

BRUCELLOSIS SEROPOSITIVITY IN SHEEP SLAUGHTERED AT SMALL RUMINANT SLAUGHTERHOUSE IN BOGOR REGENCY

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ABSTRACT

Brucellosis is among the important diseases in livestock because the disease infects multiple species of animals and causes economic loss. Brucellosis in sheep is generally caused by *Brucella melitensis* and/or *Brucella ovis*. This study aimed to detect seropositive brucellosis in sheep. Serological tests used in this study was a parallel test between Rose Bengal Test (RBT) and Complement Fixation Test (CFT). Samples were collected from 150 sheep slaughtered in small ruminant slaughterhouse, Sentul, Bogor Regency. Seropositive proportion of brucellosis in sheep based on parallel test RBT and CFT was 52% (78/150).

Key words: brucella, brucellosis, seropositive, sheep

ABSTRAK

Bruselosis merupakan salah satu penyakit pada ternak yang menjadi perhatian dunia karena penyakit ini dapat menyerang berbagai spesies hewan dan menimbulkan kerugian ekonomi. Bruselosis pada domba umumnya disebabkan oleh *Brucella melitensis* dan/atau *Brucella ovis*. Penelitian ini bertujuan mendeteksi bruselosis pada domba secara serologis. Uji serologis yang digunakan pada penelitian ini adalah Rose Bengal Test (RBT) dan Complement Fixation Test (CFT) secara paralel. Sampel diperoleh dari 150 ekor domba yang dipotong di tempat pemotongan hewan ruminansia kecil, Sentul, Kabupaten Bogor. Proporsi seropositif bruselosis pada domba berdasarkan RBT dan CFT secara paralel sebesar 52% (78/150).

Kata kunci: brucella, bruselosis, seropositif, domba

INTRODUCTION

Brucellosis in livestock has been a concern since it has spread almost worldwide and caused economic losses for the farmer. The female livestock will experience an abortion and decreased milk production. Besides causing a serious condition in animal, brucellosis is also a zoonosis. Advisory Committee on Dangerous Pathogens (ACDP) classified *Brucella* into Risk Group III, which means that the causative bacteria is the biologic agent that has a serious impact on human health status. The impact especially affects the worker who had direct contact with the infected cattle (HSE, 2013). There are ten identified species of *Brucella* (B) related to animal health status and six among them infect the land mammals, those of which are *B. abortus*, *B. melitensis*, *B. ovis*, *B. suis*, *B. canis*, and *B. neonatome*. Each species has different pathogenicity or host type (OIE, 2016).

The main cause of brucellosis in sheep is *B. ovis* and the three biovars of *B. melitensis*, but sporadically brucellosis in sheep is also caused by *B. abortus* and *B. suis* (OIE, 2009). The bacteria *B. melitensis* has three biovars, biovara 1, 2, and 3, and is the most pathogenic species of *Brucella* because of its ability to attack other ruminants and its zoonotic property. These three biovars are classified based on the antigen A and M which are parts of the smooth-Lipopolysaccharide-S-LPS-molecule. The presence of antigens A and M can be seen from the ability of each biovars to agglutinate monospecific antisera A and M biovar 1 is able to agglutinate mono-specific antisera M, biovar 2 is able to agglutinate mono-specific antisera A, biovar 3 is

able to agglutinate mono-specific antisera A and M (EC, 2001; Affi *et al.*, 2011). In contrast with *B. melitensis*, *B. ovis*, which only infects sheep, is not too pathogenic and zoonotic (EC, 2001; Xavier *et al.*, 2010).

The examination to diagnose brucellosis usually needs microbiology, serology, and molecular testing. Microbiology and molecular testing aim to observe the presence of *Brucella* or the deoxyribonucleid acid (DNA) of *Brucella*. Samples for bacterial isolation and polymerase chain reaction (PCR) examination were collected based on clinical symptoms shown by the patients. The most commonly used samples are vaginal swab, fetuses, fetal membrane, milk, reticulo-endothelial system (spleen and lymph nodes), and uterus. Serological examination is performed to observe the presence of antibodies against *Brucella* bacteria, and the most commonly test used is blood serum. Serological techniques used include serum agglutination test (SAT), complement fixation test (CFT), enzyme-linked immunosorbent assay (ELISA), and fluorescence polarization assay (FPA) (OIE, 2016).

Some studies stated that brucellosis detection in small ruminants using RBT technique alone is not enough. RBT technique should be performed in parallel with CFT to improve sensitivity, thus, increasing the chances of detection of infected individuals and improving disease control programs (EC, 2001).

Some studies have been done to detect brucellosis in goats. Primatika *et al.* (2016) said that seroprevalence of *Brucella* sp. in goats in Gunung Kidul District, Central Java, was at 9.6%. Mujiatun *et al.* (2017) stated that the proportion of seropositive

brucellosis in goats at Ambarawa market, Central Java and Ngawi District was at 2.3%. This study aimed to serologically detect brucellosis in sheep, especially sheep that are slaughtered cut in Animal Slaughterhouse, Bogor District.

MATERIALS AND METHODS

Sample Size Determination

The sample size was calculated based on the disease detection formula with a confidence interval of 95% (Dohoo *et al.*, 2003). There is no prevalence data for brucellosis in goat and sheep in Bogor Regency since there has been no surveillance of brucellosis for goat and sheep done yet (Sugiyanto, 2014). Based on the Decree of the Minister of Agriculture Number 828, 1998 about observation, traffic control, vaccination, and test and slaughter, an area is stated as free of brucellosis if the prevalence is less than 2%. For that reason, we used 2% as presumed prevalence and the sheep population in West Java in 2016 was 12.462.091 (Kementan, 2016), therefore the sample size was 150.

$$n = \left[1 - (1 - p)^{\frac{1}{d}} \right] \left[N - \frac{D - 1}{2} \right]$$

n = size of sample

N = size of population

D = the predicted positive-tested animal sample (prevalence x number of the animal population)

p = level of confidence (95%)

Selection and Examination of the Sample

A total of 150 ewes aged 8 month to 1.2 years were selected randomly from some regions in West Java namely Bogor District, Cianjur District, and Garut District. The sheep were placed in the same cage for 1-3 days before slaughtering. The blood was collected then, stored at room temperature until serum is formed. The serum was separated aseptically and stored at -20° C until it was used for rose bengal test (RBT) (EC, 2001) and complement fixation test (CFT) (OIE, 2016). RBT and CFT techniques were performed in parallel, a positive result of the examination techniques can be interpreted as seropositive brucellosis (Dohoo *et al.*, 2003; Coelho *et al.*, 2013).

Rose Bengal Test (RBT)

A total of 25 µL RBT antigen were added to 75 µL sample serum and then homogenized. The interpretation of the test results were negative if the agglutination of the serum-antigen mixture did not occur and the color seemed homogenous rose/pink and positive if the agglutination of serum-antigen occurred.

Complement Fixation Test (CFT)

In this test, microplate-96 well U-bottom was used. A total of 25 µL of inactivated serum was inserted to the microplate at first and second rows. A total of 25 µL CFT buffer was added to the entire microplate well, except the first rows. Serial dilution was done by transferring 25 µL of serum into the second rows. Approximately, 25 µL of antigen was added into each well, except the first rows, subsequently, 25 µL of complement was added into each well. Incubation at 37° C was done for 30 minutes or 4° C overnight. Then 25 µL of ewe red blood cell that have been sensitized with hemolysis were added to each well. Incubation was done for 30 minutes at 37° C or 4° C for one night while homogenized using shaker. Positive reaction results in the absence of hemolysis or imperfect hemolysis, characterized by the deposition of erythrocytes at the bottom of the well. The result of a negative reaction in the event of complete hemolysis is characterized by the absence of red blood cell deposits at the bottom of the well (OIE, 2016). Data analysis was done by calculated the proportion of brucellosis seropositive sample from data obtained in this study.

RESULTS AND DISCUSSION

There were 4 out of 150 samples revealed positive (0.02 %) result using RBT technique and 76 out of 150 samples were positive (50.6%) by CFT technique. From 4 positive samples with RBT technique, two of them were reveal positive result using CFT technique. A combined results from that two techniques obtained seropositive brucellosis of 52% (78/150) (Table 1).

RBT technique was used as a brucellosis filter test. *B. abortus*, *B. melitensis*, and *B. suis* were Brucella species with sharing the same lipopolysaccharide (LPS) arrangement, i.e. smooth LPS (S-LPS) with the combination of antigen A and M in lipopolysaccharide

Table 1. The numbers of sample size and the proportion of positive samples from each region

No	Origin	Sample size	Proportion of positive samples
1	Bogor District	109	70/109
2	Cianjur District	23	6/23
3	Garut District	18	2/18
Total		150	78/150

Table 2. Rose bengal test (RBT) and complement fixation test (CFT) test results on 150 serum samples

Complement fixation test (CFT)	Rose bengal test (RBT)		Total
	Positive	Negative	
Positive	2 ^p	74 ^p	76
Negative	2 ^p	72	74
Total	4	146	150

^p seropositive

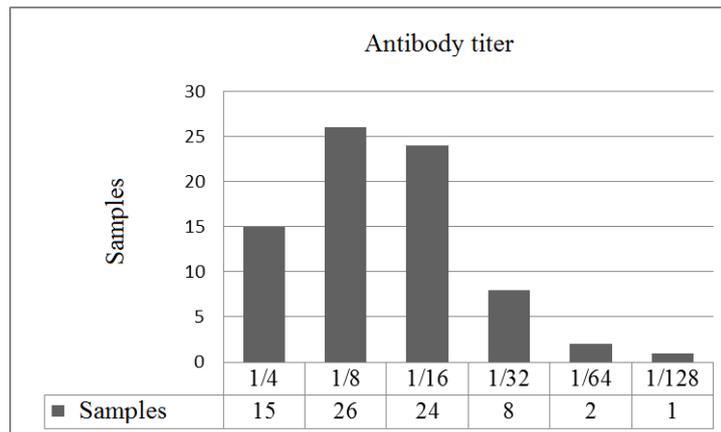


Figure 1. The results of the CFT examination revealed antibody titer obtained between $\frac{1}{4}$ to $\frac{1}{128}$

in each species or biovars. The S-LPS component was the main virulent factor of these three *Brucella* species, comprising of three main components, namely lipid A, oligosaccharide core, and o-polysaccharides (Blasco and Molina-Flores, 2011; Skendros *et al.*, 2011). To improve the sensitivity of the test, the ratio between antigen and serum of the samples examined was 1:3 (EC, 2001; Diaz *et al.*, 2011; OIE, 2016; Primatika *et al.*, 2016).

The CFT technique was used as a subsequent examination of RBT techniques. But in this study the CFT technique was done in parallel with the RBT technique, to increase the chance of detection of infected animals. This is proven that when the CFT technique was done prior to the test of RBT technique then many positive samples on the CFT technique were not detected during the examination with RBT technique. CFT technique was a quantitative test which can be used to calculate antibody titers against *Brucella*. The CFT technique performed in this study used antigen from *B. abortus*.

Figure 1 shows the distribution of antibody titers from CFT examination. Fifteen samples had antibody titer of $\frac{1}{4}$, 26 samples had antibody titer of $\frac{1}{8}$, 24 sample had antibody titer of $\frac{1}{16}$, 8 samples had antibody titer of $\frac{1}{32}$, 2 samples had antibody titer of $\frac{1}{64}$ and 1 sample had antibody titer of $\frac{1}{128}$. RBT antigen will detect positive result if the minimum serum dilution was at $\frac{1}{16}$ (OIE, 2016), negative result at RBT and positive at CFT were probably due to an antibody titer in the serum of less than $\frac{1}{16}$.

CONCLUSION

The proportion of seropositive brucellosis in sheep slaughtered in small ruminant slaughterhouse in Bogor Regency was 52% (78/150). Further studies using isolation and identification of *Brucella* species to identify the species that attack the slaughtered sheep at small ruminant slaughterhouse of Bogor Regency are required.

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