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In-House Rose Bengal Plate Agglutination Test (RBPT) for a Rapid Diagnosis of Brucellosis in Goats in Malaysia

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Abstract: The Rose Bengal Plate Test (RBPT) antigens from *Brucella melitensis* local isolates (in-house RBPT) were prepared and compared with RBPT antigen for Brucellosis in sheep and goats prepared by Veterinary Laboratory Agency, UK. Eight hundred fifty-six sera samples, of which were collected from goats were examined with the RBPT and Complement Fixation Test (CFT). The RBPT and CFT results showed that the in-house RBPT antigen was superior to the commercial prepared RBPT antigen (VLA, UK). Out of 856 sera analyzed by in-house RBPT, commercial RBPT and CFT, 30.84, 26.40 and 31.65% were found to be *Brucella* positive, respectively. The sensitivity calculated for the in-house RBPT compared with CFT was 85.24% whilst that of commercial RBPT was 78.59%. Therefore, it was conclude that in-house RBPT antigens could be prepared and used for epidemiological surveillance of *Caprine brucellosis* in Malaysia.

Key words: Goat, brucellosis, Rose Bengal Plate Test (RBPT), diagnosis, CFT

INTRODUCTION

Brucellosis is an infectious disease of worldwide distribution affecting animals and humans. Brucellosis in goats is caused by *Brucella melitensis*, the most pathogenic *Brucella* sp. In 2007, about 476 cases of caprine brucellosis were reported in Malaysia (OIE, 2008).

The Office International des Epizooties (OIE, 2008) recommended testing scheme are a combination of the Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT) (MacMillan, 1990). The RBPT was developed for the diagnosis and detection of brucellosis in animal herds, particularly for bovine brucellosis. It is a cheap, rapid and effective serologic test compared to the other serological test available. A reliable result can be obtained in large herds in a short period with the least amount of equipment. The RBPT antigens also had been implemented in diagnosis of brucellosis in small ruminants. However, the specificity and sensitivity of the RBPT in sheep and goats are still unclear (Blasco *et al*., 1994; Erganis *et al*., 2005).

The purpose of this study were to prepare in-house RBPT antigens from *B. melitensis* local isolate and compare them with commercial RBPT antigens prepared by Veterinary Laboratory Agency (VLA), UK.

MATERIALS AND METHODS

Sera sample: The blood samples from 856 adult goats were sampled by jugular venipuncture method. The sera were obtained after centrifugation of the blood at 6000 rpm for 5 min. These sera were kept at -20°C and thawed to room temperature before being analyzed.

Bacterial isolation and identification: The stock culture of *Brucella melitensis* local isolate was inoculated on *Brucella* Agar for 4 days at 37°C. The Gram staining and modified acid fast staining were carried out. Biochemical tests, which are Triple Sugar Iron, urease, nitrate reductase, basic fuschin and thionin, were exercised to ensure the identity of the bacteria.

Preparation of antigen for in-house Rose Bengal Plate Test (RBPT): The antigen was prepared as described by OIE (2008). Briefly, 3-5 colonies of the *Brucella melitensis* were inoculated into liquid media and incubated at 37°C with vigorous shaking for 48 h.

The organism is harvested by centrifugation. Later, the pellet was resuspended in 0.5% phenol saline. The mixture was heated at 80°C for 90 min to kill the organism and the suspension was stored at 4°C until used.

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Test procedures: The RBPT was performed as follows, 30 μL of test serum was added to 30 μL of the in-house or commercial rose Bengal antigen on a white porcelain plate and mixed thoroughly with a clean toothpick to produce a zone approximately 2 cm in diameter. The plate was rocked slowly for 3 min. The test was read and scored as positive if any degree of agglutination was observed.

Data analysis: For each test, sensitivity and specificity were calculated. The sensitivity was calculated from the Eq. 1:

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive} + \text{false negative}} \times 100 \quad (1)$$

and specificity was calculated from the Eq. 2:

$$\text{Specificity} = \frac{\text{True negative}}{\text{True negative} + \text{false positive}} \times 100 \quad (2)$$

RESULTS AND DISCUSSION

Caprine brucellosis is a zoonotic disease that has the public health and economic importance. The prevalence of these diseases is related to the management practices of the farm and the ability of a country to finance prevention or control programs. The diagnosis of Brucellosis is performed through the isolation of the bacteria and/or serological tests. The Rose Bengal Plate Test (RBPT) and the Complement Fixation Test (CFT) are the most widely used tests for the serological diagnosis of sheep and goats brucellosis. They are also the official tests for international trade (OIE, 2008). The Rose Bengal Plate Test (RBPT) was implemented in Veterinary Research Institute, Malaysia in 1974 for bovine brucellosis (Cheah and Arunasalam, 1977). To date, the use of the RBPT as a screening test for diagnosis of *Brucella melitensis* infection in sheep and goats was recommended by the joint FAO/WHO expert committee on Brucellosis. The test is very cheap, dependable and giving rapid results (Erganis *et al*., 2005). There is commercial RBPT antigen for screening of brucellosis in sheep and goat available but most of the Asia and Pacific region have a difficulties to obtain this diagnostic reagent is which is essential for routine laboratory research (OIE, 2008). In this study, the Complement Fixation Test (CFT) was used as a gold standard to calculate the sensitivity and specificity of commercial and in-house RBPT (Table 1 and 2). The present study found that out of 856 sera, 271 (31.66%) was found to be positive by CFT, whilst commercial RBPT and in-house RBPT detected 26.40 and 30.84% positive animal, respectively.

Table 1: Comparison of commercial Rose Bengal Plate agglutination Test (RBPT) and Complement Fixation Test (CFT)

In-house percentage	CFT+	CFT-	Total
RBPT+	213	13	226
RBPT-	58	572	630
Total	271	585	856

Table 2: Comparison of in-house Rose Bengal Plate agglutination Test (LRBPT) and Complement Fixation Test (CFT)

In-house percentage	CFT+	CFT-	Total
LRBPT+	231	33	264
LRBPT-	40	552	592
Total	271	585	856

The study found that the in-house RBPT (85.24%) is more sensitive compared to the commercial RBPT (78.59%). However, the commercial RBPT (97.77%) is more specific than in-house RBPT (94.36%).

Even though, the sensitivity of the in-house RBPT was found to be higher than commercial counterpart, simple modification such as increasing slightly the amount of sera for the test from 25-30 to 75-90 μL and maintaining the antigen volume (25-30 μL) was claimed to increase significantly the sensitivity without affecting specificity of the RBPT (Blasco *et al*., 1994). In current study, we also found that there are 58 (commercial RBPT) and 40 (in-house RBPT) sera were negative with RBPT whilst CFT were positive. Morgan (MacMillan, 1990) had mentioned a few possibilities that could lead to these false negative results, which are early stages following infection and following the ingestion of colostrums from reactors dam. In such conditions, the CFT will remain positive for a longer period compared to the RBPT. However, considering the sample population are not involving animals <1 year old the latter is unlikely be taken into account. An additional factor that could be the contributor to the false negative results is the spoilt RBPT antigen. The RBPT antigen may have lost its sensitivity due to improper storage of the reagent. Nevertheless, we also found that there are 13 (commercial RBPT) and 33 (in-house RBPT) out of the total sera samples were RBPT positive but CFT negative. These false positive results can be caused by cross-reactivity of antibodies to *Yersinia enterocolitica* type 0.9, *Escherichia coli* O:157H:7, *F. tularensis*, *Moraxella phenylpyruvica* and *Salmonella landau* (Cherwonogrodzky *et al*., 1990). The cell wall surface lipopolysaccharide of those gram-negative bacteria mentioned have been proven to share cross-reactive epitopes with *Brucella* sp. In goats, the *Y. enterocolitica* type 0.9 probably is the cause of cross reactivity in serological tests with smooth *Brucella antigen*, such as the in-house RBPT antigen developed in this present study. So far, little or to some extent no report have been published regarding the *Y. enterocolitica* type 0.9 infection in goats in Malaysia.

For that reason, there is rationale in initiating a preliminary study of those organisms that show serological cross reactivity with *B. melitensis*. Until now it can only assume that those organism especially *Y. enterocolitica* is present in goats in Malaysia. Another explanation for the RBPT positive but CFT negative cases are the presence of antibodies against *Brucella epitopes* may in the animal population. This could be due to vaccination of the animals. Nielsen *et al.* (2005) reported that half of the population was remained positive to the RBPT whilst Alton (1990) reported that the CFT is more likely to be negative after 1 year of Rev. 1 vaccination. As the agglutination test remain positive much longer than the CFT, it is possible that the false positive animal have been vaccinated more than a year.

Although, the Malaysian government practicing stamping out policy and no vaccination for *Caprine brucellosis*, we are unable to rule out that there is immunity develop by vaccination, since most of the brood stock were imported from South East Asia countries and South Africa were the disease is present and vaccination is not uncommon. Based on the information at hand, it was unable to distinguish between two possibilities; the false positive is due to cross reactivity with other bacteria with similar O-Lipopolysaccharide (O-LPS) or existence of anti-Brucella antibody following vaccination.

CONCLUSION

These findings suggest that in-house RBPT is highly sensitivity compared to the commercial RBPT antigen, plus simple, rapid and low cost. Thus, good field screening test was successfully developed in this present study. Therefore, this diagnostic test was suggested to replace the commercial RBPT, which is relative more expensive and less sensitive in detection of brucellosis in goats.

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