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Full Length Research Paper

Evaluation of four serological tests to detect prevalence of bovine brucellosis in Khartoum State

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To estimate the prevalence rate of bovine brucellosis in Khartoum State of Sudan and to compare the different serological tests, 636 stratified random samples of milk from farms and markets in the state were collected. Individual milk and blood samples were also collected from farms which showed positive reaction to the bulk milk ring test. All these samples were tested for *Brucella* antibodies using the milk ring test (MRT), Rose Bengal plate test (RBPT) and indirect ELISA test on milk and serum. The prevalence of *Brucella* antibodies was higher in milk compared with serum samples. In Enzyme-linked Immuno Sorbent Assay (ELISA) test using milk samples, it was 34.7%, followed by Milk Ring Test 32.5%, while the prevalence when using the serum was 27% with the Rose Bengal Plate Test and 24.4% when using serum-ELISA. The sensitivity and specificity of MRT were 85 and 95% and for RBPT were 92 and 94%, respectively. There were association between the MRT and milk-ELISA ($p < 0.01$) and between RBPT and serum-ELISA ($p < 0.05$). The percentage agreement among MRT and milk-ELISA was 83% Kappa ($p < 0.01$), while the percentage agreement between RBPT and serum-ELISA was 86% Kappa ($p < 0.01$). When comparing the sensitivity, specificity and percentage agreement between milk-ELISA and serum-ELISA for the same cows, sensitivity for milk-ELISA was 92.8% and the specificity was 98.8%. The percentage agreement between milk-ELISA and serum-ELISA was 79% Kappa ($p < 0.01$). Milk-ELISA will provide an easy and significant contribution to detect brucellosis compared to other serological tests.

Key words: Sudan, milk, ELISA, serology, serum-ELISA, brucellosis.

INTRODUCTION

Consumption of contaminated milk with *Brucella abortus* or other species of the genus may lead to an infectious zoonotic disease. The risk of acquiring the disease from unpasteurized milk is the major cause of public health hazard (Kang'ethe et al., 2000). Although brucellosis is an endemic disease in most African countries, the epidemiology of the disease in human and livestock is not well understood and available data are limited (McDermott and Arimi, 2002). The regulation concerning the consumption of only pasteurized milk is not implemented in many countries (Mangen, 2002). In Sudan, about 90% of milk sale is in the hands of illiterate farmers (Ministry of Agriculture and animal resources, 2006) who believe milk

is hygienic and good nutritive source under the condition they are milking and purchasing. Same percentage was reported by Bertu et al. (2010) from Nigeria. Since prevention of brucellosis in humans is mainly through prohibiting the marketing of raw milk and implementation of strict regulations for milk pasteurization (Bertu et al., 2010), the prevalence in human is expected to be high.

The prevalence of bovine brucellosis in Khartoum state was studied by many workers, Ibrahim (1973) showed that nearly all herds around Khartoum State reacted positively to the MRT. Suliman (1988) showed that almost all herds reacted positively to the MRT and when using the Rose Bengal test, the average serum prevalence of disease in cattle was found to be 15.8%. All these findings showed that bovine brucellosis is a major problem in some areas.

This study is carried out:

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Table 1. Total number of milk and blood samples from the three areas of Khartoum State.

Area	Total milk sample		Blood sample
	Individual bulk		
Oumdurman	40	154	40
Khartoum	90	93	90
Khartoum North	87	172	87
Total	217	419	217

1. To estimate the serum prevalence rate of bovine brucellosis in Khartoum State.
2. To compare the different serological tests in locating infected herds and individuals.
3. Since the sampling of milk is more easy and accessible than blood, so the use of ELISA test on milk was attempted to be compared and evaluated.
4. To advise the concerned department in the ministry of animal resources about the situation and the appropriate tests if control programs were to be attempted.

Serological diagnosis of brucellosis

Serological tests are therefore economical and reliable tools of diagnosis as there was a good correlation between isolation of *Brucella* and positive tests performed with sera and milk. When tests for detecting *B. abortus* antibodies in milk and serum are considered, the principal methods for detecting infected herds and for diagnosing brucellosis in individual animals are the serological tests which are mainly used for diagnosis of brucellosis (Noriello, 2004). The Rose Bengal plate test (RBPT) is the most widely used screening test for brucellosis in both humans and animals for its easiness and apparent simplicity of reading; however, interpretations of the RBPT results can be affected by personal experience (Cho et al., 2010).

Due to false positive and false negative results of the MRT and RBPT, several researchers recently reported the use of Enzyme-linked Immuno Sorbent Assay (ELISA) for *B. abortus* antibody in bovine milk or serum (Hunter and Allen, 1972; McGiven et al., 2003; OIE, 2004; Patel, 2007).

The indirect ELISA are highly sensitive and specific tests, and can be adapted to process a large number of samples in a short time. They are easy and economical in terms of time and effort, with sensitivity and specificity ranging between 98 to 99% for both serum and milk ELISA (Vanzini et al., 1998; OIE, 2004).

ELISA was found to be a better serological test as compared to RBT. It could be advocated for screening of animals (Ghodasora et al., 2010). The milk-ELISA for brucellosis appears to be an alternative of serum-ELISA and it was found to be more sensitive than MRT (Bertu et

al., 2010).

MATERIALS AND METHODS

Sources of samples

In this study, 636 raw milk samples were collected using the stratified random sampling from the three provinces of Khartoum State. Total number of farms in each province was supplied by the State Ministry of Agriculture and Animal Resources (Table 1). 30% of the farms in each province were selected by simple random sampling. Market milk was selected from each province. The samples were collected at two levels:

- (a) The farm level: In all farms, visited cows were milked by hand. Milk from individual cow and from farm bulk tank (Bulk) was collected.
- (b) The market level: Simple random samples from the main milk markets (Market), groceries and small vendors (Table 1).

30 farms that showed high prevalence rate of brucellosis with MRT in the three provinces of Khartoum State were selected. 217 cows were randomly chosen from these farms for collection of blood and milk samples.

Collection of samples

About 10 ml of milk were collected in sterile glass bottle either directly from the udder in cases of individual cows or from the milk tank or milk containers. Samples were then kept in an ice box and transported directly to the laboratory of the Faculty of veterinary Science in Shambat.

10 ml of blood was withdrawn using a labeled Vacutainer® type tube, put into a wire basket under shade before being taken to laboratory with minimum possible shaking.

Tests on milk samples

Milk ring test

The antigen used in this test was prepared by the Central Veterinary Research laboratory (CVRL) Soba, Khartoum. The procedure followed was as described by Alton (1988).

ELISA test on milk samples (indirect)

The test kits and the test procedure were supplied by Svanova Biotech AB-Uppsala Science Park Glunten-Uppsala-Sweden.

Tests on blood samples

Rose Bengal test

The Central Veterinary Research Laboratory (Soba) supplied the antigen. The procedure was described by Morgan et al. (1978).

ELISA test on serum

The test kits and test procedure were supplied by Svanova Biotech AB-Uppsala Science Park Glunten, Uppsala, Sweden.

Sensitivity, specificity and percentage agreement were calculated according to Gordis (2004). Chi-square and Kappa tests were used.

Table 2. Number and percentage of positive samples to different *Brucella* antibodies tests.

Area	No. of milk samples	Positive milk		No. of blood samples	Positive serum	
		MRT	ELISA		RBPT	ELISA
Khartoum	183	58 (31.7)	61 (34.4)	40	11 (27.5)	10 (24.3)
Khartoum North	259	101 (39)	111 (42.4)	90	31 (34.0)	32 (35.1)
Omdurman	194	48 (24.7)	42 (21.7)	87	17 (20.0)	11 (12.9)
Total	636	207 (32.5)	214 (34.7)	217	59 (27.0)	53 (24.4)

Table 3. The relation between ELISA results and milk ring test results.

Test		Positive	Negative	Total
ELISA (milk)	Positive	188	019	207
	%	25.5	11.8	37.3
MRT	Negative	033	396	429
	%	8.3	54.4	62.7
	Total	221	415	636

1) Sensitivity = 85%; MRT specificity = 95%. Chi square table (0.01) = 6.63; Chi square calculated = 17.9. There is an association between the two tests at $p = 0.01$.

2) Kappa Statistic- observed Agreement = 0.92. Agreement by chance = 53.4. Kappa ($p < 0.01$) = 0.83. It is above 0.75, so the agreement is excellent.

Table 4. The relation between ELISA results and RBPT.

Test		Positive	Negative	Total
ELISA (serum)	Positive	49	10	59
	%	22.6	4.6	27.2
RBPT	Negative	4	154	158
	%	1.8	71	72.8
	Total	53	164	217

Sensitivity = 92%, Specificity = 94%; Chi square (1, 0.5) = 3.84; Chi square calculated = 4.80. There is an association between the two tests at $p = 0.05$.

Kappa statistic Observed Agreement = 0.94. Agreement by chance = 55%. Kappa ($p < 0.01$) = 0.86. It is above 0.75 so the agreement is excellent.

The statistical programme SPSS was used for calculating the association results.

RESULTS

The average prevalence rate of bovine brucellosis when using milk ring test, was 32.5% in Khartoum State. The percentages of positive reactors in the three geographical areas of the state were 31.7, 39 and 24.7% in Khartoum region, Khartoum North and Omdurman, respectively (Table 2).

The percentage of positive reactors to Rose Bengal Plate test was 26.8, 34 and 20% in Khartoum, Khartoum North and Omdurman, respectively with an average serum prevalence of 27% in Khartoum state (Table 3).

ELISA results

a) Milk: When milk was used in the ELISA test, the reactors were 34.7% in Khartoum state and the prevalence rate was 34.4, 42.8 and 21.7% in Khartoum region, Khartoum North and Omdurman, respectively (Table 2).

b) Serum: the serum prevalence of bovine brucellosis in Khartoum State when serum was used was 24.4% and the percentages of positive reactors were 24.3, 35.1 and 12.9% in Khartoum, Khartoum North and Omdurman, respectively (Table 2).

Association between different *Brucella* tests

Statistically using chi square test, there is an association between MRT and ELISA test on milk at probability 0.01 (Table 3). Also, there is an association between Rose Bengal test and ELISA on serum but at probability 0.05 (Table 4). The percentage of positive reactors on milk samples to both MRT and ELISA was 25.5% and the percentage of negative samples to both tests was 54.4%. The percentage of positive reactors on serum samples to both RBPT and ELISA was 22.6%, and the percentage of negative samples to both tests was 71% (Tables 3 and 4).

The sensitivity and specificity of RBPT if ELISA on serum is regarded are 92 and 94%, respectively. The percentage agreement between ELISA on serum and ELISA on milk is 97.2%, while the sensitivity and specificity of ELISA on milk are 92.8 and 98.8%, respectively (Table 5).

DISCUSSION

The presence of *Brucella* antibodies in milk and serum in herds around the state was studied by many workers, all of them agreed to the presence of *Brucella* antibodies in milk and serum in varying percentages, Suliman (1988) found 15.8% prevalence rate using MRT in Khartoum State; Nada (2000) reported that 37% of the market milk

Table 5. The relation between milk-ELISA and blood-ELISA.

Test		Positive	Negative	Total
ELISA (serum) Milk	Positive	52	04	56
	Negative	2	159	161
	Total	54	163	217

Kappa statistics observed Agreement = 97.2%; Agreement by chance = 62.2. Kappa ($p < 0.01$) = 0.79 is above 0.75, so the agreement is excellent.

in Khartoum State reacted positive to MRT. In this study, 32.5% of the samples reacted positively to MRT on the state. David et al. (1981) and Patel (2007) used milk-ELISA and showed that considerable increase sensitivity with the ELISA as compared to MRT. When using ELISA on milk samples during this study, 34.7% reacted positively, which is higher than the result obtained by MRT; this difference was also reported by Vanzini (1998). Statistically, the two tests got a significant level of association at $P = 0.01$ with sensitivity 92.8%, and specificity 98.8% for the MRT, compared with 30% sensitivity and 93% specificity reported by Patel (2007). When the two tests MRT and ELISA tests were used together to evaluate the prevalence, only 25.5% of the milk samples were positive reactors and 54.4% were negative to both tests, while 22.6% of the blood samples were positive in both RBPT and ELISA and 71.0% were negative to both tests. This shows that the antibodies in milk are higher than those in serum which was contrary to that reported by Patel (2007).

The RBPT was described by Heck et al (1980) as more sensitive than other more common sera antibody test. During this study, the prevalence rate of Brucellosis was 27.2% when using RBPT lower than the rate obtained when MRT is used alone and higher than the 25.5% obtained when MRT and ELISA were used together. It is also higher than that reported by Suliman (1988) in Khartoum State, for no disease control program was performed from that time. It is also higher than the 15.2% prevalence reported in Ethiopia by Hunduma and Regasse (2009) and 15% prevalence reported in Nigeria Bertu et al (2010). 24.4% of the animals tested were positive to serum-ELISA which is lower than the result obtained by RBPT.

The sensitivity and specificity of RBPT were 92 and 94% respectively, which is higher than the sensitivity and specificity of MRT; if ELISA was used as gold standard test. These findings show that the use of MRT or milk-ELISA although inexpensive and many herds can be tested easily, was not very satisfactory in detecting *Brucella* antibodies and some reactors detected by the test may be false positives.

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