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LAMPIRAN

Lampiran 1. English Ver.

Brucellosis Cases in Bali Cattle in Beroangin Village, Mapilli Subdistrict, Polewali Mandar District

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ABSTRACT. Brucellosis is a disease that can cause arthritis, hygorma, orchitis in bulls, or abortion in pregnant cows due to infection with *Brucella abortus,* which is zoonotic. A case was reported by a farmer in Beroangin village, Mapilli subdistrict, Polewali Mandar district, where a 4-year-old cow on his farm had an abortion. A physical examination found no pathological changes, so blood sampling was done for the Rose Bengal Test (RBT). The results of the Brucella reactive cow test were continued in the confirmation test in the form of the Complement Fixation Test (CFT). The treatment provided was separation of reactive cows, education of farmers regarding brucellosis, and supportive treatment.

A. INTRODUCTION

Cattle are livestock that are an important source of animal protein for the nutritional adequacy of people. The demand for beef in Indonesia in 2020 was around 453,418 tons and increased in 2021 to 487,802 tons (Statistics Indonesia, 2021). Cattle, as farm animals, are no different from other animals, capable of being affected by various livestock diseases. Livestock diseases are health disorders of farm animals caused by genetic defects, degenerative processes, metabolic disorders, trauma, poisoning, parasite infestation, prions, or infection with pathogenic microorganisms. Among these diseases, parasites, prions, and pathogenic microorganisms are the most dangerous to both animals and humans (Winarsih, 2018).

Brucellosis is a disease of cattle caused by bacteria of the genus Brucella, which consists of various species according to the type of animal infected, such as *B. abortus* in cattle, *B. melitensis* in goats or sheep, and *B. suis* in pigs. However, most Brucella species are non-specific and can infect a variety of other animals. Brucellosis in cattle can cause clinical signs such as arthritis, orchitis in bulls, or abortion in pregnant females (OIE, 2022). Clinical signs that appear in cattle with chronic brucellosis are hygroma, which is a fluid-filled enlargement of the knee joint of cattle (Amaliah et al., 2022). Brucellosis is a disease that can be found globally and is zoonotic, where transmission can be through direct contact with infected animals, through eating or drinking contaminated animal products, or through the air by inhaling the causative agent. Brucellosis in humans causes symptoms similar to the flu, namely fever, weakness, malaise, and weight loss (WHO, 2020). The characteristics of fever that arise in humans infected with Brucella sp. are typical, namely undulant fever (Novita et al., 2016).

Brucellosis disease in livestock can have a very high economic impact. Losses arise due to the low productivity of affected animals and the high cost of treatment due to the long duration of treatment. The transmission of brucellosis is very fast between animals, so the eradication of brucellosis in an area is difficult, and until now, several regions of Indonesia have not been free from brucellosis (Novita, 2016). The losses caused by brucellosis in cattle in Indonesia each year reach 3.6 trillion (Wilujeng et al., 2020). The purpose of writing this final project, in addition to completing academic requirements, is also to be a reference on brucellosis cases in cattle.

B. CASE DESCRIPTION

1. Anamnesis

A case was reported by a farmer in Beroangin village, Mapilli sub-district, Polewali Mandar District. According to the farmer, the cow had aborted once, and other cows kept with the cow had also aborted. The cow had a history of giving birth before the miscarriage. The cow was ±4 years old. The cows are kept semi-intensively, where they are left loose in the farmer's field during the day and penned in the afternoon. The cow's feed comes from elephant grass. The cow's appetite was good. At the time of the abortion, the cow did not have placental retention.

2. Clinical Examination

The farmer's cattle is a Bali cattle. The examination found the animal's temperature to be 38.6°C, with a breathing frequency of 28 times per minute. Rectal palpation found that the ovaries had relatively no difference in size, accompanied by a palpable ovarian surface with relatively no corpus luteum found in both the left and right ovaries, a relatively symmetrical cornua uteri, and complete genital organs. The vaginal mucosa was pink rose in color with a little mucus. The leg joints are not enlarged.

Figure 1. Clinical Examination.

3. R*ose Bengal Test*

Blood sampling is carried out for further examination with the RBT (Rose Bengal Test) method. Blood sampling is done through the jugular vein using a venoject. As much as 2–5 ml is injected into a vacutainer tube and stored at room temperature (20–25 °C) so that the serum remains stable (Hartini and Suryani, 2016). Serum was placed in a slightly tilted position to speed up the separation process between blood components and serum. The separated serum was then transferred into an Eppendorf tube (Wilujeng et al., 2020).

The RBT process is carried out in accordance with the RBT serology testing method according to the slightly modified OIE Terrestrial Manual 2022. Serum samples were taken from as much as 25 µl and then placed on the plate. Antigen was taken in the same amount as the serum and dripped close to the serum, then immediately homogenized. The plate is then shaken on a flat surface quickly for ± 4 minutes, and then the results are read.

Agglutination Results	Interpretation
	A score of 0 (negative) if agglutination does not occur with the mixture of antigen and serum remaining homogeneous.
	A score of +1 (positive) when there is mild agglutination in the form of fine granules with edges surrounded by fine particles.
	A score of +2 (positive) when there is moderate agglutination in the form of sand-like grains with thick fringed edges.
	A score of +3 (positive) when there is complete agglutination in the form of very clear and coarse grains

Tabel 1. Interpretation of RBT results (Siswani *et al.,* 2018).

The serum sample test results were interpreted as +1 (positive) with mild agglutination in the form of fine granules at the edge of the plate.

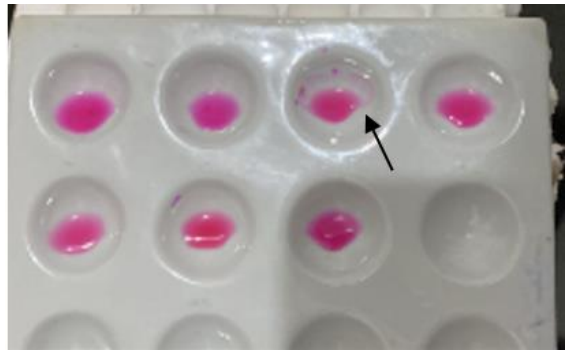

Figure 2. *Rose Bengal Test* of the sample showing +1 score, interpreting a positive result (black arrow).

4. Diagnosis

The diagnosis was based on the results of anamnesis, clinical examination, and RBT testing, with the conclusion that the cattle were diagnosed with brucellosis. Confirmation of the diagnosis was

done by referral to the CFT (Complement Fixation Test) examination, where the remaining serum in Eppendorf was stored and then sent to the Maros Verinary Center for further examination.

5. Handling Procedures

Notification of the diagnosis to farmers was done after RBT screening. Education on the zoonotic nature of the disease, handling of abortus remains, and advice were given to farmers to separate reactive cows from other healthy cows pending CFT testing results as a confirmatory test. Cows were given Vitol-140® injections containing vitamins A, D, and E with the indication of supportive therapy. Antibiotics were not given due to several factors, such as not being cost-effective (treatment costs are not proportional to the outcome), requiring a long treatment time, and the possibility of high bacterial persistence in the cow's body (CFSPH, 2018).

C. DISCUSSION

Brucellosis, or contagious miscarriage disease in bovines, is an infectious disease caused by the bacterium *Brucella abortus* (Wilujeng et al., 2020). Not only in cattle, brucellosis can also affect various animals such as goats, sheep, pigs, and dogs. Humans can also be infected with *B. abortus*, which makes brucellosis a zoonotic disease known by the symptoms of undulant fever in humans (Besung et al., 2015). Undulant fever is a fever with a temperature that increases slowly and is fixed at the peak point, then slowly drops to normal (Jamil, 2015). The name undulant is due to the characteristics of fever that show wavy formation (undulant) on the graph. Undulant fever was previously known as Malta fever, where the initial infection was identified in a soldier infected with brucellosis on the island of Malta (Galiska and Zagórski, 2013). Transmission *of B. abortus* can occur through placental fluid secretions, milk, male animal semen, droplet inhalation, open wounds on the skin, and mucosal tissues. *B. abortus* can survive in the environment for long periods and in water for up to 114 days (Kaden et al., 2018).

Initial infection occurs in the epithelial layer after the transmission process on the mucous membrane, where *B. abortus* is able to survive intracellularly on phagocytic or nonphagocytic cells and will eventually be carried to the nearest lymph node. Bacteraemia occurs after *B. abortus* actively multiplies in the lymphonodus and spreads to various lymphoid tissues such as lymph, lymphonodus iliaca, mesenterica, and supramammary. Subsequent bacteremia leads to generalized infection affecting other target organs, such as the uterus in pregnant cows, the placenta, and other lymph nodes. Lesions in the uterus are located in the placentomes, which are a composite of endometrial carancula and fetal cotyledons. Colonization of *B. abortus* on placental tropoblast cells causes placentitis, leading to abortion in pregnant cows. Inflammatory conditions in the placenta inhibit the delivery of nutrients needed by the fetus, so fetal stress conditions arise and trigger abortion (Poester et al., 2013).

The diagnosis of brucellosis in cattle is based on anamnesis, clinical examination results, and RBT results. The anamnesis that the cow had experienced abortion and was kept with cows that had experienced abortion before became the basis for the diagnosis. According to Peek and Divers (2018), *B. abortus* has a tropism for the uterus and placenta, so clinical signs in cows are often only an abortion in the last trimester of pregnancy. Abortion is associated with severe necrosis of the placenta. In certain cases, with non-severe placental lesions, the pregnancy can be maintained until parturition, but the calves will be born weak with a high neonatal mortality rate.

Clinical examination did not reveal any pathological clinical signs in the cattle. This is because cattle with *B. abortus* infections are often subclinical. Clinical signs that can be found in cows may include placental retention or metritis, but these findings are complications of abortion. Other clinical signs can be found in long-standing infections, such as arthritis and hygroma (CFSPH, 2018). *B. abortus* not only causes disorders of the reproductive system but also causes clinical signs that are nonspecific and diverse, as in osteoarticular system disorders, namely arthritis or hygroma. These clinical signs arise due to the nature of *Brucella sp*., which is able to infect and replicate various cell types (Prescott et al., 2023). Previous studies have revealed that *B. abortus* is able to invade and replicate in osteoblasts, osteocytes, and osteoclasts, as well as synoviocytes. The manifestation of infection in these cells can lead to clinical signs such as bursitis, arthritis, or hygroma (González-Espinoza et al., 2021). Hygroma is often a clinical sign of chronic brucellosis infection and is

immediately visible during a clinical examination. The hygroma is a sac structure usually filled with a yellowish-transparent fluid (Mamatova et al., 2020).

The Rose Bengal Test (RBT) is a serological test against *B. abortus* that is easy to use and do. RBT has a working principle to see the reaction between *B. abortus* antigens and blood serum that has antibodies to *B. abortus.* Antibodies to Brucella are non-specific, so they have the possibility to cross-react or give positive results against several enteric bacteria (Dwi et al., 2018). Cross-reactions can occur between Brucella species and the bacteria *Yersenia etherocolitica, Francisella tularensis, E. coli, Vibrio cholerae*, and *Stenotrophomonas maltophilia*. Cross-reaction occurs because the O-chain of the lipopolysaccharide (LPS) on the surface of Brucella is very similar to that of those bacteria, so the RBT is only used as an initial test (screening test) for brucellosis in cattle. Confirmation of the diagnosis of RBT can be continued with a confirmation test in the form of the Complement Fixation Test (CFT), which is the most sensitive serological test for brucellosis (Kartini et al., 2017). The working principle of CFT is to use a complex reaction of antibodies and homologous antigens that attract complement to bind so that the indicator in the form of erythrocytes is not lysed and shows a positive result or contains Brucella antibodies. A negative reaction indicates the lysis of complement-mediated erythrocytes that cannot bind due to the lack of antibody and antigen complex reactions (Wilujeng et al., 2020).

The finding in this case, +1 to the RBT result, indicates mild agglutination between the antibody and the test antigen. The interpretation of RBT results indicates the concentration of antibodies to Brucella in the tested serum. A score of 0 indicates the absence of antibodies in the test serum; a score of +1 indicates the presence of few antibodies in the test serum; a score of +2 indicates antibodies in the serum in the moderate range; and a score of +3 indicates very high antibodies in the serum (Rohyati et al., 2018).

Polewali Mandar District is one of the brucellosis-endemic areas. The seroprevalence of brucellosis in Polewali Mandar District in 2022 was 24.4%. The measurement results have increased from the previous year, namely in 2021, with a result of 8.4%. The high prevalence can be caused by brucellosis control efforts in Polewali Mandar District, which faced many obstacles. The absence of a brucellosis vaccination program in Polewali Mandar District is considered to be one of the risk factors for the increase in prevalence. The management of the remaining abortions by farmers can also be a risk factor, as some farmers do not know about the proper handling of the remaining abortions and only throw the remaining abortions into the environment or river. The tendency of farmers to keep their cows even though they have experienced abortion is also considered a risk factor for the increase in prevalence in Polewali Mandar District (Amaliah et al., 2022).

The handling carried out based on the findings of brucellosis with the RBT screening method is to advise farmers to separate reactive cows from non-reactive cows while waiting for CFT results as a confirmation test. This is in accordance with the reference literature, where cattle that have tested positive on RBT results must be tested with CFT before further treatment is carried out because RBT still has the possibility of false positives. The recommended handling is "test and slaughtering", which means that after CFT confirmation, cattle are advised to be slaughtered. This is due to the nature of *Brucella abortus*, which can remain in the lymphatic system of cattle, causing persistent subclinical infection, which can lead to an increase in the possibility of the spread of *B. abortus* on a farm, decreased production, and zoonotic factors for farmers (Noor et al., 2016).

Vitol-140® injections containing vitamins A, D, and E are indicated as supportive therapy. Vitamin A has two functions in the animal body, namely maintaining eye health and, in general, playing a role in the formation and protection of epithelial tissue and mucous membranes, so it is important in the processes of growth, reproduction, and immune response. Vitamin A, together with vitamin E, which is an antioxidant, plays a role in protecting cell membranes, including cells of the reproductive tract, from free radical damage. Vitamin E also plays a role in the process of preventing inflammation by preventing the oxidation of polyunsaturated fatty acids, which are precursors to the formation of prostaglandins. Vitamin D plays a role in calcium absorption and regulates the activity of immune cells (McDonald et al., 2022). One of the manifestations

of disorders after cattle abortus in brucellosis is metritis. Administration of vitamins A, D, and E is expected to maintain the mucosa of the reproductive tract of cows, increase the body's immunity, and prevent the inflammatory condition from continuing. Treatment with antibiotics is not carried out because it is considered less efficient in terms of costs incurred and time used in handling brucellosis with antibiotics (CFSPH, 2018).

D. CONCLUSION

Brucellosis is a disease that causes last-trimester abortion in cattle and is zoonotic. The treatment provided in brucellosis cases in cattle in Beroangin Village, Mapilli Subdistrict, and Polewali Mandar District is RBT and further CFT examination if the cattle are reactive to RBT, then education to farmers regarding brucellosis, separation of reactive cattle, and education in the form of testing and slaughtering. Antibiotics are not given in brucellosis cases, but supportive treatment can be given with vitamins related to the manifestation of further disorders after abortion, namely metritis.

E. RECOMMENDATION

Brucellosis vaccination should be programmed in Polewali Mandar District, West Sulawesi, along with further education to farmers regarding the handling of aborted remains, the risk of brucellosis in a farm, and so on.

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RIWAYAT HIDUP

Penulis dengan nama lengkap Arief Gautama Sirajuddin, lahir pada tanggal 4 Januari 2000 di Kota Palopo, Sulawesi Selatan, merupakan anak dari pasangan Ayahanda Drs. Sirajuddin (Alm.) dan Ibunda Nurlianah Sukirman, serta saudara dari kakanda Irsyad Saputra Sirajuddin. Penulis menempuh pendidikan dari TK Pembina Palopo kemudian melanjutkan ke jenjang sekolah dasar di SDN 75 Surutanga (sekarang dikenal dengan SDN 3 Palopo), lalu ke tingkat sekolah menengah di SMPN 3 Palopo. Penulis menyelesaikan pendidikan di SMAN 3 Palopo pada tahun 2017 kemudian diterima di Program Studi Kedokteran Hewan, Fakultas Kedokteran Universitas Hasanuddin pada tahun yang sama melalui jalur SBMPTN. Selama Perkuliahan penulis aktif di organisasi internal kampus yakni Himpunan Mahasiswa Kedokteran Hewan (HIMAKAHA) FK-UH dan menjabat sebagai Anggota Dewan Perwakilan HIMAKAHA selama dua periode, yakni 2019-2020 dan 2020-2021. Pada bidang akademik, penulis pernah menjabat sebagai koordinator Asisten Lab. Diagnosa Klinik, serta sebagai anggota Asisten Lab. Anatomi Veteriner 1 & 2. Penulis juga merupakan penerima beasiswa Peningkatan Prestasi Akademik (PPA) dari pihak Kementerian Riset, Teknologi dan Pendidikan Tinggi. Penulis melaksanakan tugas akhir strata sarjana dengan judul penelitian "**Pengaruh Pemberian Jus jeruk Pomelo (*Citrus maxima*) terhadap Gambaran Histopatologi Testis Mencit (*Mus musculus*) strain *Balb/C* yang Diinduksikan Gentamisin"** dan tugas akhir strata profesi dokter hewan dengan judul "**Kasus Brucellosis pada Sapi Bali di Desa Beroangin Kecamatan Mapilli Kabupaten Polewali Mandar".**