Detection of Brucellosis in sheep intended for export and local slaughter in Khartoum State, Sudan

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This study is designed to determine the prevalence rate of brucellosis in both sheep for export and local consumption ones. Brucellosis was studied in 404 heads of sheep in Khartoum State, Sudan. 300 heads of them were prepared for export and 104 were for slaughter for local consumption. Rose bengal plate test (RBPT), serum agglutination test (SAT) and competitive enzyme linked immunosorbent assay (cELISA) were used in the study. The overall prevalence was 3 (0.74%) by the RBPT and SAT and there were no serum samples positive by cELISA as confirmatory test. The prevalence rate was 3 (1.0%) in sheep intended for export and no positive samples found in the sheep of local consumption. According to the sex, the prevalence of the disease was 0.85% in males and no positive sample observed in females. There were no clinical signs of sheep brucellosis in all animals of the study samples although there were serologically positive samples. This study confirms the presence of sheep brucellosis in low rate which requires more attention and effort to implement procedures and regulation to eradicate the disease in Sudan.

Key words: Brucellosis, sheep, export, Sudan.

INTRODUCTION

Brucellosis is a contagious disease of animals which is transmissible to man. It is caused by main six species of the genus *Brucella*. These species are further subdivided into biovars that are useful in epidemiological studies (Musa et al., 2008). Brucellosis is a disease of many animal species but especially of those that produce food: sheep (especially milk-producing), goats, cattle and pigs and, on a more localized scale, camels, buffaloes, yaks and reindeer. Five of the six currently recognized *Brucella* species cause infection and clinical signs in one or more animal hosts. Four of these also cause human disease: *Brucella melitensis, Brucella suis, Brucella abortus* and *Brucella canis* in descending order of pathogenicity. The recently recognized types associated with marine animals may also have the capacity to cause human disease (Corbel, 2006). The disease in Sudan was first reported in animals by Bennet (1943) in a dairy farm in Khartoum. Subsequently, the disease was reported by many investigators all over the country. However, the disease was reported in human in 1904 in Berber in North of the Sudan (Haseeb, 1950).

The population of sheep in the Sudan was recently estimated to be 39,296,000 heads (Anon, 2011a). But from 1999 to 2005, a total of 17 ships carrying 40,428 sheep, 5,334 goats and 2,298 camels were rejected by Saudi authorities because of detection of some cases positive for brucellosis and that had resulted in heavy financial losses and bankruptcy of some animal traders (Musa et al., 2008).

The objective of the current research was to contribute to knowledge necessary to achieve these listed goals:

1. Determine the prevalence of ovine brucellosis in those herds ready for export.
2. Determine the prevalence of ovine brucellosis in...
animals that were slaughtered for local consumption.

MATERIALS AND METHODS

Study area

This study was carried out in sheep intended for export and local slaughter in the period from October 2011 to December 2011 in Khartoum State, which is located in North Eastern part of the centre of Sudan. The state is located between latitude 15.8° and 16.45° north, longitude 31.5° and 34.45° east. The state covers 20,736 km². The climate is semi-desert, dry and hot in summer (maximum temperature of 47.1°C and minimum temperature of 22.7°C). The range of rainfall is 150 mm per year. The main sheep breeds in Khartoum state are Hamari, Kabsahi, Gazera and Zagawi sheep.

Study population

The study population was sheep collected from different herds that were prepared for export from the Alkadru quarantine and from sheep brought to Alkadru slaughter house for local consumption.

Sampling method

The sampling method used in this study was stratified random sampling according to the purpose of keeping the animals. The two purposes were animals for live export and animals for local consumption. Simple random sampling had been carried out in each stratum.

Samples for serological examinations

Convenient blood samples were taken, cleanly, by venipuncture. The jugular vein is selected. The skin at the site of venipuncture was shaved and swabbed with 70% alcohol and allowed to dry. 5 ml of blood was been taken by needle and plain vacu um tube (OIE, 2008). The blood tubes placed in racks and left to stand at ambient temperature for 1 to 2 h in slanting position until the clot begins to contract. The racks bottles placed in a refrigerator at 4°C. After overnight, sera decanted or removed with a pipette in eppendorf tubes, labeled and preserved in an ice box which is transported to laboratory. All sera samples were kept at -20°C before serological tests.

Serological tests

Rose Bengal plate test (RBPT)

*Brucella* colored antigen used in this test was donated by Division of Brucella research in Veterinary Research Institute (VRI) Soba. The antigen was diluted 1:12 using phenol saline. According to Buxton and Fraser (1977), the test was preformed as follows:

1. Eight test tubes were placed in raw 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 transferred from the first tube to the next tube, mixed well with the 5% NaCl, and then 0.5 ml was transferred to the third tube and so on.
5. Doubling the dilution was continued up to the 8th tube then 0.5 ml from the last tube was discarded.
6. 0.5 ml of the diluted 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 black 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 flocculi. 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 flocculi; this was recorded as +++. Some sedimentation as + and no clearing as negative reaction (Alton et al., 1975).

Enzyme linked immune-sorbent assay (ELISA)

Competitive ELISA


Kit contents and instructions

All the following are included in the kit before proceeding. Refrigerate kit immediately on arrival and store conjugate at -20°C.

Plates: Plates pre-coated with *B. melitensis*, LPS antigen and Lid.
Diluting buffer: Tablets of phosphate buffered saline (PBS), phenol red Indicator and Tween 20.
Wash solution: NaH₂PO₄ and Tween 20.
Conjugate: As supplied (store at -20°C).
Chromogen: OPD tablets (irritant).
Substrate: Urea hydrogen peroxide tablets (irritant).
Stopping solution: Citric acid (irritant).
Control: Positive serum and negative serum.

Equipment required

Microtitre plate reader with 450 nm filter, Single and multichannel variable volume pipettes, disposable tips for the foregoing, Reagent troughs for multichannel pipetting, 10 L container for wash fluid, 4 ± 3°C refrigerator, rotary shaker, capable 160 Rvs/Min (or a 37 ± 3°C incubator), microtitre plate shaker, sterile distilled de ionized water, bottles tubes and beakers for storage of sera and reagents, absorbent paper towels, and freezer for storage of conjugate.

The microtitre plate reader is not essential as an assessment of
the results can be performed visually. The use of an incubator and or shaker is preferable, but by adapting the method their use is not essential.

Reagent preparation

1. Reagents provided are sensitive to changes in temperature and light. They must be prepared and stored as per instructions if they are effective in the test.
2. Very clean glassware and pure distilled water are vital for the preparation and storage of reagents.

Diluting buffer

Prepare diluting buffer by adding 5 tablets of PBS, 0.5 ml phenol red indicator and 250 µl of Tween 20 to 500 ml distilled water. The pH must be between 7.2 and 7.6 - phenol red will turn yellow below pH 7.2 and violet above pH 7.6. The buffer should be discarded if this happens and store at 4 ± 3°C. Do not keep for more than 1 month.

Wash solution

Prepare the wash solution by adding the contents of an ampoule of NaH2PO4 (0.14 g) and 1 ml of Tween 20 to 10 L of distilled water. This can be stored at room temperature (21 ± 6°C). Do not keep for more than 1 month.

Conjugate

Prepare the conjugate by adding 1 ml of the content of the conjugate ampoule to 11 ml of diluting buffer to give 12 ml of the conjugate. Once the conjugate has been prepared according to instructions on the ampoule, it must not be stored.

Stopping solution

Prepare the stopping solution by diluting the contents of the ampoule of citric acid (2 ml) with 38 ml of distilled water and store at 4 ± 3°C. Do not keep for more than 1 month.

Controls

Reconstitute each of the positive and negative control samples included in the kit with 1 ml sterile distilled water. Allow to stand until an even suspension is obtained. Ensure the entire contents are completely resuspended before use. Store at 4 ± 3°C. If the control samples are to be kept more than 1 week, store at -20 ± 5°C in aliquots.

Procedure

1. Prepare the conjugate solution. Dilute to working strength with diluting buffer according to instructions on the ampoule label.
2. Add 20 µl of each test serum per well. Leave columns 11 and 12 for controls.
3. Add 20 µl of the negative control to wells A1, A12, B1, B12, C11 and C12.
4. Add 20 µl of the positive control to wells F11, F12, G11, G12, H11 and H12.
5. The remaining wells have no serum added and act as the conjugate controls.
6. Immediately, dispense into all wells 100 µl of the prepared conjugate solution. This gives a final serum dilution of 1/6.
7. The plate is then vigorously shaken (on the microtitre plate shaker) for 2 min in order to mix the serum and conjugate solution. Cover the plate with the lid and incubate at room temperature (21 ± 6°C) for 30 min on a rotary shaker, at 160 revs/min.
8. Shake out the contents of the plate and rinse 5 times with washing solution and then thoroughly dry by tapping on absorbent paper towel.
9. Switch on microplate reader and allow the unit to stabilise for 10 min.
10. Immediately before use prepare the substrate and chromogen solution by dissolving one tablet of urea H2O2 in 12 ml of distilled water. When dissolved add the OPD tablet and mix thoroughly. This can take a few minutes, the use of a magnetic stirrer greatly increase the speed with which it dissolves. Add 100 µl to all wells. This solution cannot be stored.
11. Leave the plate at room temperature for a minimum of 10 min and a maximum of 15 minutes.
12. Slow the reaction by adding 100 µl of stopping solution to all wells.
13. Remove condensation from the bottom of the plate with absorbent paper towel. Read plate at 450 nm.

Analysis of results

The lack of colour development indicates that the sample tested was positive. A positive/negative cut-off can be calculated as 60% of the mean of the optical density (OD) of the 4 conjugate control wells. Any test sample giving an OD equal to or below this value should be regarded as being positive.

Plate rejection

The results considered invalid if any of the following apply;

1. The mean OD of the 6 negative control wells is less than 0.70.0.
2. The mean OD of the 6 positive control wells is greater than 0.100.
3. The mean OD of the 4 conjugate control wells is less than 0.700.
4. The binding ratio is less than 10.

\[ \text{Binding ratio} = \frac{\text{Mean of 6 negative control wells}}{\text{Mean of 6 positive control wells}} \]

RESULTS

This study was planned to investigate the prevalence of Brucellosis in sheep intended for export and the sheep of local consumption in Khartoum state. A total of 404 serum samples collected from different sheep herds in Khartoum state, 300 (74.3%) of them were for the purpose of live export and 104 (25.7%) for the local slaughter (Table 1). Out of the 404 serum samples 3 (0.74%) positive for brucellosis by the Rose Bengal plate (RBPT) and serum agglutination test (SAT) and there were no serum samples positive by competitive enzyme linked immuno sorbent assay (c ELISA) as confirmatory
test (Table 2).

Regarding the purpose for keeping the animals, the positive samples were 3 (1.0%) in animals prepared for live export by RBPT and SAT, while there were no positive serum samples in animals that were intended for local slaughter, and both were negative in testing by cELISA (Table 2).

According to the breed, 2 (0.87%) of Hamari breed and 1 (0.79%) of Kabashi breed were positive by RBPT and SAT.

Neither carpal hygroma nor orchitis were observed in this study, there were no data for history of abortion or retained placenta in the females that were brought for slaughter (Table 2). The effect of castration was also studied by RBPT; there were 1 (1.16%) positive in castrated animals and 2 (0.64%) positive in non castrated animals. However, by SAT, the international units (IU/ml) of the three positive samples were 160 IU/ml and two samples were 67 IU/ml (Figure 1).

**DISCUSSION**

Sudan exports sheep, goats, camels and cattle to many countries especially to Saudi Arabia and other Arabic Gulf countries (Anon, 2011a). These exported numbers influence with epidemics that emerge spontaneously. The number of tested animals in different species for Brucellosis depends on this exportation movement.

This study revealed that the overall prevalence of sheep Brucellosis in Khartoum State was 0.74% by RBPT and SAT. However, the prevalence in sheep that were kept for live export was 1% while there were no positive results in sheep that brought for slaughter for local consumption. All cELISA results were negative.

In this study, the prevalence of sheep Brucellosis in export animals was relatively low. That may be due to the good healthy status of these animals (well fed and treated with antibiotics and anthelmintics) and the highly selectivity of them for export. The negative result of sheep Brucellosis in the animals for local consumption may be explained by the low proportion of the sample size (0.0158%) to the total heads of sheep that slaughtered in that year (Anon, 2011b). Corbel (2006) stated that the ELISAs are more sensitive than the RBPT, but sometimes they do not detect infected animals which are (RBPT) positive. This may explain the miss detection of cELISA for the positive results that found by RBPT and SAT in this study. El Sanousi (2012) attributed the low prevalence rates of brucellosis among animals of the study to several factors that might reduce the spread of the disease, these factors include the climatic conditions of the Sudan (persistence of the sun light at the most hours of the day, dry desert weather and low humidity) which may not favour survival of brucella organisms for long periods. In addition to that, the management breeding system of most sheep in the Sudan is nomadic pastorialist which prevents clustering of animals and herds. More over that, the harvest of the sheep herds in the Sudan usually take place early before sexual maturation and so favors elimination of both infection and contamination of the pasture.

In spite of the relatively small sample size of this study because of limited support and materials available, the low prevalence rate (1.0%) is in agreement with Osman and Adlan (1968) in that Brucellosis is prevalent among cattle and camels but rare among goats and sheep. This result is similar to the result of Abdalla (1966) who found the prevalence rate of sheep Brucellosis was 1.7% in Wadi Halfa, North Sudan which can be attributed to the low density of animal population in the area and the desert climate.

Omer et al. (1989 to 1990) screened 33,591 castrated sheep males that were ready for export from (Alkdru) quarantine, Khartoum state and (Portsudan) quarantine, Red sea state by RBPT and found the prevalence rate of sheep Brucellosis is 0.01%. This low prevalence may have relationship to the previous restricted regime of castration of all exported males sheep.

The lowest prevalence rate of sheep Brucellosis was found by Ginawi (1997) who screened 500 heads in Blue Nile District. There were no positive sample in his study, although he used RBPT, SAT and CFT. Another low result found by El-Ansary et al. (2001) who found the
The prevalence rate of sheep Brucellosis is 1.0% but his research was carried out on sera of sheep brought for slaughter to Kassala abattoir. Another low prevalence rate of sheep Brucellosis reported by Ahmed (2004) in the Red sea State who found the rate was 0.3% in 2,050 heads. Omer et al. (2007) found that the prevalence rate is 0.9% in 61,266 heads of sheep in Kassala State. The similarity of these results of Eastern Sudan and this study might be due to similarity of the desert and semi desert conditions between that states and North Kordofan State where these sheep came from.

Other researchers screened the disease in sheep in different parts of the Sudan and found different results. Musa (1995) investigate the disease in Darfur States and found the prevalence rate was 3.6% in 2,324 heads of sheep. Musa (2005) reported a prevalence rate of 3.3% in the same area in 2,628 heads of sheep. This relatively high rate of infection may be attributed to the sheep management systems in Darfur states where sheep are mixed with either cattle or goats. Cattle, sheep and goats may be infected by *B. abortus* or *B. melitensis* (Aitken, 2007). Another reason may be the use of other 3 tests [modified SAT (mSAT), complement fixation test (CFT) and milk ring test (MRT)] more than in this study, among which (CFT) has good sensitivity and specificity. Mohamed and Salih (2007) screened the disease in Blue Nile state and found the prevalence rate was 3.3% by RBPT in only 60 heads of sheep. This result cannot be generalized over all the state because of the tiny sample size and one test used without confirmatory one. Omran (2011) investigated the disease in Sinnar state and got 4.1% prevalence rate in 585 heads of sheep. He used modified RBPT, SAT and ELISA.

### Conclusion

1. Sheep brucellosis has low prevalence rate in the
exported sheep from Khartoum State. 
2. There is no brucellosis in the sheep that are slaughtered for local consumption in Khartoum State.

RECOMMENDATIONS

1. More surveys are required to investigate the sheep brucellosis in the production states.  
2. There is need to plan, implement and monitor national eradication strategy for brucellosis in the country based on epidemiological reality.  
3. Follow up on capacity building of laboratory technical staff as well as of strengthening of national laboratories should be favorably considered in all programs of eradication and can be achieved by collaborating international organizations including OIE, FAO, WHO and Codex Alimentarius, where possible.  
4. In addition to effective veterinary services; (i) educational programmes to other stakeholders such as farmers, (ii) effective enforcement of legislation in conjunction with animal disease control, (iii) public awareness and preparedness of the disease which is transmissible to humans, are all essential for the disease control and the development of long-term disease control strategies.

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