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With 4 tables and 2 figures in the text

Some Epidemiological Aspects of Bovine Anaplasmosis in South Kordofan State, Sudan

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ملخص البحث

لقد أجريت هذه الدراسة لتحديد نسبه وجود الأضداد لطفيلى الأنابلازما الطرفية فى الأبقار بولاية جنوب كردفان. جمعت 575 مسحات دم من سلالات محلية هى بقارة و كنانة و أمبررو بالإضافة إلى أبقار مهجنة (محلية x فريزيان) مختلفة الجنس و الأعمار. أوضح الفحص المجهرى نسبة تطفل منخفضة تمثلت فى 8 % فقط من عينات الأبقار التى تم فحصها أثناء هذه الدراسة. إعتمادًا على نتائج الفحص المجهرى فإن أعلى نسبة لتواجد الطفيلى كانت فى محلية الدلنج 16 % و لكن بإستخدام التحليل الاحصائى لم تحدد علاقة وطيدة بين حدوث المرض و المحليات المختلفة. ايضا لم يوجد فرق معنوى فى نسبه حدوث المرض فى سلالات الأبقار المحلية. وعلى كِل فقد وجد فرقاً معنوياً بين الأعمار المختلفة. بإستخدم المقايسة غير المباشرة للأمتصاص المناعى المرتبط بالإنزيم فى عينات سيرم جمعت عشوائياً لتحديد نسبة تواجد الأضداد للأنابلازما الطرفية، فحصت 208 عينة سيرم جمعت من محليتى الدلنج و كادقلى و قد كان تواجد الأضداد الكلى حوالى 66 %. أعلى نسبة سجلت فى الدلنج 89 %. خلافاً لنتائج المسحات الدموية فإن مقايسة الأمتصاص المناعى المرتبط بالانزيم قد أوضحت وجود فرق معنوى بين حدوث المرض وكل من العمر و السلالة مع وجود النسبة الأعلى من الأضداد فى السلالة المحلية. أشارت الدراسة كذلك إلى ان الأضداد لطفيلى الأنابلازما الطرفية واسعة الإنتشار فى ولاية جنوب كردفان.

Summary

This study was carried out to determine the prevalence of *Anaplasma marginale* among cattle in South Kordofan State. A total of 575 blood smears was collected from different eco-types of indigenous (zebu) cattle including Baggara, Kenana, and Umbararow in addition to cross breed cattle (zebu x friesian) of both sex and different age groups. Microscopic examination revealed low parasitaemia detected in only 8 % of cattle examined during this study. Based on microscopic examination, the highest prevalence was reported in Dilling locality (16%). Statistically, there was no significant difference between prevalence rates in the different localities and among different types of indigenous cattle ($P \geq 0.05$). However, significant difference ($P \leq 0.05$) was found among different age groups. A total of 208 selected serum samples from Dilling and Kadugli localities was subjected to ELISA test to assess antibodies against *Anaplasma marginale.* The overall seroprevalence rate of *A. marginale* was found to be 66 %. The highest seroprevalence was reported in Dilling Locality (89%). Unlike the results of the blood smears, ELISA test showed significant correlation ($P \leq 0.5$) between the occurrence of *A. marginale* and both age and two eco-types of indigenous cattle with very high percentages of antibodies. This study reveals that antibodies to *A. marginale* infections are widely distributed in South Kordofan State.

Introduction

Anaplasmosis, also known as yellow bag or yellow fever (Zaugg, 1985), is a tick-borne intra-erythrocytic rickettsial disease that causes significant morbidity, mortality and life long persistent infection in ruminants (Palmer *et al,* 1999; Futse *et al*, 2008).

It is an important vector-borne rickettsial disease of ruminative livestock in the tropical and sub-tropical regions of the world (Palmer *et al*, 1986) including South America (Barros *et al*, 2005). Clinical anaplasmosis occurs most often in cattle but other ruminants including water buffalo (*Bubalus bubalis*), American bison (*Bison bison*), white-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus hemionus*), black-tailed deer (*Odocoileus hemionus columbianus*) and Rocky Mountain elk (*Cervus elaphus nelsoni*) can be infected

(Kuttler, 1984; Zaugg *et al*, 1996). Transmission of *A. marginale* occurs mechanically by biting flies or blood-contaminated fomites and biologically by ticks (Dikmans, 1950; Kocan *et al*, 2003). *A. maginale* is among tick-borne diseases (TBDs) which represent a threat to exotic cattle and their crosses in the Sudan causing economical impacts as substantial losses and costs of control (Mohammed, 2003). However, knowledge about anaplasmosis in the region is still fragmentary and far from complete. The long term studies under the auspices of FAO (1983) had thrown considerable lights on TBDs, namely theileriosis and babesiosis, with only little information on anaplasmosis. Even in the latter studies, no serological tests were used for detection of anaplasmosis, though the parasite was routinely seen in blood smears of experimental calves. In the Sudan, there is a diversity of climatic and ecological conditions that serves to accommodate the biological requirements of a variety of tick species and consequently, creates the chance of spreading a vector-borne disease (Elghali and Hassan, 2012). This fact attracted a great attention to these diseases, but still in the Sudan few published work on anaplasmosis could be cited (Suliman and El malik, 2003).

The objective of this study was to estimate the prevalence and assess the magnitude of cattle anplasmosis in South Kordofan State and to provide data necessary for future intervention.

Materials and methods

Study area and sample collection

Cross-sectional survey was conducted during the period of September 2010 to May 2011 in South Kordofan State localities, including Kadugli the capital of the state, Dilling (130 Km north Kadogli), Talodi (80 Km south Kadugli), Alabasia located in east Nuba Mountains and Abojubaiha locality (south east the state) (Fig.1).

Five hundred and seventy-five blood smears were taken from randomly selected animals of different local ecotypes from nomadic and resident herds, including Bagaara, Umbararow, Kenana cattle and cross breed (zebu×Friesian) of both sex and different age groups. Two hundred and eight sera were also collected from Kadugli and Dilling localities. All samples were labelled indicating location, breed, sex, age, date of collection and animal number. Blood for serum was collected in plain tubes, while samples for smears collected in tubes containing EDTA, then all samples were transferred to Kadugli Veterinary Research Laboratory. Thin blood smears were made on clean grease-free slides, air dried and fixed in absolute methyl alcohol for 2 minutes and kept in slides box. The sera were kept overnight in the lab then separated and preserved in -20 °C till used. All tests were read at the Institute of Veterinary Research (IVR), Soba, Sudan.

Laboratory examination

Blood smears were stained with Giemsa′s stain and examined with light microscope under oil-immersion lens (100×). Microscopic fields were extensively searched and the presence of one or more infected red blood cell was considered positive.

The two hundred and eight serum samples were subjected to ELISA test. The ILRI method (1994-1998) for ELISA was followed.

A recombinant immunodominant antigen for detection *A. marginale* antibody provided from ILRI lab. Kenya. The +ve and -ve controls are part of kit (Cat. No 10.2960-02 Svanova/Uppsala Sweden) used. The materials and buffers which were involved in preparing ELISA are:

1-Phosphate citrate tablets: one tablet dissolved in 100ml deionized water to obtain 0.05 M phosphate citrate buffer.

2- ABTS 2, 2-Azino-bis (3-Ethylbenzthiazoline 6-sulfonic Acid) tablets: one tablet dissolved in 100ml of 0.05M phosphate citrate buffer, pH 5.0.

3- H_2O_2 (hydrogen peroxide).
4- PBS (phosphate buffer saline) tablets: one tablet dissolved in one litre of distilled water, pH 7.2.
5- Skim milk powder: one gram dissolved in 100ml of PBS to make dilution and blocking buffers by adding tween 20.
6- carbonate/ bicarbonate buffer substrate capsules: one capsule dissolved in 100 D.D H_2O to give 0.05M solution, pH 9.6, as coating buffer.
7- Tween 20 one ml dissolved in one litre of PBS to make washing buffer.
8- Conjugate: sheep anti-bovine IgG (HFF) sheep polyclonal antibody (www.Thermo Scientific.com/pierce).
9- Microtitre 96-well/plates.
10-Double distilled water (D.D. H_2O)
11- Sera in 1.5ml eppendorf tubes.

Indirect ELISA protocol

The test was performed by diluting the *Anaplasma marginale* antigen at the rate 1:5000 in coating buffer of 0.5 M carbonate/ bicarbonate pH 9.6 and 150 µl were delivered onto 96-well flat-bottomed polystyrene plate (Nunc-immun Maxisorb, Kopenhagn, Denmark), sealed with aluminum foil and placed in the refrigerator at 4 °C overnight. The coating buffer was removed. The non-specific binding sites were blocked with a solution of 250 µl blocking buffer PBSTM (PBS pH7.2 +0.1% Tween 20 + 1% Skimmed milk) and incubated at 37 ºC for 20 minutes before the plates were washed 3 times using 250 µl of washing buffer (PBS PH 7.2+ 0.1% Tween 20). Test sera were then thawed at 37 ºC for 5-10 minutes and diluted in duplicate by adding 5 µl of each test sera to 195 µl of 1% skimmed milk in PBST to obtain dilution rate 1: 200 for all wells containing serum in the diluting plates; then the plate was shaked gently to mix the content. The control sera were treated with the same manner as the test sera. Then 150 µl of diluted test sera were transferred to the blocked ELISA plates and incubated for 40 minutes at 37 ºC. The plates were then washed 5 times and all wells were soaked with washing buffer and incubated for 10 minutes. Then 5µl of conjugate (sheep anti-bovine IgG, sheep polyclonal antibody) were added to 5ml of 1% skimmed milk in PBST to make a dilution of 1/1000 then 1ml of diluted conjugate was taken to make 1/10000 dilution. 150 µl of the diluted conjugate was thereafter transferred to each blocked well, incubated for 30 minutes at 37 ºC. Then the plate was washed 5 times, filled with washing buffer for soaking and incubated for 10 minutes. Immediately prior to use, 80 ml amount of the buffer substrate was warmed at 37 ºC for 10 minutes and 400 µl of ABTS and 20 µl of 30% hydrogen peroxide (H_2O_2) were added to; then 150 µl was transferred to each well and incubated for 30 minutes. Mean optical density (OD) was read at 405 nm filter on an ELISA plate reader. The cut off point was calculated as average OD of negative control sera increased by two standard deviations (mean negative + 2SD).

Statistical analysis

Chi-square was used to test the null hypothesis that there are no correlations between the seropositivity and parasite detection in blood smears among different localities, age groups and breeds; *P* values < 0.05 were considered significant (Stata Corporation 2000).

Results

Microscopic examination of blood smears

The microscopic examinations revealed that out of 575 samples, 45 (8%) were positive for *Anaplasma marginale* (Fig. 2; Table 1). Prevalence of *Anaplasma marginale* in Kadugli, Dilling, Abojubaiha, and Talodi amounted to 10%, 16%, 5%, and 5.5%, respectively (Table

1). The prevalence rates among the ecotypes were found to be 7.3 % in Bagara cattle, 11.4 % in Umbararow cattle, and 11% in Kenana cattle, while the parasite was not detected in cross bred animals (Table 2). Among the age groups, the prevalence rates of the disease were 6.7 % (4/59), 7.2 % (13/180), and 8.3 % (28/336) in animals of < 2years, 2-5 years, and more than >5 years of age, respectively (Table 2).

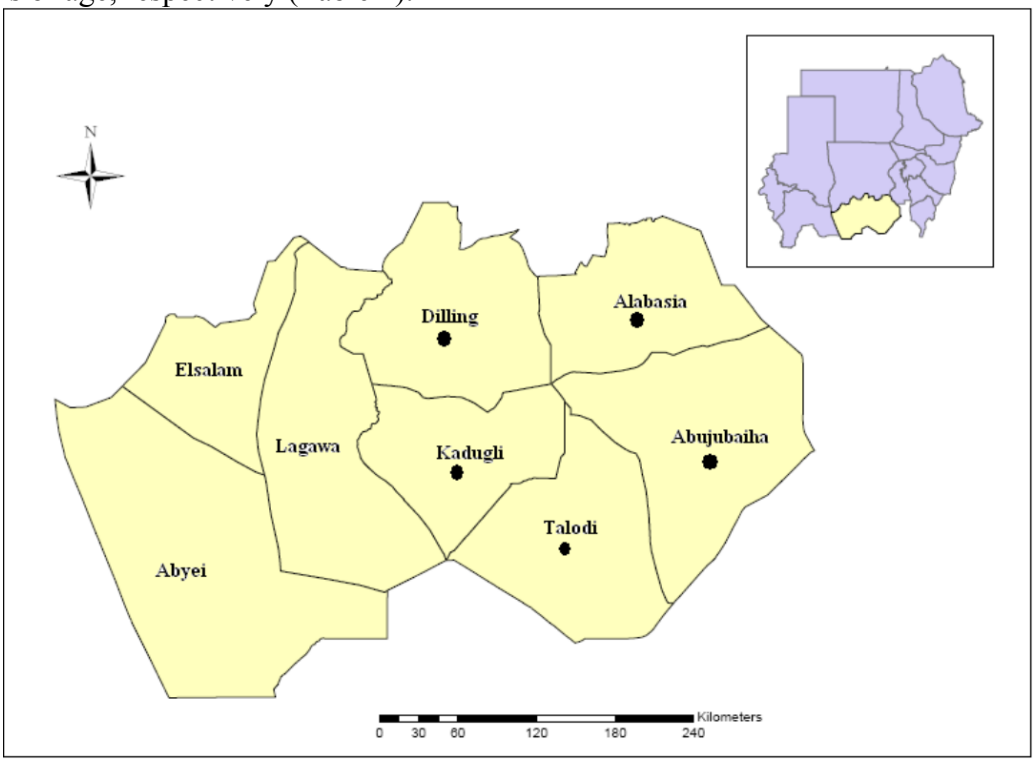

Fig. 1: Map of South Kordofan State showing sites of samples collection (•)

Seroprevalence

The overall seroprevalence of *Anaplasma marginal* in Kadugli and Dilling as shown by ELISA test was 66%. With respect to location, the seroprevalence rates for *A. marginale* were 89% in Dilling locality and 59% in Kadugli locality (Table 1). The prevalence rates among the ecotypes of cattle in the two localities were 71 %, 66.6 %, 44 % and 50 %, for Baggara, Kenana, Umbararow ecotypes and cross bred cattle, respectively (Table 3). In regard to the age groups, the seroprevalence rates for *A. marginale* were 75 % in cattle of more than 5-year-old, 63.3 % in cattle between 2 to 5-year-old and 50 % in cattle less than 2-year-old (Table 3). As regards the breeds, the prevalence of antibodies to *A. marginale* in indigenous breeds was 66%, while in cross bred animals was 50% (Table 4).

Table 1: *Anaplasma marginale* detected in cattle surveyed in some localities of South Kordofan State as shown by blood smear and ELIZA examinations during September 2010-May 2011.

	Blood Smears		**ELISA**	
Location	**No. examined**	**No. Positive** (%)	**No. examined**	**No. positive** (%)
Kadugli	307	31 (10)	160	94(59)
Dilling	50	8 (16)	48	43(89)
Abojubaiha	100	5 (5)	-	-
Alabasia	100	0 (0)	-	-
Talodi	18	1 (5.5)	-	-
Total	**575**	**45 (8)**	**208**	**137(10)**

- = not done.

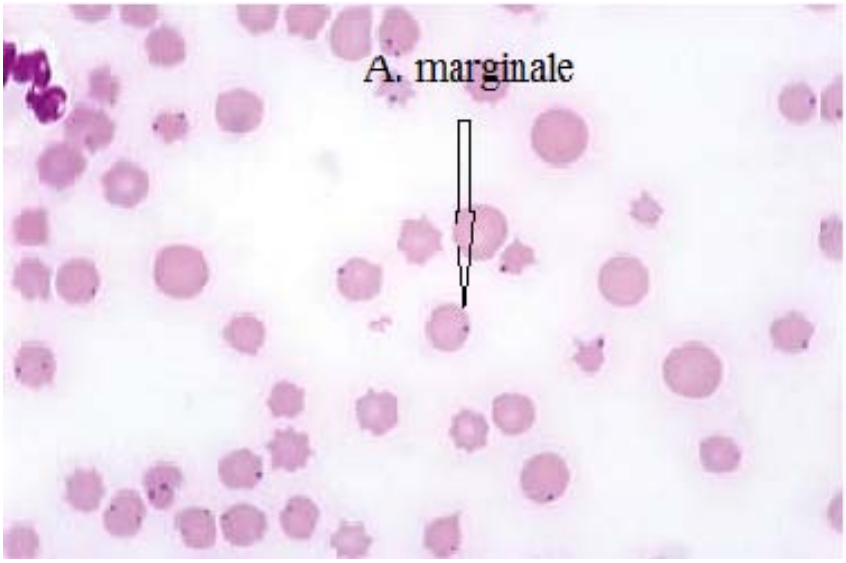

Fig. 2: Bovine erythrocytes infected with *Anaplasma marginale* (arrow).

Table 2: Prevalence rate of *Anaplasma marginale* as detected by blood smears in different ecotypes and age groups of cattle in South Kordofan State during September 2010-May 2011

	Ecotype			Age groups	
	No. examined	**No. positive (%)**		**No. examined**	**No. positive (%)**
Baggara	490	36 (7.3)	< 2 years	59	4 (6.7)
Umbararow	61	7 (11.4)	2-5 years	180	13 (7.2)
Kenana	18	2 (11)	< 5 years	336	28 (8.3)
Cross breed	6	0 (0)			
Total	**575**	**45 (8)**		**575**	**45 (8)**

Table 3: Prevalence rate of *Anaplasma marginale* as detected by ELISA test in different ecotypes of cattle and age groups of cattle in two localities of South Kordofan State during the study period September 2010-May 2011

	Ecotype			Age groups	
	No. examined	**No. positive (%)**		**No. examined**	**No. positive (%)**
Baggara	150	107 (17)	< 2 years	34	17 (50)
Umbararow	34	15 (44)	2-5 years	90	57 (63.3)
Kenana	18	12 (66.6)	< 5 years	84	63 (75)
Cross breed	6	3 (50)			
Total	**208**	**137 (66)**		**208**	**137 (66)**

Table 4: Prevalence of *Anaplasma marginale* in local ecotypes and cross breed cattle as detected by ELISA test in Dilling and Kadugli localities of South Kordofan State during September 2010-May 2011

Breeds	**No. examined**	**No. positive (%)**
Local ecotype	202	134 (66)
Cross breed	6	3 (50)
Total	208	137 (66)

Discussion

The objective of this study was to estimate the prevalence and to assess the magnitude of cattle anaplasmosis in South Kordofan State using microscopic and serological tools. Few serological investigations have been conducted to provide essential information on anaplasmosis in the Sudan. The long term studies done under the auspices of FAO (1983) covered only a triangle between the Blue Nile and White Nile in Central Sudan. Later Suliman and El malik (2003) studied the disease in Khartoum State using parasitological and IFA test and they have reported that Khartoum is an endemic area for *A. marginale.* Salih *et al* (2008) studied TBDs in northern Sudan using ELISA technique; they found that *A. marginale* antibodies were higher (38.9%) than those of *Theileria annulata*, *Theileria mutans* and *Babesia bigemina*. They have concluded that anaplasmosis is the most prevalent tick-borne disease, but the lack of research on the disease led to the unclear picture. Cattle reared under extensive system of nomadism, become infested with different tick vectors and exposed to other biting insects during their long migratory journeys between the dry areas in the north of the state and the wet areas at south of the state. Consequently, cattle become naturally infected and they distribute the diseases in different areas of the state.

Blood smears show that the highest prevalence of *A. marginale* is in Dilling followed by Kadugli, Talodi, and Abojubaiha localities, while the parasite was not detected in samples collected from Alabasia locality. This is probably due to the fact that animals in the latter locality are resident in rain-irrigated schemes; so they are well nourished and thus have good tolerance. The prevalence rate of *A. marginale* in the state is 8 %. As regards to the age groups, the prevalence rates were significantly different being higher in elder animals indicating a carrier status.

The findings of the current study reveal a high prevalence of antibodies against *A. marginale* in the two representative localities, which may reflex that cattle anaplasmosis is widely distributed in the state. Compared with the results of Suliman and El malik (2003) who reported a prevalence of 37% in Khartoum State using IFA test, and those of Salih *et al* (2008) who reported 38.9% in northern Sudan, the current study reports a higher prevalence (66%). The climatic conditions and vegetation cover of South Kordofan State afford better chances for vector build-up. High ticks infestation, presence of biting insects in addition to crowds of hosts reared together may be responsible for the high prevalence rate compared with the aforementioned studies.

The prevalence of the disease was high in both indigenous and cross breeds but significantly higher in indigenous animals. This finding is in line with de Echaide *et al* (1998) who encountered a prevalence of infection of 93%in cattle in endemic areas using competitive ELISA. The high prevalence in indigenous animals might be due to the long lasting antibody response in the more resistant indigenous breed, to the good management provided for cross breed compared with indigenous ones or to small number of cross breed animals tested. Despite the high prevalence of *A. marginale* antibodies, fortunately no clinical signs are observed. This is probably due to the mass usage of oxytetracycline in the area. Oxytetracycline is used as treatment of sick animals and as a prevention method during vector breeding season and in temporary and prolonged protection during outbreaks of anaplasmosis (Richey and Palmer, 1992).

This study concludes that South Kordofan State is an endemic area for *Anaplasma marginale* because of high prevalence of its antibodies (66%). The need for a further investigation is most recommended to detect carrier animals with antibodies titres not detectable by serological methods. Epidemiological studies on *A. marginale* using more advanced

diagnostic techniques are needed in order to define more precisely the complexity of anaplasmosis in the state. Determination of the disease magnitude and its contribution to retardation of livestock industry and development in Sudan is vitally importance.

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