Prevalence of Ovine Brucellosis and Isolation of *Brucella melitensis* biovar 1 in South Kordofan State, Sudan

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ملخص البحث

تُقدر أعداد الأغنام (الضأن) فى ولاية جنوب كردفان بأربعة مليون رأساً تقريباً. لم يتم مسح مرض البروسيلا (Brucellosis) فى أغنام هذه الولاية من قبل بالرغم من إنتشار هذا للمرض فى الأبقار. لذلك هدفت هذه الدراسة لمعرفة مدى إنتشار هذا المرض فى الأغنام. جُمعت 1229 عينة مصل و 128 عينة لبن (حليب) و فُحصت حسب ماهو ملائم من إختبار لنوع العينة: الأمصال بإختبارات الروز بنغال الصحنى و الروز بنغال الصحنى المعدل و المقايسة التنافسية للإمتصاص المناعى المرتبط بالإنزيم، والحليب بإختبار اللبن الحلقى. أستخدم إختبار التراص الأنبوبى لمعرفة تركيز الأضداد فى كل مل من الأمصال الموجبة. لقد أوضحت الدراسة أن إنتشار المرض فى أغنام المحليات الخمس كالأتى: 13.4% فى كالوقى ، 7.9% فى كادقلى ، 3% فى أبوجبيهه ، 1% فى كل من الدلنج والعباسية وبإنتشاركلى بواقع 5.5%. قُورنت نتائج إختبارى الروزبنغال وقورنت نتائجهما بنتائج المقايسة التنافسية للإمتصاص المناعى المرتبط بالإنزيم. أوضح التحليل الإحصائى وجود إختلافاً معنوياً فى معدل إنتشار المرض فى مختلف المحليات. أُستزرعت مسحات مهبلية و عينات عقد ليمفاوية و لبن (حليب) و إفرازات و أنسجة خصية فى وسط آقار مصل ودكستروز. عزلت عزلات (معزولات) لبكتيريا البروسيلا المالطية ذات النمط 1 (واحد) من عقدة ليمفاوية آربية داخلية و إفرازات خصية. هذه هى المرة الأولى التى سجل فيها وجود هذا النمط الحيوى فى السودان. شددت الدراسة على ضرورة إحاطة المسؤلين ومربى الأغنام و الأطباء البيطريين بموقف المرض فى الولاية وأوصت الدراسة أيضاً على ضرورة تعاون الأطباء البيطريون و أصحاب المصلحة للحد من المرض بالطرق المختلفة و السيطرة عليه بأستخدام لقاح البروسيلا المالطية عترة ريف 1 لتطعيم الأغنام و الماعز.

Summary

Sheep population in South Kordofan State was estimated to be four million head. Sheep brucellosis was not studied in this state despite its widespread in cattle. The aim of this study was to investigate prevalence of brucellosis in sheep in South Kordofan State. A total of 1229 blood for serum samples and 128 milk samples were collected during January 2009 to December 2010, the former were examined by the Rose Bengal Plate Test (RBPT), the modified Rose Bengal Plate Test (mRBPT), and the Competitive ELISA (cELISA) and the latter by the Milk Ring Test (MRT). Serum antibody concentrations of the positive samples were measured in international units per ml, besides vaginal swabs, lymph nodes, testicular tissues and orchitis exudate were obtained and cultured together with the milk on Serum Dextrose Agar supplemented with antibiotics (Oxoid, Brucella antibiotic supplement) and *Brucella* was isolated from an internal inguinal lymph node and testicular tissues of a ram. Both organisms were found to be *B. melitensis* biovar 1 and this is the first report of this biovar in the Sudan. The results of RBPT, mRBPT and cELISA were compared. The disease was found prevalent in sheep in 21 areas in five localities of the state. The prevalence in the five localities amounted to 13.4% in Kalogi, 7.9% in Kadugli, 3% in Aboujbaiha, 1% in Dilling and 1% in Abbasia. The overall prevalence of the disease was 5.5%. The statistical analysis showed that the prevalence of the disease was significantly different in the aforementioned localities ($p < 0.001$). Competitive ELISA detected more positive cases than both, the RBPT and the mRBPT. It is recommended that government officials, farmers and veterinarians must be aware of the disease situation in the state. Veterinarians and all stakeholders must cooperate to contain the disease by different means. Mass vaccination of sheep and goats with *B. melitensis* vaccine strain Rev.1 vaccine is recommended for the disease control.

Introduction

Brucellosis is widespread in domestic and wild animal species in Darfur states (Musa, 1995). Meagre information is available about the disease in sheep of South Kordofan State.

Sheep population in five eastern localities in South Kordofan State was estimated to be 1900000 (Anon, 2008). Sheep are reared separately or often mixed with goats in the state throughout. All livestock species in the state share the same natural pastures. Sheep are essential sources of income and food to people of the state and contribute effectively to the country′s economy by their exportation to the Arabian Peninsula. The main constraint on their export is brucellosis.

The aim of this work was to study the prevalence of brucellosis in sheep in five localities of South Kordofan State.

Materials and Methods

A data sheet that shows serial number, age, sex, area of the study, locality in South Kordofan State, history of abortion and clinical sings pertinent to brucellosis, for each sheep sampled, was designed. Sample size was estimated according to Israel (1992). A total of 1229 serum samples and 128 milk specimens were collected from sheep from 21 areas in five localities of the state, besides 28 lymph nodes which included internal iliac, submaxilary, supramammary and internal inguinal. Two udder specimens were collected from sheep found positive by the RBPT at Kadugli Abattoir. Five vaginal swabs and 21 testicular exudate were collected in MacCarteny bottles from rams in the field using sterile syringes. All samples were kept on ice and transported to Kadugli Veterinary Research Laboratory. Serum samples were preserved at -20^0C and the milk samples were kept in the refrigerator at 4^0C for 48–72 hours till used.

Serology

Rose Bengal Plate Tests

A total of 1229 serum samples were examined by the RBPT. The same serum samples were re-examined by mRBPT according to the OIE (2008).

Milk Ring Test

Milk samples (n= 128) were examined by the MRT (OIE, 2008).

Serum Agglutination Test

Forty-two, mRBPT-positive serum samples were retested with the standardized Serum Agglutination Test (SAT). The test kits were performed as described by Morgan *et al* (1978).

Competitive ELISA

The serum samples positive by the RBPT and mRBPT were retested by the c-ELISA. The test was supplied by the Animal Health Veterinary Laboratories Agency UK.

Bacteriology

Lymph node samples were homogenized with a sterile pestle and mortar. The vaginal swabs, orchitis exudate and milk cream and sediment were used to prepare slide smears stained with the modified Ziehl-Neelsen (mZN) stain. Partially acid fast organisms were cultured on Serum Dextrose Agar or Farrell's medium and incubated at 37 ^{0}C under 10% CO_2 using candle jars. The cultures were examined daily for 10 days. Colonies which resembled *Brucella* were used for slide smears preparation and stained with mZN stain for detection of partially acid fast organisms. Organisms suggestive of *Brucella* were characterized to species and biovar levels according to Corbel and Hendary (1983).

Statistical analysis

Statistical analysis was performed using SPSS version 13. Chi-square test was used for analysis of the prevalence of brucellosis in sheep in different areas of the state.

Results

Serological tests

Of the 1229 serum samples, 65 (5.3%) were positive by the RBPT, 66 (5.4%) by the mRBPT and 68 (5.5%) by the cELISA (Table 1). Of 42, mRBPT- positive serum samples, 39 (93%) were positive by the SAT. The antibody concentrations (per ml) of the 39 samples ranged between 30 and 640 IU/ml.

Milk Ring Test

The results showed that 9 (7.03%) of the 128 milk samples examined were positive for *Brucella* antibodies.

Prevalence of brucellosis in sheep in different localities

There was statistical significant difference between the prevalence of brucellosis in sheep in the five localities ($p < 0.001$) (Table 1).

Isolation of *Brucella* organisms

Brucella organisms were isolated from the internal inguinal lymph nodes and the testicular exudate of a ram.

Modified Ziehl-Neelsen stain

Smears from the lymph nodes of the 65 sheep serologically positive for brucellosis showed that only one lymph node (1.5%) was positive by the mZN stain.

Identification of the isolates to genus *Brucella*

The *Brucella* like isolates were partially acid fast, coccbacilli, gram negative, stained pink with the mZN. Oxidase, catalase and nitrate positive, but they were indole, glucose, MR, VP and koser's citrate negative. They were thus diagnosed as belonging to the genus *Brucella*.

Characterisation of *Brucella* isolates to biovar level

Characterisation of the *Brucella* isolates, from a ram lymph node and orchitis exudate, to the species and biovar levels is presented in Table 2.

Discussion

Brucellosis was reported in cattle in South Kordofan State since 1958 (Dafalla and Khan, 1958), this is the first study of brucellosis in sheep in the state. Serum samples were screened by the RBPT and the mRBPT and confirmed by the cELISA. The SAT was used for determination of *Brucella* antibody concentrations per ml in positive sera. RBPT is the main screening test of brucellosis for all livestock species in the country, because of its sensitivity, simplicity and availability although its sensitivity does not exceed 98% (OIE, 2008). The mRBPT which is more sensitive than RBPT (Blasco *et al.*, 1994) was used for comparison. The mRBPT detected more positive samples (0.1%) in sheep than the RBPT. Results of both tests were then confirmed with the more sensitive and specific cELISA (OIE, 2008) which has detected more positive cases than the former two tests. The SAT antibody titres of the 7 (17%) samples were under the diagnostic level and 32 (82%) were above the diagnostic level, and this confirms that SAT is less sensitive than both the RBPT and the mRPBT (OIE, 2008). The mRPBT can be a useful supplemental test for export animals to satisfy importing countries requirements.

The overall prevalence of brucellosis in sheep is 5.5%, the highest prevalence is 13.4% in Kalogi. Because livestock intermix at water points and pasture lands in summer season the prevalence is more than that at other localities. The prevalence of the disease was significantly different in sheep ($p < 0.05$) in the different areas of the five localities. This finding is in agreement with those of El-Ansary *et al* (2001) in eastern Sudan. The prevalence of sheep brucellosis in this state is higher than that of the neighbouring Darfur states where the prevalence was 3.3% (Musa, 2005).

One strain of *B. melitensis* biovar 1 was isolated from the internal inguinal lymph node and orchitis exudate of a ram for the first time in the Sudan. The isolation of the causative agent confirms the results of the serological tests used for diagnosis of the disease. *B. melitensis* biovar 3 was isolated

from a mixed herd of sheep and goats from the neighbouring South Darfur State (Musa *et al*, 1990; Buthina, 2009). Sheep brucellosis in South Kordofan State poses a public health hazard to the local inhabitants who consume raw sheep′s and goat′s milk, besides locally processed cheese from this milk. The disease remains a hazard to abattoir workers; since Omer *et al* (2010) found that the prevalence of brucellosis in people who are in contact with camels was 60% and 12% in abattoir workers.

Table 1: Prevalence of brucellosis in sheep in five localities in the South Kordofan State

Locality	No. examined	cELISA No. +ve (%)	Chi-square	df	P-value
Kadugli	480	38(7.9)	41.143	4	0.001
Dilling	200	2(1)			
Abbasia	200	2(1)			
Aboujbaiha	200	6(3)			
Kalogi	149	20(13.4)			
Total	**1229**	**68(5.5)**			

Table 2: Characterisation of the *Brucella* isolates from a ram to species and biovar level

Growth characteristics						Nonspecific antiserum			Phages at RTD						10^4	interpretation
	urease	H_2S	Co_2	BF	TH	A	M	R/C	WB	Tb	BK_2	Fi	Iz	R/C	Tb	*B. melitensis* 1
L.N.	++	-	-	+	+	-	+		NL	NL	PL	NL	PL	NL		
O.E.	++	-	-	+	+	-	+		NL	NL	PL	NL	PL	NL		

N.B. Both isolates are from the same animal and are the same strain
L.N. = Lymph node; O.E. = Orchitis exudate
BF = Basic fuchsin at 20µl/ml (1/50,000w/v); TH = Thionin at 20µl/ml& 10µl/ml (1/50,000 w/v)
CL = Confluent lysis; PL = plaque form lysis; NL = No lysis; O = Ovine;
- = negative test ; + = positive test; RTD= Routine test dilution

The present situation of the disease in South Kordofan State, threatens animals health, and endangers economy and food security for the people of the state. The disease needs immediate intervention by mass vaccinations of sheep and goats with *B. melitensis* Rev 1 vaccine.

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