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Detection of Brucellosis in Bali Cattle (*Bos Sondaicus*) in Bolo District, Bima Regency

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Abstract

This study aims to determine the incidence of Brucellosis in Bali cattle in Bolo district, Bima regency, based on serological tests (seroprevalence). The sample used in this study was cattle serum. Serum samples were obtained from 70 Bali cattle aged over one year. The detection method, namely the Rose Bengal Test, detects the incidence of Brucellosis. Samples in the form of serum are reacted with Brucella sp. antigen to see antibodies in the serum. The working principle of the Rose Bengal Test is to see whether there is agglutination or not. The results of this study will be an indicator to determine the presence or absence of Brucellosis in Bali cattle in Bolo district, Bima regency. Then the monitoring and control can be established by relevant stakeholders, especially the Livestock and Animal Health Service of Bima Regency. The data obtained from serum samples that describe the positive or negative for Brucellosis is presented descriptively by explaining each sample origin's seropositive and seronegative results. The results showed that in all samples from Bali cattle in the District of Bolo, as many as 70 samples reacted negatively to the Rose Bengal Test. This result indicates that the cattle on Sumbawa Island are not infected with Brucellosis.

Keywords: Brucellosis, Reproductive Disorders, Bali Cattle, Rose Bengal Test

Introduction

Cattle are livestock which quite in demand because of the high potential in economic value. Cattle commodities play a significant role in increasing income and fulfilling daily community nutrition. This is due to the high protein content produced by cows in meat and milk.

One such effort made by the government to satisfy the needs for meat and milk for the community is to develop the cattle farms in several districts, including in Bima regency, West Nusa Tenggara. Bima regency, especially the Bolo district, is an excellent agricultural area for cattle farming uses because it has suitable climate conditions, vast land and crop resources for animal feed supply, and farmers' ability in livestock farming.

Nevertheless, the development of production and productivity of cattle in Bolo district, Bima regency, has hardly progressed, which is usually caused by various diseases that infect cattle, such as Brucellosis. Brucellosis is a highly contagious venereal disease caused by bacteria of the Brucella genus.

The literature study by Djaenudin and Riza (2013) stated that currently, there are seven species of Brucella sp. known, namely B. abortus (cow), B. melitensis (goat), B. suis (pig, hares, wildebeest, caribou, (rodent), B. canis (dogs species), B. ovis (sheep), B. neotomae (desert wood rat), B. maris (marine mammal). Of the various Brucella species, most of which are pathogenic to humans, only B. ovis and B. neotomae are not pathogenic to humans, while B. maris is unknown. The Brucella genus is categorized as a zoonotic disease and classified as a microorganism of the BSL III group (Biosafety level 3) (Mujiantun, et al., 2016). This disease is one of the strategic contagious animal's diseases that has received a national priority in control and prevention by the government under the Minister of Agriculture Decree, Number 4026/Kpts/OT.140/4/2013.

Brucellosis transmission can be through direct contact with animals and consuming dairy products, so it is categorized as a zoonotic disease (Dwi, et al., 2018). Brucella sp. are the cause of Brucellosis, which is a facultative intracellular gram-negative bacterial pathogen of vertebrate species, including humans. These bacteria are gram-negative, small, aerobic, coccobacillus-shaped, non-motile, and do not produce spores (Mugabi, 2012).

Brucellosis in female cattle can cause spontaneous abortion in the third trimester of gestation, but after 1-2 abortions, birth and milk production return to normal. In some cases, the infected cattle become carriers or infertile (Sharma et al., 2016). Brucellosis has a high economic impact related to the low productivity of infected animals and the high cost of treatment in humans due to the long duration of treatment (Aparicio, 2013).

Brucellosis generally infects cattle, buffalo, goats, sheep, and pigs. This disease can also infect various animals and is transmitted to humans or zoonotic (Moreno, 2014). In female animals, the disease is characterized by abortion and retention of the placenta, whereas in males, it can cause orchitis and infection of the accessory glands (Manish et al., 2013). Brucellosis in humans is known as undulant fever due to the bacteria Brucella that causes fluctuating fever. Brucellosis was first recorded in Indonesia in 1953. Since then, Brucellosis reactors have been found widely on major islands in Indonesia, such as Sumatra, Java, Kalimantan, Sulawesi, and Timor Island, except for Bali (Dirkeswan, 2015).

The Rose Bengal Test (RBT) and Complement Fixation Test (CFT) are standard tests applied by OIE. The RBT test serves as an initial screening test. Therefore, detection of Brucellosis in Bali cattle in the district of Bolo, Bima regency, West Nusa Tenggara, can be done by using the RBT test. This test strongly supports the purpose of this study, namely to measure the incidence of Brucellosis in Bali cattle in the Bolo district based on serological tests (seroprevalence) using a test method in the form of the Rose Bengal Test (RBT).

Meterials and Methods

Materials

The study was conducted at the regional farm of Bolo district, Bima regency, West Nusa Tenggara Province, as a place for blood sampling. This study used 70 Bali cattle from 8 villages in Bolo district, Bima regency. The blood samples were collected as much as 5 ml through the jugular vein and centrifuged for several minutes. The RBT was conducted at the Vocational Laboratory of PDD Bima University of Mataram, with 49 smallholder farmers applying intensive maintenance livestock systems. The data case of reproductive disorders in cows in the form of a history of abortion and repeated breeding is based on the number of services per conception (S/C).

Method

Sample collection

70 Serum was collected from centrifugation of blood samples from Bali cattle in 8 villages (Rasabou, Bontokape, Tambe, Ngembe, Leu, Daru, Sonco, Kananga) in Bolo sub-district, Bima district, West Nusa Tenggara, Indonesia.

Equipment for taking serum and blood samples is plain venoject, venoject with Ethylen Diamine Tetra Acetic Acid (EDTA) and Non EDTA, needle veno and gloves. The equipment used for RBT is the RBT plate, stirring rod, and 3 ml syringe (sterile).

The materials used for sampling were 70% alcohol and cotton. The materials used for RBT in this study were RBT antigen and negative serum.

Sample Examination with Rose Bengal Test (RBT)

Before the RBT test, serum samples and RBT antigen were placed at room temperature (22 ± 4°C). Serum samples and negative controls were taken using a 75 L pipette and placed on the RBT plate. The antigen was shaken slowly before use, and then 75 μl of the antigen was added to the serum and control specimens. The serum and antigen mixture were homogenized to form a circle or oval with a diameter of 2 cm using a stirring rod for each sample and then incubated at room temperature for 4 minutes.

The reading of RBT agglutination after 4 minutes of incubation, the sample was declared positive if there was lump-like sand. Positive RBT results consist of positive results (+++), namely: complete agglutination (clear and clear fluid); Positive (++), namely: agglutination in the form of fine sand with slightly clear liquid and fairly clear boundaries; and positive RBT (+), namely: agglutination in the form of fine sand, not clear liquid with borderlines (OIE, 2009).

Data analysis

We used a descriptive analysis technique to analyze the results of antibody tests for Brucella abortus bacteria using the RBT.

Results and Discussion

Brucellosis

Brucellosis is a disease transmitted from animals to humans (zoonosis) that occurs through

direct contact with infected animals, drinking milk from infected animals, secretion of uterine fluid, placental tissue, fetus, colostrum, and breathing air polluted by bacteria that cause Brucellosis, namely Brucella sp. Brucellosis affects public health in almost all countries (Acha and Boris, 2003), and makes Brucellosis is one of the strategic infectious to animals because its transmission is very fast between borders and across regions, thus requiring strict animal traffic regulations (Chin, 2006 and Bosilkovski, et al, 2015).

The most common sign and symptoms of Brucellosis in female cattle are abortion with or without retention secundines (retention of the placenta), lethargy, decreased appetite, thinness, discharge of purulent fluid from the vagina, hygroma, arthritis, and orchitis. Post-mortem changes that are seen are the thickening of the placenta with patches on the surface of the chorion layer. Fetal fluid looks cloudy yellow-brown and sometimes mixed with pus (Alnemri et al., 2017).

The main symptom of Brucellosis in male cattle is a suppuration process in the testes, followed by necrosis. Animals that can survive will become carriers and have the potential to transmit the bacteria. The secondary infections of Brucella are persistent in the mammary and supra-mammary duct and genital lymph nodes (Alnemri et al., 2017).

The pathogenesis of Brucellosis is the bacteria entering the body through the entry port mucosa and then penetrating the lymphoepithelial cells and phagocytosed by neutrophils and macrophages and then infecting the lymphoglandula.

Phagocytosis does not occur because these bacteria have antiphagocytic substances, namely protein guanine 5-monophosphate, which can survive and replicate in neutrophil cells. If the defense system cannot overcome the infection, bacteremia will appear after 10-20 days and persist for 30 days to 2 months after infection. After bacteremia in pregnant animals, the bacteria will enter the placenta of pregnant animals and the udder area.

Infection in non-pregnant animals will extend to the udder area and is often without clinical signs or lesions. Bacteria in macrophages circulate in lymphoid tissue and are localized in the reticuloendothelial system of the liver, spleen and spinal cord, kidneys, and joints, and then resulting in arthritis and hygroma. Hygroma occurs due to infection in the joint membrane so that it contains transparent fluid, acute fibrillae illness, or pus so that a very conspicuous lump is seen (Mohamed et al., 2014).

The Brucellosis infection disease greatly impacts economic prosperity in various countries and adversely affects the animal industry and human health (Gwida et al, 2010). The effects of Brucellosis on livestock are fatal, namely permanent or temporary loss of cow offspring, infertility, and reduced milk production. According to Thakur et al, (2002), farmers and consumers in developing countries do not know about Brucellosis infection, the possible impacts and risks, and how to handle this disease.

Bali cattle farm in the Bolo district

The Balinese cattle rearing system in Bolo sub-district, Bima district, currently has many breeders who maintain intensive care, with some of the pens having cement mats. However, the condition of the base varies, some are in good condition, namely flat and robust, and some are not. Sources of animal feed, especially forage, are obtained from the land around the local area. Meanwhile, the feed in the form of tofu dregs was obtained from tofu producers in Bolo District. There are varies in the status and ownership of livestock, namely privately owned or as custodians with a population of 1 to 50 animals

with an average of 2-5 animals/breeder.

Identification of Brucellosis in Bali cattle in Bolo district

Bali cattle blood samples were collected in 8 villages of Bolo District, namely 6 samples from Rasabou, 8 samples from Bontokape, 8 samples from Tambe, 16 samples from Nggembe, 2 samples from Leu, 11 samples from Daru, 6 samples from Sanolo, and 13 samples form Kananga. The collecting samples were conducted in August 2021 with a total of 70 samples. The blood sample was then centrifuged, and part of the serum was taken for serological testing.

Serological testing of all samples was tested by the Rose Bengal Test (RBT) method, and the results showed that all samples in Bolo district, Bima regency, showed the negative results for Brucellosis (table 1). This result indicated by no signs of agglutination on the RBT plate in serum mixed with Brucella antigen (Figure 1). The findings are directly in line with the decision of the minister of agriculture number: 97/kpts/po.660/2/2006 regarding the statement that Sumbawa Island, West Nusa Tenggara Province, is free from the infectious animal disease Brucellosis in cattle and buffalo (Decree of the Minister of Agriculture, 2006).

Table 1. The sample size and the results of the *Rose Bengal Test*(RBT)

No	Villages	Sample Size	Positive RBT	Negative RBT
1	Rasabou	6	0	6
2	Bontokape	8	0	8
3	Tambe	8	0	8
4	Ngembe	16	0	16
5	Leu	2	0	2
6	Daru	11	0	11
7	Sonco	6	0	6
8	Kananga	13	0	13

The Brucella antigen used in the RBT examination was the Brucella antigen which had smooth colonies stained with Rose Bengal, with a buffer solution up to pH 3.65. Neutral pH conditions in the RBT test can measure the presence of IgM and IgG. The Rose Bengal Test, which has a pH level of 3.65, can prevent agglutination with IgM and appears to measure only IgG (Kaltungo et al, 2014). The pH is 3.65 from the RBT antigen, and results in temporary inactivation of IgM and leaves only intact Brucella IgG antibodies so that in the RBT test, the antibody titer of IgG reacts with antigens from RBT, which can cause agglutination reactions (Klein, 1991).

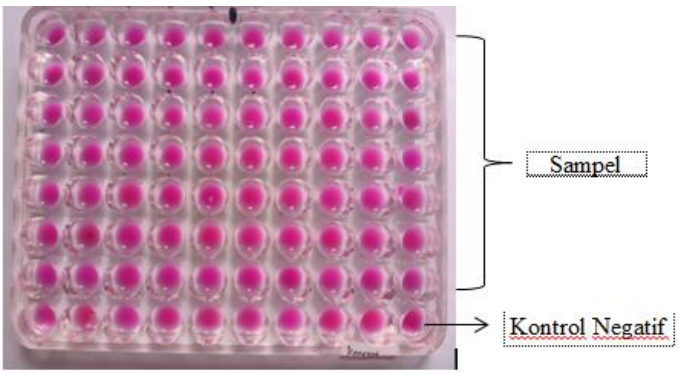

Figure 1. The serum examination results using the *Rose Bengal Test* (RBT)

In immunology, agglutination refers to the joining of particles together. This process is essential as part of the immune system, the response process that organisms use to fight disease. Agglutination is the process of binding antigens by antibodies. The body will recognize antigens derived from bacteria as foreign so that the body triggers the formation of immunity in the form of antibody products specific to the triggering antigen. The presence of bonds between antibodies with specific antigens forms large clumps. The test by RBT is to detect the presence of antibodies to Brucella in the serum. If a cow is infected with Brucella, the body will produce antibodies useful for body protection.

If the examined serum sample contains antibodies, then the addition of the Brucella antigen will cause a bond to the antibody. The presence of this bond is indicated by the formation of precipitation or clots in the serum. If there is no antibody to Brucella, then there is no bond with the antigen so that the sample remains homogeneous. In the absence of agglutination in the entire sample, there are no antibodies in the sample. In other words, the cows used are free from Brucella infection.

Sumbawa Island is free against Brucella disease not just happen by nature but because of the hard work of the relevant agencies supported by farmers and breeders. Disease control and monitoring are carried out regularly and continuously. In the last 5 years, the government has implemented the Technical Guidelines for the Eradication and Control of Brucellosis in Indonesia from the Directorate General of Livestock in 1998, namely by utilizing the testing method and slaughter in lightly infected areas (prevalence <2%). Incidence above 2% is vaccinated for 5 consecutive years. This program is also followed by continuous monitoring of the condition of the livestock. The RBT test is routinely carried out, and if a positive result is found, it is cut.

Disease monitoring and control are also carried out on islands adjacent to Sumbawa Island. A test and slaughter program is also implemented. Sumbawa Island, which in the early 1990s was a Brucellosis area, has now been declared free. The Defense Minister decree No 52/Kpts/PD.630//1/2015 stated Sumbawa Island as a Brucellosis-free area, proves successful in handling brucella disease. In the future, all areas in East Nusa Tenggara will be declared Brucella-free areas.

Brucellosis is generally asymptomatic in pregnant cows. After Brucella abortus and Brucella militants infection, pregnant cows will develop placentitis, which usually undergoes abortion between the fifth and ninth months of gestation.

In the absence of abortion, bacterial excretion occurs in the placenta, placental fluid, and vaginal discharges. The udder and associated lymph nodes may also become infected, and bacteria may be excreted in the milk. Milk that comes from infected cows and contains bacteria will potentially be a source of infection when given to calves or other cows, possibly even for human infection (Naim and Hidayat 2007).

Selection of Bali cattle breeds should always be made to reduce the number of cases of Brucellosis. Newly purchased seeds or cattle should be tested first, or there is a certificate that states Brucellosis is negative. Newly imported animals outside the cage or local area must be placed in a separate cage or quarantine area before being put in the cage/area with existing animals. Then an examination is carried out to make sure the animal is not positive for Brucellosis or other diseases. The transfer of animals from one cage to another should be carried out under animal health workers' supervision to prevent disease transmission due to animal transportation.

Conclusion

Based on the results of the Brucella antibodies detection in Bali cattle in Bolo District, Bima Regency, with the Rose Bengal Test method, it can be concluded that there were no samples that were positive for Brucellosis, which was indicated by no agglutination or sand formation. The declared negative sample was 100%, with a total sample of 70 samples.

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