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Case Study

Management of An Outbreak of Brucellosis in A Multiple Species Ruminant Farm in Malaysia

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ABSTRACT

This case report describes the management of an outbreak of brucellosis in a mixed ruminant farm in Selangor, central region of Peninsular Malaysia. Two cows with a history of abortion and endometritis were presented to the University Veterinary Hospital, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Based on the history, physical examination and results of Rose Bengal Plate Test (RBPT), brucellosis was highly suspected. This led to the screening of all the animals (n = 384) in the

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affected farm. The Rose Bengal Plate Test (RBPT) revealed that 23 cattle were positive, while no agglutination was observed in goats and deer. A confirmatory test was performed on all the (n = 384) animals using the complement fixation test (CFT) and the results showed that two (2) cows and five (5) deer tested positive. The CFT positive animals were culled. Preventive steps were then taken where the remaining cattle in the herd were vaccinated once using the RB51 vaccine, while the deer and goats were not vaccinated. All animals in the herd were also recommended to be subjected for blood sampling every 4 months and the serum samples to be tested using competitive ELISA to distinguish between serological responses due to vaccination from infection. To overcome outbreak scenario of brucellosis in a farm, a prompt action and a concerted team work among relevant stakeholders are crucial to curb the situation.

Keywords: Brucellosis, multiple species, outbreak, ruminant farm

INTRODUCTION

Cattle, goat and deer are considered as the main ruminant livestocks in Malaysia due to the increased local demand for milk and meat (Department of Veterinary Services [DVS], 2015). However, these livestock are constantly threatened by many infectious diseases and one of such disease is brucellosis. Brucellosis is a serious socio-economic and public health problem in many developing countries (Bamaiyi, Hassan, Khairani-Bejo, & Zainal Abidin, 2014). The prevalence of the disease among livestock in Malaysia is considerably low in relation to other neighboring countries (Bahaman, Joseph, & Khairani-Bejo, 2007; Bamaiyi et al., 2014).

Brucellosis was first confirmed in Malaysia in the 1950s when *B. abortus* was first isolated from large ruminants. However, subsequent studies have reported the presence of brucellosis in small ruminants such as goat and sheep, pets and humans (Sam et al., 2012). For years, the Department of Veterinary Services, Malaysia (DVS) have conducted an active serosurveillance on bovine brucellosis as a part of a serious and committed effort in controlling the disease. The exercise involved culling of infected animals and payment of compensation to owners of the affected farms (Bahaman et al., 2007). However, the status of bovine brucellosis has shifted in the last decade and anecdotal evidence suggests an increase in brucellosis among cattle (Anka et al., 2013), similar pattern was also reported among goats. This case report describes the management of an

outbreak of brucellosis in a multiple species ruminant farm in Malaysia.

Clinical History/Examination

Two cows with a history of abortion and endometritis from a multiple species ruminant farm in Selangor (central Peninsular Malaysia) were presented to the University Veterinary Hospital, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Farm records showed evidence of recent introduction of new cows into the herd. Based on history, physical examination and positive result**s** by Rose Bengal Plate Test (RBPT), brucellosis was suspected. This led to the screening of all the animals (n = 384) in the farm. The decision to screen the whole herd was made due to the farm's proximity to Negeri Sembilan state (central south Peninsular Malaysia), which was reported to have several outbreaks of brucellosis.

Diagnostic Workup

Blood sample collection and Extraction of serum. Approximately 5 mL of blood samples were collected from each animal in a pre-labelled plain vacutainer tube via jugular venipuncture. The blood samples were allowed to clot by keeping the tubes to stand overnight at room temperature. Serum was extracted by centrifugation at 3000 x g for 20 min. The sera were then stored at -20 ºC until used.

Rose Bengal Plate Test (RBPT). Serum samples were screened for brucellosis using the RBPT method described by Swai and Schoonman, (2010). Briefly, 30 µL of RBPT antigen (VLA Weybridge, UK) and 30 µL of test serum samples were placed together on a plate. The plate was then shaken for 4 min and the level of agglutination was evaluated and recorded. The samples were categorized as positive following the presence of agglutination and negative when there was no agglutination. Serum samples that were positive for brucellosis using RBPT were confirmed with CFT.

Clinical Management and Outcome. The result**s** showed that a total of 23 (5.9%) cattle were positive for brucellosis by RBPT. However, none of the goats and deer were positive using RBPT. The Complement Fixation Test (CFT) revealed 2 (0.5%) cattle and 5 (1.3%) deer were positive for brucellosis while none of the tested goats were positive. Following results from the CFT test, which is the confirmatory test for brucellosis, all CFT positive animals (two cattle and five deer) were subjected to mandatory culling as recommended by the Malaysian Veterinary Protocol set by the Department of Veterinary Services of Malaysia. Preventive measures were taken immediately following culling where all cattle in the herd were vaccinated once using the RB51 vaccine, while deer and goats were not vaccinated. All the remaining animals in the herd were recommended to be subjected for repeated blood sampling every 4 months. The serum samples were to be tested using competitive enzyme-linked immunosorbent (ELISA) that differentiates between serological responses due to vaccination from infection until none of the animals were positive for infection.

DISCUSSION

Brucellosis is a major socio-economic problem of the livestock industry. It is one of the most important zoonotic diseases associated with reporductive failures in livestock (Jajere, Atsanda, Bitrus, Hamisu, & Ayo, 2016). The disease is widespread from country to country; however, it is restricted to certain geographical locations. Brucellosis is still considered a major animal health problem affecting the livestock industry in Asia, Africa, Latin American and the Mediterranean (Yahaya, Khairani-Bejo, Zunita, Omar, & Bitrus, 2016). As in 2014, the overall prevalence of bovine brucellosis (*Brucella abortus*) reported in some ASEAN countries were (1%) Thailand, (2%) Indonesia, (4%-5%) in Malaysia (Zamri-Saad & Kamarudin, 2016). In this report, the prevalence was 6% using RBPT as compared to 1.8% with the CFT. The RBPT test is a more sensitive but less specific assay in detecting infected animals, while the CFT test is more specific in detecting animals infected with the disease. The management of the outbreak described in this case report is in accordance with the recommendations by Gürbilek, Tel and Keskin (2017) where the authors reported that successful control programs for brucellosis is based on the combined use of serological tests such as RBPT and CFT. In Malaysia, test-and-slaughter policy is the protocol of choice that was adopted for the control of brucellosis. The protocol was adopted and implemented in 1979 as the national program for "Area-Wise Eradication of Bovine Brucellosis". The test- and- slaughter policy involved culling of all individual animals that tested positive for brucellosis by CFT and corresponding payment of compensation to the affected farm or animal owners. Even though the protocol is considered expensive and recommended only in countries that have efficient surveillance program and excellent laboratory facilities which in this case may not be suitable approach to be practiced in Malaysia. However, the country's cattle population which was estimated to about 800,000 makes the adoption of the program feasible due to less cost incurred on the of execution of the program. Interestingly, the test-and-slaughter policy in Malaysia has achieved tremendous success in reducing the prevalence of brucellosis from 3.3% (1979) to 0.23% (1988). The prevalence however increases from 1% in 1998 to 5% which is the current status of bovine brucellosis in Malaysia. This was attributed to the indifference attitude of the farmers towards the program (Bahaman et al., 2007; Plumeriastuti & Zamri-Saad, 2012; Zamri-Saad & Kamarudin, 2016).

In cattle, the main causative agent of brucellosis is *B. abortus,* infection in animal usually occur as result of contact with the contents of abortion from other infected animals in the farm. Contaminated animal utensils and pasture with aborted materials are probably the most potential sources of infection. Other sources of transmission includes, inhalation, ingestion, skin contamination, conjunctival inoculation and udder inoculation from infected milking cups. Contamination via the colostrum

have also been reported in newborn calves (Blasco & Molina-Flores, 2011; Godfroid et al., 2013). Brucellosis have also been reported in cows that were inseminated with contaminated bull semen (Aparicio, 2013; Xavier, Paixao, Hartigh, Tsolis, & Santos, 2010). In this case report farm history showed evidence of recent introduction of new cow into the farms. Thus, indicating the possible transmission of the disease to other animals in the farm probably due to latent infection in the newly introduced animals.

Greater Yellowstone Ecosystem (GYE) is the classic example and still remains the major reservoirs of B. *abortus* in the United States, where the historical infection in wildlife such as bison (*Bison bison*) and elk (*Cervus elaphus*) are believed to be introduced by domestic cattle in the early 20th century. Recent studies have reported that free-range elks are still serving as reservoirs of infection to livestock (Kamath et al., 2016). Seroprevalence against *B. abortus* recorded in several elk herd units in GYE (Wyoming portion) ranged from 9% to 42% (Scurlock & Edwards, 2010). In some areas of China, the prevalence of brucellosis among deer was 28% (Li, Yao, & Wang, 2007). Medrano et al. (2012) reported 0.9% antibody detection in white-tailed deer (*Odocoileus virginianus*) in northen part of Mexico. Brucellosis is thought to have a role in the list of infectious diseases in deer by the across region distribution and under reported incidence, this eventually could influence the development of deer farming and breeding industry. In Malaysia, as the best of authors knowledge would be the first reporting on seroevident of antibody against *B. abortus* in deer. The deer and other cattle and goat in this farm were not in direct contact, however the roles of indirect transmission such as contaminated fodder, fomites or personnel should not be excluded. In this context, further investigation is needed to establish the status of *Brucella* infection in farmed as well as free-ranging deer in Malaysia with a view to systemizing for prevention and control of the disease among and between wildlife and livestock.

Mixing of animals from different herds or flocks belonging to different owners especially at the markets contribute significantly to the transmission of the disease. Many factors influence the prevalence of brucellosis, which includes contact with wildlife, production systems, management factors, agro-ecological zones and husbandry practices (Godfroid et al., 2013). However, mixing of livestock species is one of the most important factors that contributes to the spread of brucellosis in animals (Godfroid et al., 2013). In this report, the mixing of multiple species in the farm might have contributed to the seropositivity in other species of animals such as deer in this case. Based on the CFT results, it can be suggested that the either the cattle or deer may be the source of infection. It has been reported that mixed farming, especially raising of sheep and/or goats together with cattle to be a risk factor for transmission of brucellosis among different animal species (Ocholi, Kwaga, Ajogi, & Bale, 2004). However, this transmission does not equally occur in both directions.

For deer with cattle, the risk factor for transmission of brucellosis has not been reported or studied yet in Malaysia.

The increase in livestock population and investment along with limited resources have made the prevention and control of brucellosis difficult (Corbel, 2006; Al-Majali, Majok, Amarin, & Al-Rawashdeh, 2007).The re-emergence of brucellosis has been reported in many countries especially in sheep and goats (Blasco & Molina-Flores, 2011). The disease has been eradicated in most technologically advanced countries following years of qualitative investment, vaccination and culling of infected animals. Similar approach was utilized in this case report, the animals that tested positive for CFT were culled, while the remaining seronegative animals were all vaccinated. Zamri-Saad and Kamarudin (2016) stated that vaccination helped to reduce shedding of the organism in the environment and was mostly practiced in areas where the disease was endemic and hence, this move was implemented in this case. Control or eradication strategies for outbreak of brucellosis in a farm needs a prompt action and a concerted team work among relevant agencies to curb the situation as reported in this case report. This will in turn, reduce the burden of brucellosis on human health and the livestock industry (Blasco & Molina-Flores, 2011).

CONCLUSION

This case report describes the seroprevalence of brucellosis in a multiple species farm through the use of two different assays; RBPT and CFT. The CFT used in this case report showed high discriminatory power to brucellosis in deer than RBPT. The main aim of the control program of brucellosis is to reduce the impact of the disease on both human and animal health and the economy.

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