Ovine Brucellosis Traditional Farming Systems in Darfur States, Sudan

Musa, M. T*

Nyala Veterinary Research Laboratory,P. O. Box 24, Nyala, Sudan

ملخص البحث

أجريت دراسة لمرض البروسيلا في 2628 رأساً من الضأن من مصادر تربية تقليدية مختلفة في ولايات دارفور ، غرب السودان بإستخدام إختبارات البنقال الصحنى والتلازن المصلي للمعدل الأنبوبي و المثبت المتمم والحليب الحلقي والمقايسة المناعية التنافسية المرتبطة بالانزيم. . ولقد وجدت نسب إنتشار المرض في ولايات جنوب، وغرب وشمال دارفور

3% (65/ 2315) ، 12.9% (19 / 147) و 1.8 % (3/ 166)على التوالي . ولقد أوضحت الدراسة نسب الإصابة في 14 محافظة في الولايات الثلاث. نسب الإصابة في الضأن تحت التربية المستقرة والشبه مترحلة والمترحلة والمشتراه من الأسواق في الولايات الثلاث كانت ، 6.6%(317/21) ، 3%(17 / 620)، 2.5%(37 / 383) و 4.5% (10/ 244) على التوالي . أما نسبة الإصابة العامة فكانت 3.3%(87/ 2628). كان الإجهاض من الأعراض السريرية الرئيسة للمرض ووجد أن 12(15.9%) من النعاج المجهضة موجبة للمرض وحالة واحدة من خمس حالات إلتهاب الخصية نتيجة للمرض و 41.7%(60/25) مراحاً و 22%(18/4) مجموعة خليط من الضأن وحيوانات أخرى مصابه بالبروسيلا. بالرغم من أن المرض وجد في حالات قليلة ومتفرقة لكنه منتشر في معظم المحافظات وربما يسبب مشكلة في المستقبل. توصى الدراسة بإستمرار المسح لتحديد المناطق الموبوءة بالبروسيلا مليتنسس(*B. melitensis*) والبروسيلا المجهضة(*B. abortus*) وإتخاذ التدابير اللازمة للسيطرة على المرض بالتطعيم وإتباع الأساليب الصحية المناسبه.

Summary

Brucellosis was studied in 2628 sheep raised under traditional farming systems in Darfur states, western Sudan. Rose Bengal Plate test (RBPT), Serum Agglutination test (SAT), modified SAT (mSAT), Complement Fixation Test (CFT), Milk Ring test (MRT) and competitive Enzyme linked Immunosorbent Assay (cELISA) were used in the study.

The prevalence rates of the disease in South, West and North Darfur States were 3% (65/2315), 12.9% (19/147) and 1.8%(3/166), respectively. The rates in 14 provinces of the states were given. The prevalence rates under sedentary, semi-nomadic and nomadic husbandry methods and in stocks from animal markets in the three states were 6.6% (21/317), 3% (17/620), 2.5% (37/1383) and 4.5% (10/244), respectively. The overall prevalence rate was 3.3% (87/2628).

Abortion was the main clinical manifestation of the disease and 15.8 % (12 / 76) of the aborted ewes reported to the Veterinary

* *Present address: Animal Resources Research Corporation, Khartoum, Sudan.*

Authorities were positive for Brucellosis. One of the five cases of orchitis examined was due to brucellosis. Twenty-five out of 60 herd (41.7%) and 4 out of 18 flock of sheep (22%) and other domestic animal species owned by different owners were found infected.

The disease was widespread, occurred in most of the provinces and may constitutes a future problem. This work should be continued to delineate areas infected with *Brucella melitensis* and those infected with *B. abortus* (if any) and prompt measures should to be taken to control the disease by vaccination and hygienic practices.

Introduction

The population of sheep in the Sudan was recently estimated to be 48,036,000 head (Anon, 2003). This species is reared throughout the country separately or often mixed with goats and coexist in the same conditions with cattle and camels. It contributes effectively to the economy of the country by exportation to the Arabian Peninsula. Occasionally, the occurrence of infectious diseases including brucellosis curtails sheep trade and many consignments were rejected because of claims of the disease detection within them. Consequently, sheep destined for export are examined for freedom from brucellosis and other transboundary diseases.

In Darfur Region, believed to hold about 25% of the country's livestock, sheep are reared under sedentary, semi-nomadic and nomadic husbandry methods. Under such circumstances, brucellosis was found to be widely spread in different domestic animal species; *B. abortus* was isolated from cattle (Musa *et al*., 1990) and goats (Anon, 1992), and *B. melitensis* from a mixed infected flock of sheep and goats (Musa and Jahans, 1990). Brucellosis constitutes a major public health hazard in many countries (FAO/WHO Report, 1986).

This work was intended to study the seroepidemology of brucellosis in sheep in 14 provinces in Darfur states.

Materials and Methods

El Daein, Adyla, Buram, Tulus, Iddel Firsan, Rehaid el Birdi, Nyala, Shaeria and Kass provinces in South Darfur, Gabal Marra and Zalingei provinces in West Darfur and El Fasher, Milleet and Kutum provinces in North Darfur State, were visited at different times. Collection of 174 milk and 2454 serum samples was made from sheep kept under sedentary, semi-nomadic and nomadic husbandry system and from animal in markets for serological diagnosis of brucellosis. Fig.1 shows areas of samples collection.
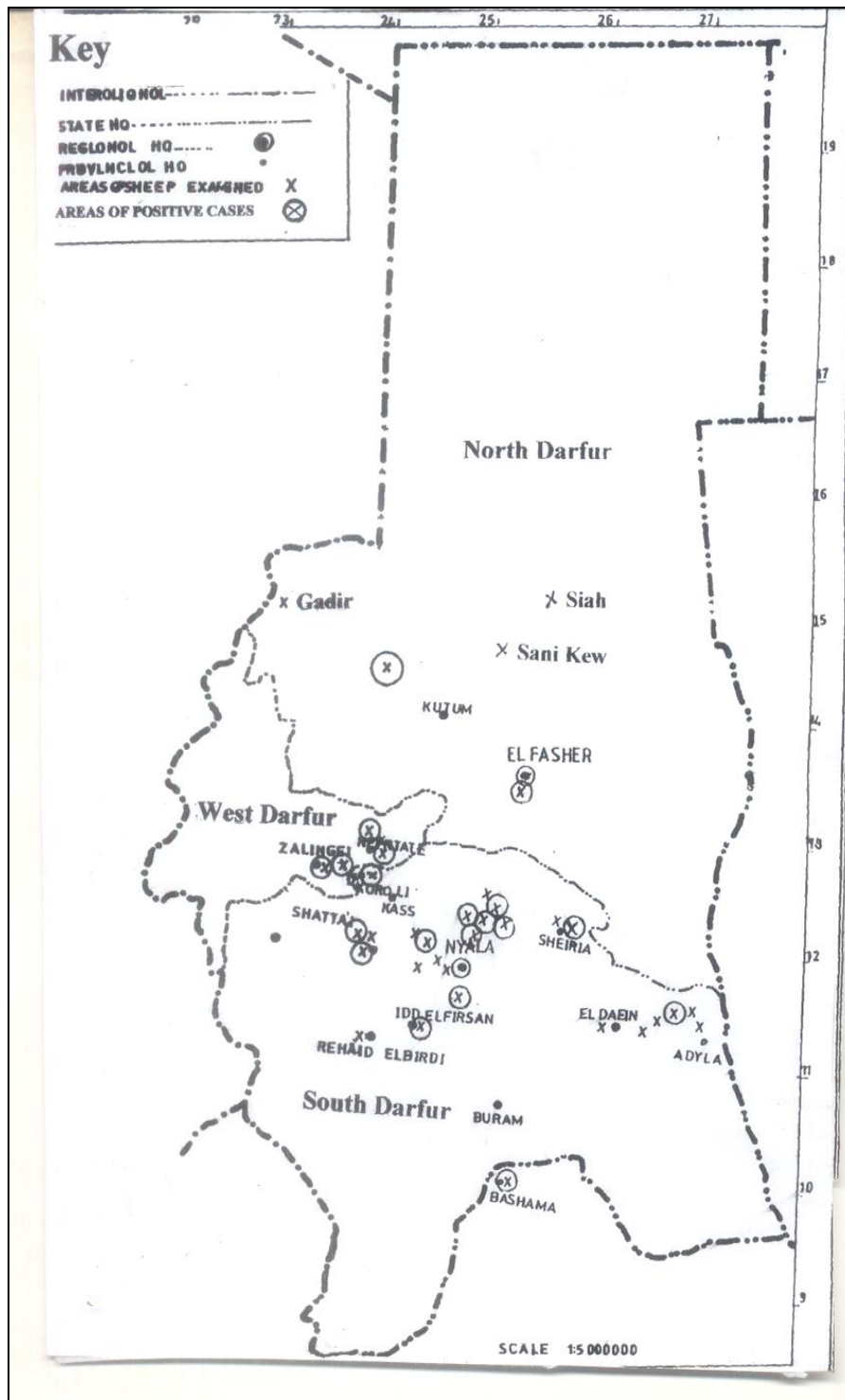
Fig. 1: South, West and North Darfur, Areas of sheep examined.

Samples obtained were transported in ice boxes to Nyala Veterinary Research Laboratory and stored at -20 C. The 174 milk samples were tested by the Milk Ring Test (MRT). Of the total 2454 serum samples 1639 were tested by RBPT, SAT and CFT according to Morgan *et al.* (1978). RBPT-positive samples were subjected to CFT irrespective of SAT results. In circumstances where CFT could not be performed, serum samples were considered diagnostic when they were positive for RBPT and SAT at titers ≥ 31iu. When both SAT and CFT could not be carried out, samples were considered positive when they reacted strongly positive for RBPT. 514 serum samples were examined by RBPT and cELISA (Brew *et al.*, 1992) and the samples were interpreted positives when the percentages of their optical densities (O.D) was ≤75%. The remaining 301 samples were examined by RBPT, SAT and mSAT (Kolar, 1989). Standardized antigens were used in all tests.

Statistical Analysis:-

Data obtained from different husbandry methods were analysed using a statistical prog-ramme (SAS, 1996).

Results

Serological results:

Of the 174 milk samples examined, 15 (8.6%) were positive for MRT. Sixty-four of the 1639 (4%) serum samples were positive for the conventional tests: three were confirmed positive by strong RBPT, 23 by SAT, and 35 by CFT. Out of the 301 serum samples examined by RBPT, SAT and mSAT, three were positive for mSAT. Of the 514 serum samples examined by RBPT and cELISA, three (0.6%) were positive for the latter test. Eighty-seven (3.3%) of the total samples examined were positive. The MRT results are persented in Table 1. Fig. 2 shows SAT antibodies titres of the positive cases.

Table 1: Pattern of MRT results of the 174 milk samples xamined

MRT readings	-	±	+	++	+++
No. of cases	159	00	7	6	2

Key: -= Negative for brucellosis; ± to +++ = Positive for brucellosis **;** - to +++ = depend on the colour of cream ring and milk column

Sensitivity rates and Specificity of RBPT:

Two hundred and seventy-seven of 2271 (12.2%) serum samples screened by RBPT were positive. Only 68 (24.5%) of the former were confirmed positive. Those not confirmed were weak positive samples. One hundred and eighteen RBPT-positive samples were negative by SAT but only three of them were positive by mSAT.

Prevalence rates of brucellosis in 14 provinces in Darfur States:

Table 2 summarises the prevalence of brucellosis and husbandry methods in the 14 provinces studied.

The Prevalence rates of brucellosis according to husbandry methods:

Table 3 shows the prevalence rates of brucellosis in South, West and North Darfur States in relation to the sheep husbandry methods and animal markets.

Clinical manifestations of the disease:

Out of 2628 sheep examined, 76 ewes had aborted, and five rams had orchitis. The serological diagnosis showed that 12 of the 76 (15.8 %) ewes and one of the five rams were positive for brucellosis. They were as follows: In Adyla Province, an aborted ewe that had a serum antibody titrte of 31 iu/ml was positive. The other four aborted ewes and a ram with orchitis were negative. In Eddel Firsan Province, 10 females had aborted of which five were positive. In the Range and Pasture Department flock, Nyertate, Jabal Mara Province, nine of 35 females had aborted; four of them had titres within the positive range. In EL Fasher Province, the *Brucella*-aborted ewe (2.2%) was from a semi-nomadic flock.

Prevalence rates of sheep brucellosis in stocks of different communities:

The sheep examined were mixed with 60 herds of cattle and camels and from eight flocks owned by sedentary, semi-nomadic and nomadic owners. Twenty-five herds (41.7%) and three flocks (37.5%) were infected.

Statistical analysis:

Table 4 shows the results of statistical analysis of brucellosis prevalence rates in the three states and under different husbandry methods. The results were highly significant and P values for all the parameters were < 0.001.

Discussion

Different types of serological tests were used in this study because limited materials were available at each stage of the work. mSAT was used because it abrogates prozone phenomenon, which prevents agglutination in the conventional SAT. The weak positive RBPT results, which could not be confirmed by SAT, CFT or cELISA were probably due to antibodies of cross-reacting genera (OIE, 2000).

In the 56% (33/59) of the positive sheep, SAT antibody titres were > 100 i.u. and in 20% were high high indicating recent infections. However, in 44% (26/59), the titres were <50 i.u. indicating that SAT alone is not suitable for the diagnosis of the disease (Stack *et al.,* 2003)

The overall prevalence rate of brucellosis in Darfur States is low. Higher prevalence rates were previously reported in Southern Sudan and Gazira (El Nasri, 1960; Dafalla and Khan 1962). However, later on, low rates were reported in export animals examined in

Khartoum (Osman and Adlan, 1986; Fayza *et al.,* 1989). In North Darfur State, the prevalence rates are the lowest. This could be attributed to low density of animal population, desert and semi-desert conditions, dryness, direct sunlight, lack of dense vegetation, besides watering of animals from dug wells rather than common watering points; these factors may not favour survival of *Brucella* organisms for long periods (Wray, 1975). On the other hand, high prevalence rates of the disease in some instances, under sedentary and nomadic conditions in South and West Darfur States, may be attributed to the existence of *brucella-* infected large herds of cattle with a high turnover of animals (Musa, 1995); besides keeping flocks of sheep in close contact with each other and with goats especially under sedentary conditions.

Table 2: Prevalence of ovine brucellosis in 14 provinces in Darfur States, Western Sudan.

State	Province	No. of Males exam.	No. +ve	No. of Females exam.	No. +ve	Total No. exam.	No. +ve	Husbandry method
(1) South Darfur State	El Daein	016	00	060	2 (3.3%)	076	2 (2.6%)	Nomadic
	Adyla	029	00	303	3 (≈ 1%)	332	3 (0.9%)	Semi-nomadic
	Buram & Tulus	117	2 (1.7%)	770	17 (2.2%)	887	19 (1.7%)	Nomadic
	Aedel Firsan	005	3 out of 5	010	5 (50%)	015	8 (53.3%)	Nomadic
	Rehaid el Birdi	001	00	046	00	047	00	22 semi-nomadic 25 nomadic
	Nyala	110	11 (10%)	172	1 (0.6%)	282	12 (4%)	1 nomadic 57 sedentary 224 from markets
	Kass	030	00	327	13 (≈ 4%)	357	13 (3.6%)	54 semi-nomadic 303 nomadic
	Shaeria	151	4 (2.6%)	168	4 (2.4%)	319	8 (2.9%)	161 Semi-nomadic 158 Sedentary
	Total	**459**	**20 (4.3%)**	**1856**	**45 (2.5%)**	**2315**	**65 (3%)**	
(2) West Darfur State	Jabal Marra	18	2 (11.1%)	85	15(17.6%)	103	17(16.5%)	96 sedentary 7 semi-nomadic
	Zalingei	00	00	44	2(4.5%)	044	2(4.5%)	Semi-nomadic
	Total	**18**	**2(11.1%)**	**129**	**17(13.2%)**	**147**	**19(12.9%)**	96 sedentary 51 semi-nomadic
(3) North Darfur State	El Fasher	02	00	044	1(2.2 %)	046	1 (2.2%)	9 Sedentary + 37 semi
	Kutum and milleet	19	00	101	2 (2%)	120	2(1.6%)	76 Nomadic + 33 semi + 11 sed.
	Total	**21**	**00**	**145**	**3(2.1%)**	**166**	**3(1.8%)**	
Total	**Total 1,2,3**	**498**	**22**	**2130**	**65(3.1%)**	**2628**	**87(3.3%)**	

Key: Semi= semi-nomadic**;** Sed.= sedentary.

Table 3: Prevalence rates of ovine brucellosis in the three states according to husbandry practices and other sources.

States	Sedentary			Semi-nomadic			Nomadic			Animal Markets		
	Total	+	%	Total	+	%	Total	+	%	Total	+	%
South Darfur	215	06	02.8	569	13	2.6	1307	36	2.8	224	10	4.5
West Darfur	096	15	15.6	051	04	7.8	-	-	-	-	-	-
North Darfur	020	00	00.0	070	01	1.4	0076	01		-	-	-
Total	**331**	**21**	**6.3**	**690**	**18**	**2.8**	**1383**	**37**	**2.**	**224**	**10**	**4.5**
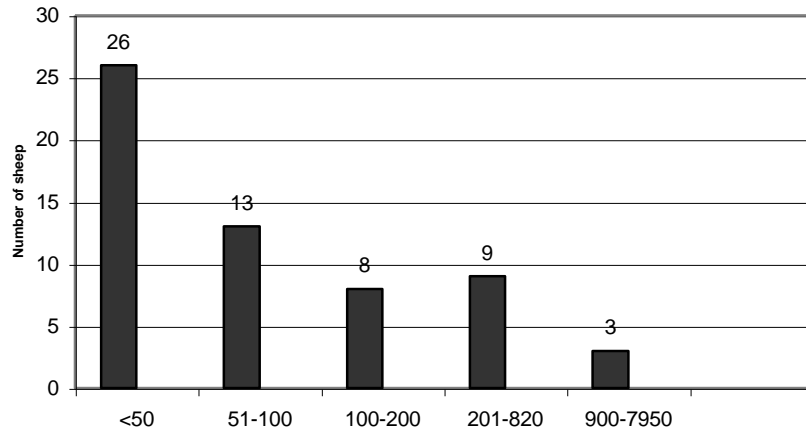
Antibody levels in international units/ml; ■ = No. of sheep

Fig. 2: Serum antibody titres (i.u) of samples confirmed positive by CFT

In Darfur states, the disease is widespread, under all conditions, and is not subjected to any control measures. This may complicates the situation and necessitates prompt actions to curb the problem by vaccination and/or other recommended procedures.

It is clearly noticeable that the disease is widely spread in sheep mixed with either cattle under nomadic conditions or goats under the sedentary ones. Sheep and goats are primary hosts for *B. melitensis* but sporadic natural ovine infections with *B. abortus* were also reported (Okoh, 1980). The associatin of *B. abortus* with abortion in ovines was also documented (Shaw, 1976). *B. abortus* biovar 6, the major cause of bovine brucellosis in southern Darfur (Musa *et al.,* 1990), was isolated from an aborted nany goat. Accordingly, it may be speculated that brucellosis in sheep mixed with infected nomadic cattle is mainly attributed to *B. abortus*. However, when both sheep and goats were infected as in the case of Range and Pasture farm, in Neyrtate, involvement of *B. melitensis* is more likely expected rather than *B. abortus* because the latter occurs less frequently in goats

(Corbel, 1989). Further work is needed to determine areas infected with each species. Camels are not primary hosts for *B. abortus* and *B. melitensis*, yet they rank second among the domestic animal species that suffer from brucellosis in Darfur (Musa, 1995). As a result, cross transmission between sheep, camels and other animal species is likely to occur. It was clearly evident that sheep owned by camel nomads were less infected by the microbe than those owned by cattle nomads. The facts that 41% of the sheep flocks examined were infected, the high prevalence rate of the disease in different domestic animal species in Darfur (Musa ,1995) and the high density of animal poplaton in different localities deserve special attention. Control of brucellosis in cattle, sheep, goats and camels by immunization with *B. abortus* S19 and *B. melitensis* Rev.1 vaccines, is deemed essentially vital in Darfur States

Acknowledgments

References

Anon. (1992). Annual report, Nyala Veterinary Research Laboratory.

Anon. (2003). Sudan in Figures, Central Bureau of Statistics, Council of Ministers, Khartoum – November, 2003, Pp. 15.

Brew, S.; Perrett, L. and Stack, J. (1992). *Guide to the EIA techniques used in the diagnosis of brucellosis at the* CVL, weybridge, pp

Corbel, M. J. (1989). Brucellosis: epidemiology and prevalence wordwide "In E. J.Young and M. J. Corbel (ed.). *Brucellosis: Clinical and Laboratory Aspects*, (eds) CRC Press, Boca Raton, Florida, pp**. 26 – 40.**

Dafalla, E. N. and Khan, A. (1962). *Sudan J. Vet. Sci. Anim. Husb.,* **3***: 80 – 89.

El Nasri, M. (1960). *Vet. Rec.*, **72:** 1200 – 1210.

FAO/WHO Report (1986). *Expert Committee on Brucellosis, 6th Report, WHO Technical Report series* 740, 101, World Health Organization, Geneva, Pp. 7.

Fayza, A. O.; El Sheikh, O. H.; Zakia, A. M.; Halima, M. O., Suliman, H. B. and Osman, A. Y. (1989–1990). *Sudan J. Vet. Res.*, **9:** 36 – 40.

Kolar, J. (1989). Brucellosis in Eastern European Countries "In: E. J. Young and M. J. Corbel (eds.). *Brucellosis*: *Clinical and Laboratory Aspects*. CRC Press, Boca Raton, Florida, Pp. 163 – 172.

Morgan, W. J. B.; Mak Kinnon, D. J.; Gill, K. P. W.; Gower, S. G. M. and Norris, P. I. W. (1978). *Brucellosis Diagnosis Standard Laboratory Techniques*. Ministry of Agriculture, Fisheries and Food, London. U.K. Pp. 1-35.

Musa, M. T. (1995). Brucellosis in Darfur States: The Magnitude of the Problem and Methods of Diagnosis and Control. *PhD. Thesis*. Faculty of Veternary Medicine, University of Khartoum,Khartoum Sudan.

Musa, M. T. and Jahans, K. L. (1990). *J. Comp. Pathol.,* **103:** 467-470.

Musa, M. T.; Jahans, K. L. and Fadlalla, M. E. (1990). *J. Comp. Pathol.,* **102:**49-54.

Okoh, A. E. J. (1980). *Trop. Anim. Hlth Prod.,* **12:** 11- 14.

OIE. (2000). Manual of Standards for Diagnostic Tests and Vaccines. World Organization for Animal Health, 4th edn, 12 rue de Prony, Paris Pp**. 335.**

Osman, A. M. and Adlan, A. M. (1986). The incidence of brucellosis in the Sudan. 54th General Session of the International Committee of the O. I. E., Paris. May 1986.

Shaw, W. B. (1976). *Br. Vet. J.*, **132***:* 18 – 26.

Stack , J.A. and Mac Millan, A.P. (2003). *Burcella* serology .http:// www.fao.org/livestock /ajah/id/ brunet- main/brunet /public-sub 4-91. html P-8

SAS (1996). Statistical analysis system, version 612, SAS User's Guide. Statistics. SAS Inst. Inc. Cary. NY., USA**.**

Wray, C. (1975). *Vet. Bull.,* **45**(3):543-550.