Sero-prevalence of Bovine Brucellosis in Kuku Dairy Scheme, Khartoum North, Sudan

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Summary

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 aborted cows 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 *Brucella species* that infect 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). 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).

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). 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 competitive enzyme-linked immunosorbent assay (c-ELISA) for the detection of serum antibodies to *Brucella* is capable of differentiating vaccinal and cross-reacting antibodies from those elicited by field infection in cattle (Lucero *et al.*, 1999; Poester et al.,2003). Marín *et al.* (1999) have found that the most sensitive tests were i-ELISA and RBPT, and the most specific are AGID-NH and c-ELISA. The situation of the disease in resource poor countries looks gloomy, however, the adaptation of control measures to their local situations together with the application of improved diagnostic methods and techniques could provide immediate cost-effective benefits (Roth *et al.*, 2003).

This sero-prevalence study was conducted in Kuku Dairy Scheme to elucidate
the prevalence rate of bovine brucellosis in the cattle of that major milk supplier to Sudan capital.

Materials and Methods

The primary source data was 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 randomly identified. 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 α =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 actual sample size. The animal identification relied on the owner name, number and name of the animals.

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. It was allowed to clot before serum was separated into small clean tubes for serological testing. The laboratory diagnosis relied mainly on two serological tests namely, RBPT and c-ELISA. 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 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. Accordingly herd prevalence rate, individual animal prevalence, average within herd prevalence and the sero-positive aborted were estimated.

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 head, out of which 6056 were mature cows, 143 bulls, 2838 calves less than one year and 1269 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 aborted cow 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 (Nielsen *et al*.,1995; McGiven *et al*.,2003; Portanti *et al.,*2006), it was concluded that RBPT was more reliable due to it's 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% (c-ELISA) and 93.3% (RBPT) are in agreement with Bakheit (2004, Personal communication) who believed that in Khartoum State, brucellosis herd prevalence approaches 100%. The result is 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. The range of within herd prevalence reported in this study (0 -55.5%) is much wider than that obtained by Asfaw (1997) who reported a range of 0 to 16.7%.

The present prevalence rate, even when using c-ELISA test, is higher than that reported from 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 5.7% in Gabu, Guinea Bissau and 3.8%in Forehcariah. The prevalence rate in Kuku, Sudan (24.9%) is 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). All serum samples examined for the estimation of brucellosis prevalence, in the latter and present studies, were subjected to RBPT as screening test. However, in this study c-ELISA was used as confirmatory test while Complement Fixation Test (CFT) was used the West African countries study. 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 antibodies could lead to false RBPT positive results (Stack and McMilland, 2003).

According to Nakavuma (1994), RBPT provides more likely false positive results. The results of this study are 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 individual positive samples from 178 to 143 resulting in 80.3% confirmation rate.

 The use of CFT as confirmatory test is recommended by OIE (2001). However, in the West African study, 26% of RBPT positive samples could not be confirmed in CFT compared with 9.7% RBPT positive reactors 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 prevalence reported in this study is 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 at least aborted once indicating that 21.92% of the herd experienced abortions while in this study the percentage of aborted cows is (12%).

Gen *et al.* (2005) obtained a very high prevalence of brucellosis in aborted dairy cows in Turkey. The antibodies against *B. abortus* were detected in 68.1%, 65.6%, 58.9% and 55.2% in serum samples by c-ELISA, CFT, RBPT and Serum Agglutination Test (SAT), respectively.

Conclusion

It may be concluded that Kuku Dairy Scheme should be considered as endemic with bovine brucellosis. Infection with Brucella has 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 vast, 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 of the Ministry of Animal Resources and Fisheries for the development of livestock sector aims at eradicating animal diseases associated with livestock and livestock products trade. This, together with zoonotic diseases combating 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.

Acknowledgement

References

Abbas, B. and **Agab, H**. (2002). A review of camel brucellosis**.** Prev. Vet. Med. 10, 55(1): 47-56.

Alton, G.G.; Jones, I.M. and Pietz, D.E. (1975). Laboratory Techniques in

Brucellosis. World Health Organization. Monograph Series No. 55, 2nd edition.

Asfaw, Y. (1997). The epidemiology of bovine brucellosis in intra- and peri-urban dairy production systems in and around Addis Ababa, Ethiopia. M.Sc. Thesis. Berlin**.**

Bakheit, M.R. (2004). Head, Department of Brucellosis, Veterinary Research Laboratory, Khartoum. Personal communication.

CDC. (2002). Public Health Fact Sheet – Brucellosis, Massauchsetts, USA.

Chivandi, E. (2006**).** Sero-prevalence of bovine brucellosis in the Gokwe Smallholder Dairy Project Herd of Zimbabwe. Southern African Journal of Education, Science and Technology. Vol. 1 (2): pp. 65- 69.

Corbel, M.J. (1997). Brucellosis: an Overview. International Conference on Emerging Zoonoses . Emerging Infectious Diseases. **Vol. 3: N0 2**

Elnour, A. (2003). Ghabis Vetrinary Service Centre. Khartoum North, Sudan (Personal communication**).**

Enright, F. (1990). *Brucella abortus*: The pathogenesis and pathology of *Brucella* infection in domestic animals. In: Animal Brucellosis, Edited by Nielson, K.H., Duncan, R. Vet. Bull. 1992. Boca raton, Florida, USA, CDC Press. Pp. 301-320.

Forbes, L.B.; Nielsen, O.; Measures, L. and Ewalt, D.R. (2000).Brucellosis in ringed seals and harp seals from Canada. J. Wildlife Dis., 36(3): 595-598.

Gonzalez-Guzman, J. and Naulin, R. (1994). Analysis of a model of bovine brucellosis using singular per-turbations. J. Math. Biol. 33(2): 211-223.

Gen, O.; Otlu, S.; Fiahn, M.; Aydin, F. and Gökce, H. (2005). Sero-prevalence of Brucellosis and Leptospirosis in Aborted Dairy Cows. Truk J. Vet. Anim. Sci. (29): 359-366

Hegazy, A.A.; El Dughaym, A.; Al Eknah, M.; Housawi, F. M.T. and Hatem, M.E. (2004). Studies on Mastitis in Female Camel with Special Reference to Brucellosis, J. Camel Science. 1: 96-102.

Langomi, H.; Silvio, M.; Aristeu, V.; Renata, B.P.; Flavia, B.T.; Lia Jeanne, P.M. and Jose, A.D. (2000). Isolation of *Brucella* spp. from milk of brucellosis positive cows in São Paulo and Minas Gerais states. Braz. J. Vet. Res. Anim. Sci. Vol. 37 (6) Sao Paulo Print ISSN 1413-9596.

Lucero, N. E.; Foglia, L.; Ayala, S. M.; Gall, D. and Nielsen, K. (1999**).** Competitive Enzyme Immunoassay for Diagnosis of Human Brucellosis. Journal of Clinical Microbiology, Vol. 37 (10); 3245-3248.

Marín, C. M.; Moreno, E.; Moriyón, I.; Díaz, R. and Blasco, J. M. (1999). Performance of Competitive and Indirect Enzyme- Linked Immunosorbent Assays, Gel Immuno-precipitation with Native Hapten Polysaccharide, and Standard Serological Tests in Diagnosis of Sheep Brucellosis. J. Clin Diagn Lab Immunol. 6 (2) : 269–272.

McGiven,J.A.;Tucker,J.D;Perrett,I.L;Stack,G.A.;   Brew,S.D.   and McMacMillan,A.P.(2003). Validation of FPA and c-Elisa for the detection of antibodies *to Brucella abortus* in cattle sera and comparison to SAT, CFT and i-ELISA.J. Immuol Methods,78(1-2):171-178

Nakavuma, J. (1994). A serological survey of *Brucella abortus* in cattle and goats in

the central and southern regions of Uganda. M.Sc. Thesis, Faculty of Veterinary Medicine, Makerere University, Kampala, Uganda.

Nicoletti, **P.** (1982). Diagnosis and vaccination for the control of brucellosis in the Near East. FAO Animal Production and Heath Paper No. 83, Rome, Italy.

Nielson, K. and Duncan, J.R. (1990). Animal Brucellosis. Boca Raton.Florida, USA. Press, Vet .Bull. 454.

Nielsen, K.H.; Kelly,L.; **Nicoletti, P.** Kelly,W **(1995). Improved competitive** enzyme-Linked Immunoassays for the diagnosis of bovine brucellosis. .J. Immuol. And Immunopathol. 46(2-3): 285-291.

Poester, F. P.; Ramos, E.T. T. and Hiesen, S.V. (2003). Application Of Enzyme-Linked Immunosorbent Assays For The Diagnosis Of Bovine Brucellosis In Rio Grande Do Sul, Brazil. Centro de Pesquisa Veterinária "Desiderio Finamor", http://www-naweb.iaea.org/nafa/aph/public/poester-indirect-1055.pdf

Portanti,O.;Tiffarelli,M,;DiFebo,T.;Luciani, M.;Mercante,M.T.;Cote,A and Lelli,R.(2006). Development and Validation of competitive ELISA kits for the serological diagnosis of ovine,caprine and bovine brucellosis.J.Vet.Public Health,53(10):494-498.

Roth, F.; Zinsstag, J.; Orkhon, D.; Chimed-Ochir, G.; Hutton, G. O.; Cosivi, O.; Carrin, G. and Otte, J. (2003). Human health benefits from livestock vaccination for brucellosis: Case study: Bulletin of the World Health Organization, 81(12): 867.

Robinson, A. (2003). Guidelines for coordinated human and animal brucellosis surveillance, FAO Animal Production and Health Paper No. 156, Rome, Italy.

OIE (1996). Updated: 23.07.2004 http://www.oie.int/eng/normes/mmanual/A_00064.htm

Samartino, L.E. (2002). Brucellosis in Argentina**.** Vet Microbiol. 90 (1-4):71 - 80.

S**tack, J.A. and McMilland, A.P.** (2003). FAO/WHO Collaborating Centre for Reference and Research on Brucellosis. Central Veterinary Laboratory, New Haw Addlestone, Surrey, KT15 3NB, United Kingdom.

Teshome, H.; **Molla, B**. and **Tibbo, M**. (2003). A seroprevalence study of camel brucellosis in three camel-rearing regions of Ethiopia. Trop. Anim. Hlth. Prod. 35(5): 381-90.

Unger, F.; Munstermann, S.; Goumou, A.; Apia C. N.; Konte M. and Hempen, A. M. (2003). Risk associated with bovine brucellosis in selected study herds and market places in four countries of West Africa. Animal Health Working Paper No. 2. ITC (International Trypanotolerance Centre), Banjul, The Gambia, 37. pp. 3.

Upadhyay, S. R.; Singh, R.; Chandra, D. ; Singh, K. P. and Rathore, B. S. (2007) . Seroprevalence of bovine brucellosis in Uttar Pradesh. Journal of Immunology and Immuno-pathology, Volume 9 (1-2): 561- 672.

Zinsstag, J.; Roth, F.; Orkhon, D.; Chimed-Ochir, G.; Nansalmaa, M.; Kolar, J. and Vounatsou, P. (2005). A model of animal–human brucellosis transmission in Mongolia. Preventive Veterinary Medicine. pp. 1 - 19. www.scienedirect.com

Blood, D.C. and Hinderson, J.A. (1983). Diseases caused by *Brucella spp.*
In: Veterinary Medicine. 6th edition. Bailliere Tindall (Publisher-
England). Pp: 605-620.

Bikas, C.; Jelastopulu, E.; Leotsinidis, M. and Kondakis, X. (2003).
Epidemiology of human brucellosis in a rural area of north-western
Peloponnese in Greece. Euro. J. Epidemiol. 18: 267-274.

Defra, **G.** (2004). Department of Environment, Food and Rural Affairs. Disease Fact
Sheet.

Elsanousi, E.M . (2004). Department of Brucellosis. Central Vet. Research Laboratory**.**
Personal communication.

Elzer, P.H. (2002) Brucellosis Vaccines for the 21st Century. NIAA Annual Meeting
Proceedings: Louisiana State University, Ag Center and School of Veterinary
Medicine, Department of Veterinary Science, 111 Dalrymple Building, Baton
Rouge, LA; 70803.

Henk, L.; Smitt, I. and Sally, J. (2004). Contributions of biotechnology
to the control and prevention of brucellosis in Africa. African
Journal of Biotechnology. Vol. 3 (12): 631-636.
15
Hurtado, R.M.D. (2001). Brucellosis: new and old issues regarding diagnosis
and management. Rocio Hurtado, January 31, 2001.

Minas, A.; Minas, M.; Stournara, A. and Tselepidis, S. (2004). The
"effects" of Rev-1 vaccination of sheep and goats on human
brucellosis in Greece. Prev. Vet. Med. 64: 41-47.