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SEROLOGICAL DETECTION OF BOVINE LEPTOSPIROSIS IN SUKABUMI AND CIANJUR REGIONS

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ABSTRACT

This study aims to detect leptospirosis serologically and serovars found in cattle in the Sukabumi and Cianjur areas. 165 cattle serum samples were taken randomly in the Sukabumi and Cianjur region. Microscopic Agglutination Tests (MAT) were used for serological testing using the antigens of Leptospira serovar Icterohaemorrhagiae, Javanica, Celledoni, Canicola, Ballum, Pyrogenes, Cynopteri, Rachmati, Australis, Pomona, Grippotyphosa, Hardjo, Bataviae, and Tarassovi. The MAT results showed that positive serum leptospirosis in cattle in Sukabumi region was 18.75%, with serovars Hardjo 16.25%, Tarassovi 1.25%, and Hardjo and Tarassovi (1.25%). Meanwhile, in Cianjur region, positive serum was 30.60% with serovar Hardjo 22.35%, Tarassovi 2.35%, Icterohaemorrhagiae 1.18%, Grippotyphosa 1.18%, Hardjo and Tarassovi 1.18%, Cynopteri and Hardjo (1.18%), Rachmati, Pomona, Bataviae and Tarassovi (1.18%). Leptospira serovar Hardjo was the dominant serovar in both regions. Overall, one serovar was detected in 22.42% of cattle, two serovars in 1.82% and four serovars in 0.61% cattle.

Key words: bovine, leptospirosis, microscopic agglutination test, serological

ABSTRAK

Penelitian ini bertujuan mendeteksi secara serologis leptospirosis dan serovar yang ditemukan pada sapi di wilayah Sukabumi dan Cianjur. Pengambilan sampel serum sapi sebanyak 165 sampel dilakukan secara acak (random sampling) di wilayah Sukabumi dan Cianjur. Microscopic Agglutination Test (MAT) digunakan untuk uji serologis dengan menggunakan antigen Leptospira serovar Icterohaemorrhagiae, Javanica, Celledoni, Canicola, Ballum, Pyrogenes, Cynopteri, Rachmati, Australis, Pomona, Grippotyphosa, Hardjo, Bataviae, dan Tarassovi. Hasil MAT menunjukkan serum positif leptospirosis pada sapi di wilayah Sukabumi adalah 18,75% dengan positif serovar Hardjo 16,25%, Tarassovi 1,25%, serta Hardjo dan Tarassovi 1,25%. Sedangkan di wilayah Cianjur sebesar 30,60% dengan dengan positif serovar Hardjo 22,35%, Tarassovi 2,35%, Icterohaemorrhagiae 1,18%, Grippotyphosa 1.18%, Hardjo dan Tarassovi 1,18%, Cynopteri dan Hardjo 1,18%, Rachmati, Pomona, Bataviae, dan Tarassovi 1,18%. Leptospira serovar Hardjo merupakan serovar yang dominan di kedua wilayah tersebut. Secara keseluruhan sapi terdeteksi satu serovar sebesar 22,42%, dua serovar 1,82% dan empat serovar 0,61%.

Kata kunci: sapi, leptospirosis, microscopic agglutination test, serologis

INTRODUCTION

Leptospirosis is a zoonotic disease caused by the pathogenic Leptospira bacteria, which can infect humans and animals. Leptospirosis generally occurs in tropical and subtropical areas with high rainfall and humidity (Widiasih *et al*. 2021), including Indonesia. The incidence of leptospirosis in the world reaches more than 1 million, and approximately 60,000 deaths are reported each year. Leptospirosis is a neglected disease, and cases have been reported in urban and rural areas (Sunaryo and Priyanto 2022).

Leptospira bacteria can infect various animal species, consisting of 66 known species, and are genetically classified into three groups: pathogens, intermediates, and saprophytes (OIE 2021; Browne *et al*. 2022). The pathogen group includes a group of Leptospira species that are able to cause illness in both humans and animals. The intermediate group is Leptospira species with moderate pathogenicity or can cause disease under certain conditions. The saprophyte group is a species that lives freely in the environment and does not cause disease. Many pathogenic and saprophytic Leptospira species are isolated from the environment, so they are able to survive in environments such as soil and water for long periods (Vincent *et al*. 2019; Casanovas-Massana *et al*. 2020). The Leptospira bacteria have more than 300 serovars that have been serologically identified, but naturally, each serovar tends to live in specific hosts (OIE 2021). Leptospirosis has many reservoir hosts, both wild and domestic animals. Animals infected with Leptospira act as a source of infection for other animals and humans. Transmission of the disease can occur through direct or indirect contact with the environment contaminated by Leptospira, which is excreted in the urine of infected animals (Widjayanti 2019). Pathogenic Leptospira resides in the kidney tubules of infected reservoir animals as immunoprivileged sites (Sholichah *et al*. 2020). Humans can be infected through wounds on the skin and mucous membranes (Widjayanti 2019).

Leptospirosis has been reported in humans, animals, and the environment. The presence of livestock or pets as a reservoir, as well as the presence of rats, is a source of leptospirosis infection. According to Putri *et al*. (2019), there is a significant relationship between the presence of garbage, standing water, Leptospira bacteria, and the presence of pets with the incidence of leptospirosis. Cattle infected with Leptospira have clinical manifestations ranging from asymptomatic to severe conditions. Acute leptospirosis may present with the sudden onset of agalactia, pyrexia, infertility,

abortion, meningitis, jaundice, and hemoglobinuria, especially in young animals. In chronic conditions, the symptoms that arise are abortion, stillbirth, premature birth, and infertility (OIE 2021; Aliberti *et al*. 2022; Zamir *et al*. 2022). Chronically infected animals are carriers for years or a lifetime and have the potential to serve as reservoirs of infection for humans and other animals (OIE 2021).

Domestic animals, such as cattle, have been reported in several areas of Indonesia to be positive for leptospirosis. Susanti (2015) stated that the prevalence of leptospirosis in cattle was 18.66% in Kulon Progo Regency and 14.5% in Bantul Regency. According to another study, 43 samples (13.03%) out of 330 samples tested positive for Leptospira (Mulyani *et al*. 2016). The seroprevalence of Leptospira in cattle at the Yogyakarta Giwangan abattoir was reported 20% positive for Leptospira (Noach and Noach 2020). Leptospira prevalence was 63.64% in Bantul Regency cattle and 50.00% in Gunungkidul Regency (Sunaryo and Priyanto 2022). Nevertheless, there has not been any thorough information available regarding the prevalence of leptospirosis in cattle in Sukabumi and Cianjur regions. In order to implement disease control and prevention strategies, this study aims to identify the serological forms and serovars of leptospirosis in cattle residing in the Sukabumi and Cianjur areas.

MATERIALS AND METHODS

The sample in this study was bovine serum from the Sukabumi and Cianjur regions which had been collected in 2012. Eighty bovine serum samples were collected from the Sukabumi region and 85 samples were collected from the Cianjur region using a random sampling method. Leptospirosis was tested serologically using the Microscopic Agglutination Test (MAT) method.

Microscopic Agglutination Test (MAT)

The MAT method is an agglutination test using live antigens of pathogenic Leptospira bacteria. The MAT testing procedure was carried out as previously described by Susanti (2015).

Leptospiral Antigen

The live antigens for Leptospira bacteria used were serovar Icterohaemorrhagiae, Javanica, Celledoni, Canicola, Ballum, Pyrogenes, Cynopteri, Rachmati, Australis, Pomona, Grippotyphosa, Hardjo, Bataviae, and Tarassovi (reference strain from Koninklijk Institut Voor de Tropen (KIT) Amsterdam, The Netherlands). The bacterial cultures used were between 5-9 days old with antigen concentrations ranging from $2x10^8$ Leptospira/mL, which were grown on liquid Ellinghausen-Mc Cullough Johnson-Harris (EMJH) medium and incubated at 28-30° C.

Screening Test

The serum sample was diluted with phosphate buffer saline (PBS) in a ratio of 1:25. Fitfy µL of the diluted serum was taken and put in the microplate hole and added 50 µL of pathogenic Leptospira antigen, then incubated at 28-30° C for 2 hours. Using PBS, the serum-antigen mixture was transferred to an object glass (without cover glass) and read using a phase contrast microscope with 10x magnification. Serum samples that show an agglutination reaction of 50% or more were proceeded for titration.

Serum Sample Titration

Serum samples that show an agglutination reaction of 50% or more in the MAT screening test were diluted using PBS with ratio of 1:50, 1:100, 1:400, and 1:1600. As much as 50 µL of each of these serum dilutions was then put into the microplate holes, and each of these dilutions was added with 50 µL of Leptospira antigen. Then the samples were incubated at 28-30° C for 2 hours. Readings were carried out as performed in the screening test. The endpoint of the reading was 50% or more agglutination (estimated from the amount of free Leptospira, i.e., 50% or less), and the titer was defined as the highest final dilution of serum in the serum-antigen mixture that showed 50% or more agglutination. The sample is considered positive if the titer is more than or equal to 100.

Data Analysis

The results of using a serum with the MAT method were analyzed descriptively.

RESULTS AND DISCUSSION

A total of 165 bovine serum samples, consisting of 80 samples from Sukabumi and 85 samples from the Canjur regions, were tested with MAT. A total of 41 samples showed seropositive results for Leptospira. The results of the MAT test can be seen in Table 1.

The percentage of samples seropositive for Leptospira from Sukabumi was 18.75% and 30.60% from Cianjur. Bovine serum with seropositive results indicated that the cattle had been infected with Leptospira. However, seronegative animals were not necessarily infected. This might be due to low titers antibodies are not detected , infection occurs in the early or late stages, or animals are chronically infected (Susanti 2015; OIE 2021; Ngasaman *et al*. 2022). The Leptospira serovar detected in the Sukabumi region were Hardjo and Tarrasovi serovar, while in Cianjur were Icterohaemorrhagiae, Cynopteri, Rachmati, Pomona, Grippotyphosa, Hardjo, Bataviae, and Tarassovi. The percentage of serovars detected in the samples is presented in Figures 1 and 2.

From the Figure 1 and 2, it can be seen that the dominant serovar detected in the samples tested was serovar hardjo, which was 16.25% (13/80) for the Sukabumi region and 22.35% (19/85) for the Cianjur region. MAT results showed that cattle can be seropositive for one or more than one serovar. Mulyani *et al*. (2016) reported that 38% of cattle in the Progo River watershed in Yogyakarta were positive for serovar Hardjo, 18% Rachmati, 15%

Icterohaemorrhagiae, 9% Bataviae, 7% Javanica, 4.5% Canicola, 4.5% Pyrogenes, 2% Tarassovi and 2% Celledoni. Research by Widiasih *et al*. (2021) stated that 1.78% cattle from Kulon Progo Yogyakarta were positive for Hardjo, 0.85% Icterohaemorrhagiae, 0.71% Rachmati, 0.21% Celledoni, 0.21% Bataviae, 0.14% Tarassovi, 0.14% Javanica, and 0.007% Pomona. Research in Chile by Montes and Monti (2021) stated that bovine serum tested with MAT showed a positive reaction to serovar Hardjo by 46.1%, Pomona 26.05%, Ballum 9.1%, Tarassovi 7.7%, Canicola 3.8%, Autumnalis 2.4%, Icterohaemorrhagiae 0.3%, Bratislava 0.3%, and Grippotyphosa 0.3%.

Naturally, cattle are maintenance hosts for serovar Hardjo (Suprayoga *et al*. 2021; Aliberti *et al*. 2022), whose transmission does not depend on environmental conditions (Martins and Lilenbaum 2017). However, cattle can incidentally be infected with other serovars, such as Icterohaemorrhagiae, Canicola, Hebdomadis, Sejroe, Pyrogenes, Autumnalis, Australis, Javanica, Tarassovi, Grippotyphosa, and Pomona. Incidental infections are caused by serovars carried by other wild or domestic species, and transmission occured due to poor management and environmental conditions. Maintenance hosts generally do not show clinical symptoms of disease but can act as a source of natural pathogens (Correia *et al*. 2017; Aliberti *et al*. 2022). Leptospira bacteria will develop in the host's kidney reservoir and be excreted through urine for a long time (Haake and Levett 2015). Leptospires are able to survive in moist soil and water for weeks or even months. Humans can become infected with Leptospira through direct contact with infected animals and environments contaminated with Leptospira. However, humans cannot act as carriers that transmit it to other animals or humans (Sholichah *et al*. 2020). Leptospirosis in humans is a terminal infection, a dead-end infection referred to as a dead-end host (Mohd Ridzuan *et al*. 2016).

Cattle where more than one Leptospira serovar detected may occur due to interactions with maintenance or other incidental hosts, both domestic animals or wild animals that enter the cattle barn. These animals can contaminate feed, livestock drinking water (Barnabé *et al*. 2023), equipment, and the enclosure environment with Leptospira bacteria excreted in the urine. As reported by Aliberti *et al*. (2022), farms in one region in Italy raise livestock besides cows; there are also other livestock such as sheep, goats, pigs, donkeys, and horses; extensive or semi-extensive rearing systems; and contact with other animals such as dogs, wild boars and wild animals. After carrying out serological tests with MAT, seropositive results were obtained for various Leptospira serovars, such as Bratislava, Pomona, Icterohaemorrhagiae, Australis, Tarassovi, Hardjo, Sejroe, Ballum, and Grippotyphosa.

Mice are maintenance hosts of serovar Icterohaemorrhagiae, pigs serovar Pomona and Tarassovi, rodents serovar Grippotyphosa, dog serovar Canicola and bats serovar Cynopteri (Quinn *et al*. 2011; Aqib *et al*. 2019; Koizumi *et al*. 2020; Aliberti *et al*. 2022). However, these animals can be incidentally infected with other serovars. Incidentally, infected animals will usually show clinical symptoms and are associated with acute infection (Quinn *et al*. 2011; Ellis 2015). In recent years, many serovars have appeared detectable in wild and domestic animals. This indicates a change in the epidemiology of leptospirosis from time to time (Aliberti *et al*. 2022).

Incidental occurrences of leptospirosis in livestock and pets have been reported. Pimenta *et al*. (2018) reported the incidence of leptospirosis in Brazil. Cows that were detected as seropositive for serovar Tarassovi and Grippotyphosa showed clinical symptoms of abortion and recurrent estrus. Aliberti *et al*. (2022) reported that cows experienced abortion, retained placenta, and decreased milk production after being tested serologically reactive to serovars Pomona, Australis, Icterohaemorrhagiae, and Tarassovi. Research conducted in Yogyakarta stated that *Leptospira interrogans* serovar Bataviae was the dominant serovar detected as causing leptospirosis in dogs with clinical symptoms of anorexia, fever, and vomiting accompanied by or without impaired liver and kidney function (Mulyani *et al*. 2019). Apart from being found in animals, wild animals such as bats have been detected

Table 1. MAT test results for bovine serum samples from the Sukabumi and Cianjur regions

Region	Seropositive	Seronegative	Number of samples	Positive serovar
Sukabumi	15 (18.75%)	65 (81.25%)	80	Hardjo and Tarassovi
Cianjur	26 (30.60%)	59 (69.40%)	85	Icterohaemorrhagiae, Cynopteri, Rachmati, Pomona, Grippotyphosa, Hardjo, Bataviae, and Tarassovi

Table 2. Antibody titer of the Leptospira serovar used in the MAT

Serovars	Sample from Sukabumi			Sample from Cianjur		
	1:100	1:400	1:1600	1:100	1:400	1:1600
Icterohaemorrhagiae				1		
Cynopteri				1		
Rachmati				1		
Pomona				1		
Grippotyphosa				1		
Hardjo	11	3		6	12	3
Bataviae				1		
Tarassovi	2			4		

to be reactive to Leptospira in wells, rivers, rice fields, and irrigation canals (Astuti *et al*. 2019; Setyaningsih *et al*. 2022).

The large number of pathogenic Leptospira serovars detected in livestock, domestic and wild animals can act as a source of transmission to humans due to contact with these animals. Farmers, agricultural workers, ranchers, or jobs in a wet environment are groups at high risk for Leptospira exposure because these bacteria can survive in a wet environment (Chadsuthi *et al*. 2017). Previous studies have reported the incidence of leptospirosis in humans in contact with farm animals. In New Zealand, it was reported that workers on cattle farms were positive for serovar Hardjo and Pomona. Furthermore, serological tests were carried out on cattle, and positive results were obtained for serovar Hardjo, Pomona, Copenhageni, Ballum, and Tarrasovi (Yupiana *et al*. 2019).

Antibody titers in the MAT result against the Leptospira serovar used in the test are shown in Table 2. Based on Table 2, antibody titres for positive serum varied from 1:100, 1:400, and 1:1600. One serum examined by MAT can react positively to several serovars used in the test. The working principle of MAT is the agglutination reaction of antigens and antibodies (Riyadi and Sunarno 2019). The serum is declared positive if the antibody titer test is ≥1:100. High titer results (≥1:1000) or a fourfold increase in titer in serum samples indicate a peak antibody reaction, a convalescent stage after infection or an acute infection (Ellis 2015; Tabo *et al*. 2018; OIE 2021). Meanwhile, according to Balamurugan *et al*. (2021) the presence of

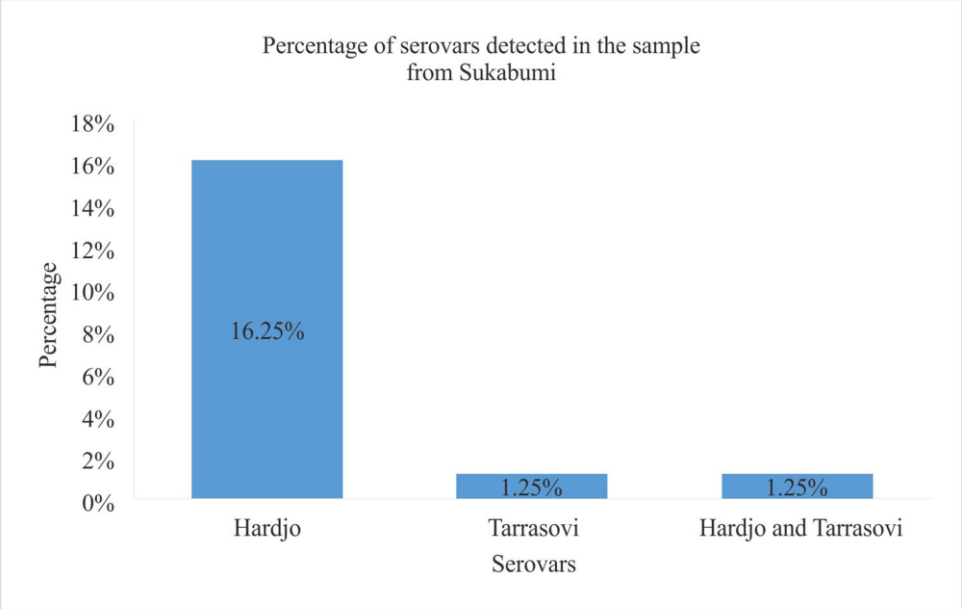

Figure 1. Leptospira seropositive percentage of serum samples from Sukabumi

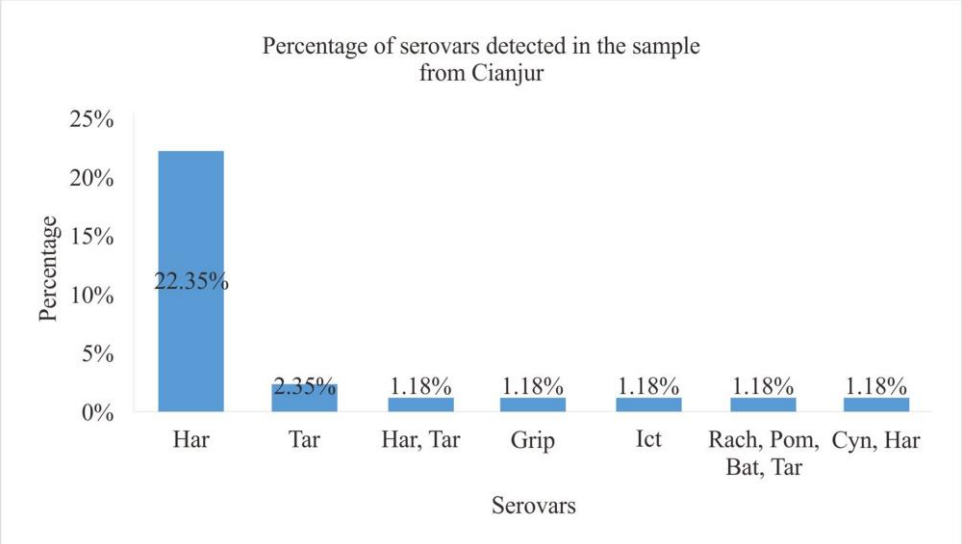

Figure 2. Percentage of seropositive Leptospira serovar of serum samples from Cianjur. Har= Hardjo, Tar= Tarassovi, Grip= Grippotyphosa, Ict= Icterohaemorrhagiae, Rach= Rachmati, Pom= Pomona, Bat= Bataviae, Cyn= Cynopteri

seropositivity with a high titer may indicate a cross-reaction with a different serovar, the possibility of a new infection with the same serovar, a previous infection, or multiple infections. Clinical signs of reproductive problems, including miscarriage, stillbirth of calves, and repeated estrus, were demonstrated by the MAT more than 1:1000 titer results from bovine serum that had positive reactivity to multiple Leptospira serovars (Pimenta *et al*. 2018). However, the level of disease pathogenicity depends on the type of animal, age, type of infecting serovar and number of infecting bacteria (Diarmita *et al*. 2006; Mulyani *et al*. 2021).

In accordance with the research reports mentioned above, serovars detected in humans were also detected in livestock, domestic animals, and wild animals. The epidemiology of leptospirosis is complex and dynamic; therefore, it is necessary to control and prevent leptospirosis in livestock through environmental management, vaccination, and antibiotic therapy (Martins and Lilenbaum 2017).

CONCLUSION

The percentage of positive for leptospirosis in cattle in the Sukabumi was 18.75%, with positive for serovar Hardjo 16.25%, serovar Tarrasovi 1.25%, and both serovar Hardjo and Tarrasovi were 1.25%. Meanwhile in Cianjur, leptospirosis positive serum was 30.60% with positif of one serovar, Hardjo (22.35%), Tarrasovi (2.35%), Icterohaemorrhagiae (1.18%), Grippotyphosa (1.18%), two serovars, Hardjo and Tarrasovi (1.18%), Cynopteri and Hardjo (1.18%), also four serovars, Rachmati, Pomona, Bataviae, and Tarrasovi (1.18%). The most dominant serovar found in both regions is serovar Hardjo. Cattle can be infected with one or more serovars so that they can act as a source of infection in other animals and humans. Cattle infected with more than one serovar will pose a higher risk and disorder.

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