

CANINE LEPTOSPIROSIS IDENTIFICATION IN SMALL ANIMAL PRACTICE

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ABSTRACT

Leptospirosis is an infectious disease caused by bacteria from the genus *Leptospira* with various serovars and often presents with a wide range of clinical signs from mild to severe conditions, particularly it presents with a mild fever to asymptomatic infection-causing animal death. The purpose of this study was to identify the characteristics of canine leptospirosis through laboratory examinations supported by medical record data in a small animal practice at the drh Cucu K. Sajuthi Joint Veterinary Practice, in Jakarta. The study was conducted on serum samples of sixteen dogs from drh Cucu K. Sajuthi Joint Veterinary Practice patients, which presented varied clinical signs of leptospirosis, and which had been accompanied by hematological examination, blood biochemistry, and Microscopic Agglutination Test (MAT) against various serovars such as *Bataviae*, *Icterohaemorrhagiae*, and *Javanica*. The collected data was analyzed descriptively. The results showed that canine leptospirosis was more commonly derived from *Bataviae* serovar and was accompanied by clinical signs such as vomiting, jaundice, anorexia, abdominal pain, lethargy, pale mucosal membrane, diarrhea, dehydration, dyspnea, and polyuria/polydipsia. A predisposition of gender and age was commonly found in male dogs less than 5 years old. Abnormalities found from hematological examination were leukocytosis, granulocytosis (neutrophilia), anemia, thrombocytopenia, and lymphopenia, while blood chemistry showed azotemia, an increase in liver enzymes (AST, ALT, ALP), hyperbilirubinemia, hyponatremia, hyperphosphatemia, and hypoalbuminemia.

Key words: blood biochemistry, clinical sign, dog, *Leptospira* sp., MAT

ABSTRAK

Leptospirosis merupakan penyakit infeksius yang disebabkan oleh bakteri dari genus Leptospira dengan berbagai serovar. Leptospirosis menimbulkan berbagai bentuk tanda klinis dengan berbagai tingkat keparahan, mulai dari demam ringan yang dapat kembali normal tanpa penanganan serius atau infeksi asimtomatik hingga kondisi parah yang dapat menyebabkan kematian. Penelitian ini bertujuan mengidentifikasi karakteristik leptospirosis pada anjing melalui pemeriksaan laboratorium yang didukung oleh data rekam medis pada pasien-pasien praktisi hewan kecil di Praktek Dokter Hewan Bersama (PDHB) drh Cucu.K Sajuthi, Jakarta. Penelitian dilakukan pada enam belas serum anjing dari pasien-pasien PDHB drh Cucu K. Sajuthi yang memiliki tanda-tanda klinis sangat bervariasi yang mengarah ke leptospirosis disertai dengan pemeriksaan hematologi, biokimia darah dan Microscopic Agglutination Test (MAT) terhadap berbagai serovar seperti Bataviae, Icterohaemorrhagiae dan Javanica. Data yang diperoleh dianalisis secara deskriptif. Hasil penelitian menunjukkan bahwa anjing yang terinfeksi Leptospira lebih banyak berasal dari serovar Bataviae dengan tanda-tanda klinis seperti muntah, ikterus, anoreksia, abdomen tegang, lemas/lesu, pucat, diare, dehidrasi, dyspnea, dan poliuria/polidipsia. Predisposisi jenis kelamin dan umur ditemukan banyak terjadi pada anjing jantan berumur kurang dari 5 tahun. Abnormalitas pemeriksaan hematologi yaitu leukositosis, granulosis (neutrofilia), anemia, trombositopenia dan limfopenia, sedangkan pada kimia darah menunjukkan azotemia, peningkatan enzim hati (AST, ALT, ALP), hiperbilirubinemia, hiponatremia, hiperfosfatemia, dan hypoalbuminemia.

Kata kunci: biokimia darah, temuan klinis, anjing, *Leptospira* sp., MAT

INTRODUCTION

Leptospirosis is an infectious disease caused by a bacterial infection with any of the serovars of the genus *Leptospira* (Sykes 2014). There are more than 300 serovars of *Leptospira* identified and these are arranged in 25 serogroups in the worldwide distribution (OIE 2018). *Leptospira* is a zoonotic pathogen agent and may infect any mammalian species (Ettinger *et al.* 2017). According to current research from the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Karala (India) it was found that an increase of incidences of canine leptospirosis, around 71.12% sample, were confirmed to have been positively infected by more than one serovar *Leptospira* (Ambily *et al.* 2012). Canine leptospirosis can be transmitted by direct contact (urine, bite, or ingestion from a host) and indirect (through contaminated water, soil, or host's cage) (Thammitiyagodage *et al.* 2017). The clinical

signs of canine leptospirosis are variable with varying severity, ranging from self-limiting febrile illness or asymptomatic infections to severe conditions that can cause animal death (Miotto *et al.* 2018).

Leptospirosis is found in areas with high temperatures and humidity, as well as a lot of soil and water with pH approaching neutral (Mulyani *et al.* 2017; Thammitiyagodage *et al.* 2017). In addition to temperature and humidity factors, vaccination programs are also very important in limiting the incidences of leptospirosis in humans and animals in tropical areas (Weese and Evason 2020). Unvaccinated puppies (under 1-year-old) suffer from the most severe forms of the disease typically with fatal outcomes, while vaccinated dogs can reduce the risk of infection especially from the specific serovar that is commonly found in commercial vaccines such as *L. canicola* and *L. icterohaemorrhagiae* (Thammitiyagodage *et al.* 2017). Canine leptospirosis often goes unrecognized or is misdiagnosed due to its variable presentation, and

accurate diagnosis depends on the stage of disease and non-specific clinical signs (Lizer *et al.* 2017).

The purpose of this study was to identify the characteristics of canine leptospirosis through laboratory examinations supported by medical record data at drh Cucu K. Sajuthi Joint Veterinary Practice, Jakarta. The results of this study also aim to reduce the risk of disease transmission to other dogs, animal owners, or veterinary technicians, and veterinarians.

MATERIALS AND METHODS

The study was conducted at Internal Diagnostic Laboratory of drh Cucu K. Sajuthi Joint Veterinary Practice from January 2019 to March 2020. The sample used in this study consisted of 16 cases of leptospirosis. The information regarding gender, age, and clinical signs of dog-related canine leptospirosis were obtained from the medical records of drh Cucu K. Sajuthi Joint Veterinary Practice patients, while the Microscopic Agglutination Test (MAT) serology test was conducted in a private laboratory out of the Internal Diagnostic Laboratory of drh Cucu K. Sajuthi Joint Veterinary Practice. Hematology was analyzed using Nihon Kohden Celltac α and Abaxis VetScan HM5 and blood biochemistry using Photometer 5101 V 5+. Antigens used in MAT serological tests were *Leptospira interrogans* with serovar Icterohaemorrhagiae, Javanica, Celledoni, Canicola, Ballum, Pyrogenes, Cynopteri, Rachmati, Australis, Pomona, Grippotyphosa, Hardjo, Bataviae, and Tarassovi.

RESULTS AND DISCUSSION

The rate of canine leptospirosis was found to be relatively higher in male dogs (69%) compared to female dogs (19%) (Table 1). This result is in accordance with a previous study by Rad *et al.* (2004) that MAT positive leptospirosis was found more often in the serum of male dogs compared to females. This is due to the natural behavior of wild male dogs (Azócar-Aedo *et al.* 2014). In agreement with a retrospective study by Ward *et al.* (2004), the seroprevalence of leptospirosis was found to be higher in male dogs compared to female dogs because male dogs were widely used as working or shepherding dogs and thus more at risk of disease infection.

The rates of canine leptospirosis in dogs less than 5 years of age were higher (57%) compared to dogs over 5 years old (31%) (Table 1). This can be related to the

vaccination history of the dogs. As expected, unvaccinated dogs have a higher risk of infection. Similar results were also reported by Shi *et al.* (2012) who found the prevalence of leptospirosis in dogs aged 1-4 years was 9.2%, less than 1 year as much as 6.3%, and over 4 years as much as 5.8%, although statistical analysis showed no significant difference from that age group. According to Ward *et al.* (2004), animals between 4 to 6.9 years old were at a greater risk of contracting leptospirosis compared to animals under one year of age. These results indicate that age, breed and gender were risk factors for leptospirosis, followed by environmental factors such as increased rainfall and temperature (Meeyam *et al.* 2006).

A total of 16 dogs (53%) that were diagnosed with canine leptospirosis showed clinical signs of vomiting as the highest percentage (23%) followed by jaundice (17%) and anorexia (14%) (Figure 1). These results are similar to the results of a previous study by Claus *et al.* (2008) which found that from 5 to 7 dogs that were diagnosed with leptospirosis showed clinical signs of vomiting and anorexia. These conditions were found in acute-subacute infections caused by the presence of antigenic stimulation of the gastrointestinal organs that cause pancreatitis and enteritis which was subsequently followed by kidney and liver disorders which presented jaundice (Sykes *et al.* 2011).

Clinical signs of jaundice were due to the activity of pathogenic *Leptospira* which can cause hepatocytes damage. Simultaneously with the spread of necrosis in the liver tissue this can be followed by an increased production of bilirubin as a result of extravascular erythrocyte destruction. Jaundice is also one of the symptoms that appear in response to the presence of hepatic necrosis. This condition can occur in single or multiple with other clinical signs and it can also occur due to the presence of chronic infections. If an infected dog has chronic hepatitis or hepatic fibrosis, other clinical signs that can also be found are anorexia, weight loss, ascites or hepatic encephalopathy (Schuller *et al.* 2014). This result is in accordance with the results of a previous study by Shah *et al.* (2018), that found that the clinical signs of dogs infected with *Leptospira* included lethargy, anorexia, diarrhea, vomiting, jaundice, abdominal pain, dehydration and polyuria. Animals infected with *Leptospira* will initially experience fever caused by the presence of antigenic stimulation of the *Leptospira* pathogen. This condition will be followed by anorexia, vomiting, diarrhea, lethargy, and polyuria/polydipsia. Polyuria/polydipsia

Table 1. Percentage of gender and age of dogs infected with canine leptospirosis from the medical records of drh Cucu K. Sajuthi Joint Veterinary Practice from January 2019 to March 2020

No.	Parameter	Total cases	%
Gender			
1	Male	11	69
2	Female	3	19
3	Unknown	2	12
Age			
1	< 5 years old	9	57
2	> 5 years old	5	31
3	Unknown	2	12

occurs due to a progressive decrease in renal function and/or liver insufficiency (Azócar-Aedo *et al.* 2014).

Abnormalities of clinical pathology examination results from hematology and blood biochemistry found leukocytosis, granulocytosis (neutrophilia), anemia, thrombocytopenia, lymphopenia, azotemia, increased alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) with hyperbilirubinemia, hyperphosphatemia, hyponatremia, and hypoalbuminemia. Based on Table 2, 69% of dogs show leukocytosis accompanied by granulocytosis (neutrophilia) as much as 63%.

Leukocytosis is a common change found in dogs with acute infection especially related to acute renal failure (Claus *et al.* 2008). Leukocytosis granulocytosis (neutrophilia) indicates antigenic stimulation of bacterial infections and indicates the occurrence of infection and disease development (Willard and Tvedten 2012). Shah *et al.* (2018) found that 18-68% of dogs infected with *Leptospira* were found to have anemia either in the form of non-regenerative or regenerative anemia. This can be caused by bleeding in

the gastrointestinal or urinary tract. and is also associated with thrombocytopenia, coagulopathy or vasculitis. Thrombocytopenia was reportedly found in 20-50% of dogs infected with *Leptospira*, which is also accompanied by neutrophilia and/or lymphopenia (Reagan and Sykes 2019). Thrombocytopenia can be caused by increased platelet consumption related to the presence of adhesion and aggregation of the vascular endothelial, phagocytosis of Kupffer cells, immune-mediated platelet destruction or splenic sequestration (Thrall *et al.* 2012). The infected dogs have anemia (56%), thrombocytopenia (50%) and lymphopenia (44%) (Table 2). This result was similar to a previous study by Sharun *et al.* (2019) that dogs infected by *Bataviae* serovar had hematologic results in the form of leukocytosis, granulocytosis (neutrophilia), anemia and thrombocytopenia.

An abnormality of blood biochemical examination results was almost found in all dogs infected with *Leptospira* is azotemia (94%). Azotemia may indicate kidney injury (renal failure). Renal failure can occur in cases of subacute leptospirosis due to the destruction of

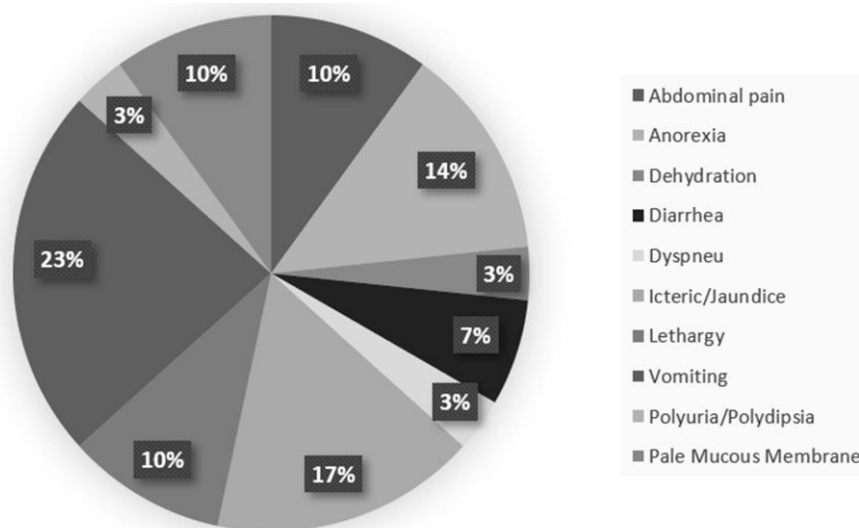


Figure 1. Percentage of the clinical signs of canine leptospirosis from the medical records of drh Cucu K. Sajuthi Joint Veterinary Practice from January 2019 to March 2020

Table 2. Percentage of blood examination results of canine leptospirosis in the Internal Diagnostic Laboratory of drh Cucu K. Sajuthi Joint Veterinary Practice from January 2019 to March 2020

No.	Parameter	Total cases	%
Hematology			
1	Leukocytosis	11	69
2	Granulocytosis (Neutrophilia)	10	63
3	Anemia	9	56
4	Thrombocytopenia	8	50
5	Lymphopenia	7	44
Blood Biochemistry			
1	Azotemia	15	94
2	Increase ALP	12	75
3	Increase AST	9	56
4	Hyperbilirubinemia	8	50
5	Increase ALT	6	38
6	Hyponatremia	6	38
7	Hyperphosphatemia	6	38
8	Hypoalbuminemia	5	31

ALT= Alanine aminotransferase, AST= Aspartate aminotransferase, ALP= Alkaline phosphatase

Table 3. Serovar that infects dogs at the drh Cucu K. Sajuthi Joint Veterinary Practice from January 2019 to March 2020

No.	Serovar	Total cases	%
1	Bataviae	10	29
2	Icterohaemorrhagiae	8	23
3	Javanica	6	18
4	Celledoni	3	9
5	Canicola	2	6
6	Rachmati	2	6
7	Tarrasovi	1	3
8	Ballum	1	3
9	Pyrogenes	1	3

renal tubular epithelial cells in the renal tubule by *Leptospira* bacteria. Moreover, acute interstitial nephritis and parenchymal swelling can cause a decrease in renal perfusion and glomerular filtration rate (GFR) (Stockham and Scott 2008). This condition can be exacerbated by the presence of renal ischemia that causes secondary hypoxia that can result in nephropathy in infected animals (Sykes *et al.* 2011). Azotemia is commonly found alongside an increase in liver enzymes ALT, AST, ALP and in cases of *Leptospira* infection in dogs (Azócar-Aedo *et al.* 2014). This statement was supported by the results of the research found in 6 samples, while 10 other samples showed only an increase in ALP and total bilirubin. Impaired liver function was caused by the presence of endotoxemia which disrupts the transport flow of the liver and hepatocyte apoptosis (Schuller *et al.* 2014). Liver function disorders accompanied by damage of hepatocytes can result from an increase in liver enzymes that are also accompanied by hyperbilirubinemia, but an increase in ALP and hyperbilirubinemia was more common than an increase in ALT (Sykes *et al.* 2011). This statement is consistent with the results of a study showing that dogs that experienced an increase in ALT (38%) was fewer than dogs with increase ALP (75%) and hyperbilirubinemia (50%). Hypoalbuminemia has also been reported in dogs infected with chronic *Leptospira* followed by albuminuria due to glomerulonephritis/renal disorders (Claus *et al.* 2008; Sykes *et al.* 2011; Shah *et al.* 2018). Dogs infected with *Leptospira* are also reported to have frequent changes in electrolyte values such as hyperphosphatemia, hyponatremia, and/or hypochloremia caused by fluid loss in the gastrointestinal or kidneys (Sykes *et al.* 2011; Schuller *et al.* 2014).

Previous research has shown a correlation between the clinical signs of the disease and the infectious serovar. Sharun *et al.* (2019) found that dogs infected by *Canicola* and *Grippotyphosa* serovar showed clinical signs more related to renal dysfunction, but in cases of infection by *Icterohaemorrhagiae* and *Pomona* serovar clinical signs were more related to liver disorders. One of the characteristics of an animal infected by *Leptospira* of *icterohaemorrhagiae* serovar is jaundice and bleeding followed by acute interstitial nephritis (Claus *et al.* 2008; Sykes *et al.* 2011). The results of a study by Mulyani *et al.* (2017) confirmed the findings of Sharun *et al.* (2019) which indicated that the presence of liver, kidney, and hemorrhagic disorders in dogs infected by *Bataviae* serovar showed symptoms in

the form of vomiting and jaundice. The results of the study were similar to this study which showed that cases of canine leptospirosis that presented clinical signs were dominated by vomiting and jaundice and were generally infected by *Bataviae* serovar (Table 3).

The results showed 29% of positive cases of leptospirosis were caused by *Bataviae* serovar, 23% *Icterohaemorrhagiae* serovar, 18% *Javanica* serovar, 9% *Celledoni* serovar, 6% *Canicola* and *Rachmati* serovars and 3% *Tarrasovi*, *Ballum*, and *Pyrogenes* serovars. The results of this study illustrate that *Bataviae* serovar is the dominant *Leptospira* serovar infecting dogs at drh Cucu K. Sajuthi Joint Veterinary Practice.

This result was in accordance with previous research that found that 100% of dogs infected with *Leptospira* in the Special Region of Yogyakarta were infected with *Bataviae* serovar (Mulyani *et al.* 2017). However, each region can have a different dominant *Leptospira* serovar that infects the dogs of that region due to several factors. As a preventive measure, vaccinations need to be tailored to different regions depending on the infection rates of a specific serovar. Conditions related to the migration of wildlife that act as a reservoir of leptospirosis also need to be taken into account in order to prevent the spread of disease (Ellis 2010). The main reservoirs for *Bataviae* serovar are dogs, rats, and mice that are found in Jakarta. This condition is suspected to cause a high prevalence of leptospirosis due to the number of *Bataviae* serovar infections found in this study.

CONCLUSION

Based on the results of the analysis of the data the highest percentage of *Leptospira* bacterial infections from MAT examinations are mostly from *Bataviae*, *Icterohaemorrhagiae*, and *Javanica* serovars. Characteristics of clinical signs of leptospirosis infection consists of vomiting, jaundice, anorexia, abdominal pain, lethargy, pale mucosal membrane, diarrhea, dehydration, dyspnea, and polyuria/polydipsia, while the clinical pathology data from hematology and blood biochemistry was leukocytosis, granulocytosis (neutrophilia), anemia, thrombocytopenia, and lymphopenia, accompanied by azotemia, increased liver enzymes (AST, ALT, ALP), hyperbilirubinemia, hyponatremia, hyperphosphatemia, and hypoalbuminemia. The predisposition of the gender and vulnerable age was in male dog less than 5 years.

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