Eco. Env. & Cons. 26 (August Suppl. Issue) : 2020; pp. (S42-S48)
Copyright@ EM International
ISSN 0971–765X

Detection of pathogenic Leptospira bacteria in the airport environment using polymerase Chain reaction

Syamsuar Manyullei[1], Hasnawati Amqam[1], Alwiyah[2] and andi Imam Arundhana Thahir[3]*

[1,2] Department of Environmental Health, Faculty of Public Health, Hasanuddin University, Indonesia, 90245
[3]Department of Nutrition Science, Faculty of Public Health, Hasanuddin University, Indonesia, 90245

(Received 21 December, 2019; accepted 28 February, 2020)

ABSTRACT

Leptospirosis, caused by Leptospira, is a disease infecting human through direct contact with the contaminated urine from the animal, including rats. This disease is mainly transmitted to humans from rats living in poor hygiene environment. This study aimed to investigate the presence of pathogenic Leptospira in rats' kidney. This was a cross-sectional study conducted in February 2019. A total of 11 rats were caught from Sultan Hasanuddin International Airport, Indonesia. Leptospira bacteria was observed using the polymerase chain reaction (PCR). The environmental indicator measured was the amount of water presented in the mousetraps. We installed 100 mousetraps and trapped 11 rats. 54.5% of them were infected by leptospirosis bacteria. Among those six rats, 33% of cages presented water inside. In conclusion, more than half of rats living at the Hasanuddin airport were contaminated by Leptospira and indicated that the means of transmission are through the sewage of the airport.

Key words : *Leptospirosis, Pathogenic leptospira bacteria, Polymerase chain reaction, Poor environment*

Introduction

Leptospirosis is a zoonotic disease that infects humans through direct contact with the urine of infected animals or with urine-contaminated environments. It occurs in vulnerable populations in countries with a subtropical or a humid tropicalclimate and has epidemic potential. In the early stages, the disease causes symptoms including high fever, severe headache, muscle aches, chills, redness of the eyes, stomach ache, jaundice, bleeding in the skin and mucous membranes, vomiting, diarrhea, and rashes (Agampodi *et al.,* 2010).

The presence of *Leptospira* bacteria, which can infect rats, is influenced by the abiotic and biotic environment. Abiotic environmental factors include rainfall index, air temperature, water temperature, air humidity, light intensity, water pH, and soil pH. Biotic environmental factors include vegetation, successful catches, rat density, and *Leptospira* prevalence in rats (Nugroho, 2015). In a warm and humid environment with temperatures of 28-30 °C and a water and soil pH 7.2-8.0, *Leptospira* can survive more than 3 weeks without a host. However, at a neutral pH *Leptospira* can survive even longer in floodwaters. Poor sanitary conditions, such as uncollected waste accumulations and the presence of rats are determining variables in the case of leptospirosis. Leptospirosis has a worldwide distribution, with a higher incidence in tropical climates, especially after heavy rains or floods due to storms. In tropical countries, the incidence of leptospirosis

***Corresponding author's email:** andiimam.arundhana@gmail.com
([1]Lecturer, [2]Student, [3]Lecturer)

usually occurs as often as 10-100 times per 100,000 people each year (Nugroho, 2015). The prevalence of the leptospirosis disease is highest in Africa (95.5 per 100,000 population), followed by the Western Pacific (66.4), North America (12.5), Southeast Asia (4.8), and Europe (0.5). Most of the reported cases have severe manifestations, with the mortality rate being higher than 10% (Erviana, 2014). Leptospirosis has been reported in Russia, Argentina, England, Brazil, Australia, Israel, Spain, Afghanistan, Malaysia, the United States, and in Indonesia (Salwani, 2016).

In Indonesia, leptospirosis prevalence occupies the third place for its mortality rate according to the International Leptospirosis Society (ILS). This is in accordance with the number of leptospirosis cases in Jakarta. Due to the large flooding that occurred in 2002, 113 leptospirosis incidents were recorded of which 20 people died (Widjajanti *et al.,* 2017). A study conducted by Sanyasi (2018) shows a probable case of leptospirosis in February 2017. The patient died on the ninth day of the onset of the disease (Sanyasi, 2018; Widowati, 2013). The case was agreed upon by the Magetan Health Service as an outbreak since it caused the death of the patient (Widowati, 2013).

South Sulawesi has areas that are at high risk of *Leptospira* infection from environmental aspects, which occur in the Wajo area for Wiringpalannae and Mattirotappareng villages that have a high risk of leptospirosis (Sanyasi, 2018). Importantly, in the Maros Regency the entrance of the country, such as the airport, has become a health concern. The airport is a gateway for people, both domestic and foreign. In addition, the airport is a meeting place for many people from all over the world and also a workplace where people offer services, such as the support flight operations. Development in transportation technology has an impact on the increasing tourism, trade and others, which facilitatesdisease transmission by people and goods. In Maros Regency, such a gateway is the Sultan Hasanuddin International Airport (Reski *et al.,* 2014).

At Sultan Hasanuddin International Airport,the location which is the highest risk factor for transmission of *Leptospira* bacteria to humans is at the cantina, which serves food for employees of theairport. The food management is a place for storing and shipping goods that has poor environmental sanitation that, thereby, provides a habitat for rats. During the research related to the presence of *Leptospira* bacteria conducted by Mulyono *et al.,* theycaught 114 of which 5 rats were *Leptospira*-positivein the perimeter and buffer regions of El Sai and Wuring ports, Maumere, Flores NTT (Budiarty, 2012). In fact, this study aimed to explore the presence of *Leptospira* bacteria in rats and determine the species of rats, i.e. *Rattus Norvegicus*, caught at Sultan Hasanuddin International Airport.

Materials and Methods

Research design and sites

This research was driven with a descriptive approach, namely exploring the species of rats trapped at the airport of Sultan Hasanuddin Sultan, success trap, pH measurement and the serotype found in the rat's body. PCR method was used to determine the presence of leptospira bacteria in rat's kidney. pH measurements were carried out in stagnant air for installation of mouse traps. pH measurement aims to know the pH value that is optimistic about the growth of leptospira bacteria, therefore a pH meter is used to measure it. This study was conducted at the Makassar Class I Port Health Office, Concordia Canteen, Avsec, Meteorology and Geophysics Agency (BMKG), Main Powerhouse (MPH), Sanitarian Treatments for Angkasa Pura Plant (STPAP), Bazarnas, and Cargo of Sultan Hasanuddin International Airport in February 2019. The selection of study locations was based on the high density of rats observed by the Class I Port Health Office of Makassar.

Data Collection

The population in this study was all species of rats at Sultan Hasanuddin International Airport, while the samples in this study were rats that were successfully caught. We installed traps at the Sultan Hasanuddin International Airport (100 traps) at eight different locations, namely 10 at STPAPs, 11 at MPHs, 12 at BMKGs, 12 at KKPs, 12 at Concordia Canteens, 22 at Cargo, 13 at Avsec, and 8 at Bazarnas. Most trapping installations were palced in Cargo because, in addition to the extensive dimensions of the location, also many items accumulate at Cargo that allow the breeding of rats. During the four days of conducting the experiment, a total of 11 traps went missing from the following locations: One in STPAP, 1 in KKP, 1 in Avsect, and 7 in Cargo.

The working principle of a single live trap is that the trap door will be closed when the bait is pulled by the rat, and the rat will be trapped. Rats were anesthetized before identification. The ethical clearance No 2372/UN4.14.8/TP.02.02/2019 from Public Health Faculty – Hasanuddin University.

Parameter Measured

The parameters observed in this study are rat morphology, such as sex, tail length (mm), species, total body length measurements (mm) of ear size (mm), number of nipple pairs, and weight (g), as well as the number of caught rats, the number of *Leptospira*-positive rats, and the water pH. The serotype was analyzed by polymerase chain reaction (PCR).

Statistical Analysis

The results of the research data were analyzed descriptively. The success trap was count by formula:

$$\frac{\text{The number of rats caught}}{\text{The number of traps installed}} \times 100$$

Results

The *Leptospira* host caught in this study was *Rattus Norvegicus*, as presented in Table 1. The 100 installed traps caught only rats of the specie *Rattus Norvegicus* (100%). Rats are important sources for leptospirosis infection and many rats are found in sewers.

In addition, we also studied stagnant water and puddles around the traps by measuring the pH of the water as listed in Tables 2 and 3.

The caught rats were examined by End Point PCR (conventional) with a gene target L Lip 32. Lip32_F (5'-ATC GCA CTC TTT TTT GC-3 'TCC)). Lip32_R (5 'ATCATCATC GTC CA-3' ATC ACC) as shown in Table 4.

Discussion

The main finding of this study is that's the airport as one of the dangerous public places in transmission of infectious diseases is leptospirosis because it is dangerous for visitors or workers who are there. Sultan Hasanuddin International Airport is one of the working areas of the Class I Port Health Office of Makassar. The airport served 480 arrivals from abroad and 40,392 from within the country and as many as 473 aircrafts departing to a destination abroad and 40,409 from within the country from

Table 1. Identification of the caught rats at Sultan Hasanuddin International Airport

No	Sex	Length Measurements (mm)				Ear size (mm)	Number of nipple pairs	Weight (gr)	Body hair color	Rat Species
		Total body length	Tailr	Skull	Foot					
1	Female	433	226	70	48	29	2 + 3	490	Top: Grayish brownBottom: Gray	Rattus Norvegicus
2	Male	296	170	48	40	28	-	110	Top: Grayish brownBottom: Gray	Rattus Norvegicus
3	Female	264	132	40	39	24	2 + 3	130	Top: Grayish brownBottom: Gray	Rattus Norvegicus
4	Female	250	128	39	35	16	2 + 3	110	Top: Grayish brownBottom: Gray	Rattus Norvegicus
5	Male	294	132	38	40	25	-	193	Top: Grayish brownBottom: Gray	Rattus Norvegicus
6	Male	261	145	38	38	20	-	157	Top: Grayish brownBottom: Gray	Rattus Norvegicus
7	Male	278	137	38	39	24	-	100	Top: Grayish brownBottom: Gray	Rattus Norvegicus
8	Female	253	121	39	38	18	2 + 3	50	Top: Grayish brownBottom: Gray	Rattus Norvegicus
9	Male	270	142	38	39	18	-	60	Top: Grayish brownBottom: Gray	Rattus Norvegicus
10	Male	435	225	51	42	25	-	400	Top: Grayish brownBottom: Gray	Rattus Norvegicus
11	Female	429	230	48	43	28	3 + 3	380	Top: Grayish brownBottom: Gray	Rattus Norvegicus

Table 2. Water Presence at Mousetrap Installation Location in Sultan Hasanuddin International Airport

Puddle presence	Frequency		Number of trapped rats		Number of rats with positive leptospira	
	Number (n)	Percentage (%)	Number (n)	Percentage (%)	Number (n)	Percentage (%)
Yes	7	7	3	27	2	33
No	98	98	8	73	4	67
Total	100	100	11	100	6	100

Table 3. pH of accumulated water at trap installation locationin Sultan Hasanuddin International Airport pH measurement

	Frequency		Number of caught rats	
	Number (n)	Percentage (%)	Number (n)	Percentage (%)
Optimal	2	28	2	67
Not optimal	5	72	1	33
Total	7	100	3	100

Table 4. Polymerase chain reaction (PCR) evaluation of rat kidney samples

No	Sample Code	Evaluation result
1	1	Positive
2	2	Negative
3	3	Negative
4	4	Negative
5	5	Negative
6	6	Positive
7	7	Negative
8	8	Positive
9	9	Positive
10	10	Positive

January to August 2018. Locations at International Airports Sultan Hasanuddin who are at risk of transmission of *Leptospira* bacteria to humans include the place of food management which serves food for employees of the airport, a place for storing and shipping goods, and places that has poor environmental sanitation that provides a habitat for rats.

Before anesthetizing the rats in rat's that were captured, their species was determined. Determination of speciesof rats used external morphological signs including colour and type of hair, colour and length of tail, as well as shape and size of the skull. In addition, measurements of body weight, measurements of total body to tail length, tail length (size from base to tip of tail), back foot length, from heel to tip of the toe without nails, ear size (from the base of the ear to the tip earlobe), weight and number of nipples in female rats. The identification showed that all caught rats were *Rattus norvegicus* and the majority of them was male. The characteristics of *Rattus Norvegicus* are as follows: Body weight ranging from 150-600 g, body length 18-25 cm, total length 31-46 cm, and a with greyish brown hair colouron the top and greyhair at the bottom (Mulyono *et al.*, 2016).

Based on the Regulation of the Minister of Health of the Republic of Indonesia Number 50 of 2017 concerning Quality Standards for Environmental Health and Health Requirements for Vector and Animal Carrying Diseases, trap success parameters are used with indicators of quality standard values <1%, if fact, the number of successful catches was 11% (>1%), which means it exceeds the specified quality standard so that the presence of rats in Sultan Hasanuddin International Airport can be categorized as crowded. For this reason, it is necessary to control the rat number and to deal with the number of rats. In the study of Joharina *et al.* in Pagedangan Ilir Village, the relative density of rats was quite high at the location of the trap installation. With 14%, their trap success rate was even higher than ours with 11% (Joharina, 2015).

Rats, especially *Rattus norvegicus*, are important sources for leptospirosis. The statistical analysis showed that the presence of rats in and around buildings or houses resulted in a 38.7 times greater risk of occurrence of severe leptospirosis (Joharina, 2015). *Rattus Norvegicus* is a large group of rats that tend to be peri domestic (living around human homes or buildings) and terrestrial, so the cruising is wider. Home range *Rattus norvegicus* is influenced by habitat type and source of feed and availability

of nesting sites. This species of rats inhabits location with proximity to water, which increases the possibility of infection with *Leptospira* bacteria through contaminated water. *Leptospira* can survive for several weeks to several months in water and in moist environments. A study showed that *Leptospira* seroprevalence in terrestrial rats, such as gutter mice and *Rattus argentiventer* (paddy rats), is higher than arboreal rats, such as *Rattus tanezumi*. Rats that are thought to have an important role at the time of the occurrence of Leptospirosis Extraordinary Events in the Special Region of Jakarta and Bekasi are of the species *Rattus norvegicus*, *Rattus diardii*, *Suncusmurinus* and *Rattus exulan* (Mulyono *et al.,* 2015).

A study reported that *Leptospira* bacterial infection in humans is incidental. Although leptospirosis can be transmitted from rats to humans through direct contact with (body parts of) rats infected with *Leptospira* bacteria, transmission often occurs by means of water contaminated with urine from infected rats. The incidence of leptospirosis is always associated with water-related things such as water sports activities, the presence of puddles, and water for daily consumption. This is due to the ability of the *Leptospira* bacteria to survive in a humid place for a long time and thus have a high risk of infection if the water source has been contaminated. *Leptospira* bacteria, especially species of *L. icterrohaemorrhagiae* infect sewage rats (*Rattus norvegicus*) and house rats (*Rattus diardii*). Whereas *L. Ballum* infects small rodents (*Mus musculus*). Rats usually urinate in puddles. Through these puddles the *Leptospira* bacteria will enter the human body (Anies, 2006).

Places with puddles of stagnant water and collected water in containerscould be observed at Sultan Hasanuddin International Airport. Of the 100 traps there were 7 (7%) locations where there was anaccumulation of water. Stagnant water was found in Avsec with trap number 023, 040 and 078, in MPH with trap number 044, and at STPAP with trap number 048 and 059. Around these stagnant water spots,we found traces of rats, used basins and gutters, and triggered tarps. Measurements of water pHshowed that two sites had optimal conditions for bacterial growth, namely pH 6.3 in Cargo (trap number 021) and pH 6.5 at STPAP (trap number 059). At pH 6.2-8.0, *Leptospira* bacteria find optimal conditions for proliferation as well assurvival for up to 16 days.

The study of Mulyono *et al.* (2015) in the city of Semarang, Central Java, showed that leptospirosis cases generally occur in proximity to stagnant water, i.e. from household waste, rainwater, stagnant waterways or a combination of these. Thus, workers, employees or visitors who come into contact with stagnant water that has optimal growth conditions for *Leptospira* bacteria are at high risk of contracting leptospirosis. During our study, we found two locations with these optimal growth conditions for *Leptospira*. In 2010, Yogyakarta Epidemiology Surveillance Agency showed that the water from 40 water and soil samples was contaminated with *Leptospira* bacteria at three locations. These water accumulations all originate from rainwater. In their study, Khairani *et al.* tested the Hardjo serotype of *Leptospira* in several types of water (Khairani-Bejo *et al.,* 2004). They explained that *Leptospira* could survive in sewage water with the pH ranging from 6.7 to 7.5 under direct sun exposure (32 °C) for up to 6 hours, whereas the bacteria could survive under sun-blocked conditions (27 °C) for up to 192 hours (Khairani-Bejo *et al.,* 2004).

Polymerase chain reaction (PCR)as a molecular biology tool that is very helpful for the development ofdiagnostic tests. This method can amplify small amounts of DNA into detectable quantities. In addition, using PCR the detection of *Leptospira* bacteria is more sensitive and also faster compared to other methods. The examination of the 11 rats caught during our study using PCR showed that six (54%) samples were positive for *Leptospira* bacteria at Sultan Hasanuddin International Airport. Once a rat is infected with *Leptospira*, the bacteria will be excreted through the urine of the rat during its lifetime. Our findingsare in accordance with a study from the Central Java Province, in which the authors found one positive rat of the species *Rattus norvegicus* using the PCR method (Khairiri, 2019). In a study conducted by Paixao *et al.,* nine samples of a total of 13 samples (69.2%) were identified positive for *Leptospira* (Paixãoa *et al.,* 2014).

However, the study by the Makassar Class I Port Health in port (2017) in Makassar Sea port is not in line with our study. In addition, Syamsuar *et al.* caught rats of the species *Rattus norvegicus* and *Rattus tanezumi* in the flooded area of Puskesmas Tempe, Wajo District, but did not use the PCR method to identify *Leptospira* infection. In animals, leptospirosis does not show clinical symptoms (subclinical), in the sense that the animal will still look

healthy even though they have been infected with *Leptospira* bacteria. So, it is necessary to identify the presence of *Leptospira* in animalsusing molecular biology tools (Manyullei *et al.,* 2018).

Leptospira can survive a monthin water with a pH between 7.2 and 8.0. Therefore, a wet and humid environment that is not directly exposed to sunlight is a potential place of leptospirosis infection for rats and humans alike. Around trap number 021 and trap number 059, which were installed in Cargo and STPPA, standing water in a ditch had pH 6.5 and behind a wood stack pH6.3. In these locations, the PCR examination also detected the presence of *Leptospira* bacteria. This is in contrast to the study by Lestari *et al.,* which found that the renal samples ofall 47 rats they caught were negative of *Leptospira* in Mijen Subdistrict, Demak Regency. However, PCR examination of water samples taken from wells located around the patient's house were positive for *Leptospirosa* (Levett *et al.,* 2001; Manyullei *et al.,* 2018).

In addition, with 11%, the successful rat catches can be categorized as dense at Sultan Hasanuddin International Airport, since it exceeded the specified quality standards. Humans can be infected with *Leptospira* through direct contact with urine, blood or tissue from diseased animals. *Leptospira* bacteria can enter the body through injured skin or through the mucosa of the mouth, nose or eyes when swimming in *Leptospira*-contaminated water. In addition, transmission also occurs via direct contact with wet soil or plants that are contaminated with urine from infected animals.

This study shows that the majority of people understand the importance of hygiene. Hygiene is one of the main aspects for a strong and healthy community. It is of importance to increase the awareness for proper sanitation in accordance with hygiene quality standards for every family in endangered regions. Due to the fact that we found *Leptospira* bacteria in the kidneys of rats, it is necessary to combat disease vectors and pests, i.e. the rats at Sultan Hasanuddin International Airport, so that transmission of *Leptospira* bacteria to humans will not occur.

Conclusion

At Sultan Hasanuddin International Airport, we found 7% stagnant water with an optimal pH (6.5 and 6.3) for the growth of *Leptospira* bacteria. In addition, with 11%, the success of rat catches exceeds the specified quality standard so that the presence of rats is categorized as dense.All rats caught at Sultan Hasanuddin International Airport belonged to the species *Rattus norvegicus*. Furthermore, we confirmed the presence of pathogenic *Leptospira* bacteria in 54% of rat kidneys at Sultan Hasanuddin International Airport by means of PCR.

Acknowledgement

The author would like to thank *Balai Besar Vektor and Reservoir B2P2VRP* Salatiga and Port Health Office Class I Makassar for the study approval and assistance in conducting this study.

References

Agampodi, S.B., Nugegoda, D.B. and Thevanesam, V. 2010. Determinants of leptospirosis in Sri Lanka: Study Protocol. *BMC Infectious Diseases*. 10(332): 1–9.

Anies, H. 2006. Leptospirosis, bukan semata penyakit banjir. *http://www.suaramerdeka.com*.

Budiarty, T.I. 2012. *Gambaran Manajemen Pengendalian Vektor di Bandara Soekarno Hatta Tahun 2012*. Universitas Indonesia.

Erviana, A. 2014. *Studi Epidemiologi Kejadian Leptospirosis pada Saat Banjir di Kecamatan Cengkareng Periode Januari-Februari 2014*. Universitas Islam Negeri Syarif Hidayatullah Jakarta.

Joharina, A.S. 2015. Identifikasi Hewan Reservoir di Daerah Peningkatan Kasus Leptospirosis di Desa Pagedangan Ilir, Kecamatan Kronjo, Kabupaten Tangerang. *Jurnal Vektora Balai Besar Penelitian and Pengembangan Vektor dan Reservoir Penyakit*. 10(1).

Khairani-Bejo, Bahaman, A., Zamri-Saad, M. 2004. The Survival of Leptospira interrogans Serovar Hardjo in the Malaysian Environment. *Journal of Animal and Veterinary Advances*. 3(3): 123–129.

Khairiri, 2019. Survei keanekaragaman tikus sebagai hewan pembawa bakteri Leptospira di Provinsi Jawa Tengah. *Jurnal BiodivIndon* 5(1).

Lestari, E. 2017. Studi Kasus Leptospirosis di Kecamatan Mijen Kabupaten Demak. *Medsains* 3(1).

Levett, P.N., Branch, S.L. and Whittington, C.U. 2001. Two methods for rapid serological diagnosis of acute leptospirosis. *Clinical and Diagnostic Laboratory Immunology*. 8(2): 349–51. DOI: 10.1128/CDLI.8.2.349-351.2001.

Manyullei, S., Daud, A. and Maria, I.L. 2018. Environmental Health Risk Assessment in Flood Prone Area; Case Study in Wajo District. *International Journal of Science and Healthcare Research* 3(4) : 9-16.

Manyullei, S., Amqam, H. and Daud, A. 2018. Study on

Identification of Rats and the Existence of Leptospira Bacteria. sp in the Flood Area of Puskesmas Tempe, Wajo District. *Asian Journal of Medical Research*. 7(3): CM01–CM06.

Mulyono, A., Ristiyanto and W.P. DA 2015. Survei Serovar Leptospira dan Inang Reservoir Leptospirosis di Banyumas. *Makalah Seminar Nasional Mikrobiologi*. 1–4.

Mulyono, A. and Ristiyanto, Rahardianingtyas, E. 2016. Prevalensi dan Identifkasi Leptospira Patogenik pada Tikus Komensal di Kota Maumere, Flores. 8(1): 31–40.

Nugroho, A. 2015. Analisis Faktor Lingkungan dalam Kejadian Leptospirosis di Kabupaten Tulungagung. *Balaba*. 11(2) : 73–80.

Paixãoa, M., dos, S., Martina, M.F.A., S., Michely da Tenórioa Wilma A., Starke-Buzettic Maria Luana Alvesc D iago da S c AG alves, F. 2014. Serology, Isolation, and Molecular Detection of Leptospira Spp. From the Tissues and Blood of Rats Captured in A Wild Animal Preservation Centre in Brazil. *Journal homepage Preventive Veterinary Medicine*. 115 : 67-73.

Pudjiatmoko, 2010. Leptospirosis. *Jurnal Atani Tokyo.*

Reski, A.R., La-Ane, R. and Manyullei, S. 2014. *Kemampuan larutan bonggol Nanas dalam menurunkan jumlah kuman pada peralatan makan*. Hasanuddin University.

S.S R. 2015. *Faktor Risiko Lingkungan yang Berpengaruh Terhadap Kejadian Leptospirosis Berat (Studi Kasus di Rumah Sakit Dr. Kariadi Semarang*. Program Pascasarjana Universitas Diponegoro Semarang.

Salwani, D. 2016. Leptospirosis.: 1–15.

Sanyasi, R.D.L.R. 2018. Laporan Kasus Kejadian Luar Bias Leptospirosis di Magetan, Jawa Timur. *Berkala Ilmiah Kedokteran Duta Wacana*. 3(1): 1–9.

Widjajanti, W., Antastasia, H. and Rosmini, R. 2017. Kewaspadaan Dini Kasus Leptospirosis di Provinsi Sulawesi Tengah. *Jurnal Vektora*. 9(2) : 59-68.

Widowati, E.W. 2013. Desain Primer Sitokrom B (cyt b) Sebagai Salah Satu Komponenen PCR (Plymerase Chain Reaction) untuk Deteksi DNA Babi. *Laporan Penelitian Individual*. 1–64.