DISEASE DETECTION OF BRUCELLOSIS IN GOAT POPULATION IN NEGERI SEMBILAN, MALAYSIA

Siti Sumaiyah Mohd Yusof, [1,3]Abd. Wahid Haron & [2]Siti Khairani Bejo
[1]Department of Veterinary Clinical Studies
[2]Department of Veterinary Pathology & Microbiology
[3]Wildlife Research & Conservation Centre
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

A serological study of brucellosis in goats caused by *Brucella melitensis* was conducted in the state of Negeri Sembilan, Malaysia. A total of 771 serum samples were collected from seven districts namely Rembau, Jelebu, Kuala Pilah, Seremban, Port Dickson, Jempol, and Tampin. At least two farms were selected and a minimum of 100 serum samples were collected from each district. All sera were tested for brucellosis using the Rose Bengal plate test (RBPT) and complement fixation test (CFT). In this study, only Rembau and Kuala Pilah showed seropositivity for *B. melitensis* with RBPT and CFT at 1.0 and 2.5%, respectively. The CFT was more sensitive than the RBPT because the serum antibodies against *B. melitensis* detected by CFT were twice higher than that detected by RBPT. As suggested by the Office International des Epizooties OIE, CFT was used as a confirmatory test for brucellosis. This test is also recommended as a prescribed test for international trade and is used in the control and eradication programmes.

Keywords: *Brucella melitensis*, RBPT, CFT, goats

INTRODUCTION

Brucellosis in goats is mainly caused by *Brucella melitensis*, a gram-negative coccobacillus or short rod. *Brucella melitensis* is pathogenic to sheep, goats and highly zoonotic (Erganis *et al.* 2005). The occurrence of the disease must be reported to the World Organization for Animal Health. Clinical manifestation of the disease is not specific. The definitive diagnosis of *B. melitensis* was usually made by isolation of the bacteria whereas the gold standard for serological methods was the complement fixation test (CFT). It was recommended by the Office International des Epizooties (OIE, 2008) to use a combination of the Rose Bengal plate test (RBPT) and the CFT for detection of brucellosis in small ruminants. The Rose Bengal plate test is being used as a screening test for brucellosis whereas the CFT is used as a confirmatory test for brucellosis. In addition, the RBPT is very cheap, dependable and give rapid results but the sensitivity and specificity of the RBPT in goats is still unclear (Blasco *et al*., 1994; Erganis *et. al*, 2005). Brucellosis in goats and sheep still occur in Negeri Sembilan as reported by the

Department of Veterinary Services (DVS). Two serological diagnostic tests of RBPT and CFT are practiced by the DVS for detection of brucellosis. The data from the DVS of Negeri Sembilan revealed that each district has seropositivity towards *B. melitensis* with Rembau and Kuala Pilah being the highest. Control and prevention programmes were implemented in order to reduce the occurrence of the disease. The DVS is also practicing a stamping-out policy to all seropositive animals in order to control the disease.

The objectives of this study were to conduct serological testing using RBPT and CFT for the detection of brucellosis in goats, to determine current status of brucellosis in goats in Negeri Sembilan, and to determine the sensitivity and specificity of the RBPT and CFT for the detection of brucellosis in goats.

MATERIALS AND METHODS

The Rembau, Jelebu, Kuala Pilah, Seremban, Port Dickson, Jempol, and Tampin districts of Negeri Sembilan, Malaysia were selected for this study. A total of 771 serum samples were collected randomly from two goat farms from each district. A minimum of 100 goat serum samples were collected randomly from animals of more than one-year old from each district. The whole blood was collected into 6 ml plain sterile tubes. The serum was separated from the clotted blood and collected into new serum collecting tubes. The serum was stored at -20°C until required for serological tests. The RBPT was performed by adding 30 µL of test serum into 30 µL Rose Bengal antigen on white porcelain plate using a pipette and sterile tips and mixed thoroughly with a clean toothpick to produce a zone approximately 2 cm in diameter. The plate was rocked slowly for 3 minutes and any apparent agglutination is interpreted as positive.

All serum samples were also sent to the Veterinary Research Institute (VRI) for a confirmatory test using CFT. Briefly, the CFT involves inactivated brucella antigen, guinea pig serum containing a complement and indicator system which consisted of sheep erythrocytes sensitised with rabbit antibodies. A positive result is revealed by the absence of haemolysis by which the specific test serum contained a specific antibody which formed a complex with the inactivated antigen to bind with the complement. A negative result is revealed by the evidence of haemolysis when the complements freely bind to the sensitised sheep erythrocytes due to absence of the antigen-antibody complex.

Specificity and sensitivity

The specificity and sensitivity were calculated by 2 × 2 tables as in the formula below:

$$\text{Specificity} = \frac{\text{Total number of comparative test negative}}{\text{Total number of relative tests negative}} \times 100$$

$$\text{Sensitivity} = \frac{\text{Total number of comparative test positive}}{\text{Total number of relative test positive}} \times 100$$

RESULTS

The results of the *Brucella melitensis* antibody detection using RBPT and CFT are shown in Table 1. The RBPT detected 8 goats positive with *B. melitensis* antibodies while CFT detected 19 goats positive with *B. melitensis* antibodies. The data also revealed that only Rembau and Kuala Pilah showed seropositivity for *B. melitensis*. The prevalence in Rembau was 2.5% by RBPT and 5.6% by CFT while the prevalence in Kuala Pilah was 3.6% by RBPT and 9.1% by CFT. The CFT (2.5%) is more sensitive in the detection of *B. melitensis* antibodies than RBPT (1.0%). Table 2 shows the RBPT and CFT sharing 1 male serologically positive for *B. melitensis* antibodies while 7 females and 18 females were serologically positive for *B. melitensis* antibodies using RBPT and CFT. Based on Figure 1, the seroprevalence of brucellosis in year 2012 is higher compared to years 2010 and 2011 because the serum samples collected were from the farms with history of brucellosis. Comparison between Tables 3 and 4 revealed that CFT (85.7%) was more sensitive than RBPT (32%) while RBPT (99.9%) was more specific than CFT (98.3%).

Table 1. Rose Bengal Plate Test and Complement Fixation Test for *B. melitensis*

Districts	RBPT				CFT			
	+ve	-ve	Total	%	+ve	-ve	Total	%
Rembau	4	156	160	2.5	9	147	160	5.6
Kuala Pilah	4	106	110	3.6	10	96	110	9.1
Jelebu	0	98	98	0	0	0	98	0
Seremban	0	50	50	0	0	0	50	0
Port Dickson	0	118	118	0	0	0	118	0
Tampin	0	117	117	0	0	0	117	0
Jempol	0	118	118	0	0	0	118	0
Total	**8**	**763**	**771**	**1.0**	**19**	**753**	**771**	**2.5**

RBPT = Rose Bengal Plate Test; CFT = Complement Fixation Test
+ve=positive; -ve=negative
+ve=positive; -ve=negative

Table 2. Seropositivity of *B. melitensis* between genders

Gender	Total	RBPT +ve (%)	CFT +ve (%)
Male	78	1 (1.3)	1 (1.3)
Female	693	7 (1.0)	18 (2.6)

RBPT=Rose Bengal Plate Test positive; CFT=Complement Fixation Test
+ve=positive; -ve=negative

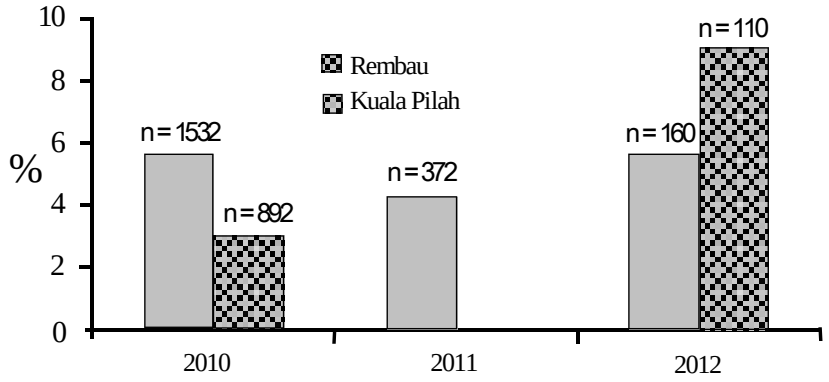

Figure 1: Serological Prevalence of Brucellosis in Goats in Rembau and Kuala Pilah using CFT (2010 – 2012) (Courtesy of Department of Veterinary Services, Negeri Sembilan)

DISCUSSION

Brucellosis in goats is caused by *B. melitensis* which is a gram-negative coccobacili. The agent causes high economic losses in the host animal and is highly zoonotic to humans. The mode of transmission between animals is mainly through the ingestion of contaminated feed and water with uterine fluid and placenta expelled by infected cows while transmission to humans is mainly via ingestion of contaminated or non-pasteurized milk products and by-products. Typical signs of brucellosis are abortion and infertility.

From the annual report of the Department of Veterinary Services (DVS), Negeri Sembilan, Rembau and Kuala Pilah showed the highest prevalence of brucellosis in goats compared to other districts. The frequency of the disease monitoring contributed towards the decline in the prevalence of brucellosis in Rembau and Kuala Pilah from 2010 to 2011. In 2012, the seroprevalence of brucellosis was the higher than the previous years because the blood was sampled from goat farms with history of brucellosis. Both the districts may be the source of infections to other districts in Negeri Sembilan through the 'Pawah' scheme. Since the disease was not detected in the districts along the border of Negeri Sembilan, there is low probability that the disease wil spreading to the neighbouring states of Pahang and Selangor.

The RBPT and CFT are the most widely used tests for serological diagnosis of brucellosis in sheep and goats (MacMillan, 1990). The CFT was used as a confirmatory test for control and eradication programmes and are also recommended as serological tests prescribed for international trade (OIE, 2000). Studies have shown that there is possibility RBPT giving negative results with CFT positive when the infection is at the early stages or the animal had consumed colostrum. However, these animals were more than one-year old, thus colostrum consumption is not the likely reason. False negative can a result of improper storage of the RBPT antigen, because the antigen loses sensitivity at temperatures exceeding 4°C. In this project, the RBPT antigen used was properly stored, thus false positive result is not expected. Furthermore, one serum sample showed RBPT positive but CFT negative. This could be due to an antigen-antibody

cross-reaction from organisms sharing structurally similar genera such as *Yersinia enterocolytica* type 0.9.

To decrease the occurrence of brucellosis in Negeri Sembilan, continuous surveillance of the positive farms need to be carried out by the DVS as a control measure. This should be done routinely and the same time restricts and control animal movement. Awareness about brucellosis and its zoonotic potential can help farmers to understand the danger of the disease. This can make them more willing to volunteer for the disease surveillance and control programmes.

Table 3. Sensitivity and specificity of RBPT compared to CFT

	CFT +ve	CFT -ve	Total
RBPT +ve	6	1	7
RBPT -ve	13	751	764
Total	**19**	**752**	**771**

Sensitivity of RBPT= 32%; Specificity of RBPT= 99.9%
RBPT = Rose Bengal Plate Test; CFT = Complement Fixation Test
+ve=positive; -ve=negative

Table 4. Sensitivity and specificity of CFT compared to RBPT

	RBPT +ve	*RBPT -ve*	*Total*
CFT +ve	6	13	19
CFT -ve	1	751	752
Total	**7**	**764**	**771**

Sensitivity of RBPT= 32%; Specificity of RBPT= 99.9%
RBPT = Rose Bengal Plate Test; CFT = Complement Fixation Test
+ve=positive; -ve=negative

CONCLUSION

The serological detection of *B. melitensis* was successfully carried out by the RBPT and CFT. The prevalence of brucellosis in goats determined by the RBPT and CFT was 1.0% and 2.5%, respectively. These results reaffirmed what have been indicated previously that the CFT is more sensitive than the RBPT. The CFT which is used as a confirmatory test for the control or eradication programmes is also recommended as a test prescribed for international trade. This study also found that in Negeri Sembilan, only Rembau and Kuala Pilah were seropositive for brucellosis.

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