Brucellosis in camels, cattle and humans: associations and evaluation of serological tests used for diagnosis of the disease in certain nomadic localities in Sudan

M.M. Omer [(1)], M.T. Musa [(1)*], M.R. Bakhiet [(1)] & L. Perrett [(2)]

(1) Animal Resources Research Corporation, P.O. Box 610, Khartoum, Sudan
(2) Food and Agriculture Organization/World Health Organization/World Organisation for Animal Health Collaborating Centre for Reference and Research on Brucellosis, Addlestone, Surrey KT15 3NB, United Kingdom
*Corresponding author

Submitted for publication: 28 November 2007
Accepted for publication: 26 August 2010

Summary
Brucellosis was studied in 2,225 camels, 20 camel nomads and 33 abattoir workers in certain nomadic localities in Sudan, using serum and milk samples. Lymph nodes, testicular tissues and udder tissues from positive camels and hygroma aspirates from three affected cows were used for isolation of *Brucella*. Serum samples were examined by Rose Bengal plate test (RBPT), modified RBPT (mRBPT), serum agglutination test (SAT) and competitive enzyme-linked immunosorbent assay (cELISA), and milk by the milk ring test.
Overall seroprevalence in camels (milk and serum samples) was 37.5%. The seroprevalence in males was 28.2% and in females 40.1%. Twelve (60%) of the 20 nomads and three (9%) of the 33 abattoir workers had positive antibody titres. *Brucella abortus* biovar 6 was isolated from two camels and three cows. Two isolates, one from each species, were atypical. The bacteriological findings suggested that camels were infected from cattle, the primary hosts of *B. abortus*. The mRBPT was suitable for screening camel sera for brucellosis, but the cELISA detected 2.1% more positives. The SAT antibody concentrations ranged between <13 and 3,282 IU/ml.

Keywords
Brucella abortus biovar 6 – Brucellosis – Camel – Cattle – Human – Sudan.

Introduction

Many tribes in different parts of the Sudan depend entirely on camels for their livelihood. Camel meat is consumed throughout the country and the animals contribute effectively to the economy by their use in agricultural practices and exportation. However, brucellosis has emerged as a major cause of abortion, hence a constraint to their breeding (1, 2, 11), and has had a negative impact on the export of camels.

Reports from veterinary laboratories have indicated that the prevalence of brucellosis in camels in some localities in Sudan is increasing (7).

The aim of this work was to study the prevalence of brucellosis in camels and the people in contact with them, and in other animals, to compare different serological tests used for diagnosis of the disease and to identify which *Brucella* species affect camels and cattle. It was also intended to investigate the epidemiological factors responsible for the spread of the disease in certain nomadic areas of Sudan.

Materials and methods

Areas of study

Camels were examined randomly for brucellosis in common grazing fields (Fig. 1) and water points (Fig. 2) in four localities in Sudan, designated A, B, C and D.

Fig. 1
Camels grazing in a common pasture in a nomadic area of Sudan

Fig. 2
Different animal species sharing a common water source in a nomadic area of Sudan

Sampling

A total of 2,225 serum and milk samples were collected for the diagnosis of brucellosis from nomadic and slaughtered camels of different sexes and ages from six months to over 15 years of age. The samples were obtained from all or most of the camels in herds that were selected randomly for examination, or from camels brought to slaughter in the main abattoir in the area. Samples included 2,000 blood specimens for serum and 225 milk samples, in addition to lymph nodes, testicular and udder tissues obtained from serologically positive camels at slaughter, three bovine hygroma aspirates from cattle presented for slaughter, and human serum samples from 20 camel nomads and 33 abattoir workers.

Serological diagnosis of brucellosis

The 2,000 serum samples from camels were screened by the Rose Bengal plate test (RBPT) and re-examined by modified (m) RBPT as described by Blasco *et al.* (5). Of the RBPT-negative serum samples, 560 were selected randomly and re-examined by competitive enzyme-linked immunosorbent assay (cELISA) at the Central Veterinary Research Laboratories, Soba, Khartoum, Sudan. One hundred random samples from the RBPT-positive sera were re-evaluated by standardised serum agglutination test (SAT) (10) for the measurement of antibody concentrations. Any sample that contained 30 international units (IU)/ml or more on the SAT was considered positive, in accordance with the guidelines of the World Organisation for Animal Health (OIE) (14). The human serum samples were also examined for the disease with the two RBPTs. Each of the 225 milk samples was supplemented with 1 ml of brucellosis-negative bovine milk to add fat globules (12) and examined by the milk ring test (MRT) (10).

Bacteriological investigations

Preparation and examination of samples

Each lymph node, udder sample and testicular sample was homogenised with a sterilised pestle and mortar using sterilised sand and phosphate buffered saline (PBS). Milk samples were centrifuged at 3,000 revolutions per minute (rpm) for 5 min to obtain sediment and cream.

Tissue homogenates, milk cream, milk sediment, and the hygroma aspirates were used separately to prepare slide smears. After staining with a modified Ziehl–Neelsen (ZN) stain, the slides were examined microscopically for weak acid-fast organisms (6).

Bacteriological culture

Samples in which weak acid-fast organisms were detected were cultured on serum dextrose agar (SDA) or tryptose agar (TA) media without antibiotics (which were not affordable), incubated at 37°C in an atmosphere of 10% CO_2 using candle jars and examined daily for 10 days for growth of weak acid-fast bacteria. Samples slightly contaminated by other bacteria were purified by subsequent subculture. Heavily contaminated samples that contained weak acid-fast organisms were emulsified in PBS and used for inoculation of guinea pigs (1 ml suspension intramuscularly). The guinea pigs were euthanised after 21 to 35 days and their sera examined for *Brucella* antibodies using RBPT. Spleens from serologically positive guinea pigs were removed, macerated by sterile forceps, cultured on SDA or TA media, and examined in much the same way as the primary cultures.

Isolates were identified as *Brucella* according to the method of Corbel (6). *Brucella* isolates were also sent to the Food and Agriculture Organization/World Health Organization/OIE Collaborating Centre for Reference and Research on Brucellosis at the Veterinary Laboratories Agency in the United Kingdom to be identified at the species and biovar levels.

Results

Serological test results

Serological data from evaluation of samples using the RBPT, mRBPT, cELISA and MRT are presented in Table I. Of the 2,000 serum samples, 797 (39.9%) were positive by the RBPTs and 809 (40.5%) by the cELISA test. Of the 225 milk samples, 38 (16.9%) were positive by the MRT. Of the 2,225 samples collected 489 were from males and 1,736 from females, with 138 (28.2%) and 697 (40.1%), respectively, testing positive. The overall prevalence was 37.5%. The prevalence of the disease in the four localities, A, B, C and D, was 36.2%, 37.8%, 36.4% and 45.5%, respectively. There were no differences between RBPT and mRBPT in terms of sensitivity. The agglutination reactions on the mRBPT occurred readily. Twelve (2.1%) of the 560 RBPT-negative samples were positive on the cELISA. Most of these cELISA-positive samples gave results only marginally above the negative cut-off values. When 100 RBPT-positive samples were evaluated using the SAT, all but one tested positive (antibody level > 30 IU/ml). Antibody titres on the SAT ranged from 30 to 100 (17%), 101 to 819 (69%), and 820 to 3,282 IU/ml (13%).

Data on the serological prevalence by camel age are presented in Table I. Seroprevalence tended to increase with camel age. Twelve (60%) of the 20 camel nomads and 3 (9%) of the 33 abattoir workers had positive antibody titres on the RBPT test.

Bacteriological examinations

Mesenteric lymph nodes from one female camel, testicular tissues and mesenteric lymph nodes from one male camel, and hygroma aspirates from three cows yielded six *Brucella* isolates. The infected mesenteric lymph nodes and the testicles were congested and haemorrhagic (Fig. 3). All the *Brucella* isolates produced oxidase, catalase, urease and hydrogen sulphide, they were CO_2-independent and nitrate-positive, but negative for indole, methyl red, Vogues Proskaur citrate and glucose.

The results of the identification of the isolates to species and biovar levels are presented in Table II. All strains were of *B. abortus* biovar 6, but two strains were atypical isolates of *B. abortus* biovar 6 due to their sensitivity to thionine at 20 µg/ml.

Table I
Prevalence of brucellosis in camels in the four nomadic localities in Sudan
A total of 2,000 serum samples were examined by the Rose Bengal plate test (RBPT), modified RBPT and competitive enzyme-linked immunosorbent assay. One hundred RBPT-positive samples were tested by serum agglutination test. The milk samples were examined by the milk ring test

Number of serum samples						Number of milk samples		Total number of samples	
Age 6 months to 1 year		Age 1 to 3 years		Age >3 to 15 years					
Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
401	56 (14.0%)	176	35 (19.9%)	1,423	706 (49.6%)	225	38 (16.9%)	2,225	835 (37.5%)

Table II
Characterisation of *Brucella* isolates, from camels and cattle, to species and biovar levels
Isolates 1 to 3 were from camels and 4 to 6 from cattle

Isolate number	Test					Reaction with monospecific antisera			Lysis with phages at RTD					Interpretation
	Urease	H_2S	CO_2	BF	TH	A	M	Wb	Tb	B2K	Fi	Iz	R/c	
1	++	+	–	+	–	+	–	CL	CL	CL	CL	CL	NL	*B. abortus* biovar 6 (A)
2	+	+	–	+	+	+	–	CL	CL	CL	CL	CL	NL	*B. abortus* biovar 6
3	+	+	–	+	+	+	–	CL	CL	CL	CL	CL	NL	*B. abortus* biovar 6
4	+	+	–	+	–	+	–	CL	CL	CL	CL	CL	NL	*B. abortus* biovar 6 (A)
5	+	+	–	+	+	+	–	CL	CL	CL	CL	CL	NL	*B. abortus* biovar 6
6	+	+	–	+	+	+	–	CL	CL	CL	CL	CL	NL	*B. abortus* biovar 6

– : negative for the test
+ : positive for the test
(A) : atypical
A : *Brucella* monospecific antiserum A
BF : basic fuchsin at 20 µg/ml (1/50,000 w/v)
B2k : Berkely 2
CL : confluent lysis
Fi : Firenze
Iz : Izatingar
NL: no lysis
M : *Brucella* monospecific antiserum M
R/C : Rough/canis
RTD: routine test dilution
Tb: Tbilise
TH: thionine at 20 µg/ml (1/50,000 w/v)
Wb: Weybridge

Fig. 3
Congested and haemorrhagic mesenteric lymph nodes and testes of a camel, from which *Brucella* organisms were isolated

Discussion

The RBPT is widely used in Sudan for brucellosis screening for regulatory control and for export requirements. The RBPT is very sensitive and is suitable for screening herds for brucellosis, but it can give false positive results due to vaccination with *B. abortus* strain 19 vaccine or cross-reactions with other bacteria. The RBPT has been reported to have high sensitivity, therefore false negative responses are reported to occur less frequently than false positive responses (14). In an effort to evaluate the potential for false negative samples to occur with the RBPT test in camels, this study used the cELISA to re-evaluate RBPT negative samples. Based on serology alone, the cELISA data indicated that the RBPT may have missed 2.1% of seropositive camels. Given that the RBPT is used widely in Sudan, false negative results could have a negative impact on brucellosis control policies. The present data suggest that the cELISA may be useful as a second screening test for confirmation of RBPT results (14). The data also indicate that the mRBPT facilitates the reading of agglutination reactions (8) and is recommended for screening camel serum samples for the disease. Others (6) have reported that ELISAs to detect brucellosis are more sensitive than the RBPT, but have only marginally greater specificity than the RBPT or complement fixation test.

The data presented here suggest that the prevalence of brucellosis in camels in the Sudan is generally low, but may be increasing (13). Previous data on brucellosis in camels in the same localities in Sudan found 12.3% and 15.5% seroprevalence (7), whereas this study found the seroprevalence in this area to be 37.5%. The results presented here are similar to those of other studies that found seroprevalence rates for brucellosis in Sudanese camels to range from 1.8% to 43.9%. Thus, Abu Damir *et al.* (1) reported seroprevalence of 2%, 3.1% and 7.5% in 750 camels in central, western and eastern Sudan, respectively; Bitter (4) found the prevalence of brucellosis to be 26.5% in camels in eastern Sudan; Yagoub *et al.* (15) found 1.82% seroprevalence in 79 young camels, 6.95% in adult males and 13.77% in adult females of 1,502 camels in the same localities as studied here; Musa (11) reported 7.76% of 1,314 camels to be seropositive in Darfur, western Sudan; and Majid *et al.* (9) found seroprevalence of 13.9% and 43.9% in camels in different localities in Sudan.

Antibodies detected in suckling calves of 6 to 12 months of age could be attributed to passive transfer of maternal antibodies (3). Antibody titres in immature camel calves (one to three years) might be due to persistent *Brucella* infections. This has also been observed in cattle (11). The higher prevalence rates observed in females than in males, and in adults than in younger camels, in this study are consistent with other reports (2, 11, 15).

Epidemiological factors that contribute to the spread of brucellosis in camels in Sudan may include mixing of infected camels with healthy animals, mixed herding of different animal species, lack of brucellosis control measures and lack of knowledge of brucellosis by nomads. Human routes of exposure to infection most likely include: consumption of raw milk, occupational exposure, and lack of adequate protective measures in abattoir workers. Use of measures such as vaccination and test and slaughter to control brucellosis in Sudan is urgently needed.

Isolation of the same biovar of *B. abortus* and the atypical variant from cattle and camels indicates that the current husbandry methods may disseminate brucellosis between cattle and other species. These data and those of others (2) suggest that the causative agent of brucellosis in Sudan is *B. abortus*.

La brucellose chez les chameaux, les bovins et l'homme : association et évaluation de plusieurs tests sérologiques pour le diagnostic de la maladie dans certaines localités nomadiques du Soudan

M.M. Omer, M.T. Musa, M.R. Bakhiet & L. Perrett

Résumé
Lors d'une étude sur la brucellose effectuée au Soudan oriental, des échantillons de lait et de sérum prélevés sur 2 225 chameaux et des prélèvements de sérum collectés sur 20 chameliers nomades et 33 travailleurs des abattoirs ont été analysés. La présence de *Brucella* a été recherchée par isolement à partir de tissus de ganglions lymphatiques, des testicules et de la mamelle de chameaux positifs, ainsi que du liquide obtenu par ponction des poches d'hygroma chez des vaches atteintes. Les échantillons sériques ont été soumis à l'épreuve au rose Bengale sur plaque (RBPT), à l'épreuve RBPT modifiée, au test de séro-agglutination (TSR) et à l'épreuve immuno-enzymatique (ELISA) de compétition ; les échantillons de lait ont été soumis à l'épreuve de l'anneau (*ring test*).
La prévalence sérologique globale était de 37,5 %. La prévalence sérologique était de 28,2 % chez les mâles et de 40,1 % chez les femelles. Douze chameliers nomades (60 %) et trois travailleurs des abattoirs (9 %) possédaient également des anticorps. *Brucella abortus* biovar 6 a été isolée de deux chameaux et de trois vaches. Deux isolats, obtenus l'un chez un chameau et l'autre chez une vache se sont avérés atypiques. Les résultats bactériologiques permettent de penser que l'infection a été transmise aux chameaux par les bovins, qui sont les hôtes primaires de *B. abortus*. L'épreuve RBPT modifiée a été jugée utile en tant qu'épreuve sérologique de dépistage chez les chameaux, mais l'ELISA de compétition s'est avérée plus sensible (2,1 % de résultats positifs détectés en plus). Les concentrations d'anticorps mises en évidence par TSR étaient comprises entre <13 et 3 282 UI/ml.

Mots-clés
Bovin – *Brucella abortus* biovar 6 – Brucellose – Chameau – Homme – Soudan.

Brucelosis en camellos, bovinos y humanos: asociaciones y evaluación de pruebas serológicas utilizadas para diagnosticar la enfermedad en ciertas localidades de nómadas de Sudán

M.M. Omer, M.T. Musa, M.R. Bakhiet & L. Perrett

Resumen
Los autores describen un estudio de la brucelosis en el Sudán oriental, en el cual se analizaron muestras de suero y leche de 2.225 camellos y muestras séricas de 20 camelleros nómadas y 33 operarios de matadero. Se trataba de aislar brucelas a partir de tejidos de ganglio linfático, testículo y ubre de camellos

positivos y del líquido aspirado de higromas de tres vacas afectadas. Las muestras séricas fueron sometidas a las técnicas siguientes: aglutinación en placa de rosa de bengala (RB); prueba de rosa de bengala modificada (RBm); prueba de seroaglutinación (SA); y ensayo inmunoenzimático de competición (ELISAc). Asimismo, se sometieron las muestras de leche a la prueba del anillo lácteo.
La seroprevalencia global resultó de un 37,5%. La seroprevalencia fue del 28,2% en los machos y del 40,1% en las hembras. Doce (60%) nómadas y tres (9%) técnicos de matadero mostraron títulos positivos de anticuerpos. En dos camellos y tres vacas se aisló el biovar 6 de *Brucella abortus*. En dos de esas muestras, una de cada especie, la cepa aislada era atípica. De las observaciones bacteriológicas se dedujo que las vacas eran el huésped primario de *B. abortus* y que ellas habían contagiado a los camellos. Se comprobó que la técnica de RBm resultaba útil para las pruebas sistemáticas de detección de brucelosis en el suero de los camellos, si bien con el ELISA de competición se detectó un número superior de positivos (un 2,1% más). Las concentraciones de anticuerpos observadas con la técnica de SA oscilaban entre < 13 y 3.282 UI/ml.

Palabras clave
Bovino – *Brucella abortus*, biovar 6 – Brucelosis – Camello – Humano – Sudán.

References

1. Abu Damir H., Tageldin M.H., Kenyon S.J. & Idris O.F. (1984). – Isolation of *Brucella abortus* from experimentally infected dromedary camels in Sudan: a preliminary report. *Vet. Res. Commun.*, **13**, 403-409.

2. Agab H., Abbas B., Ahmed H.J. & Mamoun I.E. (1996). – First report on the isolation of *B. abortus* biovar 3 from camels (*Camelus dromedarius*) in Sudan. *Rev. Elev. Méd. vét. Pays trop.*, **47**, 361-363.

3. Arunasalam V. (1977). – The transfer and duration of *B. abortus* maternal antibodies in calves. *Malay. vet. J.*, **6**, 157-162.

4. Bitter H. (1986). – Disease resistance in dromedaries with particular reference to *Trypanosoma evansi* infection. Tierarztliche Hochschule, Hannover, 150 pp.

5. Blasco J.M., Garin-Bastuji B., Marin C.M., Gerbier G., Fanlo J., Jimenez de Bagues M.P. & Cau C. (1994). – Efficacy of different Rose Bengal and complement fixation antigens for the diagnosis of *Brucella melitensis* infection in sheep and goats. *Vet. Rec.*, **134**, 415-420.

6. Corbel M.J. (2006). – Brucellosis in humans and animals. Food and Agriculture Organization, World Organisation for Animal Health, World Health Organization, Geneva, 28-35.

7. Kassala Veterinary Research Laboratory (2004, 2005). – Brucellosis in export animals. *In* Annual reports of the Kassala Veterinary Research Laboratory, Sudan.

8. Liapi M.V., Loannou I.G. & Papaprobromou M. (2003). – DO6-comparison of Rose Bengal in analogy of 1:3 with serum and Rose Bengal in analogy of 1:1 with serum interpreted in series with complement fixation test as confirmatory test for the detection of infected sheep and goat flocks with *Brucella melitensis*. A four-month field trial during the Cyprus brucellosis test and slaughter eradication programme. *In* Proc. Brucellosis 2003 International Research Conference, 15-17 September, University of Navarra, Pamplona, Spain, 62-63.

9. Majid A.A., Goraish L.A. & El Mansoury Y.H.A. (1999). – Sero-epidemiological observation of camel brucellosis in eastern and western Sudan. *Sudan J. vet. Sci. anim. Husb.*, **38**, 178-184.

10. Morgan W.J.B., Mackinnon D.J., Gill K.P.W., Gower S.G.M. & Norris P.I.W. (1978). – Brucellosis diagnosis: standard laboratory techniques. Ministry of Agriculture, Fisheries and Food, London.

11. Musa M.T. (1995). – Brucellosis in Darfur States. The magnitude of the problem and methods of diagnosis and control. PhD thesis, University of Khartoum, Sudan.

12. Musa M.T. & Shigidi M.T.A. (2001). – Brucellosis in camels in intensive animal breeding areas of the Sudan: implications in abortions and early life infection. *Rev. Elev. Méd. vét. Pays trop.*, **54** (1), 11-15.

13. Mustafa A.A. & Awad El Karim M.H. (1971). – A preliminary survey for detection of *Brucella* antibodies in camel sera. *Sudan J. vet. Sci. anim. Husb.*, **12**, 5-8.

14. World Organisation for Animal Health (OIE) (2004). – Bovine brucellosis. *In* Manual of Standards for Diagnostic Tests and Vaccines. OIE, Paris, 409-439.

15. Yagoub I.A., Mohamed A.A. & Salim M.O. (1990). – Serological survey of *Brucella abortus* antibody prevalence in the one-humped camel (*Camelus dromedarius*) from Eastern Sudan. *Rev. Elev. Méd. vét. Pays trop.*, **43** (2), 167-171.