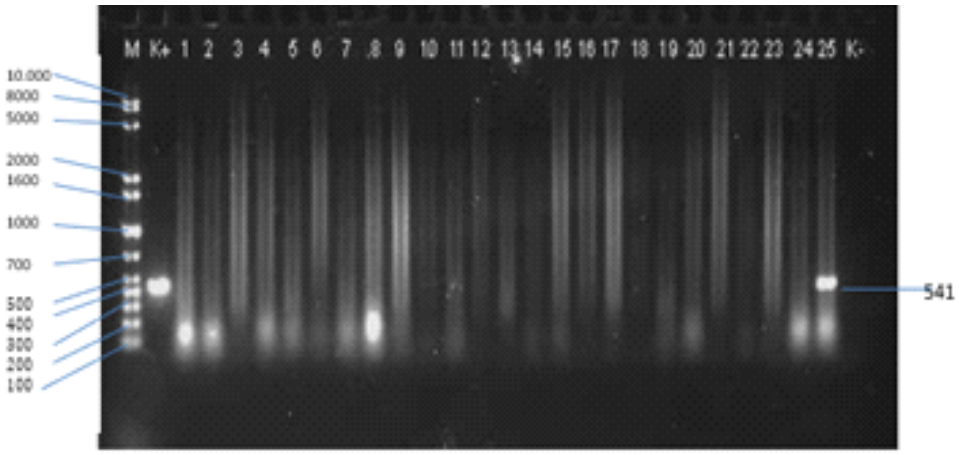

Figure 3. Electrophoresis of standard PCR product on 1% agarose gel. Marker is a 1 kb ladder. Lane 25 (sample 25) is representative of the PCR product. The product of the PCR amplification produced 541 bp.

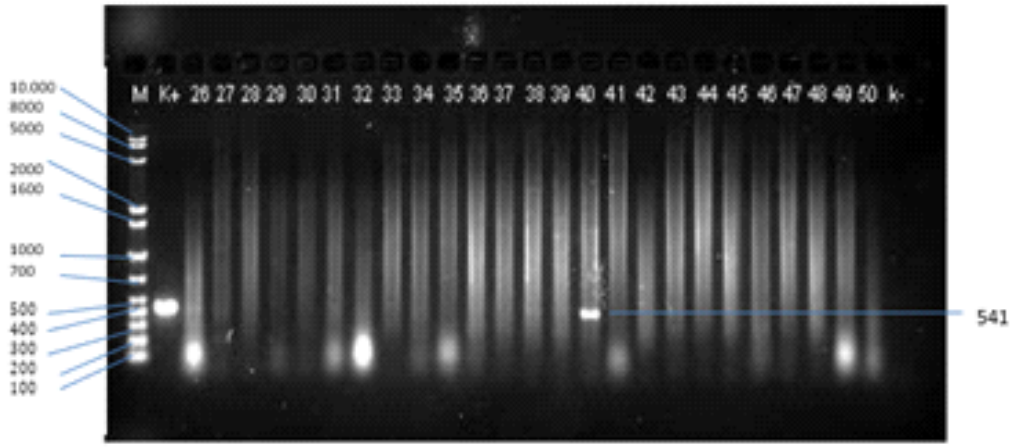

Figure 4. Electrophoresis of standard PCR product on 1% agarose gel. Marker is a 1 kb ladder. Lane 40 (sample 40) is representative of the PCR product. The product of the PCR amplification produced 541 bp.

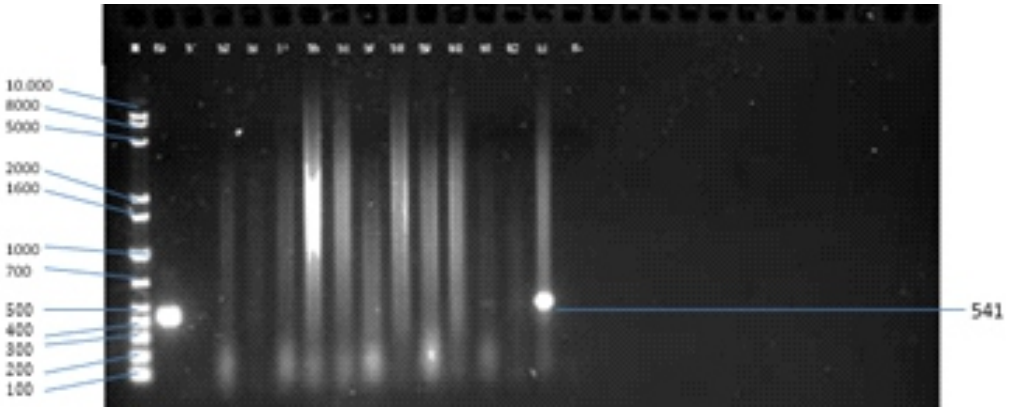

Figure 5. Electrophoresis of standard PCR product on 1% agarose gel. Marker is a 1 kb ladder. Lane 40 (sample 63) is representative of the PCR product. The product of the PCR amplification produced 541 bp.

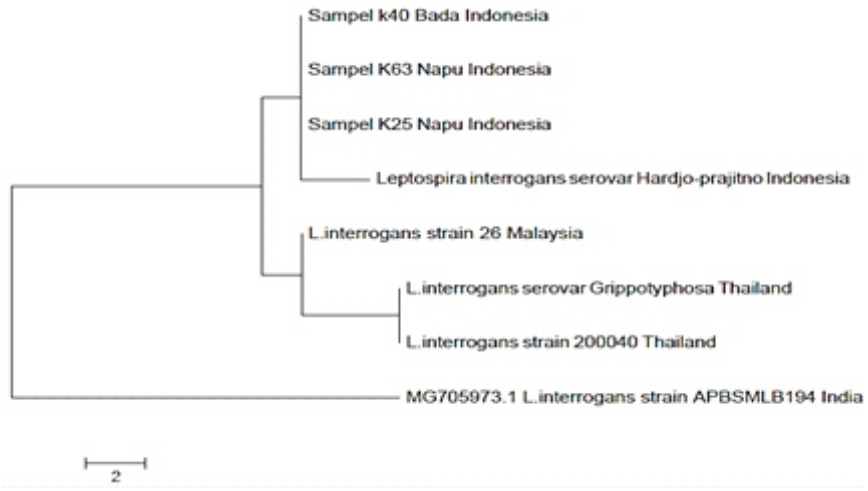

Figure 6. Leptospira Phylogenetic Tree based on 16S rRNA gene sequences. The phylogenetic tree was constructed by using Neighbor-Joining Methods with 1000 bootstrap replication.

DISCUSSIONS

The present study revealed that the number of male rats was higher than females. This result was similar to the previous study reporting that the number of male rats (57%) captured in the trap tends to be more common than females (43%) because the mobility of male rats is more active than females (Astuti, 2003). The male rats should defend their territory/territories and be a struggle in the limited feed. Besides, the male must search for females for mating. When the rat population increases in the area, it will occur a social competition and force the notch lower male rats to get out and look for another area or new territorial region.[21]

The result was in line with the rapid assessment of the host reservoir of leptospirosis in the region of Jogonalan Subdistrict, Klaten regency, Central Java after the earthquake. Captured male rats was greater than the females for 65% and 35%, respectively.[24] Most of them were caught in the gardens, fields, yards, and swamps, habitats. *R. exulans* and *R. argentiventer* were peridomestic rat species found in plantations, rice fields, and backyard.

However, the result of this study was contrary to Priyambodo's finding stating that female rats are easier to be caught compared to male rats. In a group of rat, female rats normally forage alone for their mouse cub, while male rats guards the nest or act as its territory.[22]

Based on the species of rats, the results in the present study were similar to Mauron et al., 2011 investigating a 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.*[23]

In Indonesia, *Leptospira* spp. from rats found in some species. For example, *R. norvegicus, R. tanezumi, R. exulans and S. Murinus* are mostly distributed in Jakarta, *R. hoffmani* in Sulawesi*, R. argentiventer, R. bartelsi, R. tanezumi and R. norvegicus* in West Java and *R tanezumi* in Sumatra.[24] According to research on Special Vectors and Reservoir Diseases (Vektora) in 2014 and 2015 conducted in Donggala, Tolitoli, Parigi Moutong and Tojo Una-Una districts, Central Sulawesi province that the presence of *Leptospira* spp. in rats was detected in some *species e.g. Bunomys sp, Paruromys dominator, Rattus sp., R. tanezumi, R. hoffmanni, R. exulans and Maxomys* sp.[15,16]

This study found *Leptospira* spp. in species of *R. argentiventer*, which has never been reported as a leptospirosis reservoir in Central Sulawesi Province. This result was in line with the research carried out by Kudo *et al*, in 2018 concerning the molecular epidemiological survey of *Leptospira* infection in wild rats in housing in Cambodia City. They also confirmed that *R. argentiventer* was infected with *Leptospira* pathogenic species based on PCR. The rat habitat infected with pathogenic *Leptospira* caught in the study was in residential or residential environments and paddy fields.[25]

Pathogenic *Leptospira* detection can be observed by PCR using 16S rRNA and *LipL32* genes as molecular markers. In this study, the *LipL32* genes successfully amplified one of 63 samples from rat kidneys of *R. argentiventer* species with positive pathogenic *Leptospira*. In addition, the 16S rRNA gene also successfully amplified three sample from the rats (*R. argentiventer* and *Paruromys Dominator* species) with positive pathogenic *Leptospira*.

LipL32 gene

Leptospira has a structured membrane comprising membrane cytoplasm (inner membrane), periplasm, and outside membrane (outer membrane). On the membrane outside, there are various proteins with a level expression that vary as OmpL1 (leptospiral outer membrane porin), some LipL (leptospiral outer membrane lipoprotein) such as LipL21, LipL36, LipL34, LipL41, LipL32 and Lig protein (leptospiral immunoglobulin-like protein). Another protein is flaB (flagellar protein B) found in the periplasm.[26]

Among these lipoproteins, *LipL32* is the most prominent antigen. Extract of the outer membrane of the *Leptospira* protein pathogen contains component 32-kD lipoprotein *(LipL32)*. Sequences and expressions of *LipL32* are highly conserved in pathogenic *Leptospira 6*. The membrane outside of *Spira* non-pathogenic does not contain *LipL32*.

LipL32 is only found in pathogenic strains and act as an important virulence factor. The *LipL32* gene consists of 818 bases and 272 amino acids. *LipL32* is *highly conserved* in pathogenic *Leptospira* species and able to detect pathogenic *Leptospira* with a 96.4% similarity.[26]

In this study, 63 rats kidney samples were successfully amplified. The pathogenic *Leptospira* was successfully identified from the kidneys of *R. argentiventer*. Azhari *et al.*, 2018 reported that's caught in Selangor, Malaysia were positive pathogenic Leptospira based on Lipl32 gene analysis. The Lipl32 gene was able to identify pathogenic Leptospira in 38 of 248 rats: 17 rats of species *R. rattus*, five rats of species *Maxomys whiteheadi*, four rats of species *Sundamys mueller,* two rats of species *gliss*, 1 rat of species *R. tiomanicus* and one rat of species *Suncus murinus*. Those 38 rats are positive with pathogenic *Leptospira*, indicated by the formation of DNA bands in the 1% agarose gel electrophoresis process of 474 bp.[27]

The results of the phylogenetic analysis showed that the kidney sample positive for *Leptospira* spp were in the same group as pathogenic *Leptospira*. The sample joined with *L. interrogans* serovar Hardjo from Indonesia, serovar Autumnalis from India, serovar Lai from China, serovar Icterohaemorrhagiae from India, serovar Balico from the Philippines, serovar Grippotyphosa from Malaysia, serovar Mini from India, serovar Canicola from Iran, serovar Hebdomadis from China; *L. noguchii* serovar Pomona from China and *L. kirschneri* from the USA. The result was in line with the research conducted by Jean Francois Cosson *et al*., in 2014 that of 901 rats caught, 64 of 901 caught rats positive *L. interrogans* and *L. borgpetersenii*. Serovar *L. interrogans* and *L. borgpetersenii* distribution is widespread throughout the world and has been confirmed in mice and as a cause of leptospirosis in humans.[20]

Gen *LipL32* an outer membrane protein of pathogenic *Leptospira* (OMP). *LipL32* is highly conserved in pathogenic *Leptospira* species and can be used to detect pathogenic *Leptospira 26*. Based on the results of is no difference between the samples of Napu with sequences of reference *L. interrogans* from GenBank Acc Number NC_004342.2.

16S rRNA gene

The gene was commonly used to detect both pathogenic and non-pathogenic *Leptospira 28*. Based on the traditional classification system based on the 16S rRNA sequence, the genus *Leptospira is* divided into two species namely *L. interrogans* (pathogens) and *L. biflexa* (nonpathogen) which are saprophytes in the environment. Species *L. biflexa* has 60 serovars known, while *L. interrogans* at least 250 serovars 7. Gokmen *et al.*, 2016 stated that the target gene of 16S rRNA is able to amplify both pathogenic and non-pathogenic leptospira in the serum of patients.[29]

The sequence analysis of three samples with the BLAST program shows the sample sequence has a 99% level of similarity with several serovars of pathogenic *L. interrogans*. Those serovars are serovar Canicola from Egypt, serovar Copenhageni from Brasil, serovar Autumnalis from Russia, serovar Pyrogenes from Russia, serovar Javanica from Russia, serovar Icterohaemorrhagiae from Brasil, serovar Manilae from Japan, serovar Bratislava from the USA, serovar Linhae from China, serovar Hebdomadis from Germany, and *L. kirschneri* serovar Grippotyphosa from Russia. The result corresponds to research conducted by Sumanta et al., 2015 in Yogyakarta, Indonesia, which found six rats were positive *L. interrogans* and *L. Borgpetersenii*.[30]

The first animal known as the carrier of *Leptospira spp* is a rat, because of its proximity to human life. Rats are the main reservoir capable of carrying leptospira spp during his life without showing any clinical manifestations (maintenance host). The rats reported as leptospira carriers are *R. norvegicus, R. diardii, R. bartelsi, R. argentiventer,* and *R. tanezumi.*[10-12] According to research conducted by Cosson *et al.* in 2014, rats were confirmed as reservoirs and sources of transmission of leptospirosis to humans.[20]

Serovar of pathogenic *Leptospira* reservoir has a specific host, e.g. serovar Hardjo specific host for cattle, serovar canicola specific hospes for a dog, and serovar icterohaemoorhagiae for rat.[31] *Leptospira* requires special conditions for its

development. It can survive in the alkaline ground, mud, swamps, rivers, fluid organs, animals tissues, and milk. *Leptospira* bacteria survival depends on factors such as pH, temperature, and the presence of inhibitory compounds. In general, *Leptospira* bacteria are sensitive to drought, heat, acid, and disinfectant. In laboratory conditions, *Leptospira* bacteria can survive for several months at room temperature with a pH from 7.2 to 8.[32]

Leptospira infection may be asymptomatic, mild or severe, and acute or chronic. Diseases are growing niche to be lighter in the host reservoir, and worse when leptospira serovar does not correspond to infect natural hosts. The clinical signs are associated with kidney disease, liver disease, or reproductive dysfunction, but other organs can also be infected .[33]

Humans are hosts susceptible to *Leptospira* infection. *Leptospira* infection in humans causes severe manifestations, but human does not act as a carrier/transmitter *Leptospira 20*. The incubation period ranges from 1-2 weeks, in some cases only two days incubation. Two phases characterize this disease: phase lasts 7-10 days roommates bacteremia and leptospiura phase for one week to several months.[34]

CONCLUSION

PCR using the target gene 16S rRNA and *LipL32* are to able to detect the presence of *Leptospira* bacteria in kidney samples. *Leptospira* pathogenic species is identified in two rats, *R. argentiventer* and *P. dominator* distributed in the Highlands Napu and Bada, Poso district, Central Sulawesi, particularly in fields and gardens.

ACKNOWLEDGMENT

The authors are grateful to the Natonal Institute of Health Research and Development, National Institute Of Health Research and Development Unit Donggala, the Center for Research and Development of Vector and Reservoir Diseases Salatiga, and Board for Development and Empowerment Human Resources of Health, Ministry of Health, Republic of Indonesia

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