

## Prevalence of Tropical Theileriosis in Nyala Dairy Farms, South Darfur State, Sudan

Bothina B.M. Gaafar<sup>1\*</sup>, Hassan<sup>2</sup>, S.M., Limia M. El Hag<sup>3</sup> and Salih<sup>3</sup>, D.A.

<sup>1</sup>Ministry of Animal Resources, South Darfur State, Nyala, Sudan

<sup>2</sup>Faculty of Veterinary Medicine, University of Khartoum, Sudan

<sup>3</sup>Veterinary Research Institute, P.O. Box 8067, Khartoum, Sudan

\*Corresponding author: bothinabakur@yahoo.com

### ملخص البحث

أجريت هذه الدراسة في مزارع للألبان بمدينة نيالا. جُمعت مسحات دم و عينات مصل وقراد من أبقار، مختلفة الفئات العمرية والسلالات والجنس. تمت عملية جمع العينات شهرياً و بطريقة عشوائية و إستمرت لمدة سنة واحدة إعتباراً من يونيو 2006 إلى مايو 2007. أظهرت النتائج أن معدل إنتشار التيلرية هو 3.6% (1200/43) نتيجة لفحص مسحات الدم المصبوغة بالجمسا و 31% (100/31) نتيجة لفحص عينات المصل بواسطة إختبار الأضداد المستشعة (المتألقة) غير المباشر. كما وجد أن هناك ثلاثة عشر نوعاً من القراد، تنتمي إلى أربعة أجناس تنطفل على أبقار المنطقة. نوقش تأثير القراد والحمى المدارية على الأبقار في ولاية جنوب دارفور وأوصى بعدم إدخال الماشية إلى دارفور من وسط السودان دون تطبيق تدابير وقائية صارمة للسيطرة على القراد. يُنصح أيضاً بتطبيق البرنامج المعد للسيطرة على القراد في مزارع الألبان التي تؤكد وجود الناقل الرئيسي (الهيالوما الأناضولية) للمرض فيها. كما يجب منع إنتشار هذا النوع من القراد إلى مزارع ومدن أخرى ومن عبور الحدود الدولية.

### Summary

This study was conducted in dairy farms in Nyala town, South Darfur. Cattle of different age group, breed and sex were randomly sampled for ticks, blood smears and serum samples. Sampling was carried out on a monthly basis for one year starting from June 2006 to May 2007. The prevalence rate of *Theileria* spp. was 3.6% (43/1200) based on Giemsa's stained blood smears. Using indirect fluorescent antibody test, out of 100 serum samples, 31 (31%) were positive for *T. annulata* antibodies. Four genera and thirteen species of ticks were found infesting cattle in the area. These were *Amblyomma variegatum*, *A. lepidum*, *Rhipicephalus (Boophilus) annulatus*, *R.(B.) decoloratus*, *Hyalomma anatolicum*, *H. anatolicum excavatum*, *H. dromedarii*, *H. impeltatum*, *H. impressum*, *H. marginatum rufipes*, *H. truncatum*, *Rhipicephalus evertsi evertsi* and *R. s. sanguineus*. The impact of ticks and tropical theileriosis on cattle in South Darfur State is discussed. It is recommended that introduction of cattle from Central Sudan should be accompanied by strict tick control measures. Planned tick control programme on farms where *H. anatolicum* is present is highly recommended. Spread of this tick species to other farms, towns and probably across the international borders must be prevented.

### Introduction

South Darfur State, in Western Sudan, is one of the richest states in animal resources which is estimated at 11 million consisting of 3.9 million cattle, 3.6 million sheep, 2.9 million goats, 53500 donkeys, 30700 horses and 8700 camels (Anon, 2004). Tick-borne diseases (TBDs) constitute a major constraint of livestock production and have a considerable economic impact (Jongejan and Uilenberg, 2004). TBDs are widespread, causing substantial economic losses and pose a

constant threat to the development of animal wealth (FAO, 1983). Tropical theileriosis is caused by *Theileria annulata* and transmitted by ixodid ticks of the genus *Hyalomma* (Uilenberg, 1981). The disease occurs in a wide zone of Africa, Southern Europe and a large part of Asia (Dolan, 1989). Tropical theileriosis is the most important TBD in the Sudan. Serologically, Salih *et al* (2007) reported that 14% of cattle in South Darfur were positive for *T. annulata* antibodies. However, no clinical cases

of tropical theileriosis have so far been reported in this state. The objective of this study was to elucidate seroprevalence of *T. annulata* and prevalence of its vector *Hyalomma anatolicum* in Nyala town, South Darfur State.

#### Materials and Methods

A total of 1200 blood smears (780 samples from indigenous and 420 from Friesian cross bred cattle) and 100 serum samples were collected during this study. Sampling was carried out randomly at monthly intervals from seven sites in Nyala for a period of 12 months from June 2006 to May 2007. Details on sample size in each month are provided in Table 1. Four age groups of cattle were sampled; group one: < one-year-old, group two: one year to < two-year-old, group three: two year to three-year-old and group four: older than three years. Whole body tick collection was carried out from 1200 cattle. The collected ticks were preserved in 70% ethanol. Blood smears, sera and ticks vials were labelled according to the cattle breed, date, area and age, and then submitted to Nyala Veterinary Research Laboratory and stored till processed. Ticks were identified under a dissecting microscope according to Hoogstraal (1956). Thin blood smears were made from ear vein puncture, stained with Giemsa's and examined under a microscope. At least 50 microscopic fields were examined for the presence of piroplasms. Serologically, indirect fluorescent antibody (IFA) test was performed according to FAO (1984). Statistical analysis was performed using SAS package version 6.0. Data collected on ticks were subjected to appropriate general liner model (GLM). The SAS was used to perform analysis of variance (ANOVA) and mean separations were performed using Ryan-Einot-Gebriel-Welsh multiple range test (REGWQ) (Day and Quinn, 1989).

#### Results

The prevalence rates of piroplasms in Nyala dairy farms are shown in Table

1. The study revealed that out of 1200 Giemsa stained blood smears examined microscopically, 43 (3.6%) were positive for *Theileria* spp. piroplasms. The highest prevalence rate of *Theileria* spp. piroplasms was in December (7/98; 7.1%), followed by November (6/100; 6%), June (6/103; 5.8%), and July (5/105; 4.7%). The lowest prevalence rate was in August (1/111; 0.9%), followed by April (1/99; 1.0%) and February (1/95; 1.1%) (Table 1).

*Theileria* spp. were prevalent among two breeds of cattle (Table 2). The prevalence of *Theileria* piroplasm in cross bred cattle was higher (22/420; 5.2%) than in local ones (21/780; 2.7%). However, the highest prevalence of *T. annulata* antibodies was in local cattle breeds compared with the cross breed (Table 2).

Among the age groups examined for *Theileria* spp., the highest prevalence (9/178; 5.1%) was recorded from one to two-year-old animals, and the lowest prevalence (4/123; 3.2%) was recorded among < one-year-old calves (Table 3). On the other hand, the highest prevalence of antibodies (40.8%; 20/49) was recorded in the older group of cattle (> 3-year-old) and the lowest (1.8%; 2/11) among < one-year-old calves.

Four genera and thirteen species of ticks were found infesting cattle in Nyala. These were *Amblyomma variegatum*, *A. lepidum*, *Rhipicephalus (Boophilus) annulatus*, *R.(B.) decoloratus*, *Hyalomma anatolicum*, *H. anatolicum excavatum*, *H. dromedarii*, *H. impeltatum*, *H. impressum*, *H. marginatum rufipes*, *H. truncatum*, *Rhipicephalus evertsi evertsi* and *R. s. sanguineus*. Table 4 shows mean ( $\pm$ SE) monthly total number of seven tick species infesting cattle in Nyala during the study period.

#### Discussion

Tick-borne diseases of cattle remain an implacable impediment to livestock development in the Sudan (Gamal and El Hussein, 2003).

**Table 1: Monthly prevalence of *Theileria* spp. piroplasms among cattle in Nyala during June 2006 to May 2007.**

Months	BS		IFA	
	Animals examined	Positive <i>Theileria</i> spp. piroplasms (%)	Animals examined	Positive <i>T. annulata</i> antibodies (%)
<b>2006</b>				
June	103	6 (5.8)	8	4 (50.0)
July	105	5 (4.7)	8	3 (37.5)
August	111	1 (0.9)	9	1 (11.1)
September	100	2 (2)	8	1 (12.5)
October	84	3 (3.6)	8	3 (37.5)
November	100	6 (6)	8	4 (50.0)
December	98	7 (7.1)	8	6 (75.0)
<b>Total</b>	<b>701</b>	<b>30 (4.3)</b>	<b>57</b>	<b>22 (38.6)</b>
<b>2007</b>				
January	107	3 (2.8)	9	2 (22.2)
February	95	1 (1.1)	8	1 (12.5)
March	104	4 (3.8)	8	3 (37.5)
April	99	1 (1)	9	1 (11.1)
May	94	4 (4.3)	9	2 (22.2)
<b>Total</b>	<b>499</b>	<b>13 (2.6)</b>	<b>43</b>	<b>9 (20.9)</b>
<b>Grand Total</b>	<b>1200</b>	<b>43(3.6)</b>	<b>100</b>	<b>31(31.0)</b>

**Table 2: Prevalence of *Theileria* spp. piroplasms and *T. annulata* antibodies among local and cross bred cattle in Nyala from June 2006 to May 2007.**

Breed of cattle	BS		IFA	
	Animals examined	Positive <i>Theileria</i> spp. Piroplasms (%)	Animals examined	Positive <i>T. annulata</i> antibodies (%)
Zebu	780	21 (2.7)	54	22(40.7)
Zebu × Friesian	420	22 (5.2)	46	9(19.6)
<b>Total</b>	<b>1200</b>	<b>43 (3.6)</b>	<b>100</b>	<b>31(31)</b>

**Table 3: Prevalence of *Theileria* spp. piroplasms and *T. annulata* antibodies among different age groups of cattle in Nyala during June 2006 to May 2007**

Age groups	BS		IFA	
	Number examined	Positive piroplasms (%)	Number examined	Positive <i>T. annulata</i> antibodies (%)
< 1 year	123	4(3.2)	11	2(18.1)
1 < 2 years	178	9(5.1)	22	5(22.7)
2 to 3 years	360	13(3.6)	18	4(22.2)
> 3 years	539	17(3.2)	49	20(40.8)
<b>Total</b>	<b>1200</b>	<b>43 (3.6)</b>	<b>100</b>	<b>31 (31)</b>

**Table 4: Mean ( $\pm$  SE) monthly total tick species infesting cattle in Nyala during June 2006 to May 2007**

Months	<i>R.(B.)decoloratus</i>	<i>R.(B.)mudas</i>	<i>H.romedarii</i>	<i>H.impeltatum</i>	<i>H.m. rufipes</i>	<i>H. truncatum</i>	<i>A. variegatum</i>
<b>2006</b>							
<b>June</b>	0.16 $\pm$ 0.04bc	0.13 $\pm$ 0.04cd	0.46 $\pm$ 0.06a	0.86 $\pm$ 0.07bc	0.50 $\pm$ 0.06cd	0.08 $\pm$ 0.03e	0.04 $\pm$ 0.02c
<b>July</b>	0.39 $\pm$ 0.07a	0.28 $\pm$ 0.06c	0.25 $\pm$ 0.05bc	0.60 $\pm$ 0.08c	0.05 $\pm$ 0.03e	0.52 $\pm$ 0.08ab	0.52 $\pm$ 0.08a
<b>August</b>	0.40 $\pm$ 0.06a	0.15 $\pm$ 0.04cd	0.11 $\pm$ 0.04c	0.68 $\pm$ 0.07bc	0.33 $\pm$ 0.06de	0.01 $\pm$ 0.01e	0.65 $\pm$ 0.08a
<b>September</b>	0.21 $\pm$ 0.06bc	0.13 $\pm$ 0.04cd	0.18 $\pm$ 0.05bc	0.23 $\pm$ 0.05c	0.29 $\pm$ 0.06de	0.13 $\pm$ 0.04cd	0.59 $\pm$ 0.08a
<b>October</b>	0.11 $\pm$ 0.04bc	0.11 $\pm$ 0.04d	0	0.21 $\pm$ 0.05cd	0.63 $\pm$ 0.07c	0.79 $\pm$ 0.07a	0.21 $\pm$ 0.05b
<b>November</b>	0.08 $\pm$ 0.03bc	0.78 $\pm$ 0.13a	0.06 $\pm$ 0.03c	0.86 $\pm$ 0.11bc	1.23 $\pm$ 0.11b	0.14 $\pm$ 0.04d	0.06 $\pm$ 0.03c
<b>December</b>	0.15 $\pm$ 0.04bc	0.51 $\pm$ 0.11b	0.11 $\pm$ 0.04c	0.35 $\pm$ 0.06cd	1.59 $\pm$ 0.09a	0.24 $\pm$ 0.06b	0.06 $\pm$ 0.03c
<b>2007</b>							
<b>January</b>	0.23 $\pm$ 0.05b	0.04 $\pm$ 0.05cd	0.12 $\pm$ 0.04c	0.60 $\pm$ 0.07bc	0.77 $\pm$ 0.09c	0.19 $\pm$ 0.05c	0.06 $\pm$ 0.03c
<b>February</b>	0.03 $\pm$ 0.02c	0.08 $\pm$ 0.03cd	0.17 $\pm$ 0.05bc	0.55 $\pm$ 0.09cd	0.12 $\pm$ 0.09e	0.12 $\pm$ 0.04cd	0.02 $\pm$ 0.02c
<b>March</b>	0.08 $\pm$ 0.04bc	0.33 $\pm$ 0.08c	0.71 $\pm$ 0.09a	0.68 $\pm$ 0.09cd	0	0.02 $\pm$ 0.02e	0.02 $\pm$ 0.02c
<b>April</b>	0.08 $\pm$ 0.04bc	0.08 $\pm$ 0.03cd	0.12 $\pm$ 0.04c	0.89 $\pm$ 0.08b	0.55 $\pm$ 0.08cd	0.03 $\pm$ 0.02e	0.05 $\pm$ 0.03c
<b>May</b>	0.05 $\pm$ 0.03b c	0	0.15 $\pm$ 0.05bc	1.18 $\pm$ 0.08a	0.42 $\pm$ 0.08d	0.18 $\pm$ 0.05c	0.02 $\pm$ 0.02c

Means ( $\pm$  SE) followed by the same letter in each column are not significantly different at 5% level based on Ryan's Q test.

The impact of ticks and tick-borne diseases on South Darfur State is tremendous due to an abundance of ticks at different climatic zones of the region throughout (Abdallah, 2007). Recently, due to progressive development of South Darfur State and rapid growing of human population in Nyala town, there is an increasing demand of milk and milk products. Many farmers introduced Friesian cattle and their crosses with indigenous breeds (Friesian × Kenana or Butana) from Central Sudan. However, ticks and tick-borne diseases are the main threat to these cattle in most state localities. The present study provides an epidemiological data regarding tropical theileriosis in and around Nyala town. It reveals that out of 1200 blood smears, 43 (3.6%) were positive for *Theileria* piroplasms. Abdallah (2005) reported prevalence of 2.8% in the same region. The increase in the prevalence may be attributed to the fact that introduction of cattle with foreign blood into the region may have altered the enzootic stability, as animals with foreign blood are known to attract more ticks (A.M. El Hussein, unpublished observation). It is also possible that increased prevalence is due to the introduction of the highly efficient vector, *H. anatolicum*, with the exotic breeds of cattle.

In the current study, tropical theileriosis occurred throughout the year with a minimal seasonal variation. This could be due to the fact that *H. anatolicum* is more adapted to the microclimate of the surveyed farms. El Hussein *et al* (1991) ascribed the high incidence of theileriosis during summer to heat stress and lack of adequate feeding.

There was an increased prevalence of *T. annulata* antibodies in local breed compared with cross breed although exotic cattle (*Bos taurus*) are more susceptible to tropical theileriosis than zebu ones (Osman, 1976; Shommein and Hagir, 1988). This result is substantiated by the finding that infestation of ticks was

low on exotic cattle compared with zebu despite the fact that the former are more susceptible to tick infestation (Data not shown). Nevertheless, the use of acaricides did not prevent these animals from attracting the disease probably because of their inappropriate application. In the Sudan, indigenous cattle are normally resistant to tick-borne diseases, but they may be severely affected or even die if are stressed (Osman, 1976).

The results indicate high seroprevalence of theileriosis in groups of old cattle than those of young. This finding is in agreement with those of Flach *et al* (1995) and Darghouth *et al* (1996) who conducted epidemiological investigations into tropical theileriosis in Morocco and Tunisia, respectively. They commented that calves are less heavily infested because of low attachment or high detachment rates caused by grooming whereas adults become more tolerant as a result of repeated infestation. It is also possible, in the current study, that calves/ticks contact is less since they are tethered or housed away from their dams; hence they become less infested with ticks. Punyua and Hassan (1992) have found that cattle tethered up to midday have less tick infestation than another group which was allowed to graze for whole day.

Four genera and thirteen species of ticks were found infesting cattle in Nyala. This result is more or less similar to previous reports from Darfur. Osman (1978) reported presence of twenty tick species from the same region on domestic animals under nomadic system. Recently, Abdallah (2007) collected 15 tick species from sedentary and semi sedentary cattle in South Darfur State. This may be attributed to the occasional control of ticks by chemical acaricides in the former system. The most predominant genus encountered in this survey was the *Hyalomma*. Among the *Hyalomma* spp., *H. anatolicum* was found in several farms; this is a further confirmation that

*H. anaticum*, the most efficient tropical theileriosis vector, is established in Nyala and probably in other towns of South Darfur. Outbreaks of tropical theileriosis are currently not common in Nyala as evidenced by the 22 cases of theileriosis in the current study where macroschizonts are detected in farms where *H. anaticum* was present (Data not shown). To make the situation even worse, cattle in search of water and pasture cross the international borders to the neighbouring countries and, thus, it would not be surprising to find that this tick species has reached and established itself in the far west African countries.

It is strongly recommended that introduction of livestock from Central Sudan into South Darfur should be accompanied by strict tick control measures. At the same time, there must be a planned programme of tick control in farms where *H. anaticum* is found for the purpose of eradicating this newly introduced tick species. Spread of this species to other farms, other towns and probably cross the international borders must be prevented.

#### References

- Abdallah, M.E.A. (2005).** *Prevalence of blood parasites in domestic animals in South Darfur State of Sudan*. M.V.Sc. Thesis. University of Khartoum.
- Abdallah, M.M. (2007).** *Studies on ticks and tick-borne diseases on cattle in South Darfur State of Sudan*. M.V.Sc. Thesis. University of Khartoum.
- Anon. (2004).** *Statistical Bulletin for Animal Resources*. Ministry of Animal Resources and Fisheries, Sudan Government, Khartoum. Issue No. 14, pp. 34-42.
- Darghouth, M.A.; Bouattour, A.; Ben Miled, L.; Kilani M. and Brown, C.G.D. (1996).** *Vet. Parasitol.*, **95**: 199–211.
- Day, R.W. and Quinn, G.P. (1989).** *Ecolog. Monog.*, **59**: 433-463.
- Dolan, T.T. (1989).** *Rev. Sci. Tech. Off. Int. Epiz.*, **8**: 11-36.
- El Hussein, A.M.; Mohammed, S. A.; Osman, A.K. and Osman, O.M. (1991).** *Sudan. J. Vet. Res.*, **10**: 51–56.
- FAO (1983).** Tick and tick-borne diseases control. The Sudan: Studies on important tick-borne diseases of cattle. Technical Report No.2 AGGCP/SUD/ 024/ DEN/ROME
- FAO (1984).** Tick and tick-borne diseases control. A practical field Manual. Vol. II. Food and Agriculture Organization of the United Nations, Rome.
- Flach, E.J.; Ouhelli, H.; Waddington, D.; Oudich, M. and Spooner, R.L. (1995).** *Vet. Parasitol.*, **59**: 117–188.
- Gamal, A. and El Hussein, A.M. (2003).** *Sudan J. Vet. Sci. Anim. Husb.*, **42**: 268–273.
- Hoogstraal, H. (1956).** African Ixodoidea. I. Ticks of the Sudan (with special reference to Equatoria Province and with preliminary reviews of the genera *Boophilus*, *Margaropus*, and *Hyalomma*). Department of the Navy, Bureau of Medicine and Surgery. Washington, D.C., U.S.A.
- Jongejan, F. and Uilenberg, G. (2004).** *Parasitol.*, **129**: S3-S14.
- Osman, A.M. (1976).** *Bull. Off. Int. Epiz.*, **86**: 81–87.
- Osman, O.M. (1978).** *Bull. Anim. Prod. Afr.*, **26**: 329–333.
- Punyua, D.K. and Hassan, S.M. (1992).** *Exp. Appl. Acarol.*, **14**: 61-65.
- Salih, D.A.; Hassan, S.M. and El Hussein, A.M. (2007).** *Prev. Vet. Med.*, **81**: 323-326.
- Shommein, A.M. and Hagir, B.S. (1988).** Theileriosis in the Sudan. In: Int. Symp. Develop. Anim. Resour. Sudan. University of Khartoum. Khartoum, 3<sup>rd</sup>–7<sup>th</sup> January 1988. Arab Organization for Agriculture. Pp. 28-32.
- Uilenberg, G. (1981).** Theilerial species of domestic livestock. In: Irvin, A.D. Cunningham, M.P. and Young, A.S. (eds.) *Advances in the Control of Theileriosis*. Martinus Nijhoff Publishers. The Hague, The Netherlands pp. 4-37.