African Journal of Microbiology Research Vol. 6(31), pp. 6086-6090, 16 August, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.653
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Serological survey of cattle brucellosis in Eldein, eastern Darfur, Sudan

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Accepted 11 May, 2012

The study was carried out to determine the prevalence of the brucellosis in cattle in Eldein area, Eastern Darfur state, Western Sudan. Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and Competitive Enzyme Linked Immuno Sorbent Assay (cELISA) were used for the diagnosis of the disease. RBPT showed 21 (8.4%) positive results, SAT showed 50(20%) positive sample and cELISA showed 5(2%) positive samples. Twenty four (9.6%) cows had a history of abortion, 20 (8%) had histories of retained placentas and 3 (1.2%) had knee hygromas. The study revealed that sex, age and breed were not associated with the brucella seropositivity (P>0.05). In this study, three types of serological test were used, namely RBPT, SATand cELISA and there was significant difference between any two given tests (P<0.05). Using agreement between tests (Kappa Statistic) indicated that RBPT and SAT had moderate agreement (Kappa=0.547), RBPT and cELISA had fair agreement (Kappa=0.364) and SAT and cELISA had slight agreement (Kappa=0.158). cELISA is the most sensitive and reliable test in field.

Key words: Brucellosis, cattle, serological tests, prevalence, Sudan.

INTRODUCTION

Brucellosis is a one of the highly contagious and most important zoonotic diseases in tropical area and a significant cause of reproductive losses in animals (OIE, 2009). Animal brucellosis poses barrier to trade in animals and animal products and could seriously impair socio-economic development, especially for livestock owners (Corbel, 2006). Losses due to abortion or stillbirths, irregular breeding, loss of milk production and reduced human productivity are some of the economic consequences of the disease. The reduced human productivity can hardly be measured in medical care. Brucella considered as a possible bio-terrorist agent. However, it has never been successfully used in this manner. *Brucella abortus, Brucella melitensis* and *Brucella suis* considered as “agents of mass destruction” and as category B organisms. In animals, bovine brucellosis is characterized by reproductive failure which can include abortion, birth of weak, unthrifty calves, orchitis and/or epididymitis in male. The organism causes abortion in cattle after the fifth month of pregnancy with retention of placenta, metritis and subsequent period of infertility. The proportion of cows that abort within a herd is variable and small percentage of infected cows abort more than once. The objectives of this study was to investigate the seroprevalence of brucellosis among cattle in El-Ddein area, using Rose Bengal Plate Test, Serum Agglutination Test and Competitive Enzyme linked Immunosorbant Assay and to evaluate the sensitivity of these test in diagnosis of the disease in the field.

MATERIALS AND METHODS

Study area

The study area was Eldein area, South Darfur state in western Sudan. The region lies between latitude 12˚ and 9.5˚ N and longitude 28˚and 25.5˚E.

Sampling methods

Two sampling methods were used in this study as described by

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Table 1. Herd composition in different areas of Eldein locality in 2011- 2012.

Location		Eldein	Abujabra	Bahar Elarb	Asalaia	Elfardoas	Total
Breed	Rizigat	48(63.2%)	39(79.6%)	50 (92.6%)	5(11.9%)	23(79.3%)	165(66%)
	Kenana	6(7.9%)	2(4.1%)	2(3.7%)	12(28.6%)	3(10.3%)	25(10%)
	Butana	13(17.1%)	7(14.3%)	2(3.7%)	25(59.5%)	3(10.3%)	50(20%)
	Ambrarrow	1(1.3%)	1(2%)	0(0%)	0(0%)	0(0%)	2(0.8%)
	Cross	8(10.5%)	0(0%)	0(0%)	0(0%)	0(0%)	8(3.2%)
	Total	76(100%)	49(100%)	54(100%)	42(100%)	29(100%)	250(100%)
Sex	Male	27(35.5%)	7(14.3%)	13(24.1%)	3(7.1%)	29(100%)	79(31.6%)
	Female	49(64.5%)	42(85.7%)	41(75.9%)	39(92.9%)	0(0%)	171(68%)
Age	1-3 years	5(6.6%)	6(12.2%)	5(9.3%)	3(7.1%)	0(0%)	19(7.6%)
	4-6 years	20(26.3%)	15(30.6%)	16(29.6%)	15(35.7%)	10(43.5%)	76(30.4%)
	7-9 years	48(63.2%)	27(55.1%)	25(46.3%)	18(42.9%)	19(65.5%)	137(54.8%)
	>9years	3(3.9%)	1(2%)	8(14.8%)	6(14.3%)	0(0%)	18(7.2%)

Thrusfield (2007). The multi-stage cluster sampling (cluster refer to region, herd size and animals). However, selection of clusters (region, herd size and animals) were done according to support or willingness of the owner and this called a non-probability sample methods.

Surveys carried out

To collect samples for the study, many places were visited in Eldein area, South Darfur state:

(1) Eldein Locality: Eldein vaccination cruch (Nomads), Eldein slaughter house, and Eldein cattle market, and others.
(2) Abujabra Locality: Abujabra cattle market and others.
(3) Bahr Elarab Locality: Bahr Elarab vaccination cruch (Nomads) and others.
(4) Alfardoce Locality: Alfardoce cattle market.
(5) Asalaia Locality: ELGhazala Gawazet, OmElghora, and Asalaia market.

Sample collection

A total of 250 serum samples were collected from 89 herds of cattle (herd size ranged between 20-500), by veinpunture of the jugular vein using vacutainer tubes with needle holders (Becton and Dickson). After obtaining blood, and to facilitate separation of sera, racks containing blood tubes were either placed inside a small box on top of ice or were being put under a shade of a tree either on damped sand or in a trough of cold water. After four to five hours, the clots were separated from sera by a straight, clean and sterile wire, taking care not to carry over traces from one sample to another. Later when sera separated from the clots, the formers were collected in the plastic vials with caps, labelled appropriately, placed in the sample plastic bags, preserved in a large ice box and transported to the laboratory. The distribution of the samples within the herd composition are shown in Table 1.

Serological tests

Rose bengal plate test (RBPT)

The antigen used in the RBPT was obtained from (Research of Veterinary Institute), Soba, Sudan.

It was prepared and standardized as described by Alton et al. (1975). The serum samples and the antigen were removed from the refrigerator and placed at room temperature for an hour then the test was done by dispensing 0.025 ml of each serum to be tested to an enamel plate. The same amount of RBPT antigen was added to each serum and both were mixed together, rocked by hand for four minutes after which the test was immediately read. Agglutination appeared as weak positive, positive, strong positive or very strong positive (Alton et al.,1975).

Serum agglutination test (SAT)

The antigen used for SAT was standardized concentrated antigen supplied by (Research of Veterinary Institute), Soba. The antigen was diluted to 1 to 12 using 5 ml phenol saline. The test was preformed as follows:

(1) Eight test tubes were placed in row in a rack for each sample.
(2) 0.8 ml of 5% NaCl solution was added to the first tube and 0.5 ml into each of the remaining seven tubes using 1 ml graduated pipette.
(3) 0.2 ml of serum was added to the first tube of each raw mixed well with the 5% NaCl by sucking and expelling gently to avoid producing bubbles.
(4) 0.5 ml of mixture as transferred to the next tube, mixed well with the 5% NaCl, then 0.5 ml was transferred to the third tube.
(5) Doubling the dilution was continued up to 8 tube then 0.5 ml from the last tube was discarded.
(6) 0.5 ml of the antigen was added to each tube.
(7) Control positive tubes containing equal amounts of antigen and known positive serum were included in the test.
(8) Control negative tubes containing equal amounts of antigen and known negative serum were included in the test.
(9) After shaking, the tubes were incubated at 37°C overnight.

The test was read by examining the tubes against a back background with light coming from behind the tubes A positive reaction is one in which the serum –antigen mixture is clear and agglutinated antigen appears at the bottom of the tube. Gentle shaking does not disrupt the floculi. This is a complete agglutination and is recorded as ++++. In partial agglutination serum-antigen mixture is partially clear and gentle shaking does not disrupt the

Table 2. Clinical signs indicating brucellosis in different areas in Eldein locality.

Variables		Eldein	Abujabra	BahrEl Arab	Asalia	Elfardoas	Total
History of abortion	Yes	10(13.2%)	4(8.2%)	5(9.3%)	5(11.9%)	0(0%)	24(9.6%)
	No	66(86.8%)	45(91.8%)	49(90.7%)	37(88.1%)	29(100%)	226(90.4)
History of retention of placenta	Yes	6(7.9%)	1(2%)	5(9.3%)	8(19%)	0(0%)	20(8%)
	No	70(92.1%)	48(98%)	49(90.7%)	34(81%)	29(100%)	230(92%)
Presence of Hygroma	Yes	0(0%)	2(4.1%)	0(0%)	1(2.4%)	0(0%)	3(1.2%)
	No	76(100%)	47(95.1%)	54(100%)	41(97.6%)	29(100%)	247(98.8%)

Table 3. Distribution of brucella positive reactors in animals in different areas in Eldein (2011-2012).

Location	No. of positive sample		
	RBPT	SAT	cElisa
Eldein	5(6.6%)	15(19.7%)	1(1.3%)
Abujabra	6(12.2%)	10(20.4%)	4(8.16%)
Bahr El Arab	3(5.6%)	10(18.52%)	0(0%)
Asalia	4(9.5%)	55(11.9%)	0(0%)
Alfardoas	3(10.3%)	10 (11.9%)	0(0%)
Total	21(8.4%)	50(20%)	5(2%)

Table 4. Association between some factors and occurance of the brucell seropositivity.

Factor	Chi- Square	P value	Interpretation
Breed	5.249	0.0263	Not significant, P > 0.05
Age	1.629	0.599	Not significant, P > 0.05
Sex	0.32	0.858	Not significant, P > 0.05

floculi, this was recorded as +++ or ++. Some sedimentation as + and no clearing as negative reaction (Alton, 1975).

Enzyme linked immunosorbant assay (ELISA)

Competitive ELISA

The test was done according to COMPLISA (Veterinary Laboratoty Agency, New Haw, Addlestone, Surrey KT 15 3NB United Kingdom. Version 2.0, June 2009).

Data analysis

SPSS version 19 was used for the data analysis. Descriptive statistic was used for the results as count and percentage, while, Chi-square was employed for assessing the relationship between various factors and presence of brucellosis. It was difficult to use sensitivity and specificity for evaluation of serological tests in this study because the gold standard was absent then agreement between tests (Kappa Statistic) was used without assuming one test was best. Kappa statistic ranges from one (Complete agreement) to zero (no agreement). Other point estimates according to Thursfield (2007) are:

0-0.2: slight agreement
0.21-0.40 fair agreement
0.41-0.61: moderate agreement
0.61-0.80: substantial agreement
>0.81: almost perfect agreement

RESULTS

A total of 250 serum samples from 89 herds were examined in different areas of Eldein locality. Twenty four cases of abortion, 20 cases of retention of placenta and 3 cases of knee hygromas were observed in different (Table 2). A total of 250 cattle were examined for brucellosis in Eldein locality and 21 (8.4%) serum samples were positive for the Rose Bengal Plate (RBPT), 50 (20%) serum samples were positive for Serum Agglutination Test (SAT) and 5 (2%) serum samples were positive for competitive ELISA (C ELISA) as confirmatory test (Table 3). Sex, age and breed were not associated with brucella seropositivity (P<0.05)(Table 4).

A high prevalence rate of the disease was recorded in

Abujabra area and out of 49 serum samples tested, 6 (12.24%) were positive by RBPT, 10 (20.4%) by SAT, and 4 (8.16%) by cELISA. A low prevalence rate was recorded in Afardoas area and out of 29 serum samples tested, 3 (10.34%) were positive by RBPT, 10 (11.9%) by SAT.

In this study, three types of serological tests were used, namely RBPT, SAT and cELISA and there was significants difference between these tests ($P<0.05$), using agreement between tests (Kappa Statistic) indicated that RBPT and SAT had moderate agreement (Kappa=0.547), RBPT and cELISA had fair agreement (Kappa=0.364) and SAT and cELISA had slight agreement (Kappa=0.158).

DISCUSSION

In this study, the results showed that the prevalence rate of cattle brucellosis in cattle in Aldein area was 8.4% based on (RBPT), 20%, based on SAT and 2% based on cELISA (Table 3). And this disagreement between tests could be attributed to less sensitivity of (RBPT) and SAT due to antibobies against cross reacting but cELISA was found to be more specific than the other tests (Table 4). The high prevalence rate was in Abujabra in Eldein area and this could be attributed to the fact that it has a very large numbers of livestock and Abujabra is bordering West Kordofan and shares international boundaries with Republic of South Sudan and a large transboundary movements of animals between these areas. But the low prevalence rates was in Alfardoas and this might be attributed to fewer number of animals compared to other areas. The prevalence rate was similar to those reported by other investigators in the country especially in Eldein area and areas with similar husbandry methods. Musa (1995) reported 15.75% prevalence rates of the disease in cattle in Eldein area. The findings of this work are in some difference with Musa (1995) this could be attributed to the large sample size (946) compared with 250 samples in this study but small herds used by Musa (about 23) compared with this study 89. Other point Musa reported the prevalence rate from different tests and he found 65 positive by CFT; 16 by SAT; 18 by MRT; 8 by Competitive ELISA; 39 by RBPT and Competitive ELISA and three by strong or very strong RBPT positive results. Similar results from Musa reported 15.8% prevalence rates of the disease in cattle in Bram area and this locality bordering Eldein area. Also Musa found that the prevalence rate of the disease in cattle of some close locality in great Darfur state is 22.2% in Idd El Firsan locality, 9.6% in Nyala locality, 12.8% in Wadi Salih locality, 8.8% in Zalingei locality. Yousif (2010) reported 10.3% prevalence rates of the disease in West Darfur state. Shigidi (2010) reported in different states of the Sudan and prevalence rate of the disease ranges from3-40%.

The study revealed that, brucellosis appears to be widely spread in Eldein area. The existence of brucellosis in the Eldein area coupled with the lack of control measures especially in the traditional sector which maintain the vast majority of animal wealth in Darfur. Methods of husbandry were found to be the major factors responsible for the spread of the disease. And the highest prevalence rate of brucellosis was found in nomadic cattle, and this is attributed to the fact that millions of cattle move continuously throughout the year, along particular routes making contacts between infected cows and susceptible ones.

In this study, three types of serological tests were used, namely RBPT, SAT and cELISA. RBPT and SAT were used as screening tests for Brucella antibodies in serum, while the cELISA were used as confirmatory test. The RBPT has been recommended by many investigators to be used for screening test for brucellosis. In this study 21 samples were positive for RBPT, but this was confirmed by SAT and cELISA. Furthermore, the test was reported to be more sensitive but less specific. cELISA in this study used as confirmatory test and more sensitive with high specificity than two other test, although the results obtained by cELISA.

Conclusions

According to this study; it could be concluded that, the prevalence of brucellosis in cattle in Eldein area is similar to that reported in the other parts of the country.

(i) Close vicinity to Republic of Southern Sudan and North Kordofan state resulted in high incidence rate of brucellosis, due to free animal movement.

RECOMMENDATION

(1) Number of samples used in this study were too small compared to the animal populations sampled (target population), so, it is recommended that, sample size should be representatives in further researches.

(2) There should be coordination with the related authorizes in the Republic of Southern Sudan to determine the magnitude of spread of the disease in the areas around the borders to adopt effective control programmes in these areas.

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