Prevalence of *Brucella abortus* antibody in serum of Bali cattle in South Sulawesi

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Abstract. Brucellosis in cattle is a zoonotic disease that causes abortion. This disease is highly contagious and difficult to control. Clinical symptoms Brucellosis is not pathognomonic and often asymptomatic thereof laboratory assessment is needed for serological detection of Brucellosis. A total of 235 blood samples were obtained from Bali cattle farms in three regencies, Enrekang, Barru, and Soppeng. Serological detection was carried out by the RBT method and further confirmed by the CFT method. Results showed that Brucellosis seroprevalence was 12.76%. The highest incidence rate was in Enrekang Regency with a value of 15.60%, followed by Soppeng and Barru Regency, of 9.09% and 2.50%, respectively. These results indicated that Brucellosis is still enzootic in the South Sulawesi region.

1. Introduction

Brucellosis is the main zoonotic disease that causes abortion in animals throughout the world. This disease is highly contagious and difficult to control, causing huge economic losses for farmers. Brucellosis is caused by the bacterium *Brucella* which is an intracellular microorganism. The main transmission of Brucellosis in animals occurs through contact with materials contaminated with the placenta, fetus, fetal fluid, and vaginal fluid from abortion or parturition. Other transmissions also occurred through contact with the skin, conjunctival membranes, inhalation (respiratory tract mucosa), per-oral (gastrointestinal/oropharyngeal mucosa), vertical transmission, and contact with contaminated milk. The disease could be asymptomatic after the first abortion, but the infected animals became carriers and released the organism through milk. This bacteria also released through urine, feces, hygroma fluid, saliva, nasal secretions and ocular fluid [1,2].

Transmission of brucellosis from animals to humans may occur through the digestive tract, lungs, mucosa, and skin. The infection then spread to other organs through the blood circulation and the lymphatic system [3]. Symptoms of brucellosis in humans are greatly varied, from non-specific, flu-like symptoms in an acute condition, undulant fever, arthritis, orchitis, and epididymitis in males. This occupational disease often infects slaughterhouse workers, butchers, and veterinarians. The disease usually transmitted through contact between injured skin and infected animals or contaminated materials [4].

Word Health Organization (WHO) categorized *Brucella* as a risk group III microorganism [5]. Brucellosis had spread in 26 provinces in Indonesia and with a lack of control, the country was losing 385 billion/year [6]. A study conducted by Muflihana et al. [7] detected *B. abortus* in Bali cattle in Pinrang Regency, South Sulawesi with seroprevalence of 18.3%. Clinical symptoms of infected cattle Brucellosis
is non-pathognomonic and often asymptomatic, which cause difficulty in early detection for Brucellosis control. Thus, laboratory testing is very urgent for the serological detection of Brucellosis.

2. Materials and Methods

2.1. Blood sampling and serum preparation
Blood samples were collected from cattle farm at three regencies in South Sulawesi province, Enrekang, Barru, and Soppeng. Blood was obtained aseptically from the jugular vein using plain vacutainer without anticoagulant. Further, sera were separated and stored at the temperature of -20°C.

2.2. Serological test
The serological test was carried out by Rose Bengal Test (RBT) and Complement Fixation Test (CFT) method. For the RBT method, a total of 25 μl serum was taken using a 10-100 μl micropipette and a 25 μl RBT reagent was placed in the well plate (WHO haemagglutination tray). The plate then placed on top of the shaker to mix well. The agglutination reactions were observed after 4–5 minutes. The serum which showed positive results then confirmed by the Complement Fixation Test (CFT) test. Serum with CFT titers 1: 4 (1/4) or more determined as positive for brucellosis.

3. Results and discussion

3.1. Blood sampling
A total of 235 blood samples were collected from Enrekang, Barru, and Soppeng; each of 173, 40, and 22, respectively.

3.2. Serological test results
The serological test for Brucellosis diagnosis has been carried out since more than a decade ago, with a simple agglutination test. This test was cheap, easy, and rapid even though the assessment of results was quite subjective. The test was also known to give false-positive results due to cross-reactions with other microorganisms [8]. The Rose Bengal Test (RBT) is an acidified antigen modification test recommended by the OIE for screening Brucellosis in cattle [5]. For confirmation of diagnosis, the RBT test result must be followed by other serological tests, one of which is the Complement Fixation Test (CFT). Nielsen [8] suggested that the CFT test is very valuable as a confirmatory test for an eradication program and is a serological test method recommended by OIE for international trade.

Serologic test results were presented in table 1. The RBT test showed 38 of 235 samples were positive for Brucellosis. The confirmation test by CFT showed that 30 of 38 samples were positive for Brucellosis. The RBT and CFT test showed that of the 235 Bali cattle tested, 30 were infected giving a seroprevalence of 12.76%. These results indicated that Brucellosis was still enzootic in the South Sulawesi region.

| Regency  | Sample number | RBT positive result | CFT positive result |
|----------|---------------|---------------------|---------------------|
| Enrekang | 173           | 35                  | 27                  |
| Barru    | 40            | 1                   | 1                   |
| Soppeng  | 22            | 2                   | 2                   |
| Total    | 235           | 38                  | 30                  |

The study also showed that the incidence rate of Brucellosis was varied in sample origins. The highest number of positive results, 15.60%, was obtained from Enrekang regency. In this area, samples were collected from cattle with extensive system farming; the cattle were raised on pasture. The male and the female cattle were grazing together; thus natural mating often occurs. The condition enabled the transmission of *Brucella* from infected animals to other animals in the grazing field. Besides, if abortion
occurred, the fetus and the placenta will also become the source of transmission to other cows in the grazing field. In contrast to Enrekang Regency, the samples from Soppeng and Barru Regency were collected from cattle farms with intensive and semi-intensive systems. In intensive and semi-intensive systems, the risk of disease transmission is lower. The serological test showed the incidence of Brucellosis in Barru and Soppeng regency was 2.50% and 9.09%, respectively.

Male animals infected with *Brucella* also showed symptoms of epididymitis, seminal vesiculitis, orchitis or testicular abscesses [9]. Brucellosis is a reproductive disease that attacks animals with predilection in the reproductive tract, especially the adult uterus. Allantoic factors, such as erythritol, steroid hormones, and several other substances were known to stimulate the growth of *Brucella* [10]. *Brucella* tropism in male and female reproductive organs is caused by erythritol which stimulates the growth of the organism. However, *Brucella* has also been isolated in the reproductive organs where erythritol levels were not detected. Erythritol is a sugar alcohol compound synthesized in the ungulate animal placenta and is able to stimulate the growth of virulent strains of *B. abortus* [11].

Rose Bengal Test (RBT) is the main serological test method for diagnosing Brucellosis. The method has high sensitivity, but low specificity. Barroso et al. [12] stated that RBT test sensitivity reaches more than 99%. Therefore, confirmation of results is needed with other more specific methods, such as ELISA and CFT. The CFT test is more commonly used as a confirmation test following RBT. Research conducted by Chisi et al. [13] on evaluating serological test methods for the diagnosis of Brucellosis showed that the RBT + CFT test combination provided 100% specificity.

4. Conclusion

The Serological tests on the blood serum of Bali cattle in three regencies in South Sulawesi showed a seroprevalence rate of 12.76%. The disease incidence was varied among samples origin. Samples from cattle reared with extensive farming systems showed a higher incidence than those from intensively reared cattle.

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