SEROSURVEILLANCE OF EHRLICHIA RUMINANTIUM ANTIBODIES OF SHEEP USING MAP1-B ELISA IN SENNAR STATE, SUDAN

Mohammed S. Mohammed[*,1], Shawgi M. Hassan[2] and Magdi B. Abdel Rahman[3]
[1].Faculty of Veterinary Medicine, University of Gezira, P.O.Box 155 Tambool, Sudan.
[2].Faculty of Veterinary Medicine, University of Khartoum, private P.O. Box 3288 Khartoum, Sudan.
[3].Central Veterinary Research Laboratories, P. O. Box 8067 Khartoum, Sudan.

المستخلص

تم جمع عدد 300 عينة من مصل الضأن من ولاية سنار في كل من مدينة سنار، سنجة، الدندر، أم بنين وأبونعامة بواقع مزرعتين من كل منطقة ومرة واحدة كل أربعة أشهر لفترة سنة وذلك باعتبار مر ة واحدة في كل فصل من فصول السنة ابتداء من فصل الخريف (سبتمبر 2002) ثم الشتاء (يناير 2003) ثم الصيف (مايو 2003). تم فحص العينات باستخدام اختبار (ELISA) لتحديد نسبة انتشار الأجسام المضادة لجرثومة ارليخيا رومينشيوم. وقد دلت نتيجة الفحص أن 230 عينة تحمل الأجسام المضادة لجرثومة ارليخيا رومينشيوم بنسبة انتشار كلية بلغت (76.6%). وقد بلغت نسبة الانتشار كأعلى نسبة (85.1%) في أم بنين وأدنى نسبة (63.3%) سجلت في سنار. وقد لوحظ أن الأجسام المضادة للجرثومة متواجدة في مصل الضأن على مدار السنة . وأن معدل نسبة انتشار الإصابة مرتفع خلال موسمي الخريف (80%) والشتاء (82%) مع وجود انخفاض طفيف خلال موسم الصيف (68%) ولا يوجد اختلاف إحصائي معنوي ($P<0.05$).

Key words: Ehrlichia ruminantium, Amblyomma, serosurveillance, Sudan.
Corresponding Author: Mohammed S. Mohammed, Department of Parasitology, Faculty of Veterinary Medicine, University of Gezira, private P.O. Box 3288 Tambool, Sudan.
E. mail: mohammedsayed09@ Hotmail .com, Tel: +249 (0) 910979940.

Abstract

A total of 300 serum samples were collected from adults sheep in Sennar State at five localities namely Sennar, Singa, Dinder, Um Banein and Abu Naama from two farms in each location three times a year, during September 2002 (autumn), January 2003 (winter) and May 2003 (summer). Indirect Enzyme-Linked Immunosorbent Assay (ELISA) was used to determine the sero-prevalence of Ehrlichia ruminantium. The test was performed with recombinant major antigenic protein 1 (MAP1-B). Out of 300 sheep sera tested, 230 (76.6%) were found positive for E. ruminantium antibodies. According to locations, sero- prevalence ranged between 85.1% at Um Banein and 63.3% in Sennar. The E. ruminantium antibodies persisted throughout the year in each location, and seasonality prevalence of the disease showed an increase in sero-prevalence during autumn (80 %) and winter (82 %) while a slight reduction was observed in summer (68 %) but statistically not significant ($P < 0.05$).

Introduction

Heartwater is a tick-borne disease of domestic and wild ruminants caused by the obligate intracellular bacterium Ehrlichia ruminantium which has significant economic and developmental impact on livestock health and production in area of Sub-Saharan Africa where vector ticks of the genus Amblyomma are present (Uilenberg, 1983; Walker and Olwage, 1987). The disease is considered to be one of the most important tick-borne disease of sheep and goats in the Sudan, which was reported in the early sixties in eastern parts and later in central and western parts of the country (Osman, 1997).

Studies on epidemiology of heartwater and implementation of disease control have been hampered by the lack of reliable serodiagnostic tests (Camus et al., 1996; Semu et al., 2001) and most of these serological tests are hampered by their low specificities (van Velit et al., 1995) because of cross reactivity with other Ehrlichia species. Later, however, the production of large amounts of E. ruminantium made it possible to the use of these antigens in ELISA tests (Camus et al., 1996). The major antigen protein 1(MAP1-B) indirect ELISA based on the recombinant MAP1-B fragment of the immunorecombinant MAP1-B protein of E. ruminantium is considered to be the most sensitive and specific assay for the serodiagnosis of heartwater (Peter et al., 2001).

However, knowledge on the epidemiology of heartwater in domestic ruminants (sheep, goats, cattle and camels) in the Sudan is still very scanty. Hence, only about 5% of the infected animals are reported (Abdel Rahman et al., 2003b). This study was carried
out to investigate the prevalence of E. ruminantium antibodies in sheep during different seasons in Sennar State, Sudan.

Materials and Methods

Sera collection:

Serum samples were collected from sheep at five localities in Sennar State. These included the towns of Sennar (13º 33` N: 33º 37` E), Singa (13º 09` N: 33º 57` E), Dinder (13º 44` N: 34º 12` E), Um Banein (13º 04` N: 33º 57` E) and Abu Naama (12º 44` N: 34º 08` E) (Fig. 1). Ten adult sheep of different types from two farms in each location were randomly sampled three times a year; September 2002 (autumn), January 2003 (winter) and May 2003 (summer). Whole blood for serum was withdrawn from the jugular veins using plain vacutainers and needles. The collected blood was allowed to clot at room temperature for 2-3 hours. Then the tubes were kept overnight in refrigerator at 4ºC. Serum was separated and transferred into clean serum containers, labelled indicating farm, location, date of collection then stored at -20ºC till use.

ELISA kit:

Ehrlichia ruminantium ELISA kit with a recombinant major antigen protein 1(MAP1-B) were kindly provided by ICTTD2 stand for: "Integrated Control of Tick¸¸¸¸¸¸¸¸s and Tick-borne Diseases".

ELISA Test procedure:

The test was performed according to van Vliet et al. (1995) protocol.
MAP1-B was diluted in coating buffer [15 mM Na2Co3, 35 mMNaHCo3 (pH 9.6)] at rate of 1: 1000.
The ELISA plates (Nunc, Maxisorp) were coated with the MAP1-B and each well-received 100 µl. The plates were incubated for one hour at 37ºC and then kept in refrigerator at 4º C. The plates were then incubated for 15 minutes at 37ºC with 100 µl /well blocking buffer (Phosphate buffered

saline (PBS) pH 7.2, supplemented with 0.1% Tween 20 and 1% skimmed milk (PBSTM)) (Oxoid; Basingstoke, Hampshire, England). Thereafter, the plates were washed three times with PBS supplemented with 0.1% Tween 20 (PBST) and subsequently incubated for 1 hour at 37ºC, with sera in a volume of 100 µl / well (diluted 1: 200 in PBSTM). All samples were prepared in duplicate on the same plate. Each plate contained one positive, one negative reference serum samples and a third well was left as blank. Plates were washed three times with PBST and incubated at 37ºC for 1 hour after adding 100 µl /well rabbit anti- sheep antibodies conjugated with horseradish peroxidase [Heavy and light Chain (Nordic, Tilburg, The Netherlands)] diluted in PBSTM (1: 1000). They were washed three times with PBST. Thereafter, substrate solution composed of citrate phosphate buffer and ABTS [2,2'- azinobis (3-ethylbenzthiazoline sulfonic acid)] was prepared freshly and added in a volume of 100 µl/ well. Colour was allowed to develop for 30 minutes in dark, and absorbance was measured at 450 nm with an ELISA reader (Labsystem, Multiskan, RC).

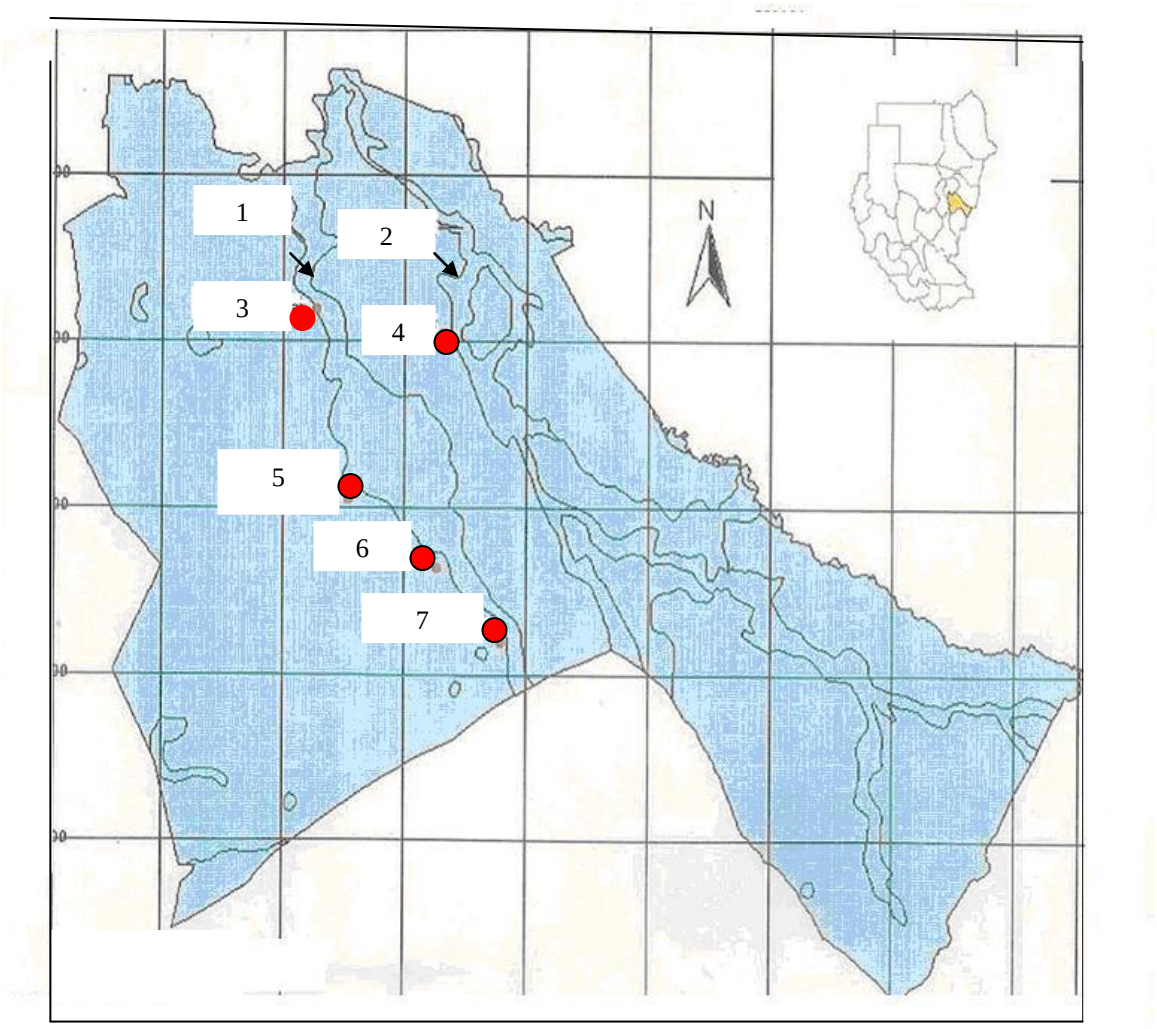
Determination of cut off value:

The negative sera were collected from Barber town (17º 0` N: 34º 0` E) Northern Sudan where it is known to be free from Amblyomma species (Jongejan et al., 1987). The collected sera were tested by indirect MAP1-B ELISA; all the samples proved to be negative for E. ruminantium antibodies. The cut off value was calculated as an average of optical density of negative control ± 2 standard deviation (van Vliet et al., 1995).

Statistical analysis:

The data were analyzed using SPSS for Windows Release 14.0 and MLn multi-level analysis (MLn, Institute of Education, London) software. Associations between the two farms in each locality, the five locations and the three seasons were assessed using the Chi-square test.

FIG. 1 Sennar State, Sudan and localities at which serum samples were collected



(1)Blue Nile- (2) Dinder River- (3) Sennar- (4)Dinder- (5)Singa- (6)Um Banein- (7)Abu Naama.

Results

Out of 300 sheep sera collected from 5 locations (Sennar, Singa, Dinder, Abu Naama and Um Banein) and tested by indirect ELISA MAP1-B, 230 (76.6 %) were found to be positive with E. ruminantium antibodies (Table 1). The prevalence of E. ruminantium antibodies according to location and farm is shown in Table 1. E. ruminantium antibodies were more prevalent at Um Banein (85.1 %) and Abu Naama (81.2 %) than at Sennar town (63.3 %). The prevalence of the disease did not significantly ($P < 0.05$) vary among farms in each location. The antibodies persisted throughout the year at each location, and seasonality prevalence showed no significant ($P < 0.05$) difference between autumn (September), winter (January) and summer (May) but a slight reduction in seroprevalence was observed in summer (Table 2).

Table 1: Seroprevalence of Ehrlichia ruminantium antibodies of sheep tested by MAP1-B ELISA in different locations in Sennar State in the year 2002-2003.

Location	Farm	No.+ve (%) in each farm	No.+ve (%) in each locality
Um Banein	1	26 (86.7)	51 (85.1)
	2	25 (83.3)	
Abu Naama	1	24 (80)	49 (81.2)
	2	25 (83.3)	
Singa	1	24 (80)	47 (78.3)
	2	23 (76.7)	
Dinder	1	23(76.7)	45 (75)
	2	22 (73.3)	
Sennar town	1	18 (60)	38 (63.3)
	2	20 (66.7)	
Total			**230 (76.6)**

Number of serum samples = 30 in each farm.

Table 2: Seroprevalence of Ehrlichia ruminantium antibodies of sheep tested by MAP1-B ELISA in different seasons and locations in Sennar State in the year 2002-2003.

Location	Winter		Autumn		Summer	
	No. tested	No.+ve(%)	No. tested	No.+ve(%)	No. tested	No.+ve(%)
Um Banein	20	18 (90)	20	17 (85)	20	16 (80)
Abu Naama	20	19 (95)	20	16 (80)	20	14 (70)
Singa	20	17 (85)	20	17 (85)	20	13 (65)
Dinder	20	15 (75)	20	16 (80)	20	14 (70)
Sennar town	20	13 (65)	20	14 (70)	20	11 (55)
Total	**100**	**82 (82)**	**100**	**80 (80)**	**100**	**68 (68)**

Discussion

Antibodies against E. ruminantium detected by means of MAP1-B indirect ELISA in the present study indicated a high prevalence of the disease with an overall prevalence of 76.6 %. The result corresponds closely with similar studies carried out in Kassala and Gadarif (Eastern Sudan) by Abdel Rahman et al. (2003a) and in Zimbabwe in sheep and goats (Mahan et al., 1998).

The disease prevalence was lower in Sennar town (63.3 %) and Dinder (75 %) than in Singa (78.3 %), Abu Naama (82.2 %) and Um Banein (85.1 %). These differences were associated with the abundance of the tick vector A. lepidum (Mohammed and Hassan, 2007). This finding is in agreement with Abdel Rahman et al. (2003a) in Kassala and Gadarif. In Cameroon, Awa (1997) confirmed the presence of heartwater using cELISA in an area where A. vaerigatum was found to be the most predominant tick species. In Ghana, Bell- Sakyi et al. (1996) reported that heartwater among sheep, goats and cattle was widespread in areas where the vector was prevalent. De Vries et al. (1993) found a correlation between E. ruminantium antibodies and distribution of Amblyomma spp. In Amblyomma species infested areas, 52% and 26% were positive by

cELISA and IFA, respectively, while in Amblyomma- free area (11% and 10%) of sera positive by cELISA and IFA, respectively were reported (De Vries et al., 1993).

Seasonally, there was a high level of antibody prevalence during autumn and winter with a reduction in summer. This finding was also associated with the abundance of the vector during these periods. The peak of heartwater corresponded with the rainy season, as there is an increase in the adult tick population (Mohammed and Hassan, 2007). The animals are also in poor condition due to the preceding dry season (Camus et al., 1996). However, the disease persists until the end of the rainy season in the Sudan (Karrar, 1968) and may even prevail throughout the year in Zimbabwe (Akafekwa, 1976) as nymphs and adults can persist in the first half of dry season. In parts of Senegal, the challenge is even higher during the first half of the dry season, due to the high nymphal population of A. variegatum in this period (Camus et al., 1996).

The advancement in understanding the epidemiology of heartwater in the Sudan in domestic ruminants has undoubtedly been hampered by the poor diagnostic tests available to detect the presence of E. ruminantium in both animals and the tick vectors. However, serological evaluation using recombinant MAP1-B antigen revealed wide distribution of E. ruminantium antibodies in Central Sudan. The test is handy and can be used to carry out countrywide serosurveillance of heartwater antibodies in the Sudan to properly delineate geographic distribution of the disease.

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References

Abdel Rahman, M.B., Abdel Rahim, A. I, El Hussein, A.M., Hamid, M. E., El Eragi, A. M., Zakia, A., Bakhiet, M. A, Hind, E, A. El Gadal, A and Jongejan, F, 2003a. Sero-surveillance of cowdriosis (heartwater) in sheep in Eastern Sudan using MAP1-B ELISA. Sud. J. Vet. Sci. Anim. Husb., 42: 167-175.

Abdel Rahman, M. B., El Hussein, A.M, Abdel Rahim, A. I, Hamid, M. E, El Gadal, A. A and Majid, A. A, 2003b. Heartwater in the Sudan: Current status. Sud. J. Vet. Sci. Anim. Husb., 42: 159-166.

Akafekwa, G. I., 1976. Control of tick- borne diseases of cattle in Zambia with special emphasis on East Coast fever. Bull. Off. Int. Epiz., 86: 105-116.

Awa, D. N., 1997. Serological survey of heartwater relative to distribution of the vector Amblyomma variegatum and other tick species in north Cameroon. Vet. Parasitol., 68: 165-173.

Bell-Sakyi, L., Koney, E. B. M, Dogbey, O., and Sumption, K. J., 1996. Heartwater in Ghana: implications for control of ticks. Trop. Anim. Health Prod. 28:59S-64S.

Camus, E., Barré, N, Martinez, D and Uilenberg, G, 1996. Heartwater (cowdriosis), a review., Office International des Epizooties. 2nd edition. 12 rue de prony 75107 Paris. France. pp. 177.

De Vries, N., Mahan, S. M, Ushewokunze-Obatolu, U, Norval, R. A. I. and Jongejan, F, 1993. Correlation between antibodies to Cowdria ruminantium (Rickettsiales) in cattle and the distribution of Amblyomma vector ticks in Zimbabwe. Exp. Appl. Acarol., 17: 799-810.

Jongejan, F., Zivkovic, D, Pegram, R. G, Tatchell, R. J., Fison, T, Latif, A. A and Paine, G, 1987. Ticks (Acari: Ixodidae) of the Blue and White Nile ecosystems in the Sudan with particular reference to the Rhipicephalus sanguineus group. Exp. Appl. Acarol., 3: 331-346.

Karrar, G., 1968. Epizootiological studies on heartwater disease in the Sudan. Sud. J. Vet. Sci. Anim. Husb., 9: 328-343.

Mahan, S.M., Semu, S.M, Peter, T.F and Jongejan, F , 1998. Evaluation of the MAP-1B ELISA for cowdriosis with field sera from

livestock in Zimbabwe. Annals of the New York Academy of Sciences., 849: 259-261.

Mohammed, M.S. and Hassan, S. M, 2007. Distribution and population dynamics of ticks (Acari: Ixodidae) infesting sheep in Sennar State, Sudan. Onderstepoort Journal of Veterianry Research, 74:301–306

Osman, A. M., 1997. Major tick-borne diseases of sheep and goats in the Sudan. Parasitologia., 39: 143-4.

Peter, T. F., O'Callaghan, C. J, Medley, G.F, Perry, B. D, Semu, S.M and Mahan, S.M, 2001. Population-based evaluation of the Ehrlichia ruminantium MAP1-B indirect ELISA. Exp. Appl. Acarol., 25: 881-897.

Semu, S. M. T. F. Peter, Mukwedeya, D, Barbet, A. F, Jongejan, F. and Mahan, S. M, 2001. Antibody response to MAP1-B and other Cowdria ruminantium antigens are down regulate in cattle challenged with tick transmitted heartwater. Clin. Diagn. Lab. Immunol., 8: 338-396.

Uilenberg, G., 1983. Heartwater (Cowdria ruminantium infection): Current status. Adv. Vet. Sci. Comp. Med., 27: 427-480.

van Vliet, A. H., van der Zeijst, B. A. M, Camus, E, Mahan, S. M and Jongejan, F. 1995. Use of a specific immunogenic region on the Cowdria ruminantium MAP1 protein in a serological assay. J. Clin. Microbiol., 33: 2405-2410.

Walker, J.B. and Olwage, A, 1987. The tick vector of C. ruminantium (Ixodoidea, Ixodidae), genus Amblyomma and their distribution. Onderstepoort J. Vet. Res., 54: 353-379.