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DEVELOPMENT OF AN IN-HOUSE ROSE BENGAL PLATE TEST FOR DIAGNOSIS OF BRUCELLOSIS IN GOAT

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ABSTRACT. Brucellosis, caused by *Brucella melitensis*, is a significant problem for both public and animal health worldwide. The Rose Bengal plate test (RBPT) antigen from *Brucella melitensis* local isolates were developed in this study. The performance of the assay was investigated using serum samples collected from goats. A total of 1063 serum samples obtained from goats were examined for the presence of antibodies against Brucella by in-house RBPT (LRBPT), commercial RBPT (Veterinary Laboratory Agency – VLA, UK) and Compliment Fixation test (CFT). The sensitivity and specificity was calculated using CFT as the gold standard. Out of 1063 goats sera analyzed 364 (34.24%), 335 (31.51%), and 373 (35.08%) were found to be positive by LRBPT, commercial RBPT and CFT, respectively. The sensitivity calculated for the LRBPT, was 90.1% compared to commercial RBPT 85.0%. However, the specificity of the LRBPT was lower (95.9%), than the commercial RBPT (97.4%). Furthermore the LRBPT has better value of NPV (94.7%) than commercial RBPT NPV (92.3%). While the PPV, of the commercial RBPT is higher (94.6%) than LRBPT (92.3%) respectively. High sensitive and low cost LRBPT compared to cRBPT *B. melitensis* RBPT test was successfully developed in this present study. Therefore it was concluded that this diagnostic test kit can complement and replace the available commercial RBPT which is relatively more expensive and less sensitive in detection of brucellosis in goats. It could also be used for epidemiological surveillance of caprine brucellosis in Malaysia.

Keywords: Brucella melitensis, Rose Bengal plate test, complement fixation test, LRBPT positive predictive value, negative predictive value

INRODUCTION

Brucellosis is a disease of economic importance in Animal production worldwide (Corbel, 1997; Godfroid *et al.*, 2011). The main effects of brucellosis in livestock are abortion, infertility,

decreased in animal production and cost replacement animals which contribute to the economic losses (Corbel, 2006). Brucellosis in cattle is usually caused by *Brucella abortus* the most pathogenic Brucella specie. Nevertheless, infection caused by *B. melitensis* have also been found when cattle are in close proximity with sheep and goats (OIE 2009a, Aff *et al*., 2011). On the other hand, *B. melitensis* is the main causative agent of caprine and ovine brucellosis. (Garin-Bastuji *et al*., 1998: OIE, 2009b). The occurrence of these must be reported to the world organization for animal health because of its zoonotic potential. In humans, the disease is called Malta fever. Some of the common route of infection is ingestion of contaminated meat or unpasteurized milk from infected animals. The clinical manifestation of brucellosis is not specific and diagnoses were achieved by isolation of bacteria and serological methods. The isolation of bacteria is considered to be the gold standard. The prescribed serological tests are the Rose Bengal Plate Test (RBPT) and Complement fixation Test (CFT) (OIE, 2009). They are being used as a screening and confirmatory test respectively. This study was designed to develop an in-house RBPT antigen from local isolate of *Brucella melitensis* and compare them with commercial RBPT antigen Veterinary Laboratory Agency (VLA), UK.

MATERIALS AND METHODS

Serum samples

A total of 1063 goat serum were collected.

Bacterial strain and re-identification

Cultures of the Brucella isolates obtained from the bacteriology laboratory, Faculty of Veterinary Medicine, Universiti Putra Malay were used in this study. Isolates were reconfirmed as *Brucella melitensis* by culturing on Brucella agar for 4 days at 37 °C. Pure cultures were identified as *B. melitensis* using colony morphology, gram-staining, modified acid fast staining, biochemical test and PCR amplification of specie specific gene for *B. melitensis.*

Preparation of Antigen for In-house Rose Bengal Plate Test (RBPT)

Preparation of antigen was carried out as described by OIE (2009). A few colonies of *Brucella melitensis* were inoculated into Brucella broth and incubated at 37 °C with vigorous shaking for 72 hours. The culture was harvested by centrifugation, and then pellets were re-suspended in 0.5% phenol saline. The mixture was then heated at 80 °C for 90 minutes to kill the organism and the suspension was stored at 4 °C in a dark bottle until used.

Test procedures

The test was performed as described by Shahaza *et al.*, (2009). Thirty microliter (30 µL) of test serum was added into 30 µL of the Rose Bengal antigen on a white plate or tiles. The mixture was then thoroughly mixed with a clean stirrer in order to produce a zone approximately 2 cm in diameter. The plate was then rocked slowly for 3 to 4 minutes. The test was read and scored as positive if any degree of agglutination was observed.

Determination of Analytical Sensitivity, Analytical Specificity and Repeatability of the In-house RBPT

Thirty positive and thirty negative goat sera were used in order to assess the of ASe, ASp, and Repeatability using reference sera that were confirmed previously by culture and CFT.

RESULTS

Determination of Analytical Sensitivity, Analytical Specificity, and Repeatability of the In-house RBPT

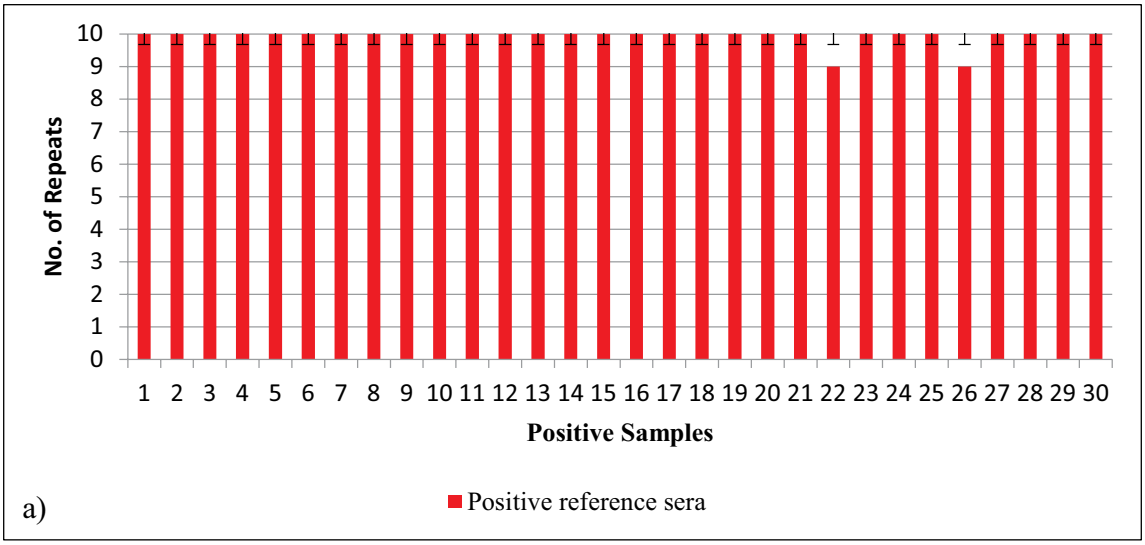
Out of the total number of 30 positive and 30 negative reference sera of goats (Table 1). The in-house RBPT ASe and ASp were recorded as 96.6 % and 96.8% respectively. Nevertheless the in-house RBPT have consistent repeatability which was recorded 99.3% and 100% means for positive and negative reference sera, respectively (Figure 1).

Determination of Diagnostic Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of the in-house RBPT

The result obtained reveals that LRBPT has higher value of (90.1%) sensitivity as compared to the (85.0%) sensitivity in the commercial Rose Bengal Plate Test

Table 1. Cross tabulation of the LRBPT compared with CFT as gold standard using reference sera of goats showing ASe and ASp

		CFT		Total
		Positive	Negative	
In-house RBPT	Positive	28	1	29
	Negative	1	30	31
Total		29	31	60
		ASe 96.6%	ASp 96.8%	



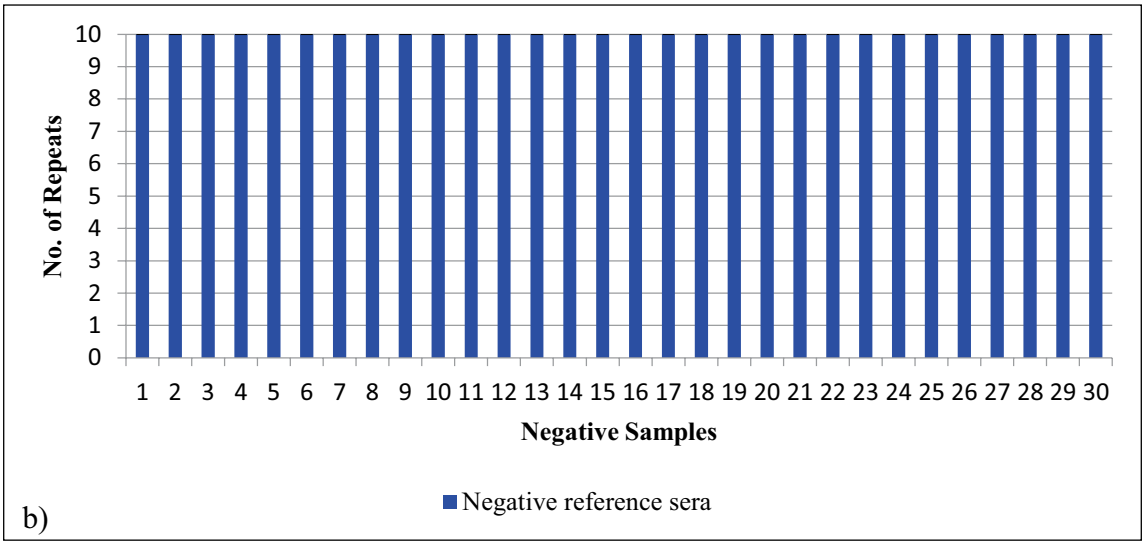


Figure 1. Mean (±MSD) of repeatability results the LRBPT. (a) using positive sera of goats, (b) using negative sera of goats.

Table 2. Cross tabulation of the LRBPT compared with CFT as gold standard using field sera of goats showing DSe, DSp, PPV, NPV and PI

		CFT		Total	
		Positive	Negative		
In-house RBPT	Positive	336	28	364	PPV 92.3%
	Negative	37	662	699	NPV 94.7%
Total		373	690	1063	
		DSe 90.1%	DSp 95.9%		
		PI 186.0			

Table 3. Cross tabulation of the cRBPT-B. melitensis compared with CFT as gold standard using field sera of goats showing DSe, DSp, PPV, NPV and PI

		CFT		Total	
		Positive	Negative		
cRBPT-B. melitensis	Positive	317	18	335	PPV 94.6%
	Negative	56	672	728	NPV 92.3%
Total		373	690	1063	
		DSe 85.0%	DSp 97.4%		
		PI 182.4			

as shown in Tables 2 and 3. While the specificity (97.4%) of the commercial Rose Bengal Plate Test is higher than that of the in-house Rose Bengal Plate Test (95.9%). However, the PPV of the in-house Rose Bengal Plate Test (92.3%) is lower than the PPV of the commercial Rose Bengal Plate Test (94.6%). In addition, the NPV of the in- house RPBT (94.7%) is higher than the NPV of the cRPBT (92.3%).

DISCUSSION

Brucellosis is a specific contagious disease of humans and animals caused by bacteria of the Brucella group. The disease is considered by FAO, WHO and OIE as the most widespread zoonosis in the world. The importance of the disease is due both to its economic impact on the animals and to the severe hazard it causes to human health. The diagnosis of Brucellosis can be through isolation of bacteria and serological tests. The Rose Bengal Plate Test (RBPT) and Complement Fixation Test

(CFT) are the most commonly used tests for the serological diagnosis of bovine and caprine brucellosis. Of the 1063 goat sera tested 364 (34.24%), 335 (31.51%), and 373 (35.08%) were positive by LRBPT, cRPBT, and CFT respectively. In this study, CFT was used as the gold standard to calculate the sensitivity and specificity of LRBPT and cRPBT as well as to compare their PPV, and NPV respectively Table 2 and 3. In addition, the Analytical performance characteristic of the developed in-house RBPT reveals excellent performance of analytic sensitivity (ASe) of 96.6% and analytic specificity (ASp) of 96.8%. This indicates the in-house RBPT has the ability to measure small amounts of a substance in a sample. The study found that the in-house RBPT (90.1%) is more sensitive compared to cRPBT (85.0%). However the cRPBT (97.4%) has higher specificity than in-house RBPT (95.9%). While the PPV and NPV of the in-house RBPT was (92.3%) and (94.7%) compared that of cRPBT, which was (94.6%) and (92.3%) respectively However, in this current study, it was found that there are 37 (LRBPT) and 56 (cRPBT) samples that were negative with RBPT while positive with CFT. There are a few possibilities that could lead to these false negative results as mentioned by MacMillan (1990) following early stages of infection and the ingestion of colostrum from reactor dam. Spoilt RBPT antigen could be another cause as the RBPT antigen may lose its sensitivity due to improper storage of the reagent. 28 (LRBPT) and 18 (cRPBT) number of sera samples were found positive with RBPT but negative with CFT. The false positive results could be caused by cross-reactivity of antibodies to *Yersinia enterocolitica* type 0.9, and other Gram-negative bacteria like *Escherichia coli* 0:157H; *Salmonella landau* (Cherwonogrodzky *et al.,* 1990). This study also found better value of NPV (94.7%) than cRBPT (94.3%). While the PPV of the cRBPT was higher (94.6%) than that of LRBPT (92.3%) respectively. This study also found that the sensitivity and specificity of the developed in-house RBPT was better or higher than those obtained by Shahaza *et al.* (2009). which was 85.2% sensitivity and 94.4% specificity. The reason behind this could be due to the optimization procedures that was achieved using international standard *Brucella melitensis* serum (ISaBmS) to determine the analytical sensitivity and specificity of the developed in-house RBPT.

CONCLUSION

The findings suggest that the in-house RBPT, which is rapid and low cost, has higher sensitivity compared to the commercial RBPT antigen. Therefore, this diagnostic test was suggested to replace the commercial RBPT, which is relatively more expensive and also less sensitive in the detection of brucellosis in goats.

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