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SHORT PAPER

Brucellosis in Camels (*Camelus dromedarius*) in Darfur, Western Sudan

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Summary

In a field outbreak of brucellosis in 21 camels mixed with cattle, sheep and goats, five camels, three of which showed clinical signs, were serologically positive. In a subsequent abattoir survey of apparently healthy camels, six animals were seropositive, albeit with titres that tended to be lower than those found in the field outbreak. Of the six seropositive slaughtered camels, five were shown to have lymph nodes (prescapular and supramammary) infected with brucellae (*Brucella melitensis* biovar 3, two camels; *Brucella abortus* biovar 6, three camels). Infection of camels with *B. abortus* biovar 6 had not previously been reported. Infection of the supramammary lymph nodes presents a potential hazard to those who consume raw camels' milk, a common practice in nomadic camel owners.

Keywords: bacterial infection; *Brucella abortus*; *Brucella melitensis*; brucellosis; camel; *Camelus dromedarius*

Introduction

Sudan has about 3.6 million camels (Report, 2003), which are farmed in wooded savannah lands together with sheep, goats and occasionally cattle, there being no attempt at segregation. Under these circumstances, brucellosis has spread between these species (Musa *et al*., 1990a,b; Musa, 1995) and has emerged as a major constraint to camel breeding (Musa and Shigidi, 2001). Brucellosis of camels in Darfur has been studied mainly by serological methods, without isolation of the causative agent (Abu Damir *et al*., 1984; Musa and Shigidi, 2001).

The aim of this work was to define clinical manifestations associated with the disease and to isolate *Brucella* organisms from camels and characterize them to biovar level.

Materials and Methods

Samples from Camels

These were obtained from (1) a field outbreak of brucellosis, and (2) an abattoir survey. They consisted of 83 serum samples and a single hygroma aspirate, all of which were transported to the laboratory on ice and stored at −20°C until examined. In addition, lymph node samples were collected from seropositive camels.

Field outbreak. Samples were obtained in August 2002 from a herder in South Darfur State, who kept camels mixed with cattle, sheep and goats of both sexes and various ages, with no attempt at segregation (Fig. 1). There had been a history of abortion and lameness in the camels, and cases of abortion in other species. Serum samples were collected from 21 camels and a single hygroma aspirate was collected from a male animal.

Abattoir survey. Female camels ($n = 62$) aged 5 to ≥12 years from different areas in the Darfur region were brought to Nyala abattoir over the period March to June, 2003. Ante-mortem serum samples were

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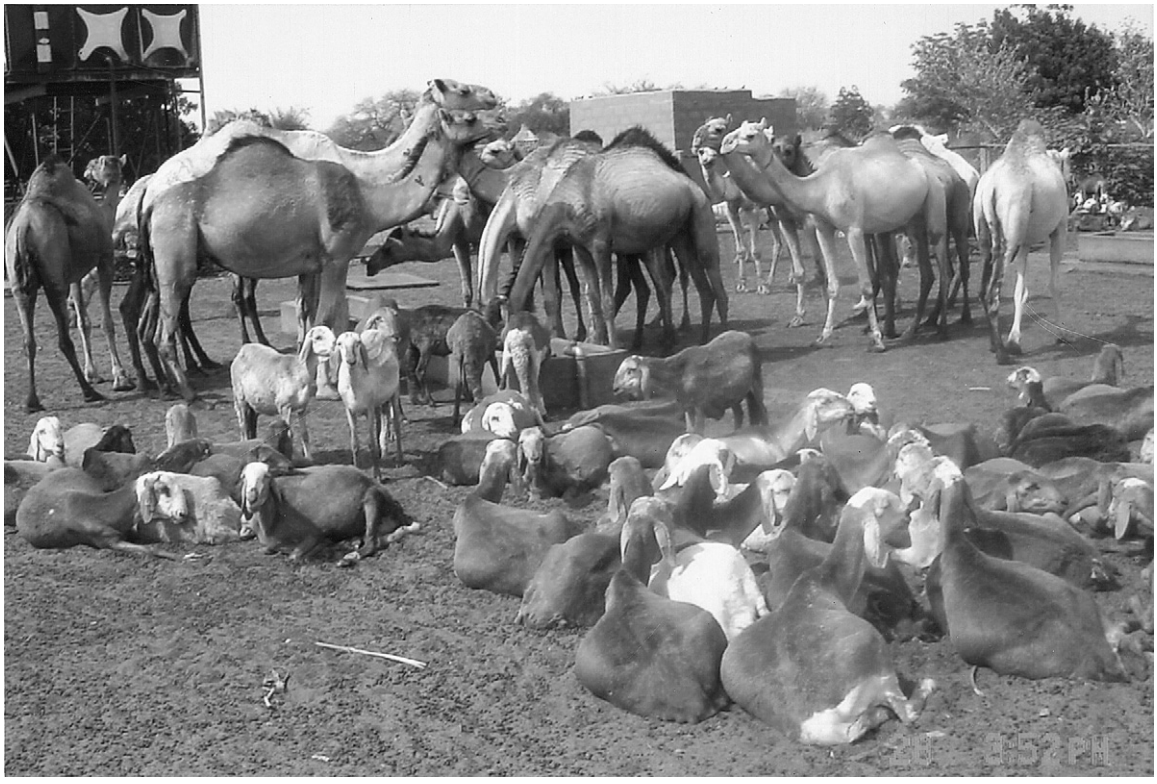

Fig. 1. Mixed farming of camels and other species in Darfur.

collected at the rate of 1–3 per day and examined for brucellosis. At necropsy, the right and left prescapular and supramammary lymph nodes were collected from each serologically positive camel for isolation *of Brucella*.

Serology

The hygroma aspirate and serum samples from camels were examined for brucellosis by the Rose Bengal Plate Test (RBPT), and antibody concentrations in the positive samples were measured in international units (i.u.) per ml by the serum agglutination test (SAT). Standardized antigens from the Veterinary Laboratory Agency (VLA), UK were used in the tests, which were performed as described by Morgan *et al.* (1978).

Isolation and Characterization of Brucella *Isolates from Lymph Nodes*

The lymph nodes collected, which were enlarged, were freed from fascia by aseptic procedures. Material was then collected through the seared surface and homogenized. Smears from each homogenate were stained by modified Ziehl–Neelsen (ZN) stain (Stamp *et al.*, 1950) and those giving positive results were cultured on serum dextrose agar plates for isolation of *Brucella*. The cultures were incubated at 37°C in an atmosphere containing CO_2 10% and examined after 3–5 days for bacterial growth. Smears were prepared from each culture, stained and examined as for the homogenates. Cultures positive for *Brucella*-like bacteria were purified by subculture or by inoculation of guinea-pigs according to the methods described by Morgan *et al.* (1978). The isolates were characterized to the species and biovar levels at the Veterinary Laboratories Agency, UK, again by the methods of Morgan *et al.* (1978).

Results

Field Outbreak

Nine (42.9%) out of the 21 camels (nos 1–21) examined serologically showed clinical signs suggestive of brucellosis and one (4.8%) was affected with "wry neck syndrome". The clinical manifestations and their association with brucellosis are presented in Table 1. Five (23.8%) of the camels were serologically positive for brucellosis and three of the positive animals had clinical signs of the disease. The fourth positive camel (no. 5) was a female with wry neck signs, and the fifth (no. 3) was a male used for breeding. The

Table 1
Field outbreak: clinical signs and serology in 21 camels

Camel no.	*Sex*	*Age (years)*	*Clinical symptoms*	*RBPT*	*SAT (i.u./ml)*	*Remarks*
1	M	5	Knee hygroma	+ve	1641	Hygroma aspirate +ve by RBPT, SAT (410.5 i.u./ml) and culture
2	F	11	Aborted recently, arthritis	+ve	1641	Aborted twice
3	M	11	None	+ve	31	Used for breeding
4	F	5	Aborted	+ve	205	–
5	F	8	Wry neck syndrome	+ve	410.5	–
6,7	F	6,12	Arthritis	−ve	−ve	–
8,9	M	4,6	None	−ve	−ve	–
10–12	F	6–l2	Abortion	−ve	−ve	
13–21	F	0.5 ≥ 12	None	−ve	−ve	–

M, male; F, female.

male camel (no. 1) with hygroma of the right knee was used for riding. It exhibited acute lameness due to infection of the joint. According to the owner, the animal initially suffered from an acute left shoulder pain, then from severe lameness of the right knee, and finally from the hygroma. The serum antibody level of the female camel (no. 2) which aborted twice, the second occasion being 3 months before sampling, and which showed severe arthritis, matched that of the hygroma case (Table 1). The hygroma aspirate was also serologically positive for brucellosis. Smears from the hygroma aspirate and its culture contained bacteria which were shown to be weakly acid-fast by the modified ZN method. These findings confirmed the association of the clinical manifestations with brucellosis.

Abattoir Survey

Of the 62 camels examined, six were serologically positive for brucellosis (Table 2), the SAT titres being generally lower than in the field outbreak (see above); and of these six positive animals, five yielded *Brucella* organisms on culture (Table 2). The two *B. melitensis* biovar 3 strains isolated were agglutinated with monospecific antisera A and M, were CO_2-independent, and produced H_2S, urease, catalase and oxidase. They grew in the presence of safranin O (100 μg/ml), basic fuchsin 20 μg/ml and thionin 20 μg/ml; and they were partly lysed by bacteriophage Bk_2 but not by Wb, Tb, Fi or R/C at the routine test dilution. The three *B. abortus* biovar 6 strains were agglutinated with monospecific antiserum A but not M, were CO_2-independent, and produced H_2S, urease, catalase and oxidase. They grew in the presence of safranin O (100 μg/ml), basic fuchsin 20 μg/ml and thionin 20 μg/ml but not in thionin 40 μg/ml; and they were lysed by bacteriophages Wb, Tb, Bk and Fi but not by R/C at the routine test dilution.

The isolates were obtained from four different localities (previously provinces) in North, South and West Darfur States (Table 2). The isolation of *B. melitensis* in North Darfur State was the first on record from this area, in which sheep, goats and camels are the predominant domestic species.

Discussion

Camels are very susceptible to brucellosis and under extensive farming conditions high prevalence rates of the disease have been reported in this species. Bitter (1986) examined 948 camels from different herds in eastern Sudan and reported a prevalence of 16.5–32.3%. Musa (1995), who examined 416 camels from seven herds owned by nomads of the same clan

Table 2
Survey of 62 slaughtered female camels: results of serological and bacteriological tests

Camel no.	*Source of animal*	*Serology*		*Smears* of*		Brucella *isolated*
		RBPT	*SAT (i.u.)*	*PLN*	*SLN*	
8	Zalingei	+ve	–	–	–	–
12	Saraf Omra	+ve	287	+	+	*B. melitensis* biovar 3
13	El Qoz, Bram	+ve	72	+	++	*B. melitensis* biovar 3
26	Idd El Firsan	+ve	102.5	+	++	*B. abortus* biovar 6
39	Wadi Salih	+ve	287	+	+	*B. abortus* biovar 6
41	Idd El Firsan	+ve	51.5	+	++	*B. abortus* biovar 6

PLN, prescapular lymph node; SLN, supramammary lymph node.
*Density of *Brucella* organisms in smears indicated by ++, +, or – not tested.

in western Sudan, found a 23.3% prevalence rate and concluded that camels ranked second only to cattle in the rate of infection with brucellosis.

Spread of brucellosis in camels depends on the *Brucella* species prevalent in other animals sharing their habitat and on the husbandry methods of the different species. Zowghi and Ebadi (1988) isolated *B. melitensis* biovar 1 from several camels in Iran, Al Khalaf and El Khaladi (1989) isolated *B. abortus* biovar 1 from aborted fetuses of camels in Kuwait, Radwan *et al.* (1995) isolated *B. melitensis* biovars 1, 2 and 3 from camels in Saudi Arabia, Gameel *et al.* (1993) isolated *B. melitensis* biovar 1 from camels in Libya, and Agab *et al.* (1995) isolated *B. abortus* biovar 3 from camels in eastern Sudan. The present report represents the first record of the isolation of *B. abortus* biovar 6 from camels. In the Sudan, Musa and Jahans (1990) also isolated *B. melitensis* biovar 3 from a mixed flock of sheep and goats. Musa *et al.* (1990a) also isolated *B. abortus* biovar 6 from cattle in different provinces of South and West Darfur States. The present report illustrates the role of animal husbandry methods in dissemination of the disease. Camels are mainly browsers and occasionally grazers. In the present study the camels may have been infected directly from other animals or indirectly *via* factors such as water, feed, premises, infected dust, inhalation, flies and ticks (Report, 1986; Moreno and Moriyon, 2002). In the investigation of the field outbreak the sheep and goats were serologically negative for brucellosis, and cattle were probably the source of infection for the camels.

Brucellosis in camels causes abortion, placental retention, fetal death and mummification, delayed sexual maturity and infertility (Musa and Shigidi, 2001). In the present study it also caused repeated abortion, arthritis and hygroma. However, the clinical signs were milder and the antibody concentrations were lower in camels than in cattle (Musa *et al.*, 1990b). Lymph nodes from seronegative camels were not cultured in view of the lack of evidence of infection, and even one RBPT-positive animal (no. 8; Table 2) was negative for *Brucella* organisms.

The antibody concentrations in the field outbreak tended to be higher than in the survey of slaughtered camels, in which infection was in the more chronic stages. There was no proof that the wry neck syndrome seen in one camel was associated with brucellosis. Indeed, Schwartz and Dioli (1992) stated that the syndrome is of unknown cause. As in cattle and other species, in camels *Brucella* organisms localize in the lymph nodes. Localization in the supramammary node is of public health significance in view of the consumption by nomads of raw camels' milk. The study emphasizes the need for the control of brucellosis in camels and other species in the Darfur region.

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