Veterinary Research 5 (2): 13-15, 2012
ISSN: 1993-5412

Investigations on Seroprevalence of Bovine Brucellosis in Northeastern, Sudan

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Abstract: A total of 377 serum samples were collected from un-vaccinated cattle brought for slaughter in Northeastern, Sudan to determine the seroprevalence of Bovine Brucellosis (BB) and to evaluate preliminarily the in-house Rose Bengal Plate Test (RBPT). The RBPT and Indirect Enzyme Linked Immunosorbant Assay (I-ELISA) were used to screen all serum samples. The RBPT was carried out in two approaches, the standard RBPT and modified RBPT (mRBPT). The seroprevalence of BB based on I-ELISA and RBPTs were respectively 15.4% (58/377) and 14.3% (54/377). A total of 6.9% (4/58) of true positive was misdiagnosed as negative by both RBPTs whereas 37% (20/54) of strong positive reaction (+3) by mRBPT was agglutinated weakly (+1) by RBPT. There was significant difference between RBPT and mRBPT in respect of degree of agglutination at $p<0.05$ level. A good agreement between I-ELISA and RBPTs tests was observed ($k = 0.96$). The study showed that application of mRBPT enhanced the clarity of test reading compared with RBPT and the I-ELISA was more sensitive than both RBPTs.

Key words: Bovine, brucellosis, prevalence, sensitive, misdiagnosed, Sudan

INTRODUCTION

Brucellosis is an infectious zoonotic bacterial disease responsible for considerable economic losses and serious threat to public health. Outbreaks in cattle are associated with abortion, reduced milk yield, weak new born and infertility (WHO, 1971). Human infection is due to consumption of contaminated dairy products or exposure to infected animals (Neta *et al*., 2010).

The definite diagnosis of brucellosis is performed by isolation and identification of the causative agent. Serological tests are normally preferred because isolation is time consuming and hazardous (Poester *et al*., 2010). There are numerous serological tests have been used for the diagnosis of brucellosis in animals as screening or confirmatory tests (OIE, 2009). The Rose Bengal Plate Test (RBPT) and Indirect Enzyme Linked Immunosorbant Assay (I-ELISA) are ones of those initially used as screening tests and were found to be most sensitive for serodiagnosis of brucellosis (Marin *et al*., 1999).

In Sudan, the disease is prevalent in different regions affecting cattle, camel, sheep and goats (Musa and Jahans, 1990; Musa *et al*., 1990a, b; Hashim *et al*., 2007). But comparable data regarding the prevalence of Bovine Brucellosis (BB) in Northern East of the country are not available. The present study was formulated to determine the seroprevalence of BB in Red Sea state, Northeastern Sudan and to evaluate preliminarily the in-house RBPT which is usually used extensively for different diagnostic purposes.

MATERIALS AND METHODS

Study population and design: Cattle belonging to Zebu breed which brought for slaughter to the main abattoir of Red Sea state, Northeastern Sudan were investigated serologically for BB from September, 2009 to September, 2010. Adult and un-vaccinated cattle were only included in the study.

Because the expected prevalence of BB was unknown in the area of study, it was assumed to be 20% with an absolute precision of 5% at the 95% level of confidence. Accordingly, 246 sample of sera were actually required but up to 377 samples were collected randomly using systemic procedure (Thrusfield, 2005).

Collection of samples: The blood samples were withdrawal aseptically from jugular vein into sterile vaccutainer tubes conveyed immediately to the laboratory and allowed to stand at upright position at room temperature. The separated sera were transferred to sterile microfuge and stored at -20°C until needed.

Serological tests: The in-house RBPT obtained from Central Veterinary Research Laboratories, Soba, Khartoum

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was used initially to screen cattle for the presence of *Brucella abortus* antibodies. The RBPT was carried out in two approaches, the standard RBPT according to procedure described by OIE (2009) and modified RBPT (mRBPT) as described by Blasco *et al.* (1994). The positive results of both RBPTs were graded from +1 (Weak positive) to +3 (Strong positive). The negative reactions were recorded as 0 (No agglutination). All serum samples were retested by I-ELISA according to the manufacturer's instruction (Brucella-Ab I-ELISA kit, Svanova Biotech-Uppsala, Sweden).

Data analysis: The seroprevalence of BB was defined as proportion of seropositive cattle out of the total tested animals. Sensitivity and specificity of RBPTs was calculated, respectively as proportion of cattle that were seropositive or seronegative in relation to I-ELISA corresponding findings. Difference in degree of agglutination between RBPT and mRBPT was analyzed statistically by using McNemar's χ^2-test and considered significant at $p<0.05$ level. Comparison of agreement between I-ELISA and RBPTs was determined by kappa coefficient.

RESULTS AND DISCUSSION

The results obtained by different serological methods were shown in Table 1. Based on I-ELISA, the overall seroprevalence of BB was 15.4% (58/377). Of which, 6.9% (4/58) was false negative by RBPT and mRBPT. Only 14.3% (54/377) of tested cattle was diagnosed positive by both RBPTs. A total of 37% (20/54) of weak (+1) reactions by RBPT was agglutinated strongly (+3) by mRBPT.

There was significant difference between RBPT and mRBPT in respect of degree of agglutination at $p<0.05$ level (χ^2 = 18.05, 95% Cl; 0.37±0.13). Sensitivity and specificity of both RBPTs were found to be 93.1 and 100%, respectively (Table 2). A good agreement (k = 0.96) between I-ELISA and RBPTs tests was observed (Table 2).

Table 1: Seroprevalence of bovine brucellosis diagnosed by different serological tests in Northeastern, Sudan

Test	Positive	Negative	Total	Seroprevalence (%)
I-ELISA	58	319	377	15.4
mRBPT	54	323	377	14.3
RBPT	54	323	377	14.3

Table 2: Sensitivity and specificity of RBPTs in relation to I-ELISA for diagnosis of bovine brucellosis

	Indirect ELISA			Sensitivity specificity		
Test	Positive	Negative	Total	Positive	Negative	Kappa coefficient
RBPTs						
Positive	54	0	54	93.1%	100%	0.96
Negative	4	319	323	-	-	-
Total	58	319	377	-	-	-

The nomadic and seminomadic population in Sudan rely mainly on livestock breeding and dairy products. Brucellosis in cattle can pose a considerable potential risk to their animal welfare and public health. The absence of control and hygiene measures which represented by grazing of animal from different species and sources either in pasture or corrals, crowding at water points and markets, illiteracy among herders, animals and owners coexistence, consumption of raw dairy products and inadequate application of vaccination programme had played a major role in spread of the disease. The present study showed that the seroprevalence of BB is relatively high either based on RBPTs (14.3%) or I-ELISA (15.4%). Approximately similar prevalence rate of 16.9% was reported recently in cattle in Kassala state by the milk ring test (Omer *et al.*, 2010). The spread of the disease in the area of study is expected to increase as long as the previous mentioned factors exist. This was concluded in review of previous data reported from Kassala state that showed seroprevalence of BB was progressively increased from 5.1-17.1% during the period 2004-2006 (Ahmed *et al.*, 2007).

The study also explained that the two approaches of RBPTs provided satisfactory result for detecting positive cases compared with I-ELISA findings (k = 0.96) and they were capable to identify 93.1% of seropositive samples of infected cattle. But it should be noted that application of mRBPT in cattle is only preferred to enhance the clarity of test reading as any visible agglutination is considered to be positive (OIE, 2009). This observation support the report of Omer *et al.* (2010) who reported that mRBPT facilitated the reading of agglutination and was recommended for screening camel sera for brucellosis.

The present data also suggested that seronegative results obtained by RBPTs need confirmation with other test such as I-ELISA which was found more sensitive. The sensitivity and specificity of I-ELISA was found previously comparable if not superior with rose bengal, buffered plate antigen, 2-mercaptoethanol agglutination and complement fixation tests (Saravi *et al.*, 1995).

CONCLUSION

In this study, the results explained that BB in the Northeastern of Sudan is relatively high. Serological screening of the disease in cattle by mRBPT and I-ELISA may provide, respectively clear agglutination and detection of more positive serum samples.

ACKNOWLEDGEMENTS

The researchers greatly appreciated the helps provided by technician staff in processing of samples throughout the period of study.

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