Prevalence of *Babesia bigemina* and Ticks Infesting Cattle in Kassala State, Eastern Sudan

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ملخص البحث

أجريت هذه الدراسة في ولاية كسلا - شرق السودان فى الفترة من يناير 2009 إلى ديسمبر 2010 لتحديد مدى إنتشار مرض البابيزيا التوأمية والقراد المتطفل على الأبقار. جمعت مسحات دم وعينات دم فى أوراق الترشيح من 334 رأس في حين تم جمع القراد من 105 رأس. تم تشخيص البابيزيا التوأمية في ثلاثة عينات (0.90٪) فى أبقار قادمة من خارج الولاية من أصل 334 عينة باستخدام مسحات الدم ومن أصل 25 عينة (شاملة تلك العينات الموجبة) فحصت بواسطة إختبار البلمرة المتسلسل ، كانت ثلاث عينات (12٪) إيجابية. عُرفت ثلاث أجناس من القراد هى الهيالوما، مروحية الرأس والأمبليوما تنتمى إليها ثمان أنواع هى الهيالوما الأناضولية، الهيالوما الإبلية ، الهيالوما الشعراء و الهيالوما الحمراء ، مروحية الرأس الإيفيريستية الإيفيريستية، مروحية الرأس العلس الدموي، العلس الناصل والأمبليوما اللبدية. هذه الدراسة تشير إلى أن هذا المرض نادر الحدوث بالولاية و ذلك لعدم إستيطان القراد الناقل و عليه فإن الأبقار معرضة الى خطر الإصابة بهذا المرض متى ما إستوطن القراد الناقل. ومع ذلك، هناك حاجة ماسة لدراسة أكثر تفصيلاً لمدة سنتين متعاقبتين على الأقل من أجل مراقبة موقف المرض في مواسم مختلفة من السنة.

Summary

This study was carried out in Kassala State, Eastern Sudan, during January 2009 to December 2010 to determine *Babesia bigemina* prevalence and tick infestation among cattle. Blood smears and blood spotted on filter papers were collected from 334 head while ticks were collected from 105 head. *B. bigemina* was detected in three (0.90%) out of 334 samples using blood smears and out of 25 samples subjected to PCR, three samples, including the three positive cases detected by blood smear examination, (12%) were positive. Three genera of ticks, *Hyalomma, Rhipicephalus,* and *Amblyomma* were identified including eight species; *Hyalomma anatolicum, H. dromedarii, H. rufipes, H. impeltatum, Rhipicephalus evertsi evertsi, R. sanguineus, R.* (*Boophilus*) *decoloratus* and *Amblyomma lepidum*. This study suggests that the low prevalence of disease is due to fact that the vector tick does not occur in the state, hence cattle are at risk whenever the vector tick is introduced and established. However, more detailed study is highly needed based on at least two successive years in order to monitor the disease situation in different seasons of the year.

Introduction

Ticks and tick-borne diseases (TBDs) are widespread in the Sudan constituting serious constraint on production of sound milk and development of meat industry. More than 70 species of ticks, representing the Sudanese fauna had been identified (Hoogstraal, 1956).

Bovine babesiosis is mainly found wherever the tick vectors exist, but it is most common under tropical and sub-tropical climates. *Babesia bigemina* and *B. bovis* are more widely distributed and are of a major importance in Africa, Asia, Australia, Central and South America and parts of Southern Europe (Dolan, 1989). The early incident of babesiosis in Kassala State was reported by Mohammed and Yagoub (1990).

The exact picture of the ticks and tick-borne diseases in Kassala State is not yet clear. This study was carried out to highlight the current situation of bovine babesiosis and ticks infesting cattle in Kassala State.

Materials and Methods

Study area

The study was carried out in Kassala State (15°-17¯–15°-24¯N; 35°-55¯ 37°-

55¯E) during the period of January 2009 to December 2010.

Collection of samples

Three hundred and thirty-four cattle (316 ♀ and 18 ♂) were examined in this study. Blood smears and blood spotted on filter papers were collected from Kassala (*n*=151), Atbara River (*n*=83), Elgash River (*n*=45) and Seteit River (*n*=55). The cattle composed of cross-bred (*n*=201) and indigenous ecotype (*n*=133). The sampled cattle were categorized according to the age group as <1 year (*n*=25), 1-<2 years (*n*=27), 2-<3 years (*n*=38), 3-<4 years (*n*=28) and ≥ 4 years (*n*=216).

Total body tick collection (Macleod *et al*, 1977) was done on 105 cattle using a pair of blunt metal forceps. The age groups of cattle were as follows, <1 year (*n*=4), 1-< 2 years (*n*=6), 2-< 3 years (*n*=6), 3-<4 years (*n*=11) and ≥ 4 years (*n*=78). A total of 820 ixodid ticks was collected during this study. These ticks were collected from four farms in Kassala locality (*n*=34) and two farms in Atbara River locality (*n*= 20) as well as from nomadic cattle in Seteit (*n*= 22) and El-Gash (*n*=29) localities. Ticks collected from each animal, were kept separately in a universal bottle containing 70% ethanol and labelled indicating locality, animal number and date of collection. In the laboratory, the ticks were identified according to Hoogstraal (1956) and Walker *et al* (2003). Mean infestation rate (MIR) was calculated by dividing the total number of ticks by the total number of animals.

Preparation of blood smears

Thin blood smears were made from ear vein puncture, stained with Giemsa's stain and examined under a microscope. At least 50 microscopic fields were examined for presence of piroplasms. The presence of greater than or equal to one piroplasm was considered positive.

Deoxyribonucleic acid (DNA) extraction

DNA was extracted from 25 blood spotted on filter papers using the MiniPrep extraction kit (Qiagen, Germany) following the manufacturer's instructions.

Polymerase chain reaction (PCR)

PCR was performed in a final volume of 50 µl as follows: 25 µl Green master mix, containing dNTPs, PCR buffer and Taq polymerase (Fermentas, Germany), 16 µl H_2O, 2 µl of each primer at a dilution of 10 pmol and 5 µl genomic DNA. The primers used were forward primer: GCGAATGGCTCATTACAACAG and reverse primer: GGACGTAATCTGC ACAAGCTG. The design of primers was based on the *Babesia bigemina* gene small subunit rRNA (GenBank accession number X59604.1). The expected band was 1433 bp. The thermo-cycler programme was run at 94°C for 3 minutes, then 35 cycles at 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute. A final extension step at 72°C for 10 minutes, and then held at 4°C. In each run, positive and negative controls were included. The positive control was *Babesia bigemina* DNA kindly provided by the International Livestock Research Institute, Nairobi, Kenya, while the negative control was distilled water.

Detection of PCR product

Samples as well as 100 bp DNA markers were loaded into 1.5% agarose gel and the power supply was adjusted to 100 V for 30 min. The gel was transferred to a UV illuminator for visualization and documentation.

Statistical analysis

Data collected on ticks infesting cattle at different localities were subjected to appropriate general linear model (GLM) procedure of statistical analysis system (SAS package). Mean separations were performed using Ryan-Einot-Gabriel-Welsh (REGW) multiple range test (Day and Quinn, 1989).

Results

Microscopic examination of blood smears

Intra-erythrocytic organisms morphologically comparable with *Babesia* piroplasms were observed in three out of 334 (0.90%) thin blood smears examined. They belonged to two cows (4.4%; *n*=45) from El-Gash locality and one cow (1.8%; *n*=55) from Seteit River locality.

Polymerase chain reaction

Three out of 25 samples (12%) were positive showing amplification DNA bands of 1433 bp for *B. bigemina* (Fig. 1).

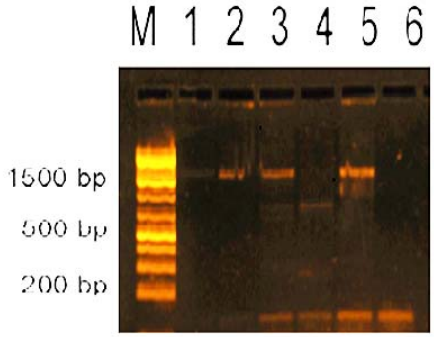

Fig. 1: PCR of *B. bigemina* DNA random samples from Kassala State. Lanes: M= 100 bp DNA ladder marker; 1–4= test samples; 5= positive control; 6= negative control.

Ticks infestation

Three tick genera were identified including eight species, *Amblyomma lepidum, Hyalomma anatolicum, H. dromedarii, H. impeltatum, H. rufipes, Rhipicephalus* (*Boophilus*) *decoloratus, R. evertsi evertsi, R. sanguineus. H. impeltatum* was the dominant tick species (38.7%) followed by *H. anatolicum, R. e. evertsi* and *A. lepidum* (Table 1). *H. impeltatum* and *H. anatolicum* as well as *A. lepidum* were recorded in all localities, while *R. e. evertsi* was found in three localities. *H. dromedarii* and *R.* (*B.*) *decoloratus* were found in Seteit River and El Gash localities, while *R. sanguineus* was found in El-Gash and Kassala localities. *H. dromedarii, R.* (*B.*) *decoloratus* and *R. sanguineus* were of low infestation rates, while *H. rufipes* which was collected from two localities was the least recorded tick species. The majority of ticks (56.8%) were collected from El Gash locality where the eight tick species were encountered (Table 1).

According to the age groups, the highest mean infestation rate (MIR) was reported on heifers at 2 to < 3-year-old followed by those of 3 to < 4-year-old. Animals of age groups 1-< 2 years and ≥ 4 years had the same MIR (7.5 ticks/animal), while calves less than one-year-old were the least tick infested group (5 ticks/animal) (Table 2).

The statistical analysis showed that the general mean infestation rate varied according to the year of collection, being significantly higher for the second year than that calculated for the first year (Table 3).

Discussion

Data on bovine babesiosis in the Sudan is meagre and fragmented; thus, this study is meant to cover Kassala State, Eastern Sudan as a part of a research programme destined to determine prevalence of bovine babesiosis in Sudan. According to Barnett (1977), there are two aspects of disease diagnosis; firstly to identify the agent in an animal or herd, secondly to survey a county or a zone for the presence and distribution of the disease agent.

Detection of parasites by microscopy is not easy and it is generally impossible to discriminate pathogenic from non-pathogenic species that may occur simultaneously within the same host. Although this technique is inexpensive and portable, the accuracy of diagnosis relies on the training and skill of diagnostician. Giemsa's stained blood smear examination remains the quickest and cheapest technique that has traditionally been used for diagnosis of piroplasmosis. It is, however, insufficiently sensitive to detect chronic carriers and not always sufficiently specific, as different species of piroplasms may morphologically resemble each other.

Table 1: The occurrence of tick species in different localities of Kassala State during January 2009 to December 2010.

Location	No. of animals	*H. anatolicum*	*H. impeltatum*	*H. dromedarii*	*H. rufipes*	*R.e. evertsi*	*A. lepidum*	*R. sanguineus*	*R. (B.) decoloratus*	Total
Kassala	34	46	73	0	0	23	6	2	0	**150**
Seteit	22	12	14	3	0	0	13	0	8	**50**
Atbara River	20	47	56	0	8	15	29	0	0	**155**
El-Gash	29	112	174	27	3	65	46	22	16	**465**
Total	**105**	**217**	**317**	**30**	**11**	**103**	**94**	**24**	**24**	**820**

Table 2: Mean infestation rate (MIR) of tick species according to the age groups of cattle in Kassala State during January 2009 to December 2010.

Tick species	<1Y (4)	1-<2Y (6)	2-<3Y (6)	3-<4Y (11)	≥4Y (78)	Total (105)
H. anatolicum	2	11	17	11	176	217
H .impeltatum	16	19	33	43	206	317
H. dromedarii	0	0	0	5	25	30
H. rufipes	0	2	3	0	6	11
R.e. evertsi	0	5	9	4	85	103
A. lepidum	2	8	10	19	55	94
R. sanguineus	0	0	0	10	14	24
R.(B.) decoloratus	0	0	0	9	15	24
Total	**20**	**45**	**72**	**101**	**582**	**820**
MIR	**5**	**7.5**	**12**	**9.2**	**7.5**	**7.8**

Number between parentheses is the number of animal surveyed in each age group.

Table 3: Mean (±SE) numbers of ticks per head of cattle in two years in Kassala State during January 2009 to December 2010.

Years	*H. anatolicum*	*H. impeltatum*	*R. e. evertsi*	*A. lepidum*	*R. sanguineus*	*H. dromedarii*	*H. rufipes*	*R(B.) decoloratus*
First	0.85±0.18b	2.01±0.39b	0.04±0.18b	0.72±0.34a	0.15 a	0.17±0.11a	0.06±0.05a	0.09±0.05a
Second	3.05±0.50a	3.82±0.59a	1.45±032a	1.03±0.39a	0.29±0.10a	0.36±0.12a	0.10±0.07a	0.34±0.20a

Means (±SE) followed by same letter in each column are not significantly different at 5% level based on Rayan´s Q test (REGWQ).

No of animals survey in the first year is 47 and in the second year 58

In cases that are difficult to diagnose by blood smear or when the detection of carrier animals with very low parasitaemia is required, the direct detection of *Babesia* spp by PCR based assays is used. According to Oliveira-Sequeira *et al* (2005), PCR is superior in detection of infections and proved to be far more sensitive. By this technique, three positive samples which were also positive by blood smear were detected from 25 samples. The low prevalence finding is in agreement with that of Awad *et al* (2011) who reported only seven out of 150 (4.67%) were positive samples in Kassala State using semi-nested PCR.

It is highly desirable to have knowledge on prevalent tick species in each county or zone (Barenett, 1977). In the present study, the results agree with those of Karrar *et al* (1963) who reported that ticks fauna in Kassala Province included *A. lepidum, R.* (*B.*) *decoloratus, H. excavatum, H. dromedarii, H. impeltatum, H. marginatum, H. rufipes, H. truncatum, R. e. evertsi, R. sanguineus* and *R. praetextatus. H. excavatum, H. marginatum, H. truncatum,* and *R. praetextatus* were not encountered in present study. The *R.* (*B.*) *annulatus* and *R.* (*B.*) *decoloratus* are affected by the climatic factors (Salih *et al.,* 2008). In the present study, few numbers of *R.* (*B.*) *decoloratus* were collected. According to Karrar *et al* (1963), low tick occurrence is either due to low population density of *R.* (*B.*) *decoloratus* in the area, or slight breeding activity at the time of collection. To correlate the findings of Karrar *et al* (1963) with these of the present study, it should be noted that the eastern region at that time was a large area including Kassala, Gedarif, and Red Sea Provinces, which is now divided into three States; one of them is Kassala State. However, low occurrence of *R.* (*B.*) *decoloratus* and low prevalence rate of *B. bigemina* may indicate that this tick species is not well established in the area.

From knowledge of tick species distribution, one can broadly predict the potential distribution of disease (Barnett, 1977). This statement is typified by a finding of the current study as *R.* (*B.*) *decoloratus* was identified in El-Gash and Seteit localities where bovine babesiosis cases were detected. The number of ticks in the environment is a crucial factor to the epizootiology of babesiosis. In areas where tick populations are large, the incidence is stable; the parasites persist in the host and rarely cause clinical signs (enzootic stability) (Losos, 1986). When this interplay of host-parasite-vector becomes disturbed, it engenders outbreaks of babesiosis (Losos, 1986).

According to this study, the prevalence of *B. bigemina* is very low; active search for the vector tick demonstrate even the absence of the tick species. However, few *R. (B.) decoloratus* were collected from cattle entering the state during early rainy season. The positive cases of babesiosis were detected in these cattle.

During this study, unsuitable climatic conditions such as low rainfall (approximately 1.9 mm) have led to reduction of tick population. This result agrees with Mohammed and Yagoub (1990) who reported an outbreak of babesiosis. Furthermore, they claimed that during 1985-1988, the Sudan was seriously affected by drought and desertification. Subsequent to these harsh conditions, the tick population was considerably reduced and it is possible that the enzootic stability of indigenous animals might also have been affected. In 1988, extremely high rainfall accompanied by widespread floods was recorded in the Sudan. Environmental conditions became suitable again for tick survival and development and their population had tremendously increased leading to outbreaks of TBDs in livestock in the eastern region of Sudan.

This study concludes that since the vectors *R.* (*B.*) *decolaratus* is present

in low occurrence in certain localities of Kassala State, the animal population is at risk whenever the vector ticks are established. However, a two successive years study is highly needed to monitor the disease situation in different seasons of the year.

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