Prevalence of Brucellosis in Different Animal Species and Man and Isolation of *Brucella abortus* biovars 1 and 6 from Cattle in Sennar State

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ملخص البحث

الإهتمام بخطورة مرض البروسيلا في ولاية سنار لم يتم إلاّ حديثاً بالرغم من قِدم تاريخ حدوثه. الهدف من هذه الدراسة معرفة مدى إنتشار مرض البروسيلا في الحيوانات المختلفة والإنسان فى ولاية سنار. حُدد عدد العينات بالتقدير المسبق. جُمِعت 2429 عينة مصل ، 1547 من الأبقار، 585 من الأغنام، 169 من الماعز، 100 من الخيول و 28 من الإنسان. كما جُمِعت 24 مسحة مهبلية من الأبقار والأغنام والماعز و 3 عينات للسائل الزليلي من الورم الرطب في الأبقار. أُستخدمت إختبارات الروزبنغال الصحني والروزبنغال المعدّل و المقايسة التنافسية للإمتصاص المناعي المرتبط بالإنزيم كما إستخدم إختبار التراص الأنبوبي لقياس مستويات الأضداد فى أمصال الحيوانات الموجبة. زرعت سوائل الورم الرطب والمسحات المهبلية لعزل بكتريا البروسيلا. نتائج الإختبارات المصلية لإنتشار المرض كانت كالآتي: 24.1% في الأبقار و 4.9% في الأغنام و 4.7% في الماعز و 3.6% في الإنسان. بينما لم تسجل أى حالة موجبة فى عينات الخيول. تم عزل عترتين للبروسيلا المجهضة من ذوات النمط الحيوي 6 والنمط الحيوي 1 من سائل الورم الرطب في الأبقار. تشترك مختلف الحيوانات في الولاية في نفس المرعى ومصادر المياه، تحفظ الحيوانات حول المنازل في حظائر مختلطة مساءاً مما يؤدي إلى إنتشار المرض بينها وإنتقاله للإنسان من خلال الإتصال المباشر والغير مباشر بالحيوانات المصابة ومنتجاتها. كانت الإختلافات بين نتائج الإختبارات المستخدمة ضئيلة لأن المرض مستوطن في المنطقة. أعلى نسبة إنتشار للمرض وجدت في أبقار اللبن. تُوصي الدراسة بإستمرار المسح لمرض البروسيلا في الولاية لإظهار مدى إنتشار المرض في الأبقار و الماعز و الضأن و الخيول والأشخاص المخالطين للحيوانات المصابة ومنتجاتها. بناءاً على النتائج الحالية نوصي ببدء السيطرة المباشرة على المرض وذلك بنشر الوعي بخطورة المرض بين أصحاب الحيوانات ومعرفة آثاره الصحية والإجتماعية والإقتصادية وحثّهم على المشاركة في السيطرة عليه بالتطعيم ومحاولة القضاء على عليه بإتباع سياسية الفحص وذبح الحيوانات المصابة.

Summary

Recently an attention was paid to the seriousness of brucellosis in Sennar State in spite of its back-dated occurrence. A total of 2429 serum samples was collected from cattle (*n*=1547), sheep (*n*=585), goats (*n*=169), horses (*n*=100) and man (*n*=28). In addition, 24 vaginal swabs and 3 hygroma aspirates were collected from cattle, sheep and goats. The study was conducted during the period from May 2008 to April 2009. Serological tests used were the Rose Bengal Plate (RBPT), Modified Rose Bengal Plate test (mRBPT) and competitive ELISA (cELISA). Serum Agglutination Test (SAT) was used to measure antibody levels in the positive serum samples. Prevalence of brucellosis reached 24.1% in cattle, 4.9% in sheep, 4.7% in goat and 3.6% in man. No positive sample was reported from equine samples. The difference between the results of the different tests was marginal because of the disease endemicity in the state. The highest disease prevalence was found in dairy cattle. Two strains of *Brucella abortus,* biovar 6 and biovar 1, were isolated from hygroma aspirates. Different animal species, in the state, share the same pasture and water sources and those of households share the same premises at night. Therefore, spread of brucellosis is most likely to occur among different animals, and in man, through direct and indirect contacts with infected animals and animal products. This study recommends continual surveillance of brucellosis in the state to determine the magnitude of the problem and the prevalence of the disease in cattle, sheep, goats, horses and man. Adoption of immediate disease control measures accompanied by raising public awareness of its economic impact and hazards to public health are highly recommended. Participation of animal owners in routine animal vaccination to bring down the disease prevalence and adoption of an eradication programme via test and slaughter policy are strongly encouraged.

Introduction

Sennar State is part of El Gezira Irrigated Scheme; as a result, agriculture is the major activity of the people in the state where livestock breeding is an integral part. The state has about 4,666,598 head of animals which are composed of cattle, goats, sheep and camels. These animal species share the same pastures and water points throughout the state.

Brucellosis was reported in cattle, sheep and goats in El Gezira State in 1953 (Dafalla and Khan 1958) and received a limited attention in the neighbouring Sennar State. This study was intended to investigate the prevalence of brucellosis in animals and man and to isolate *Brucella* from infected cases.

Materials and Methods

Sampling:

Sample sizes were calculated according to Thrusfield (1991) and Israel (1992) at 5% precision and 95% confidence levels. Serum samples were collected from 1547 cattle, 585 sheep, 169 goats, 100 horses and 28 abattoir workers and people in-contacts with animals. In addition, 24 vaginal swabs (from aborted animals; three from goats, five from sheep and 16 from cattle) and three hygroma aspirates from three cattle, were obtained for isolation of *Brucella* species.

Serology:

The RBPT, mRBPT and cELISA were used for serological diagnosis of brucellosis; the former two are screening tests and the latter was used as a confirmatory one. The results of the tests were compared. SAT was used for measuring antibodies concentration per ml of positive samples.

Isolation of *Brucella*:

The vaginal swabs and hygroma aspirates were used for isolation of *Brucella* organisms. Smears were prepared from these samples and stained with the modified Ziehl-Neelsen stain (mZNS) (Stamp *et al,* 1950). The positive samples were cultured on Serum Dextrose Agar (SDA) supplemented with antibiotics (Oxoid Brucella antibiotic supplement). Cultures were incubated at 37 ºC in an atmosphere of 10% CO_2 and examined daily from day 3 till day 10 post inoculation. Slide smears were prepared from colonies resembling those of *Brucella* and examined for partially acid-fast bacilli using mZNS.

Results

Serological findings:

The results of the 2429 serum samples tested with the RBPT and cELISA are presented in Table 1. The result of mRBPT showed no difference compared to RBPT. The mRBPT reacted quicker and showed clear agglutination reactions. There were no statistically significant differences between RBPT and cELISA ($p > 0.05$). The 399 cattle, sheep, goats and human positive serum samples showed different levels of antibodies concentration per ml.

Bacteriological investigations:

Vaginal Swabs:

All smears made from the vaginal discharge swabs of aborted sheep and goats were negative by the mZN stain. Cultures form these samples were also negative for *Brucella* organisms.

Hygroma aspirates:

Examination of slide smears prepared from the hygroma aspirates from one bull and two cows were mZN stain-positive. The cultures from the three samples yielded pure *Brucella* like colonies. The pure cultures were identified as *Brucella* organisms according to Corbel and Hendary (1983). *Brucella* was characterized to the species level at the Veterinary Research Institute, Khartoum, Sudan. Further characterization to biovar level was made at the Animal Health Veterinary Laboratories Agency (AHVLA), Weybridge, UK.

Table 1: Comparison between RBPT and cELISA results

Species	No. examined	RBPT	cELISA
		No. positive (%)	No. positive (%)
Cattle	1547	366 (23.7)	372(24.1)
Sheep	585	24 (4.1)	29(4.9)
Goats	169	8 (4.7)	8 (4.7)
Horses	100	0 (0)	0 (0)
Humans	28	1 (3.6)	1(3.6)
Total	**2429**	**399 (16.4)**	**410 (16.8)***

*P value = 0.983

Discussion

Sennar State is part of El Gezira Irrigated Scheme. Apart from crop cultivation, livestock rearing is an integral part of agricultural activities where most of the people own more than one animal species. The different species of animals in the state share the same natural pastures or harvested crop fields. At home, they are kept together in an enclosure or a shed at night. Under these circumstances, spread of brucellosis is most likely to occur between different animal species as well as man. This speculation is known since a long time ago, and the prevalence of the disease was investigated (Bakhiet, 1981; El sawi *et al,* 1981; Shallali *et al,* 1982). However, all these investigators examined only cattle for brucellosis and ignored other domesticated animal species. Moreover, their surveys included a limited number of animals without isolation of any *Brucella* species and they used only RBPT for diagnosis of the disease.

In this study, RBPT, mRBPT and cELISA were compared. No differences were found between RBPT and mRBPT, whereas cELISA is more sensitive than the former two tests and should be used as a second screening test or confirmatory test. The marginal differences among RBPT, mRBPT and cELISA may be due to endemicity of the disease in the state. The prevalence in cattle was 24.1%, which is high compared with that reported by Bakhiet (1981). This study also shows that the disease occurs in sheep (4.9%), goats (4.7%) and humans (3.6%). The close similarity in disease prevalence rates in sheep and goats is probably due to their small population and open husbandry system practised in the state, under which they are reared.

Uncontrolled movement of animals, shortage of veterinary support services, lack of proper vaccination policy and improper husbandry practices have favoured the spread of brucellosis among animals. Human cases occur due to their direct and indirect contact with animals.

There were no previous reports of isolation of *Brucella* in Sennar State. In this study three *B. abortus* strains were isolated from cattle with hygromas which indicate that the disease is chronic and have existed in the area since a long time ago. One of the isolates was *B. abortus* biovar 1 which has a limited distribution in the Sudan (Musa, 1995). The other two isolates were *B. abortus* biovar 6 which is widespread in indigenous cattle (Musa, 1990). No isolation attempts were made from sheep, goats and humans; consequently, further work is needed to isolate *Brucella* spp. from these hosts.

Acknowledgments

Sincere thank are due to J. Stack and L. Perrett for permission to send *Brucella* isolates and for typing to species and biovar levels at the AHVLA, UK, and to Dr. S. Elshafee and all staff members of, and colleagues in, Sennar Regional Veterinary Research Laboratory for their continuous help

during the collection of samples. Thanks and appreciations are due to staff of Departments of *Brucella* and Bacteriology, Veterinary Research Institute (VRI), Soba, Khartoum. This work is published by kind permission of the Director VRI, and Director-General Animal Resources Research Corporation (ARRC), Khartoum, Sudan.

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