



# Identification of *Leptospira* Serovar in Leptospirosis Suspect Serum in Manggala Subdistrict, Makassar City

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## Abstract

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**BACKGROUND:** Leptospirosis or also commonly called Weil disease is an acute infectious disease that can attack humans and animals and is classified as a zoonotic disease. Leptospirosis is caused by bacteria of the genus *Leptospira* found in rodents. The difficulty of the diagnosis process causes the case of leptospirosis less reported and is one of the neglected infectious diseases. Patient findings are often not optimal because of underdiagnosis or misdiagnosis.

**AIM:** This study aims to determine the presence of *Leptospira* serovar bacteria in blood serum of leptospirosis suspect using the microscopic agglutination test (MAT) method.

**METHODS:** This study is a descriptive observational using accidental sampling technique. Sample collection was performed in the Antang Community Health Center, Batua Health Center, and Bangkala Health Center in Manggala District.

**RESULTS:** This study found that there were 31 serum samples. Results of MAT examination on blood serum yielded 11 positive samples of *Leptospira* (37%) with serovar Hebdomadis, Djasiman, Mini, Icterohaemorrhagiae, Bangkingan, and Bataviae.

**CONCLUSIONS:** The presence of *Leptospira* bacteria in blood serum of suspected individuals using the MAT method obtained 11 samples of positive blood serum. Special counseling activities are required about leptospirosis and diagnosis enforcement in the community or workers who experience symptoms or leptospirosis suspect.

## Introduction

Leptospirosis or also commonly called Weil disease is an acute infectious disease that can attack humans and animals and is classified as a zoonotic disease [1]. Leptospirosis is caused by bacteria of the genus *Leptospira* found in rodents. Some types of animals that can be a source of leptospirosis include rats or rodents, pigs, cows, goats, sheep, horses, dogs, cats, insects, birds, and insectivores. However, rats are the animals most often a source of leptospirosis because mice act as natural hosts of the *Leptospira* bacteria and have high reproductive power [2].

The spread of leptospirosis is throughout the world with endemic areas, namely, countries with subtropical or tropical climates [2]. The incidence of leptospirosis in tropical (warm) countries generally occurs as many as 10–100 per 100,000 population annually, whereas in temperate countries, the incidence of leptospirosis is more the least is 0.1–1 per 100,000 population annually [4]. The International Leptospirosis Society states that South and Southeast Asia, the Western Pacific, Central and South America, and Africa have the highest estimated burden of leptospirosis. This can be proven based on 1593 cases of leptospirosis

in Europe reported in 2014, including 937 which have been confirmed with a notification rate of 0.23 cases per 100,000 population [5]. Latin America has reported cases of leptospirosis in 2014 of 10,702 cases with a percentage by 95% [6].

Yunianto *et al.* (2012) states that Indonesia as a country with leptospirosis cases is quite high and is ranked third in the world. Cases of leptospirosis in Indonesia in 2010 occurred in seven provinces, namely, DKI Jakarta, West Java, Central Java, DI Yogyakarta, Bengkulu, Riau Islands, and South Sulawesi. Leptospirosis in Indonesia from 2004 to 2011 tended to increase, in 2011, there were 690 cases of leptospirosis with 62 people died (CFR 9%). This increase occurred due to outbreaks in Yogyakarta Province (Bantul and Kulon Progo Regencies), where the incidence of leptospirosis was 539 cases with 40 deaths (CFR 7.42%) and Central Java Province with 143 cases with 20 deaths (CFR 10.6%) [7].

Increased cases of leptospirosis occurred again in 2018 after in 2016 only reached 830 cases, now in 2018 to 894 cases. The number of deaths due to leptospirosis in Indonesia in 2014–2018 was 61 people, 65 people, 62 people, 108 people, and 148 people with a CFR of 11.75%, 17.76%, 7.44%, 16.88%, and 16.55% so that it can be concluded that the number of

deaths due to leptospirosis is relatively increased and the highest deaths occur in 2018 [8].

An increase in the number of leptospirosis sufferers can occur during the rainy season and can even reach epidemic proportions if there is a flood with extreme weather patterns [1]. Flood water will cause leptospires to be spread more widely, so humans can be infected with *Leptospira* bacteria through contact with water/flood, soil (mud), and plants polluted by urine of animals infected with *Leptospira* bacteria [2].

Makassar City is known to be the capital of the province of South Sulawesi which has several flood-prone areas. One area in Makassar City which is a flood prone area is Manggala District. Makassar City Health Office annual report in 2018 stated that Manggala Subdistrict was one of the districts that were prone to flooding. The difficulty of the diagnosis process causes the case of leptospirosis less reported and is one of the neglected infectious diseases. Patient findings are often not optimal because of underdiagnosis or misdiagnosis. This greatly results in delays in the management of patients which can worsen the prognosis even though the disease in general has a good prognosis [10].

## Methods

This study was a descriptive observational study conducted at Antang Health Center, Batua Health Center, and Bangkala Health Center in Manggala District on January 2, 2020–January 19, 2020, and conducted an examination at the Reservoir Center and B2P2VRP Vector Salatiga. The examination was conducted on Wednesday and Thursday February 21–22, 2020. The population in this study were all patients who visited Antang Puskesmas, Batua Puskesmas, and Bangkala Puskesmas, while the sample in this study was patients who had leptospirosis suspected criteria including fever more than 3 days, headaches, and muscle aches. The sampling technique uses non-probability sampling, namely, accidental sampling. Data obtained from primary data are symptoms of leptospirosis and microscopic agglutination test (MAT) examination results.

MAT is a test that can determine the agglutination of antibodies in the patient's serum by mixing them in various dilutions with *Leptospira* on or off that has been formulated. The antileptospiral antibodies in the serum cause the *Leptospira* to stick together and form clots. This clotting process is called agglutination which is observed using a dark field microscope [2]. The working principle of the MAT method is that the patient's serum is reacted with live suspense antigen from *Leptospira* serovar. The antigens used in this study were the antigens from the Reservoir Center and B2P2VRP Vector Salatiga made by the laboratory.

The tools and materials used in this study were the Spoit tool used for blood sampling, the centrifuge used to separate fresh blood and blood serum, Mikrotube was used as a serum container, coolbox, and film used for packing samples. In the MAT examination, the dilution and additional culture stages were carried out by adding a dilution solution and serovar of *Leptospira* bacteria, the incubation stage was carried out for 2–4 h at 37°C. After incubation, the serum/antigen mixture was available microscopically for agglutination so that the titer could be determined. The MAT is read by a dark field microscope. The end point is the highest dilution of serum where 50% agglutination occurs. Due to the difficulty in detection when 50% of *Leptospira* were agglutinated, the end point was determined by about 50% of *Leptospira* free without agglutination, as compared to the control suspension [18].

## Results

The subjects in this study were Leptospirosis suspects, the sample were selected accidentally which means that the patient met the criteria for suspicion and was willing to have blood collected and whatever amount would be used as a sample. The results of the study are shown in Table 1 as follows:

**Table 1: Microscopic agglutination test results for serum sample of leptospirosis suspects in Manggala subdistrict, Makassar City**

S. No.	Sample code	Result	Note
1	1	Negative	-
2	2	Negative	-
3	3	Negative	-
4	4	Negative	-
5	5	Negative	-
6	6	Negative	-
7	7	Negative	-
8	8	Negative	-
9	9	Positive	Icterohaemorrhagiae
			Bataviae
			Djasiman
10	10	Positive	Bataviae
			Mini
11	11	Negative	-
12	12	Negative	-
13	13	Negative	-
14	14	Negative	-
15	15	Negative	-
16	16	Negative	-
17	17	Negative	-
18	18	Positive	Bataviae
19	19	Positive	Bataviae
20	20	Negative	-
21	21	Positive	Bataviae
22	22	Negative	-
23	23	Positive	Icterohaemorrhagiae
			Djasiman
24	24	Positive	Bataviae
25	25	Positive	Bataviae
26	26	Negative	-
27	27	Negative	-
28	28	Negative	-
29	29	Positive	Hebdomadis
30	30	Positive	Hebdomadis
31	31	Positive	Bangkinang

Source: Primary Data, 2020.

The results of blood sampling conducted at three Puskesmas, namely, Antang Puskesmas, Batua Puskesmas, and Bangkala Puskesmas were 31

samples. The results of the MAT examination showed that of the 31 blood samples taken, there were 11 positive samples of *Leptospira* with positive sample codes namely sample code numbers 9, 10, 18, 19, 21, 23, 24, 25, 29, 30, and 31. It was found that there were fewer positive samples (35.5%) than negative samples (Table 2).

## Discussion

Blood samples were taken at Antang Health Center, Bangkala Health Center, and Batua Health Center. Total samples obtained during the process are 31 samples that meet the criteria for suspicion. The symptoms of leptospirosis are similar to other infectious diseases such as influenza, meningitis, hepatitis, dengue fever, dengue hemorrhagic fever, and other viral fever. Clinical symptoms of leptospirosis are divided into three phases which include leptospiremia phase, immune phase, and healing phase. In the phase of leptospiremia, sufferers will experience sudden high fever to chills accompanied by headaches, muscle aches, hyperesthesia of the skin, nausea, vomiting, diarrhea, relative bradycardia, jaundice, and ocular injection. This phase lasts 4–9 days and ends with the disappearance of clinical symptoms for a while [11].

Based on the symptoms of leptospirosis that is generally felt by respondents, the highest percentage is fever more than 3 days and headache as much as 96.8%. In addition, muscle pain also has a percentage of 80.6%. The symptoms most rarely felt by respondents were conjunctivitis or inflammation of the membrane covering the front of the eye and causing reddish eyes by 1%. Meanwhile, the symptoms of albuminuria, jaundice, and bleeding have not been found in the respondents in this study which is equal to 0%.

Leptospirosis sufferers will first show leptospirosis symptoms that are in the first phase of clinical symptoms, namely, the leptospiremia phase, where in this phase, the *Leptospira* bacteria are already in the blood and cerebrospinal fluid, causing symptoms of high fever, sudden chills and headaches, aches and pains in the muscles, especially the thighs, calves, and waist accompanied by tenderness [10].

*Leptospira* bacteria will enter the blood circulation which is marked by a fever and develops in the target organ and will show symptoms of infection in the organ. The incubation period of leptospirosis is 4–19 days, averaging 10 days. Direct transmission from human to human is rare [11].

Infection in general often occurs through the mucous membranes of the eyes, mouth, nose, or genital tract. The bacteremia period can last for a week, starting

1 or 2 days after infection. During this period, *Leptospira* can be isolated from the blood and most organs of the body and also from the cerebrospinal fluid. The primary bacteremia phase ends with the appearance of circulating antibodies and can be detected usually after 10–14 days. The period of secondary bacteremia arises after the period of primary bacteremia which is 15–26 days and is generally rarely reported [12]. After infection, *Spira* appears in the blood and attacks almost all tissues and organs. These bacteria are then cleansed from the body by the host immune response to infection. *Leptospira* can stay in the kidney tubules and in the urine for several weeks to several months and sometimes even longer. After that, *Leptospira* bacteria are cleansed from the kidneys and other organs but can stay in the eye for longer.

Factors that can affect the incidence of leptospirosis include age, sex, type of work, injury history, and personal hygiene [13,14]. Based on the type of work, leptospirosis generally attacks farmers, plantation workers, miners, sewer cleaners, abattoir workers, and military [10]. Research conducted by Dewi and Yudhastuti on risk factors for the occurrence of leptospirosis in the Gresik Regency region shows that the majority of respondents who suffer from leptospirosis are those who come in contact with water namely farmers, shellfish shells, and pond workers [15].

In Indonesia, it was reported that since 1936 various *Leptospira* serovars have been isolated, both from wild animals and pets. In Ambarawa, it was isolated from rodent *L. Bataviae* animals; *L. icterohaemorrhagiae*; *L. Javanica*; *L. Pyrogenes*; and *L. Semarang*. Abdullah 1961 can isolate *L. autwnnalis*; *L. canicola*; *L. sarmini*; *L. schu! ieri*; *L. benyamin*; *L. acid*; *L. javanica*; *L. grippytyphosa*; and *L. bovis*, from *Rattus ratus* who was captured in Bogor and its surroundings [16].

Efforts to prevent leptospirosis can be carried out by immunizing, avoiding swimming, bathing, swallowing or bowing head to potentially contaminated fresh water such as rivers, especially after the rainy season or flood, closing wounds and abrasions on waterproof bandages, avoiding contact with flood water and do not eat food contaminated with flood water, use appropriate personal protective equipment such as rubber shoes, waterproof clothing/clothing and gloves, and do not eat food that may be exposed to mice and may be contaminated with rat urine [1].

**Table 2: Frequency distribution of microscopic agglutination test results on blood serum samples with leptospirosis suspects in Manggala district, Makassar City**

Result	Total respondent	
	n	%
Positive	11	35.5
Negative	20	64.5
Total	31	100

Source: Primary Data, 2020.

## Conclusions

Based on the findings of this study, it can be concluded that the presence of *Leptospira* bacteria in the blood serum of leptospirosis suspects using the MAT method obtained 11 positive blood serum samples. Special counseling activities are necessary about leptospirosis and diagnosis enforcement in the community or workers who experience symptoms or leptospirosis suspect.

## Ethical Clearance

953/UN4.14.7.1/TP.01.02/2020.

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## Author Queries???

AQ6: Kindly cite References 3,9 and 17 in the text part and also chronological order