Distribution Serovar and Risk Factors on the Incidence of Canine *Leptospirosis* after Flood in Jakarta, Indonesia

(Taburan Serovar dan Faktor Risiko terhadap Kejadian *Leptospirosis* Anjing Selepas Banjir di Jakarta, Indonesia)

AMBAR RETNOWATI, AGUSTIN INDRAWATI*, UPIK KESUMAWATI HADI, SAFIKA & SUSAN M NOOR

ABSTRACT

Leptospirosis *is a zoonosis, which has become a serious problem, especially in areas with high rainfall. Massive* leptospirosis *occurs after heavy rains or floods. This study aims to analyze the types and distribution of leptospira bacteria serovars and risk factors for* leptospirosis *in post-flood dogs. The research was conducted from January to March 2020 at the Ragunan Animal Hospital and independent clinics in Jakarta, Bekasi, Depok and South Tangerang. This study used a cross-sectional study of serum samples from 24 dogs suspected of* leptospirosis. *Diagnostic techniques are based on questionnaires and clinical symptom evaluation, physical examination and biochemical analysis, serum and hematology, and the Microscopic Agglutination Test (MAT) test. The sample criteria were dogs with leukocytosis, thrombocytopenia, azotemia with Blood Urea Nitrogen (BUN) and high keratin. The sample then tested the MAT. Measurement of risk factors in the form of variable questionnaires with laboratory test results using the Chi-square test.* Leptospira *serovars were identified as bataviae (9 samples), icterohaemorrhagiae (6 samples), tarassovi (4 samples) and javanica (2 samples). The distribution of leptopsira serovars in the study area is quite diverse. Risk factors that increase the incidence of* leptospirosis *in dogs are the maintenance system (OR 17.50), history of contact with rats (OR 9.625), flood-affected areas (OR 3.50) and drinking water sources (OR 1.643). Floods can be a medium for transmitting leptospira bacteria to dogs. Risk factors such as rearing systems, poor sanitation and a history of contact with rats can increase the incidence of* leptospirosis *in dogs in areas with high rain intensity. The role of dog owners is needed in preventing the incidence of* leptospirosis *in dogs.*

Keywords: Dog; flood; leptospirosis; microscopic aglutination test; serovar

ABSTRAK

Leptospirosis *adalah zoonosis yang telah menjadi masalah serius, terutama di daerah dengan curahan hujan yang tinggi.* Leptospirosis *berlaku secara besar-besaran selepas hujan lebat atau banjir. Kajian ini bertujuan untuk menganalisis jenis dan taburan serovar bakteria leptospira dan faktor risiko untuk* leptospirosis *pada anjing pasca banjir. Penyelidikan dilakukan dari Januari hingga Mac 2020 di Rumah Sakit Hewan Ragunan dan klinik bebas di Jakarta, Bekasi, Depok dan Tangerang Selatan. Kajian ini menggunakan kajian keratan rentas sampel serum daripada 24 anjing yang disyaki* leptospirosis*. Teknik diagnostik didasarkan pada soal selidik dan penilaian gejala klinikal, pemeriksaan fizikal dan analisis biokimia, serum dan hematologi serta ujian mikroskopi penggabungan (MAT). Kriteria sampel adalah anjing dengan leukositosis, trombositopenia, azotemia dengan darah nitrogen urea (BUN) dan keratin tinggi. Sampel kemudian diuji dengan MAT. Pengukuran faktor risiko dalam bentuk soal selidik berubah-ubah dengan hasil ujian makmal menggunakan uji khi kuasa dua. Serovar* Leptospira *dikenal pasti sebagai bataviae (9 sampel), icterohaemorrhagiae (6 sampel), tarassovi (4 sampel) dan javanica (2 sampel). Taburan serovar leptopsira di kawasan kajian agak pelbagai. Faktor risiko yang meningkatkan kejadian* leptospirosis *pada anjing adalah sistem pemeliharaan (OR 17.50), sejarah hubungan dengan tikus (OR 9.625), kawasan yang dilanda banjir (OR3 .50) dan sumber air minum (OR 1.643). Banjir boleh menjadi medium penyebaran bakteria leptospira kepada anjing. Faktor risiko seperti sistem pemeliharaan, sanitasi yang buruk dan sejarah sentuhan dengan tikus dapat meningkatkan kejadian* leptospirosis *pada anjing di kawasan yang mempunyai keamatan hujan tinggi. Peranan pemilik anjing sangat diperlukan dalam mencegah kejadian* leptospirosis *pada anjing.*

Kata kunci: Anjing; banjir; leptospirosis; ujian mikroskopi penggabungan; serovar

INTRODUCTION

Leptospirosis is a zoonosis caused by Leptospira bacteria. Leptospira bacteria are included in the Trepanometaceae family, order Spirochaeta. These bacteria are spiral-shaped with tight coils and hook-shaped ends so that the bacteria are very active (Mohammed et al. 2011). Animals that can act as reservoirs include rats, dogs and livestock such as cows, goats, sheep and pigs (Anis et al. 2012; Desvars et al. 2011). *Leptospirosis* is still a public health problem, especially in tropical and subtropical climates with high rainfall (Bierque et al. 2020). Flood becomes a medium of transmission and can provide an optimal environment for the survival of leptospira bacteria (Haake & Paul 2015).

The results of the Bierque et al. (2020) study report that leptospira bacteria can survive in environments with a pH approaching neutral and slum environments such as muddy soil, stagnant water and poor house drainage. Rats are a reservoir capable of transmitting leptospira bacteria for life without showing clinical manifestations or as life carriers (Koizumi et al. 2019). Not all animals infected with *Leptospirosis* show symptoms of illness (Ellis 2015). Dogs and cattle are natural hosts for certain serovars which usually show no symptoms or are relatively mild after being infected with these serovars, but they can become seriously ill after becoming infected with other serovars (Guernier et al. 2018).

Reports of the incidence of *Leptospirosis* in dogs in Switzerland showed the main clinical symptoms in the kidney (99.6%), lung (76.7%), and liver (26.0%), as well as hemorrhagic syndrome (18.2%) which caused the mortality amounted to (43.3%) (Major et al. 2014). The signs of *Leptospirosis* in dogs are often nonspecific and may include fever, myalgia, anorexia, vomiting, and diarrhea (Miotto et al. 2018). Specific symptoms such as jaundice, fever, abdominal pain, muscle aches and stiffness, uveitis, dyspnea and coagulopathy also occur but the frequency is low (McCallum et al. 2019). Seven days of bacterial invasion, signs are mild and limited to lethargy and mild hyperemia. Infected dog with severe clinical symptoms at 9 days and worsening for up to 13 days. Other symptoms such as acute renal failure and liver disease are also common in dogs (Brown 1996). Acute Renal Failure (AKI) in dogs, accounting for more than 90% of reported cases is *Leptospirosis*. Risk factors that have been associated with *Leptospirosis* include water exposure, male gender, and sheepdog or working dog in several research studies (Harkin 2012; Ward et al. 2004).

Dogs are one of the animals that are able to transmit leptospira bacteria to humans through urine. The incidence of *Leptospirosis* in humans in Indonesia was reported as many as 895 cases during 2018, with a mortality rate of 17.8% and an annual mortality of 39.2/100,000 population. DKI Jakarta is an endemic area of *leptospirosis* with high flood intensity (Ministry of Health 2019).

Information on the distribution of leptopsira bacteria serovar and the risk factors affecting dogs is still very limited. In addition, data on leptospira cases in dogs in post-flood urban areas is still limited and has not even become a concern. Based on this, this research is necessary. This study aims to analyze the types and distribution of leptospira bacteria serovars in dogs in Jakarta, Bekasi, Depok and South Tangerang. Post flood 2020 and risk factors affecting the incidence of *Leptospirosis* in dogs during the study.

MATERIALS AND METHODS

STUDY AREA

The research was conducted at Ragunan Animal Hospital, South Jakarta, Indonesia from January 2020 to March 2020.

STUDY DESIGN AND SAMPLES

The study used a cross-sectional study of 24 samples of dogs suspected of *Leptospirosis* who were treated at the Jakarta Animal Hospital and independent clinics in Jakarta, Bekasi, Depok and South Tangerang. 21 dogs from flood-affected areas and 3 dogs were not affected by the flood. The samples were then evaluated for clinical symptoms, physical examination and biochemical analysis, serum and hematology, and the MAT test. Dogs showing symptoms of jaundice, hypertemia, gastrointestinal, urogenital and respiratory symptoms are then subjected to a hematology examination with the Hematology Analyzer Rayto RT-7600 machine to determine the number of red blood cells (RBC), white blood cells (RBC) and platelet count (PLT). Biochemical examination using the Photometer Rayto RT 9200 chemistry Analyzer to determine levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), serum concentration of urea nitrogen in the blood (BUN) and creatinine (CR). The research design refers to the method of Mioto et al. (2018). In general, the criteria for samples with suspected *leptospirosis* include leukocytosis, thrombocytopenia, azotemia with Blood Urea Nitrogen (BUN) and high keratin. The sample then tested the Microscopic Agglutination Test (MAT).

Measurement of risk factors in the form of variable questionnaires with laboratory test results using the Chi-square test.

<center>PROCEDURES</center>
<center>BLOOD SAMPLE</center>

The tools used are: 3 mL syringe, 70% alcohol, serum separator tube (SST) II contens clot activator and gel 4 mL. The collected serum was stored in a 2 mL micro tube and a micro tube rack for serological testing. The blood sample is taken from the cephalic vein dogs with suspect *Leptospirosis* from clinical symptoms after an examination by a veterinarian at a veterinary hospital or independent veterinary practice as much as 3-5 mL. Intake was done using a venoject tube without anticoagulant or serum separator tube (SST) II. Blood was then separated from the serum for *leptospirosis* by MAT test.

<center>MICROSCOPIC AGGLUTINATION TEST</center>

MAT method was carried out by filling 96 wells on the microtiter plate with 50 µL of serum diluted with *Phosphate Buffer Saline* (PBS) with a ratio of 1: 25 (100: 2400 µL) and the wells were then filled with the same volume to have a serum and PBS ratio of 1: 50, 1: 100, 1: 400 and 1: 1600. Live Leptospira Antigens from IVETRI (Indonesia Research Centre for Veterinary Sciences) (serovar: *ichterohaemorrhagiae, javanica, celledoni, ballum, pyrogenes, cynopeteri, rachmati,* *australis, pomona, canicola, grippotyphosa, bataviae, hardjo, tarassovi*) 0.05 mL were added, then incubated at two temperatures of 28-30 °C during 2 h (Marbawati & Ismanto 2010). The figure of the results MAT test is viewed under a dark field or phase contrast microscope. The 50% agglutinated or 50% unagglutinated Leptospira reaction is the end point of the MAT test reading. Samples with a titer > 100 shows that the test reacts positively to the serovar used (Marbawati & Ismanto 2010).

<center>DATA ANALYSIS</center>

Bivariate analysis was carried out to determine the influence of each risk factor (independent variable) on the incidence of *Leptospirosis* cases (dependent variable) by using the Chi-square test with confidence level as 95% on the dependent variable of the serological MAT Test and analyzed using IBM SPSS software 21.0. The independent variable is a type of variable that explains or influences other variables, while the dependent variable is the type of variable that is influenced by the independent variable. The strength of the association can be measured using the RR and or OR in a 2 × 2 table (Budiharta & Suardana 2002).

<center>RESULTS AND DISCUSSION</center>

<center>RESULT</center>

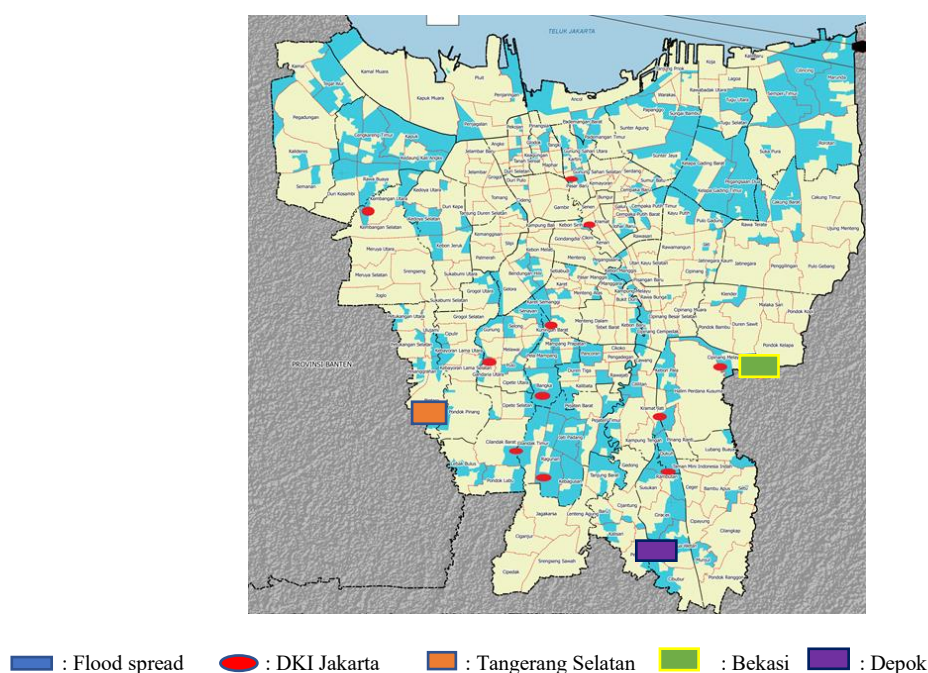Distribution of canine *leptospirosis* after flood as shown in Figure 1.

FIGURE 1. Distribution of Canine *Leptospirosis* as flood-affected January until March 2020

Based on the result, canine with positive *Leptospirosis* with MAT test laboratory were distributed in Jakarta 11 district which includes South Jakarta 5 district (Ragunan, Cilandak Barat, Bangka, Kebayoran Lama), West Jakarta 1 district (Kembangan), Center Jakarta 2 (Gunung Sahari, Kebon Sirih), and East Jakarta 3 district (Cipinang, Kampung Melayu, Kramat Jati). Other areas like Depok district (Depok), Bekasi 1 district (Bekasi) and South Tangerang 1 district (Bintaro).

Distribution cases of *Leptospirosis* after flood from dog serum samples during January until March 2020 are presented in Table 1. Total samples of dogs examined were 24, where after the MAT test, 14 were positively infected with *Leptospirosis* and 10 were not infected with *Leptospirosis*. Dog samples from North Jakarta based on the MAT test were not infected with *Leptospirosis*. The leptospira serovar bacteria found in positive samples consisted of *bataviae, icterohaemorrhagiae, javanica,* and *tarassovi.* Serovar *bataviae,* which is the serovar of leptospira bacteria, is mostly found in dog samples, while the lowest is serovar *tarassovi.*

TABLE 1. Distribution cases of *Leptospirosis* after flood from dog serum samples during January until March 2020

Sample of Origin	Amount sample	Negative Result MAT	Positive Result MAT	Serovar
South Jakarta	9	4	5	*bataviae, tarassovi*
West Jakarta	1	0	1	*icterohaemorrhagiae, javanica*
North Jakarta	3	3	0	-
Center Jakarta	2	0	2	*bataviae, icterohaemorrhagiae*
East Jakarta	3	0	3	*bataviae, icterohaemorrhagiae, javanica, tarassovi*
South Tangerang	2	1	1	*bataviae*
Bekasi	1	0	1	*bataviae*
Depok	3	2	1	*icterohaemorrhagiae*

Serovars used in the MAT test are: *icterohamorragie, javanica, celledoni, canicola, ballum, pyrogenes, cynopteri, rachmati, australis, pomona, grippotyphosa, hardjo, batavia* and *tarassovii*

Table 2 shows the MAT test titers contained in each infecting serovar, the titers in the MAT test vary, the results are said to be positive if the titer shows a positive reaction to the serovar at a 1: 100 reactions. This study resulted in varying titers, namely 1: 100; 1: 400 and the highest titer 1: 1600 for serovar *bataviae* in the Bekasi area.

The data in Table 3 shows that 75% of dogs suffering from *Leptospirosis* are working dogs, or dogs used for hunting or guarding houses and yards or gardens, 25% are pet dogs. Dogs with male sex by 75%, and female dogs by 25%. The mean age of dogs affected by *Leptospirosis* (91.7%) was more than one-year-old. Puppy dogs or less than a year only about 8.3% affected by *Leptospirosis*.

TABLE 2. Titer MAT results from dog serum samples during January until March 2020

Sample of origin	Amount sample	*Bataviae*	*Icterohaemorrhagiae,*	*Javanica*	*Tarassovi*
South Jakarta	5	1:100	-	-	-
		1:400	-	-	1:100
		1:100	-	-	-
		1:400	-	-	-
		1:400	-	-	1:400
West Jakarta	1	-	1:100	1:100	-
North Jakarta	0	-	-	-	-
Center Jakarta	2	1:400	1:100	-	-
		-	1:100	-	-
East Jakarta	3	1:400	-	1:400	-
		-	1:100	-	1:100
		-	1:100	-	1:400
South Tangerang	1	1:100	-	-	-
Bekasi	1	1:1600	-	-	-
Depok	1	-	1:100	-	-

Serum with a titer of 1: 100 or more for one or more serovars is tested positive

TABLE 3. Frequency distribution of sample data during the study through a questionnaire

Category	Description	Amount	(%)
Type of dog	Pet dog	6	25.00
	Working dog	18	75.00
Sex	Male	18	25.00
	Female	6	75.00
Age	Adult	22	91.00
	Puppy	2	8.30
History of Vaccination	Yes	16	66.67
	No	8	33.33
Maintenance system	House hold	6	25.00
	No house hold	18	75.00
History contact of rat	No	11	45.80
	Yes	13	54.20
Flood affected area	No	3	12.50
	Yes	21	87.50
Categorical of flood	(High)	4	16.70
	(Medium)	14	70.80
	No flood (Low)	3	12.50
Source of drinking water	Boiled water	1	4.20
	Raw water	23	95.80
Recovery	Life	7	29.20
	Death	17	70.80

Total sample of the study was 24 dogs

The risk factors for canine *Leptospirosis* after flooding are presented in Table 4. There is a significant relationship (P<0.05) where the strength of association (OR) is high for canine *Leptospirosis* in this study: Maintenance system, history of contact with rats, flood-affected areas, flood category and recovery phase. The risk factors that increased the incidence of *Leptospirosis* in dogs were the maintenance system (OR 17.50) with (P<0.001), history of contact with mice (OR 9.625) with (P<0.05), flood-affected areas (OR 3.50) with (P<0.05), drinking water sources (OR 1.643) with (P<0.05), and dog recovery status (OR 2.125) with (P<0.05). Dogs that were not exposed to flooding decreased the incidence of *Leptospirosis* (OR 0.286) by (P<0.05). Flood risk factors and the presence of reservoirs influenced the acute incidence of *Leptospirosis* in dogs from this study.

TABLE 4. Bivariate analysis of risk factor

Category	Crude OR	CI 95%	P-value
Type of dog (working dog/pet dog)	5.20	0.714-37.895	0.088
Sex (Male/female)	0.25	0.240-2.594	0.224
Age (adult/puppy)	0.591	0.417-0.837	0.253
Vaksinasi (Yes/No)	1.00	0.173-5.772	1.000
Maintenance system (Household/No)	17.50	1.560-196.319	0.007***
History contact of rat (Yes/No)	9.625	1.378-67.246	0.035**
Flood affected area (Yes/No)	3.50	1.780-6.883	0.021**
Categorical flood (Yes/No)	0.286	0.145-0.562	0.017**
Source of drinking water (raw water/boiled water)	1.643	1.184-2.280	0.429
Recovery (Infausta/ fausta)	2.125	1.283-3.518	0.015**

Total sample of the study was 24 dogs; 95% CI; *** very significant (P<0.01) ** more significant (P<0.05).

Case fatality rate (CRF) cases of *Leptospirosis* were calculated based on dogs that were positive confirmed by the gold standard MAT test (Table 5). The CFR of *Leptospirosis* cases during the 3-month study period showed a rate of 40.47%.

TABLE 5. Case fatality rate for *Leptospirosis* cases from January to March 2020

Time	MAT (+)	Monthly Mortality	CFR (%)	Average (%)
January	7	5	5/7 (71,42)	40,47
February	4	2	2/4 (50,00)	
March	3	0	0/3 (0,00)	

DISCUSSION

The *Leptospirosis* can be spread due to flood water to several areas where rat urine containing leptospira contaminates the stagnant water (Naing et al. 2019; Sykes et al. 2011). Leptospira in Indonesia is mainly caused by mice that release the bacteria into the environment through urine, brown mice (*Rattus norvegicus*) and garden rats (*Rattus exulans*) are the source of transmission to animals and humans (Gasem et al. 2020; Ministry of Health 2017). *Leptospirosis* is still a threat to public health in Indonesia because it is related to the presence of risk factors, namely the high population of rodents as an environmental reservoir, poor environmental sanitation and frequent flooding in 2020, which can cause acute *leptospirosis* in dogs.

The incidence of *Leptospirosis* in dogs can be influenced by the inherent conditions of the individual dog (such as the dog's age, gender, purpose of keeping the dog) and daily habits such as frequent hunting of mice. External factors such as flooding can be a medium for transmission of leptospira bacteria. Pathogenic *Leptospirosis* has the ability to form biofilms *in vitro* and survive in biofilms under natural conditions (Garba et al. 2018; Polo et al. 2019). Leptospira can also interact with other soil community members such as Amoeba, which is one of the main colonies in drinking water as a reservoir for potentially pathogenic bacteria (Boey et al. 2019). According to Collantes et al. (2016) and Levett (2001), clinical symptoms of dogs infected with *Leptospirosis* include azotemia, myalgia, vomiting, anorexia, fever, hyperemia in the area of the molle pallatum or intra-orbital, jaundice, production of cytokines by monocytes, macrophages, and other cells that have an impact on vascular permeability disorders, pressure drops blood, anorexia, and unstable body temperature.

MAT is a serological test that has high sensitivity and specificity for detecting serovars (Miotto et al. 2018; Reagan & Sykes 2019). Serovar *bataviae* dominates *Leptospirosis*-positive dogs in several areas in this study, namely South Jakarta, Central Jakarta, East Jakarta, South Tangerang, Depok and Bekasi. Exposure can occur through direct contact with infected animals or through indirect contact through soil or water contaminated with urine from infected animals (Haake & Paul 2015). Serovars *bataviae* and *icterohaemorrhagiae* are found in many cases of *leptospirosis* in humans. Serovars *tarassovi* are commonly found in cattle, but in this study 4 dogs reacted positively to this serovar. Serovars *tarassovi* are usually found in livestock, especially pigs,

because they are carriers of serovars *tarassovi* and act as reproductive disorders in pigs (Ministry of Agriculture 2012).

The antibody titer against MAT varies widely which is thought to be influenced by the different immune response of each individual dog. A total of 14 dogs positive for MAT showed agglutination antibodies against 9 dogs infected with serovar *bataviae*, serovar *icterohaemorrhagiae* infected 6 dogs, serovar *javanica* infected 2 dogs and serovar *tarassovi* infected 4 dogs. Changes in serovar prevalence are thought to be related to two main factors that greatly influence the epizoology of Leptospira serovars (Harkin 2012). Vaccination can prevent *Leptospirosis* in dogs. Serovars *icterohaemorrhagiae* and *canicola* were the most common serovars found in the field. The involvement of additional serovars such as serovar *bataviae* and *tarassovi* needs to be an important concern. Dogs that have been vaccinated can contract *leptospirosis* due to differences in the serovars that infect, commercial vaccines in the field only protect against *icterohaemorrhagiae* and *canicola* serovars, so it is necessary to add a serovar vaccine from other leptospira bacteria. According to Altheimer et al. (2020), the use of vaccines against leprospira cases in dogs needs to be done because the results of the study indicate the potential risk of zoonoses that should not be underestimated with a high infection rate.

The average case fatality rate within 3 months of the study was very high, namely 40.47% (Table 5). The calculation of CFR is based on dogs that have been confirmed by the MAT test. The highest *Leptospirosis* cases and mortality occurred in January 2020, namely 71.42%. The conditions of the research area in January were high rainfall and floods. This is thought to be a factor in the high cases of *Leptospirosis* in the study area. In addition, veterinarians still lack knowledge when diagnosing early cases of *Leptospirosis*. Dogs infected with *Leptospirosis* sometimes do not show specific symptoms such as jaundice and conjunctival suffusion, but show non-specific symptoms such as general weakness and gastrointestinal symptoms. In addition, the obstacle of MAT testing is that it takes a long time and laboratory facilities are quite expensive. Historical information such as the proximity of the animal to the rat reservoir and originating from the flooded area is expected to support the diagnosis of *Leptospirosis* cases in dogs.

Decreased cases of death were caused by several factors such as clinical accuracy and socialization

programs to veterinary hospitals and veterinary clinics regarding *Leptospirosis* cases to veterinarians so that case handling was carried out appropriately by selecting the appropriate treatment. Therapeutic antibiotics and support were provided throughout the study. Therefore, the choice of therapeutic treatment must be made appropriately in the administration of antibiotics. Ampicillin 500 mg every 6 h or doxycycline 100 mg twice daily can be used to treat mild *Leptospirosis*, whereas for severe cases Penicillin G 1.5 MU can be used intravenously every 6 h (Edwards 2004).

CONCLUSION

Leptospira *serovar bataviae, icterohaemorrhagiae, tarassovi,* and *javanica* were found in post-flood dogs by MAT testing. The risk factors that increased the incidence of *Leptospirosis* in dogs were maintenance system (OR 17.50), history of contact with rats (OR 9.625), flood-affected areas (OR 3.50), drinking water sources (OR 1.643) and status of dog recovery (OR 2.125). Dogs that were not affected by flooding decreased the incidence of *Leptospirosis* cases (OR 0.286). The factors of flood risk and the presence of a reservoir influenced the acute incidence of *Leptospirosis* in dogs from this study. Post flood leptospirosis cases in several areas need to be an important concern because they have a high mortality. Vaccination precautions in dogs and addition of local isolate serovar vaccines are also recommended.

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Ambar Retnowati
Veterinary Public Health Study Program
IPB University
Indonesia

Agustin Indrawati*, Upik Kesumawati Hadi & Safika
Department of Animal Diseases and Veterinary Public Health
IPB University
Indonesia

Susan M Noor
Veterinary Research Center
Ministry of Agriculture
Indonesia

*Corresponding author; email: indraseta@apps.ipb.ac.id