

**Sero-prevalence of bovine brucellosis
in Kuku Dairy Scheme, Khartoum North,
Sudan**

T. E. E. Angara¹ ; A. A. Ismail²; H. Agab¹ and N. S. Saeed ²

**1. College of Veterinary Medicine and Animal Production,
Sudan University of Science and Technology,
P O Box 204, Khartoum North, SUDAN**

2. National Health Laboratory, Khartoum, Sudan.

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Abstract

A sero-prevalence study was conducted in Kuku Dairy Scheme, Khartoum North, Sudan. The scheme was proved to be endemic with bovine brucellosis. Cross-reaction with other bacteria and the possibility of false positive reactor animals due to vaccination had justified the use of competitive ELISA test for serum detection as a confirmatory test.

The number of cattle examined, throughout the study, was 574 out of 845 cows kept in Kuku Dairy Scheme. All the obtained sera were screened using Rose Bengal Plate Test (RBPT). Twenty eight out of the thirty herds of the sample had at least one positive reactor, resulting in 93.3% herd prevalence rate. All sera positive to Rose Bengal Plate Test (n = 178) were subjected to further confirmatory test using Competitive Enzyme-Linked Immunosorbent Assay (c-ELISA). 143 cows (80.3%) were confirmed positive by c-ELISA. Out of 28 positive herds, 27 (96%) had at least one positive reactor.

According to the confirmatory test, the herd prevalence rate was 90%, individual animal prevalence rate was 24.9% and average within herd prevalence rate was 24.5% (± 15.7 , CI 4.088 at 95%).

The number of seropositive aborter was found to be 17 cows out of 143 (12%). It is concluded that bovine brucellosis was highly prevalent in Kuku Dairy Scheme. This fact justifies immediate adoption of an effective control policy for this zoonotic disease.

Introduction

Brucellosis is an infectious disease caused by the bacteria of the genus *Brucella*. Various *Brucella* species affect sheep, goats, cattle, deer, elk, pigs, dogs, and humans (CDC, 2002). The disease was also reported in camels (Abbas and Agab, 2002; Hegazy *et al.*, 2004; Teshome *et al.*, 2003) and in marine mammals (seals, sea otters, dolphins, propoises) (Forbes *et al.*, 2000).

Brucellosis can be a serious economic disease. 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 (Nicoletti, 1982). The Centre for Disease Control and Prevention lists *Brucella* as a possible bio-terrorist agent. However, it has never been successfully used in this manner (CDC, 2002). The centre also classifies *B. abortus*, *B. melitensis* and *B. suis* as “agents of mass destruction” and as category B organisms (Elzer, 2002). 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 (Enright, 1990). The transmission and spread of brucellosis is affected by a variety of factors and good knowledge of these is essential to the success of a control policy (Reviriego *et al.*, 2000; Bikas *et al.*, 2003; Minas *et al.*, 2004). The economic loss due to abortion can be very high if no control measures are applied. Most of the infected cows, after aborting once, remain as carriers and are not abortive (Gonzalez-Guzman and Naulin,

1994). Bovine brucellosis caused mainly by *B. abortus* is still the most widespread form of the disease (Corbel, 1997). The disease in cattle is widely distributed and has been recorded in 120 out of 175 (68.8%) countries of the world (Nielson and Dunkan, 1990). Brucellosis continues to be an important source of morbidity primarily in the Mediterranean region, Arabian Peninsula, India, Mexico, Central and South America (Hurtado, 2001). The prevalence of bovine brucellosis is variable in cattle but is generally higher among dairy cattle than range cattle due to the intensive farming practices to which these animals are subjected (Langoni, 2000).

The disease occurs worldwide except in those countries where bovine brucellosis caused by *B. abortus* has been eradicated (OIE, 1996-updated 2004). The incidence varies considerably between herds, areas and between countries (Blood and Hinderson, 1989). The Mediterranean countries of Europe, Northern and Eastern Africa, Near East countries, India, Central Asia, Mexico and Central and South America are especially affected (Robinson, 2003).

The competitive enzyme-linked immunosorbent assay (c-ELISA) for the detection of serum antibodies to *Brucella* is a multispecies assay known to be capable of differentiating vaccinal and cross-reacting antibodies from those elicited by field infection in cattle (Lucero *et al.*, 1999). There is increasing evidence that competitive monoclonal antibody (Mab)-based ELISA (c-ELISA) may offer distinct advantages over the indirect enzyme-linked immunosorbent assay (i-ELISA) and all other conventional tests used in brucellosis serology. These advantages include: detection of animals at an earlier stage of infection; differentiation between antibody responses due to infection and those due to strain 19 vaccinations and elimination of false-positive reactions due to exposure to organisms bearing cross-reacting antigens (Stack and McMilland, 2003). Marín *et al.* (1999) compared the

competitive and standard enzyme-linked immunosorbent assays (ELISAs), Rose Bengal plate test (RBPT), complement fixation test (CFT), and agar gel immunoprecipitation with native hapten (AGID-NH) by using sera from *Brucella*-free, *Brucella melitensis*-infected, and *B. melitensis* Rev1-vaccinated sheep. It was found that the most sensitive tests were indirect ELISA and RBPT, and the most specific tests were AGID-NH and competitive ELISA. Poester *et al* (online) concluded that C-ELISA is a good confirmatory test with the advantage of distinguishing the antibody response due to vaccination from that resulting from infection with *Brucella abortus*.

Treatment of bovine brucellosis is not permitted; all infected cattle and contacts, which have been exposed to infection, must be slaughtered (Defra, 2004).

Vaccination of livestock is crucial to the control of brucellosis. Effective reduction of disease prevalence in livestock through mass vaccination will eventually lead to reduction of brucellosis in the human population (Henk *et al.*, 2004).

In Argentina and other countries in South and Central America, brucellosis has been recognized as a disease problem since the 19th century, but despite control efforts started in Argentina in 1932, the disease still is not considered under control in this country (Samartino, 2002). Although the situation of the disease in resource poor countries looks gloomy, the adaptation of control measures to the local situations in these countries together with the application of improved diagnostic methods and techniques could provide immediate cost-effective benefits (Roth *et al.*, 2003).

Currently *B. abortus* strain RB51 is the only vaccinal strain that does not cause vaccination titres (Elzer, 2002). However, Nicoletti (1990)

claimed that the attenuated live *B. abortus* S19 vaccine is the recommended vaccine for bovine brucellosis.

This sero-prevalence study was conducted in Kuku Dairy Scheme, Khartoum North, which supplies a considerable proportion of Khartoum State with cattle milk, to elucidate the prevalence rate of bovine brucellosis in the cattle of that major milk supplier to Sudan capital.

Materials and Methods

The required data were obtained from both primary and secondary sources. The primary source was the data collected during a sero-prevalence survey conducted in the period January-June 2004. Samples from cattle population were selected based on the method described by Robinson (2003). The sample design was based on a cluster random sample design. In the first stage, primary statistical units (clusters = the herds = holdings) were identified randomly. Given the total number of holdings (herds) of 215 (Agricultural Department, Kuku Dairy Project, 2004), the size of the primary statistical units was calculated to be 30 with $\alpha = 0.05$ and desired accuracy of 10 and expected prevalence of 90 % (Bakheit, 2004).

With regard to the secondary statistical units, all mature cows were targeted unless there was a problem in restricting the animal. The number of animals examined was 574 out of 845 cows constituting the total population of cows in the sample. For the identification of the animals, researchers relied on owners' experience. Owners in the scheme used to give names after their animals. The milkers were quite familiar with the animals and their names. For the animals identification, the researchers relied on the owner name, number and name of the animal.

Five ml venous blood was withdrawn from the milk vein using disposable syringes. Blood samples were transferred to the National Health Laboratory,

Khartoum, in thermo flasks with minimal possible shaking. Blood was allowed to clot before serum was separated into small clean tubes for serological testing. The laboratory diagnosis relied mainly on serological tests namely; Rose Bengal Plate Test (RBPT) and Competitive Enzyme linked Immuno-Sorbent Assay (cELISA). The serum samples were first screened using standardized buffered Rose Bengal stained antigen obtained from the Central Veterinary Laboratory, Khartoum using the technique described by Alton *et al.* (1975) and then subjected to competitive enzyme linked Immuno-sorbent Assay (c-ELISA) as a confirmatory test to eliminate any positive reaction due to vaccination or cross reaction. Kits with pre-adsorbed *Brucella* smooth liposaccharide (S-LPS) antigen to polystyrene plates were imported from Svanova Biotech-Uppsala, Sweden. The kits were first tested for validity. Then the method described by the producing company was applied.

Analytical framework: The deterministic part of the Ecozoo model developed by Zinsstage *et al.* (2005) was used for data analysis. These data include the necessary results of the disease epidemiology as well as data on herd composition. Transmission of the disease, cost of the agriculture sector and data on the cost of health sector will be presented in forthcoming communications. Accordingly herd prevalence rate, individual animal prevalence, average within herd prevalence and the sero-positive aborted were estimated.

The secondary sources included the data obtained from text books, journals, relevant studies and publications such as World Health Organization (WHO), Food and Agriculture Organization (FAO), International Epizootic Office (OIE), Centre of Disease Control and Prevention (CDC), electronic sources and web sites as well as the data obtained from General Administration of Planning and Livestock Economics (GAPLE), Ministry of Animal Resources and Fisheries, Sudan.

Results

The surveillance revealed that the total number of animals in the sample (30 herds) constituted 1438 heads of cattle which were classified as follows: 845 (59%) were mature cows, 20 heads (1%) were bulls, 396 (28%) were calves less than one year and 177 heads (12%) were heifers. However, the estimated cattle population in the 215 holdings of the scheme was 10306 heads, out of which 6056 were mature cows, 143 were bulls, 2838 were calves less than one year and 1269 were heifers (Table 1).

Table 1. Herd composition in Kuku Dairy Scheme.

Source	Calves<1year	Heifers	Adult cows	bulls	total
Sample	396	177	845	20	1438
Population	2838	1269	6056	143	10306

Source: computed from the laboratory results 2005

In the RBPT, 28 out of the 30 herds of the sample had at least one positive reactor, resulting in 93.3% herd prevalence rate (Table 2).

178 out of 574 samples tested positive to Rose Bengal antigen, resulting in 31% individual animal prevalence (Table 3).

Within herd prevalence rate ranged between 0% - 55.6% with an average of 30.08% (± 19.25).

Table 2. Herd prevalence in Kuku Dairy Scheme based on RBPT and c-Elisa

Test	Positive reactors	Negative reactors	Total	Prevalence rate (%)	Confirmatory rate (%)
RBPT	28	2	30	93.3	96
c-ELISA	27	3	30	90	

Source: computed from the laboratory results 2005

All sera positive to Rose Bengal (178 samples) were subjected to further confirmatory test using c-Elisa. 143 (80.3%) of these sera were confirmed

positive by c-Elisa (Table 3). Out of 28 positive herds, 27 herds (96%) had at least one positive reactor.

Table 3. Individual animal prevalence in Kuku Dairy Scheme based on RBPT and c-ELISA

Test	Positive reactors	Negative reactors	Total	Prevalence rate (%)	Confirmatory rate (%)
RBPT	178	396	574	31.0	80.3
c-Elisa	143	431	574	24.9	

Source: computed from the laboratory results 2005

According to the confirmatory test, herd prevalence rate was 90%, individual animal prevalence rate was found to be 24.9% and average within herd prevalence rate was found to be 24.5% (sd 15.7) at 95% confidence level. The number of sero-positive aborter was found to be 17 (12%) out of 143 cows.

Discussion

In this study, the prevalence of bovine brucellosis in Kuku Dairy Scheme (Sudan) was found to be 24.9% based on c-ELISA as a confirmatory test after screening using RBPT. Depending on the results recorded by previous workers (>....), it was concluded that RBPT was more reliable due to its high sensitivity and c-ELISA was high specific in detecting of *Brucella* antibodies. The prevalence rate reported in this study was lower than that used for the calculation of the sample size which was 50% even if we considered the RBPT screening result (31.0%). The highest within herd prevalence (55.6%) based on RBPT in this study was less than that obtained earlier in some herds (60%) (Elnour, 2003). Herd prevalence rate of 90% in c-Elisa and 93.3% in Rose Bengal Plate Test come in agreement with Bakheit (2004, Personal communication) who mentioned that in Khartoum

State, brucellosis herd prevalence approaches 100%. The result was also similar to that obtained by Asfaw (1997) in the pre-urban dairy production systems around Addis Ababa, Ethiopia, where he obtained 100% herd prevalence rate. Within herd prevalence rate ranging from 0 to 55.5% was much wider than that obtained by Asfaw (1997) who reported a range of 0 to 16.7%.

This Kuku sero-prevalence result, even when using c-ELISA test, was higher than that in the District of Bafala (Guinea Bissau) with 18.6% prevalence rate and the three Districts of Guinea, Dubreka (12.7%), Boke (6.3%) and Coyah (5.9%) whereas in Gabu - Guinea Bissau the prevalence rate was found to be 5.7% and in Forehcariah it was found to be 3.8%. The prevalence rate in Kuku, Sudan (24.9%) was much higher than that reported in the Gambia (1.1%), Senegal (0.6%) and the District of Labe in Guinea where the disease was absent (Unger *et al.*, 2003).

It was noted that in the two studies (Kuku-Sudan and the 4 countries in West Africa) all serum samples examined for the estimation of brucellosis prevalence were subjected to RBPT as screening test. However, in this study (Kuku) c-ELISA was used as confirmatory test while the West African countries study relied on Complement Fixation Test (CFT) as confirmatory test. In this study, however, 9.7% of the positive samples using RBPT could not be confirmed. This might be attributed to the fact that cross reactions with other bacteria could have led to false positive results in case of RBPT test (Stack and McMilland, 2003).

According to Nakavuma (1994), the RBPT provides more likely false positive results. The results of this study were in agreement with Nakavuma (1994) since the confirmatory test (c-ELISA) reduced the number of positive RBPT herds from 28 to 27 with confirmation rate of 96% and the number of positive samples was declined from 178 to 143

resulting in 80.3% confirmation rate. In West African countries, complement fixation test (CFT) was used as confirmatory test as recommended by OIE (2001). However, in the West African study, 26% of the samples in RBPT could not be confirmed in CFT compared with the result of 9.7% positive reactors which could not be confirmed by c-ELISA in our study. Given the different level of laboratory standards in the four countries where the RBPT was carried out, possible explanation for this agreement between RBPT and CFT could be that the RBPT antigen might have become contaminated or expired, antigen and/or sera might not have been brought up to room temperature before testing, or an overestimation of the agglutination reaction by the individual investigator could be considered (Unger *et al.*, 2003).

The result reported in this study was much higher than that obtained by Upadhyay *et al.* (2007) who conducted sero-surveillance in 17 randomly selected districts of Uttar Pradesh State, India. They recorded an overall prevalence rate of bovine brucellosis of 12.77% by AB-ELISA (415 cattle were screened).

Chivandi (2006) reported a result of 4.11% prevalence rate of bovine brucellosis in the Gokwe Smallholder Dairy Project Herd of Zimbabwe. Sixteen of the 73 animals that were bled had aborted at least once translating to 21.92% of the herd experiencing abortions while in this study the percentage of aborted cows was (i2%).

Gen *et al.* (2005) obtained a higher result of brucellosis sero-prevalence in aborted dairy cows in Turkey. The antibodies against *B. abortus* were detected in the serum samples as 68.1%, 65.6%, 58.9% and 55.2% by the Competitive Enzyme-Linked Immunosorbent Assay (c-ELISA), Complement Fixation Test (CFT), Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT), respectively.

Conclusion

The study concluded that Kuku Dairy Scheme should be considered as endemic with bovine brucellosis. Infection with *Brucella* resulted in abortion of many cows. *Brucella* infection in the Scheme might have been accompanied by other infection as indicated by the higher results using RBPT compared to c-ELISA. Reaction due to vaccinal titres was excluded because there was no clear and documented history of previous vaccination in the Scheme. Brucellosis situation in Kuku Dairy Scheme should be tackled seriously considering the zoonotic nature of the disease, the heavily populated wide area (The capital city) supplied with milk produced in the Scheme and the feeding habit of in-contact people who used to drink raw cattle milk. The strategic plan for the livestock sector in the Ministry of Animal Resources and Fisheries aims at eradicating animal diseases associated with livestock and livestock products trade. This, together with combating zoonotic diseases programme in the country, implies control of the disease in the Scheme and in the country at large. If the capability of the Animal Health Research Corporation to produce S19 vaccine is considered, then combating of the disease is not impossible. The study recommended formulation of long term plan to control the disease in Sudan.

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