

Brucellosis in Camels in Intensive Animal Breeding Areas of Sudan. Implications in Abortion and Early-Life Infections

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Key words

Dromedary - *Brucella abortus* - Brucellosis - Intensive husbandry - Abattoir - Sudan.

Summary

To study brucellosis in 3413 camels raised in areas of Sudan, where cattle, sheep and goats were intensively bred, bacteriological and serological examinations were performed. Among the camels, 3275 belonged to 110 herds, 35 were reared individually or with cattle, and 103 had been slaughtered at Nyala abattoir. The infection was found in 50 (45.5%) of 110 herds, with prevalence rates ranging from 1.4 to 89.5%; in 72 (7.3%) out of 993 males and in 196 (8.1%) out of 2420 females; 75% of the positive camels were adults over 4 years old and the remaining 25% were younger, from 6 months to 4 years old. In infected herds, abortion rates associated with the disease ranged from 3.1 to 72.7% depending on the location. Other conditions caused by the disease were retention of placenta, fetal death and mummification, delayed service age and infertility. Hygromas and cases of orchitis were not shown to be caused by brucellosis. The disease in camels was found milder than in cattle. *Brucella abortus* antibodies in infected camels ranged from 31 to 1969 IU/ml (2/20 to 2/1280). The milk ring test was improved by adding bovine milk negative for the disease to camel milk. Male camels used for service were negative for the disease implying that they did not play a role in its transmission. Recommendations for brucellosis control were given.

INTRODUCTION

Camels in Sudan were previously reared in arid and semiarid lands. They moved to higher rainfall areas side by side with other domestic livestock and wildlife. This change resulted in exposure of camels to diseases that were uncommon in their natural habitat, e.g. fasciolosis, dermatomycosis, tick paralysis, trypanosomosis, theileriosis and brucellosis, which were diagnosed in our laboratory in Nyala.

The aim of this work was to study brucellosis and its impact on camels in intensive livestock rearing areas in Sudan.

MATERIALS AND METHODS

Many areas in Darfur region, Western Sudan (Figure 1) were visited to collect random samples from 3413 camels of both sexes and different ages for brucellosis diagnosis. The camels sampled were

3275 from 110 herds, 35 reared either with cattle or individually and 103 slaughtered in Nyala abattoir. With the aid of a questionnaire, information from each camel sampled was obtained including its location, owner, sex, age, pathological condition, history of abortions, breeding records, whether reared individually, with other species or in a camel herd, herd size, number of animals positive for brucellosis and status of the camel at the time of sampling (suckling, weaned, adolescent, adult, pregnant or repeat breeder).

Sampling for bacteriological diagnosis of brucellosis

Slaughtered camels

In Nyala abattoir, 103 camels were bled for serum samples before slaughter using serum vacutainer tubes. Sera were separated in plastic tubes with caps and screened for *Brucella abortus* antibodies by the rose bengal plate test (RBPT). Mesenteric, retropharyngeal and supramammary lymph nodes of positive camels were sampled at postmortem examination. Positive sera were then kept for further serological diagnosis of the disease.

Live camels

In the field, fetal stomach contents were obtained from three fetuses and a vaginal swab was taken from an aborted she-camel 12 days after abortion.

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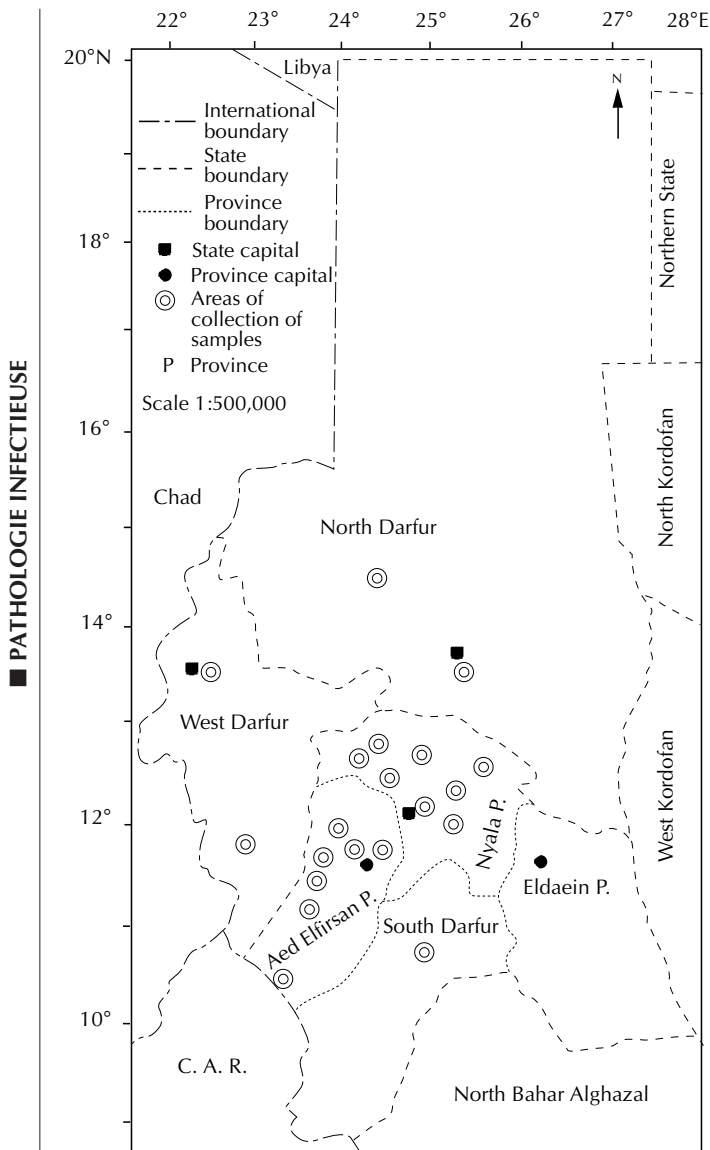


Figure 1: Areas of sample collection from camels for brucellosis diagnosis in Darfur region, Western Sudan.

Sampling for serological diagnosis of brucellosis

Beside the 103 samples from Nyala abattoir, blood for serum samples was also collected from 3200 camels, mainly in Nyala and Aed El Firsan provinces in South Darfur State, but also in West, North and other parts of South Darfur States. In addition to sera, fluids were sampled in four camels with hygromas and three with orchitis. Camels with either condition were considered positive for brucellosis when both sera and fluids or either one of them reacted positively to the tests used for the disease diagnosis. Camels, whose fetuses were sampled and vaginal swabs taken, were included in the 3200 camels sampled in the field. They were considered positive under the same conditions as those with hygromas and orchitis.

One hundred and ten milk samples were also collected with bijou bottles from she-camels in different areas in Darfur States. All samples obtained for bacteriological and serological diagnosis of brucellosis were stored and transported to the laboratory in iceboxes. In the laboratory they were stored at -20°C until required for diagnosis.

Laboratory investigations

Bacteriological diagnosis of brucellosis

Lymph nodes from camels positive by RBPT, fetal stomach contents and the vaginal swab were used to prepare slide smears stained by the modified Ziehl-Neelsen stain and examined with a microscope for *Brucella*-like organisms.

Serological diagnosis of brucellosis

Out of the 3303 sera collected from camels in Nyala abattoir and in the field, 3274 samples, as well as the seven fluids from hygromas and cases of orchitis, were examined by RBPT, the serum agglutination test (SAT) and the complement fixation test (CFT). The 110 milk samples were tested by the milk ring test (MRT). Because it is difficult to obtain ring formation in MRT with camel milk, each sample was supplemented prior to its testing with 1 ml of bovine milk negative for brucellosis to facilitate ring formation. Standardized antigens supplied by the Central Veterinary Laboratory, UK, were used according to Morgan *et al.* (3).

The complement (C) and hemolytic immune body (HIB) used in CFT were supplied by Tissue Culture Services, UK. Camel sera tested by CFT were inactivated at 60-62°C for 30 min; the test was performed with U-shape microtiter plates. CFT was performed with 25 µl of sera, antigens, C, HIB and diluted standardized red blood cells, and with the hot fixation method.

The remaining 29 sera were examined by RBPT and competitive ELISA (cELISA) according to Brew *et al.* (2). Standardized *B. abortus* lipopolysaccharide antigen extracted by the hot phenol method (2) and a conjugate of *B. abortus* monoclonal antibody isotype IgG1 conjugated to horse radish peroxidase (also supplied by the Central Veterinary Laboratory) were used in cELISA.

RESULTS

Bacteriological investigations

In the abattoir

Smears of four lymph nodes from camels positive by RBPT showed partially acid-fast organisms.

In live camels

In the field, a smear from one out of the three fetal stomach contents similarly showed partially acid-fast organisms. The smear from the vaginal swab of the aborted she-camel was also positive by the modified Ziehl-Neelsen stain. Since the samples were taken from camels serologically positive for brucellosis that also showed symptoms of the disease, the organism present in the smears was acknowledged to be *Brucella*.

Serological investigations

Sera

Out of 3274 samples examined by conventional serological tests, 256 (7.82%) were positive for brucellosis. The fluids from the four hygromas and three orchites were negative for brucellosis. Four (13.8%) out of the 29 sera samples examined by cELISA were positive for brucellosis, while only 3 (10.3%) of the same samples were positive by RBPT.

Milk

Three (2.7%) out of the 110 milk samples were positive by the conventional MRT, but, when the samples were supplemented

with bovine milk, 8 (7.3%), including the former 3, were positive improving the test results by 166.7%.

Results of the serological diagnosis of brucellosis in camels at different locations are summarized in Tables I and II. Most camels examined were from Nyala and Aed El Firsan provinces in South Darfur State and a few from North and West Darfur States (Figure 1). The two provinces were intensive animal breeding areas; Nyala province was a pathway for nomads during their migrations inside and outside the country.

Prevalence of brucellosis in camels in Nyala province and other areas

As shown in Table I, the 88 camels examined in Abu Audam were from El Daein province and driven to Nyala along routes and

pastures shared with other livestock herds. The 416 camels examined in Margoum belonged to seven herds owned by nomads from the same clan who were moving together. In these herds 22 she-camels had aborted and were positive for brucellosis. Out of these, 17 (77.3%) aborted once and 5 (22.7%) twice. Among the total 97 camels positive for the disease in Margoum, 2 (2.1%) were at the breeding age, 4 and 6 years old, and had not mated, 2 (2.1%) were repeat breeders and 1 (1.03) was aborted and retained the placenta. By SAT, prozone reactions were observed in 17 (17.5%) out of the 97 positive results, especially in the first 1/10 and occasionally in the second 1/20 dilutions. Out of the 150 males examined 23 (15.3%) were positive for brucellosis, but none of the six males used for breeding in the seven herds were positive for the disease. This indicated that the males were not playing a role in the transmission of the disease. In one herd in Wadi Kaya out of

Table I
Results of serological diagnoses of brucellosis in camels in Nyala province and at other locations in Darfur States

Location	Camels examined			Males			Females			Aborted females		
	Total num.	Pos.	%	Total num.	Pos.	%	Total num.	Pos.	%	Total num.	Pos.	%
Abu Audam	88	6	6.8	36	1	2.8	52	5	9.6	4	0	
Margoum	416	97	23.3	150	23	15.3	266	74	27.8	50	22	44
Wadi Kaya	1025	89	8.7	327	18	5.5	698	71	10.2	29	9	31
Khazan Gadid	333	20	6	64	1	1.6	269	19	7.1	21	4	19.1
Kashkur	18	2	11.1	8	2	25	10	00		NI	NI	
Camels reared with cattle	9	3	33.3	9	3	33.3	0.0	00		NA	00	
Bilail	45	4	8.9	18	00		27	4	14.8	NI	NI	
Camels from various locations	26	4	15.4	20	3	15	6	1	16.6	NI	NI	
Abattoirs	103	25	24.3	70	17	24.3	33	8	24.3	NI	NI	
Total	2105	250	11.9	702	68	9.7	1403	182	13	104	35	33.7

Pos. = number of animals positive for brucellosis; NI = no information; NA = not applicable

Table II
Prevalence of brucellosis in camels in Aed El Firsan province, South Darfur State

Location	Camels examined			Males			Females			Aborted females		
	Total num.	Pos.	%	Total num.	Pos.	%	Total num.	Pos.	%	Total num.	Pos.	%
Aed El Firsan	19	11	57.9	7	3	42.85	12	8	66.7	11	8	72.7
Asir Barcol	76	00		25	00		51	00		NI	NI	
Markundi	381	00		82	00		599	00		52	00	
Damba	513	5	1	104	00		409	5	1.2	82	3	3.7
Rehid El Birdi	319	2	0.6	73	1	1.4	246	1	0.4	57	5	8.8
Total	1308	18	1.4	291	4	1.4	1017	14	1.4	202	16	7.9

Pos. = number of animals positive for brucellosis; NI = no information

19 camels randomly examined 17 (89.5%) were positive for brucellosis. Among those 15 she-camels were aborted; 5 (33.3%) were positive for the disease and 10 (66.7%) negative. In the same area a she-camel showed signs of abortion and two months later expelled a mummified fetus. After examination it was found positive for brucellosis and its SAT antibody level was 1/320 (410.5 IU/ml). Similarly, the serum of the she-camel found positive through a vaginal swab taken 12 days after abortion was highly positive by RBPT. In Khazan Gadid one of the she-camels was reported having aborted three times. At the time of the investigation its SAT antibody level was 2/20 (31 IU/ml). At the same location, three male camels, 10, 18 and 20 years of age, were observed suffering from orchitis, but their testicular fluids were negative for brucellosis. In Kashkur two camels, a male and a female, had hygromas but were negative for brucellosis. In Bilail the herd examined was made up of 45 camels bought from different places in North, South and West Darfur States and driven to Nyala province for pasture. Two camels from North Darfur and two from West Darfur were positive. The 26 camels sampled at different locations in the three States were reared with cattle herds or individually, but shared pastures and water with other domestic animals. Those slaughtered in Nyala abattoir were mainly culls, which explained the high prevalence of the disease within that group. In Nyala province camels examined for brucellosis were from 65 herds. Out of those herds, 43 (66.2%) contained infected camels and the infection rate ranged from 1.4 to 89.5% per herd.

Brucellosis in camels in Aed El Firsan province

In the province 19 camels were examined near Aed El Firsan town (Table II). Within those 2 out of 5 adult males were used for packing and 9 (64.3%) out of 14 from a herd were found positive for the disease. Eleven (91.7%) out of 12 females from the herd were reported having aborted. Eight (72.7%) out of the 11 aborted were found positive for brucellosis. Other camels were examined in the province in Asir Barcol, Markundi, Damba and Rehid El Birdi (Table II). These camels were introduced in the province 2-3 years ago and this might explain the low prevalence rate of brucellosis within them. Abortions storms were reported from she-camels in Aed El Firsan province but only 16 (8%) out of the 202 aborted camels were positive for brucellosis. Fifteen (93.8%) of the brucellosis-positive camels aborted once and one (6.3%) twice. In an aborted she-camel the antibodies were detected as early as day 2 following abortion and were at 1/40 (51.5 IU/ml). Two cases of stifle hygromas were detected in two camels in Damba, but both of them were negative for the disease.

Other than the five camels used for packing, the camels investigated in Aed El Firsan province belonged to 45 herds. Only seven herds (15.6%) out of the 45 contained infected cases and the infection rate within them ranged from 1.9 to 64.3% per herd.

Of the total 202 female camels aborted in Aed El Firsan, 186 (92.1%) were negative for brucellosis. Out of the aborted negative she-camels, 165 (88.7%) aborted once, 15 (8.1%) twice, 4 (2.1%)

three times and 2 (1.1%) four times. As a result there were factors other than brucellosis that caused abortions in camels in Aed El Firsan province.

Overall prevalence rate of brucellosis in Darfur region

In general, 3413 camels were examined for brucellosis in Darfur, Western Sudan, and 268 (7.9%) were found positive for the disease. These included 72 (7.3%) out of 993 males and 196 (8.1%) out of 2420 females. Fifty (45.5%) of the 110 camel herds examined contained infected camels and the infection rate ranged from 1.4 to 89.5% per herd. SAT antibody levels in camels serologically positive for brucellosis ranged from 2/20 to 2/1280 (31 to 1969 IU/ml).

The distribution of infected camels among different age groups is shown in Table III.

■ DISCUSSION AND CONCLUSION

Camels are not known to be primary hosts for any of *Brucella* organisms, but they are susceptible to both *B. abortus* and *B. melitensis* (5). Consequently, infection of camels depends on the infected animals in contact with them. Thus, in Iran, Zowghi and Ebadi (7) isolated *B. melitensis* in several camels, while in Butana area, Eastern Sudan, where camels were reared with cattle, sheep and goats, Agab *et al.* (1) isolated may strains of *B. abortus* from lymph nodes of camels serologically positive for brucellosis. In Darfur region, which owns over 25% of cattle, sheep and goats in the country, brucellosis is widely spread in these animals (4) and introduction of camels in the areas led to high levels of incidence as shown in the study.

Although *Brucella* organisms were not isolated in this study because of inadequate facilities, the evidence showed beyond doubt the occurrence of the disease in camels in Darfur region.

Prevalence of *B. abortus* antibodies in all age groups in camels showed that brucellosis infection in the animals started early in life probably through sucking and persisted into adulthood. Similar patterns were found in cattle, and Oloffs *et al.* (6) reported that 30% of the positive animals in Uganda were younger than three years of age and within them was a 2-year-old bull, which was not introduced for service.

The high levels of abortions in some herds caused by brucellosis and the difficult repeated ones due to other etiological agents threatened camel breeding at some locations in Darfur region. Although other causes of abortion were not investigated trypanosomosis was speculated to be one of them.

Brucellosis in camels seemed to display less clinical signs and antibody levels than in cattle (4), probably because of a relative resistance of the former to brucellosis. The disease should be controlled by vaccination of camels and primary hosts.

Table III
Distribution of brucellosis-infected camels among different age groups

	6-9 months	1-2 years	2 ¹ / ₂ -3 years	3 ¹ / ₂ -4 years	Adults over 4 years
Num. examined	72	587	322	256	2176
Num. positive for brucellosis	3 (4.2%)	19 (3.2%)	22 (6.8%)	23 (9%)	201 (9.2%)

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Résumé

Musa M.T., Shigidi M.T.A. La brucellose chez les dromadaires en zones d'élevage intensif au Soudan. Implications dans l'avortement et l'infection précoce

Des examens bactériologiques et sérologiques ont été menés sur 3 413 dromadaires provenant de différentes régions du Soudan, où se trouvaient des élevages intensifs de bovins, de moutons et de chèvres, afin de détecter la présence de la brucellose. Parmi les dromadaires, 3 275 ont appartenu à 110 troupeaux, 35 ont été élevés individuellement ou avec du bétail et 103 avaient été abattus à l'abattoir de Nyala. L'infection a été présente dans 50 (45,5 p. 100) des 110 troupeaux, avec des taux de prévalence qui ont varié de 1,4 à 89,5 p. 100 ; elle a également été présente chez 72 (7,3 p. 100) des 993 mâles et chez 196 (8,1 p. 100) des 2 420 femelles. Soixante-quinze pour cent des dromadaires positifs ont été des adultes de plus de 4 ans d'âge et les 25 p. 100 restants ont été des jeunes de 6 mois à 4 ans. Les taux d'avortement associés à la maladie chez les troupeaux infectés ont varié de 3,1 à 72,7 p. 100 entre les régions. Les autres conditions liées à la maladie ont été la rétention du placenta, la mort fœtale et la momification, le retard de l'âge de la monte et l'infertilité. Il n'a pas été mis en évidence que la brucellose était à l'origine d'hygromas et de cas d'orchite. La maladie a paru plus atténuée chez les dromadaires que chez le bétail. Les anticorps de *Brucella abortus* ont varié de 31 à 1 969 UI/ml (2/20 à 2/1280) chez les dromadaires infectés. L'ajout de lait de bovin négatif pour la brucellose au lait de dromadaire a rendu plus précise l'épreuve d'agglutination en anneau du lait. Les dromadaires mâles utilisés pour la reproduction ont été négatifs pour la brucellose, montrant ainsi qu'ils n'ont pas joué de rôle dans la transmission de la maladie. Des recommandations pour la lutte contre la brucellose sont proposées.

Mots-clés : Dromadaire - *Brucella abortus* - Brucellose - Elevage intensif - Abattoir - Soudan.

Resumen

Musa M.T., Shigidi M.T.A. Brucelosis en camellos en áreas de cría animal intensiva en Sudan: implicaciones para el aborto y las infecciones neonatales

Se realizaron exámenes serológicos y bacteriológicos, con el fin de estudiar la brucelosis en 3413 camellos criados en zonas de Sudan, en las que se cría intensivamente ganado bovino, ovino y caprino. Entre los camellos, 3275 pertenecían a 110 hatos, 35 fueron criados individualmente o con ganado y 103 habían sido sacrificados en el matadero de Nyala. La infección se distribuyó de la manera siguiente: en 50 (45,5%) de los 110 hatos, las tasas de prevalencia de brucelosis variaron entre 1,4 y 89,5%; en 72 (7,3%) de los 993 machos; en 196 (8,1%) de las 2420 hembras; en camellos adultos de más de 4 años de edad (75% del total de animales positivos); y en camellos jóvenes de 6-9 meses a 4 años de edad (25% restante). En los hatos infectados, las tasas de aborto asociadas con la enfermedad variaron de 3,1 a 72,7% según la localización. Otras condiciones provocadas por la enfermedad fueron retención de placenta, momificación y muerte fetal, retraso en la edad de servicio y no concepción. No se observaron casos de higromas o de orquitis causados por la brucelosis. La enfermedad en camellos fue más leve que en bovinos. Los anticuerpos de *Brucella abortus* en los animales infectados variaron entre 31 y 1969 UI/ml (2/20 a 2/1280). El test de ring en leche fue mejorado mediante la adición de leche bovina negativa para la enfermedad a la leche de camello. Los camellos macho usados para servicios fueron negativos para la enfermedad, lo que implica que no jugaron un papel en su transmisión. Se dan recomendaciones para el control de la brucelosis.

Palabras clave: Dromedario - *Brucella abortus* - Brucelosis - Crianza intensiva - Matadero - Sudán.