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Some Studies on the Epidemiology of Ovine Theileriosis in River Nile State, Northern Sudan

[1]B.M. Ahmed, [2]A.M. El Hussein, [3]A. El Ghali and [2]D.A. Salih
[1]Faculty of Education, Nile Valley University, P. O. Box 136, Atbara, Sudan
[2]Central Veterinary Research Laboratories, P. O. Box 8067, Alamarat, Khartoum, Sudan
[3]Atbara Veterinary Research Laboratory, P. O. Box 121, Atbara, Sudan

Abstract: In cross-sectional survey conducted in River Nile State, Northern Sudan, 1905 blood smears from sheep were examined for the presence of *Theileria* piroplasms. Out of 800 samples from resident sheep 177 (22.1%) were found infected, while in 1105 samples from slaughterhouse 197 (17.8) were infected. Thirteen out of 210(6.2%) Hyalomma anatolicum anatolicum ticks were found infected with *Theileria* sporoblasts. The number of infected acini per salivary glands was significantly higher ($P<0.01$) in female ticks (1.56) than in male ticks (1.0). Combining infection rate in ticks with infection prevalence studies lead to improved understanding of the epidemiology of malignant ovine theileriosis in the River Nile State, Northern Sudan. The results also indicated year-round transmission of the parasite in the study area.

Key word: Blood smear, infection rate, Sheep-protozoa, *Theileria lestoquardi, H. a. anatolicum*

Introduction

Malignant ovine theileriosis (MOT) is an infectious tick-borne protozoan disease caused by *Theileria lestoquardi* (Morel and Uilenberg, 1981). It occurs in southeastern Europe, North Africa, the near and Middle East and Southern USSR and is transmitted by Hyalomma spp. (Dolan, 1989). In the Sudan, malignant ovine theileriosis was first described by Mason, (1915), then reported in Khartoum State (Nagwa, 1986; Tageldin *et al.*, 1992 and Latif *et al.*, 1994). More recently, Salih et al., (2003) found that T. lestoquardi infections were widely distributed in primary sheep grazing areas of the country. They concluded that 16.3% (51 out of 315) of the sheep were found positive at dilution 1/80 using schizont antigen in the indirect fluorescent antibody test.

In Northern Sudan the disease was reported by (El Hussein *et al.*, 1993; El Ghali and El Hussein, 1995 and and Salih *et al.*, 2003). In related studies, 21% and 12% of the total number of sheep admitted to Atbara Veterinary Hospital (AVH) were diagnosed with theileriosis during the year 1991-1992 and 1992-1993 respectively (El Ghali and El Hussein, 1995). The disease has been reported to cause high morbidity and mortality rates in sheep in the Sudan (Tageldin *et al.*, 1992; El Hussein *et al.*, 1993 and Latif *et al.*, 1994) with resultant high economic loss.

The natural infection rate of ticks with *Theileria sporozoites* is an important parameter in the epidemiology of theileriosis. If routine serological and parasitological surveys could be combined with tick infestation counts and tick infection rates then predictions of the transmission of *Theileria species* could be made and used in control schemes (Young, 1981).

The present investigation was carried out as a part of an ongoing effort to study the epidemiology of ovine theileriosis in River Nile State, Northern Sudan.

Materials and Methods

Collection of Blood Smears from Apparently Healthy Sheep

Group A: A total of 800 of blood smears were collected from resident, apparently healthy sheep during the period November 1996 to December 1997. Sheep were sampled by ear vein puncture at 17 locations along Nile in River Nile State (Fig. 1).

Group B: A total of 1105 blood smear were collected at irregular intervals by Atbara Veterinary Research Laboratory (AVRL) staff during the years 1994 to 1997 at antimortem from sheep for slaughter at Atbara abattoir. These animals were usually purchased from animal markets in other States.

Blood smears were air-dried, fixed in methyl alcohol for 2-3 minutes and stained with 10% Giemsa's stain solution for 45 minutes. Stained blood smears were then examined for blood parasites using light microscope. At least 50 microscopic fields per slide were examined for detection of blood parasites.

Assessment of *Theileria* Infection in Hyalomma Anatolicum Anatolicum Tick Collected on Sheep: Cloth ear bags were applied to 8 apparently healthy sheep infested with nymphs in one study area (3 Km south of Atbara town). The engorged nymphs were collected by shaking the ear bags in polyethylene sacs, brought to the laboratory, and allowed to moult at room temperature (20- 28 °C) at 75% RH. Emerged adult ticks were then identified according to Hoogstraal (1956). Only *Hyalomma anatolicum anatolicum* ticks were used for the assessment of infection rates. The ticks were then allowed to feed for 4 days on rabbits (day of application was considered as day zero). On day one of application, died and unattached ticks were

removed. The ticks were then removed from rabbits, dissected
and their salivary glands were applied to clean grease-free slides, fixed in methyl alcohol for 10 minutes. The fixed slides were stained using Schiff's reagent (FAO, 1993). The stained salivary glands were examined for the presence of *Theileria* sporoblasts in acini using objective X 40 lens in light microscope.

Analysis of Data: Data collected on prevalence (morbidity, mortality, case fatality rates) and salivary gland infection rates were subjected to statistical analysis using Microsoft Excel program and student t-test. Statistical Package for Social Science (SPSS), Analysis of Variance (ANOVA) was also used to calculate the differences between seasons and years.

Results

The only blood parasites encountered during examination of blood smears were *Theileria* piroplasms that commonly appeared as ring, rod or comma shaped. The parasitaemia level in these animals was usually low (< 1/1000). The result of blood smears examination is shown in Tables 1, 2 and 3. The monthly prevalence rates of *Theileria* piroplasms during the period from November 1996 to December 1997 among 800 sheep (group A) ranged from 10% in February to 36% in May (Table 1). The mean seasonal prevalence rates were almost equal being 23.1%, 22.5%, and 21.7% during winter (Nov.- Feb.), summer (Mar.-June) and autumn (July- Oct.), respectively (Table 2). The overall prevalence rate was 22.1% (Table 1).

As shown in Table 3, 197(17.8%) out of 1105 animals in group B were found infected with *Theileria* piroplasms. The average infection rate among these animals varied from 10.9% during 1995 to 26.8% during 1994 with an overall prevalence rate 17.8% Table 3. However, no significant differences ($P>0.05$) in infection rates were detected among seasons or years in this group of animals. The overall average rate among these animals was relatively higher (21.3%) in winter than during summer (16.2%) or autumn (17.6%). However, the highest peaks of infection rates were recorded during autumn 1994(41.2%), winter 1996(31.6%) and summer 1994(25.4%) (Table 3).

Out of 210 *Hyalomma anatolicum anatolicum* adults ticks that were examined for *Theileria* infection 13 ticks (6.2%) were found infected with *Theileria* sporoblasts Table 4. The number of sporoblasts per infected tick varied between 1 and 3 (average 1.28). Among 89 males ticks examined, 7 (7.84%) were infected while only 6 (4.95%) out of 121 females were infected. However, the mean number of infected acini per salivary glands was found to be significantly higher ($P < 0.01$) in female ticks (1.56 ± 0.24) than in male ticks (1.0 ± 0.00) (Table 4).

Discussion

Ovine theileriosis has been reported to cause high morbidity and mortality rates in the Sudan (Tageldin *et al.*, 1992; El Hussein *et al.*, 1993 and Latif *et al.*, 1994). High prevalence of infection with *Theileria* (68%) has also been previously reported among healthy sheep (carrier state) in Khartoum State (Nagwa, 1986). However; information on ovine theileriosis in the Sudan is meager.

In the present study 22.1% of the resident sheep in River Nile State (Group A) and 17.8% of sheep procured from other places for slaughter at Atbara abattoir were found to harbour *Theileria* piroplasms. The lower prevalence rate of Theileria infection observed in this study as compared to that conducted in Khartoum State (Central Sudan) (Nagwa, 1986) may indicate higher transmission rates in central Sudan than in the drier areas of River Nile State. Moreover, while only blood smears were examined in this study, both blood and lymph node biopsy smear were used in Khartoum study, thus increasing the probability of parasite detection.

While the prevalence rate of *Theileria* infection did not vary much ($P> 0.05$) with the seasons among resident animal Table 2, the infection rates were higher during winter (mean 22.9%) and summer (23.7%) than during autumn (7.1%) during the same year (1997) in slaughter animals (group B) Table 3. Similarly, the prevalence of patent parasitemia in resident animals during these latter seasons was higher than in group B animals Table 2 and 3. This result may indicate continuous transmission of *T. lestoquardi* in the Sudan. This is supported by the fact that *H. a. anatolicum* adult ticks were found to be active (albeit at low numbers) throughout most of the year in the present study area (Ahmed *et al.*, Submitted) as well as in other parts of the country (Jongejan *et al.*, 1987 and Osman, 1999). However, while resident animals may have opportunity of contracting the infection in their shelter and limited grazing spaces in agricultural areas, animals procured for slaughter may come from natural grazing areas where lower tick burdens exist. These latter animals were not also exposed to much tick infestation during their short stay in River Nile State except during winter when they may be confined in sheltered pens. On the other hand, differences observed in *Theileria* prevalence rates between the two groups may be due to sample size differences where group B samples were usually smaller than group A (Table 2 and 3).

It should be mentioned, however, that the infection rates with *Theileria* piroplasms in sheep in the present study were generally lower than that reported for *T. annulata* (which is transmitted by the same vector) in blood smears of healthy cattle (mean 37.5% range 34% - 74%) in the same study area (El Hussein *et al.*, 1991). Field infection rates of *H. a. anatolicum* tick

Table 1: The prevalence rate of *Theileria* piroplasma among resident sheep during the period from November 1996 to December 1997. (Group A)

Months	No. of sheep examined	No. of sheep infected	Prevalence rate (%)
Nov. 1996	69	13	19
Dec. 1996	89	25	28
Jan. 1997	75	17	23
Feb. 1997	58	06	10
Mar. 1997	59	14	24
Apr. 1997	43	05	12
May 1997	44	13	30
June 1997	44	09	20
July 1997	67	12	18
Aug. 1997	50	10	20
Sep. 1997	48	12	25
Oct. 1997	61	13	21
Nov. 1997	44	16	36
Dec. 1997	49	12	24
Total	800	177	Average 22.1%

Table 2: Seasonal variation in prevalence rates of *Theileria* piroplasms among resident (Group A) sheep during year 1997

Season	No. of sheep examined	No. of sheep infected	Prevalence rate (%) (Mean ± SE)*
Winter (Nov.-Feb.)	384	89	23.1 ± 3.56
Summer (Mar.-June)	190	41	21.5 ± 3.77
Autumn (July-Oct.)	226	47	21.7 ± 1.47

*No significant difference ($P > 0.05$) based on T test

Table 3: *Theileria* infections rates in blood smears collected antemortem from sheep at Atbara slaughterhouse (Group B)

Season	1994 Inf./total (%)	1995 Inf./total (%)	1996 Inf./total (%)	1997 Inf./total (%)	Total (%)*
Winter	15/71 (21.1)	15/111 (13.5)	24/76 (31.6)	14/61 (22.9)	68/319 (21.3)
Summer	15/59 (25.4)	25/237 (10.5)	38/249 (15.3)	33/139 (23.7)	111/684 (16.2)
Autumn	14/34 (41.2)	2/36 (5.6)	1/18 (5.6)	1/14 (7.1)	18/102 (17.6)
Total*	44/164 (26.8)	42/384 (10.9)	63/ 343 (18.4)	48/214 (22.4)	197/1105 (17.8)

*ANOVA: No significant difference between seasons or years ($P > 0.05$)
The values in parentheses are percentages.

Table 4: Infection rate of Hyalomma anatolicum anatolicum salivary gland with *Theileria* sporoblast

Ticks	No. examined	No. positive	Per cent positive	Mean No. of infected acini/gland mean ± SE*
Females	121	6	4.95	1.56 ± 0.24
Males	89	7	7.84	1.00 ± 0.00
Both sexes	210	13	6.2	1.28

*Significant difference ($P < 0.05$) based on T test

with *T. lestoquardi* are usually much lower (Osman, 1999) than those observed for the same tick with *T. annulata* (Walker *et al.*,1983; Flach *et al.*, 1993 and Sangwan *et al.*, 1994). Hence transmission of *T. annulata* to cattle may be more efficient than that of *T. lestoquardi* to sheep. However, more investigations are required to further study quantitative aspects of transmission and host-parasite relationship of *Theileria*

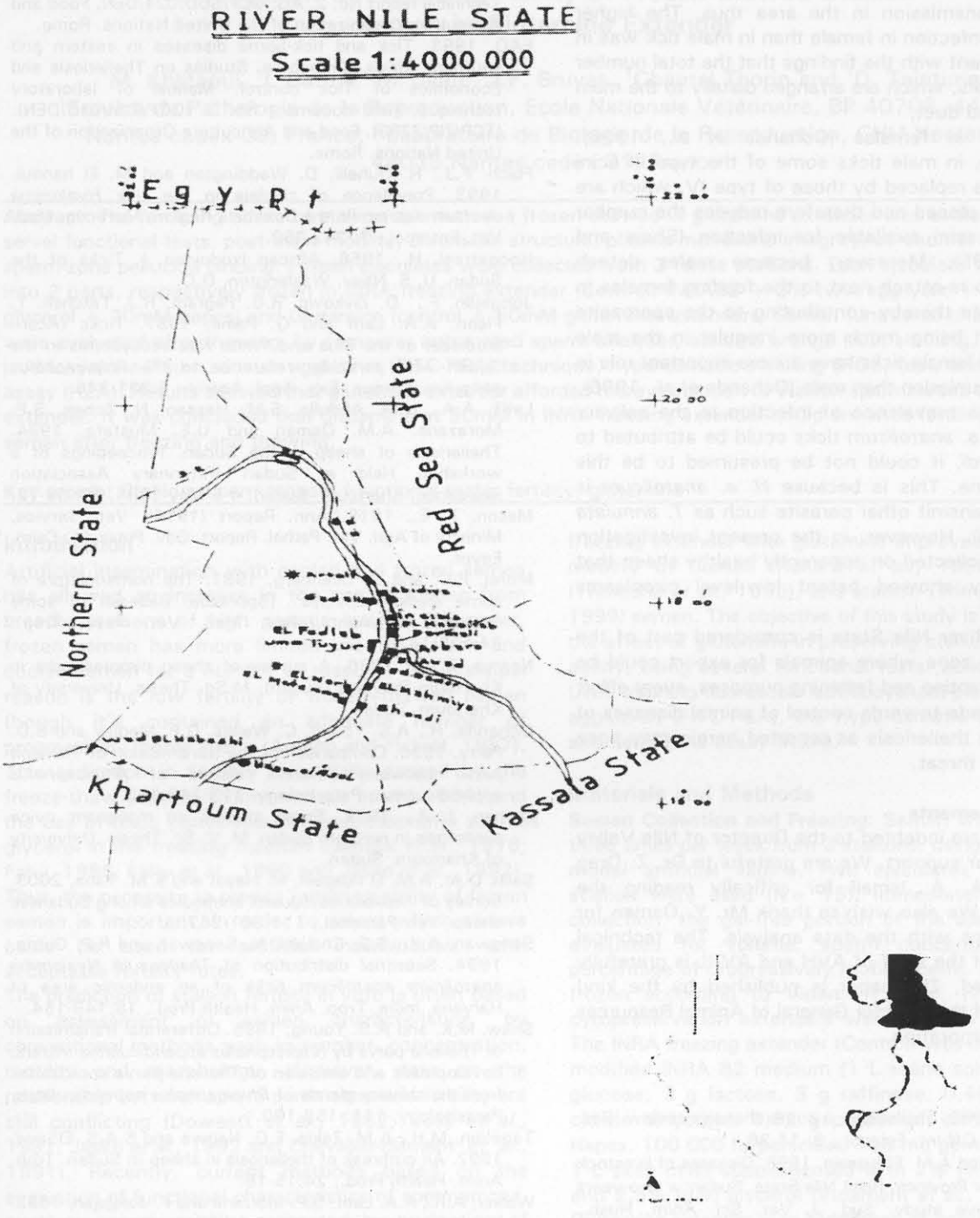

Fig.1:Map of the Sudan Showed the River Nile State (Study area)

parasites in various ruminants in the Sudan.

Since all of *Theileria* isolates from sheep in the Sudan (including those from carrier animals in the present study area) were identified as *T. lestoquardi* (Tageldin *et al.*, 1992; Latif *et al.*, 1994; Osman, 1999; Salih *et al.*, 2003 and El Hussein *et al.*, unpublished data) it is assumed that the piroplasms observed in the present study were *T. lestoquardi*. The continuous occurrence of outbreaks of MOT (Data not shown) further supports this notion and indicates that a state of endemic

stability has not yet been established. The low infection rate in ticks (Table) 4 is a further indication of unstable transmission in the area thus. The higher intensity of infection in female than in male tick was in total agreement with the findings that the total number of type III acini, which are arranged distally to the main salivary gland duct,
are greater in females (Ochanda *et al.*, 1996). Furthermore, in male ticks some of the type III acini appear to be replaced by those of type IV, which are also distally placed and therefore reduces the number of type III acini available for infection (Shaw and Young, 1995). Moreover, because males detach irregularly to re-attach next to the feeding females in order to mate thereby contributing to the sporozoite development being much more irregular in the male ticks. Thus, female ticks have a more important role in parasite transmission than male (Ochanda *et al.*, 1996). Although the prevalence of infection in the salivary glands of *H.a. anatolicum* ticks could be attributed to *T. lestoquardi*, it could not be presumed to be this parasite alone. This is because *H. a. anatolicum* is known to transmit other parasite such as *T. annulata* (FAO, 1983). However, in the present investigation ticks were collected on apparently healthy sheep that intermittently showed patent low-level piroplasms infection.

Finally, as River Nile State is considered part of the disease free zone where animals for export could be held for quarantine and fattening purposes, every effort should be made towards control of animal diseases of which ovine theileriosis as reported herein may pose the greatest threat.

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