OPEN ACCESS

J. Adv. Vet. Anim. Res., 2(1): 69-73.
Available at- *http://bdvets.org/JAVAR*

JAVAR Journal of Advanced Veterinary and Animal Research

ORIGINAL ARTICLE

Volume 2 Issue 1 (March 2015)

DOI: 10.5455/javar.2015.b53

Prevalence of ticks (Acari: Ixodidae) and *Theileria annulata* antibodies in White Nile State, Sudan

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ABSTRACT

This study was conducted to determine the prevalence of ticks infesting cattle and seroprevalence of *Theileria annulata* antibodies in White Nile State, Sudan between March to June 2012. Ticks, blood smears (n=105) and serum samples (n=82) were collected from Rabak, Kosti, El Dowaim, El Getaina, El Jabalain, Kenana and El Gezira Aba. Three tick genera and eleven species were identified. The genera belong to *Amblyomma, Hyalomma* and *Rhipicephalus*. The species were *A. lepidum* (810), *A. variegatum* (7), *H. impeltatum* (189), *H. truncatum* (78), *H. anatolicum* (255), *H. dromedarii* (19), *H. rufipes* (338), *R.* (*Boophilus*) *decoloratus* (54), *R.* (*B.*) *annulatus* (56), *R. evertsi evertsi* (476) and *R. sanguineus* group (60). Out of 105 blood smears, 7 (6.7%) were positive for *Theileria* spp. piroplasms, whereas in ELISA, out of 82 serum samples, 50 (61%) were positive for *T. annulata* antibodies. It is concluded that there are three tick genera and eleven species infesting cattle in White Nile State and the high prevalence *T. annulata* antibodies indicates that tropical theileriosis is endemic in the region. Prevalence of *Amblyomma* spp. implying that heartwater may be endemic in the area.

Keywords

Theileria annulata, Tick, *TaSP* ELISA, Blood smear, Sudan

ARTICLE HISTORY

Received : 07 July '14, **Revised**: 20 October '14,
Accepted : 23 October '14, **Published online**: 17 November '14.

INTRODUCTION

Ticks are blood sucking ectoparasites of mammals, birds and reptiles worldwide, with approximately 850 species been described (Bishop et al., 2008). They transmit a large number of pathogens than any other vector group. The most economically important ticks of livestock in Sudan belong to the genera *Hyalomma*, *Rhipicephalus, Rhipicephalus* (*Boophilus*) and *Amblyomma* (El Hussein et al., 2012). Tropical theileriosis is caused by *Theileria annulata* and transmitted by *H. anatolicum* in Sudan and by *H. scupense* in North Africa (Gharbi et al., 2011). Using IFA test Salih et al. (2005b) recorded 72.8% prevalence rate in Northern Sudan, 90.5 % in Central Sudan, 18.6 % in eastern Sudan, 20.2 % in western Sudan, 33.3 % in Blue Nile, and 33.5 % in White Nile. El Haj (2010) reported 55 % prevalence rate in dairy cattle using IFA in Khartoum State. Using enzyme-linked immunosorbent assay (ELISA), seroprevalence of tropical theileriosis was shown to be prevalent all over the Sudan ranging between 6.3% in South Darfur State and 86.5% in Khartoum State (Salih, 2003). No studies on ticks and incidence of tropical theileriosis have been, hitherto, been conducted along the White Nile. Hence, this study was conducted to determine prevalence of ticks infesting cattle and *Theileria annulata* antibodies in White Nile State during the period March to June, 2012.

MATERIALS AND METHODS

Study area: White Nile State is located in the southern part of Sudan. It borders Khartoum State in the north, North Kordofan State in the west, South Kordofan and Republic of South Sudan in the south east and Gezira

and Sinnar States in the east. It has an area of 39701 Km^2 and a total human population of 1,726,356 working mainly on agriculture and livestock rearing. The state is one of the largest states in animal population in the Sudan. It is estimated at 6298095 animals (cattle, 2506950; sheep, 1801504; goats, 1788086 and camels, 20155) (Anonymous, 2007). The mean daily maximum temperature is 41°C (April) and the mean daily minimum is 15.8° C (January). The state has a single rainy season during June – October with its peaks in July - September. The mean relative humidity ranges between 25.2 and 79.6% in April and August, respectively (El Imam, 1999).

Six sites were included in this study, which are Rabak, Kosti, El Dowaim, El Getaina in the northern part of the state and El Jabalain in the southern part of the state. The other two sites included Kenana (29 km south-east Rabak) and El Gezira Aba (30 Km north-west of Rabak). These two sites were included because they contain large population of nomadic cattle.

Sample collection: Cattle used for sampling had not been treated with any chemical acaricides during March to June, 2012. Animals were chosen according to the breed, coat color and sex. The samples were collected from five farms set apart in each locality including eight cattle in each farm. The samples collected were ticks, blood smears and whole blood in plain vacutainers. The ecotypes of cattle were Zebu (Kenana and Butana) and their cross-bred lines with Friesian cattle. The samples were collected once a month during March to June, 2012.

Tick collection: Half body tick collection was carried out from all body sites of 280 heads of cattle. A pair of blunt metal forceps was used for collection without damaging their mouth parts. The ticks of each animal were separately preserved in vials containing 70% ethanol that were labeled indicating animal, body site, date of collection, sex and coat color of the host. The ticks were identified under a dissecting microscope. The identified ticks were recorded according to their sex and developmental stages.

Blood smears: Blood smears were prepared from 105 heads of zebu and cross-bred cattle (15 heads from each site). Every sample was labeled with regard to the parameters aforementioned. The slides were air-dried and immediately fixed in absolute methyl alcohol for one to two minutes, stained with Giemsa's stain and examined for *Theileria* spp. piroplasms.

Blood collection: Whole blood samples were collected from 82 heads of cattle from all over the state. Five ml of whole blood were collected per veinopuncture in sterile plain vacutainer tubes. Sera were separated by centrifugation at 2,000 rpm for 5 min and placed in eppendorf tubes, fully labelled. The tubes were kept at -20°C until tested.

Indirect *Theileria annulata* surface protein (*TaSP*) ELISA for *Theileria annulata*: An ELISA based on a recombinant *Theileria annulata* surface protein (*Ta*SP) was used for detection of anti-*T. annulata* antibodies in the sera. The *Ta*SP ELISA procedure was carried out according to Salih et al. (2005). The cut-off value (31.6) of *Ta*SP ELISA was determined according to Salih et al. (2005).

Statistical analysis: Data collected were subjected to statistical analysis using SPSS version 13 and were expressed as mean±SD.

RESULTS AND DISCUSSION

Three tick genera and eleven species were identified. The genera belong to *Amblyomma, Hyalomma* and *Rhipicephalus*. The species were *Amblyomma lepidum* (810), *A. variegatum* (7), *Hyalomma impeltatum* (189), *H. truncatum* (78), *H. anatolicum* (255), *H. dromedarii* (19), *H. rufipes* (338), *R.* (*Boophilus*) *decoloratus* (54), *R. (B.) annulatus* (56), *Rhipicephalus evertsi evertsi* (476) and *R. sanguineus* group (60).

The results indicated that *A. lepidum, R. (B.) decoloratus* and *R. (B.) annulatus* were not found on cattle in El Getaina while *R. sanguineus* group were not encountered in El Dowaim. In general, El Jabalain has the highest mean of ticks burden (11.9±1.35) followed by Kosti (10.93±1.07), El Gezira Aba (10.13±0.98), Rabak (10.08±0.97), El Getaina (9.04±0.95), Kenana (5.75±0.51) and the lowest mean was found in El Dowaim (4.05±0.39). The highest mean (7.08±1. 0) of *H. anatolicum* was encountered in El Getaina (**Table 1**). The highest mean of ticks on cross-bred (11.6±1) followed by Butana (10.8±2.2) then Kenana (8.3±0.4). The mean numbers of ticks recovered from brown, gray and white coat color cattle were (11.6±2), (9±1), (8.25±0.5), respectively (**Table 1**). *A. variegatum* was recovered in a few numbers (2 males and 3 females) in Kosti area and 2 males in El Jabalain, whereas, *H. dromedarii* was encountered in few numbers in El Getaina (6 males and 1 female), El Dowaim (7 males and 2 females), Kosti (4 males and 3 females), Kenana (2 females) and one male in El Jabalain (**Table 2**).

Table 1: Means (±SE) of ticks collected from cattle in White Nile State during March to June 2012

Parameters	Animal (N)	*A. lepidum*	*H. rufipes*	*H. impeltatum*	*H. anatolicum*	*H. truncatum*	*R. (B.) annulatus*	*R. (B.) decoloratus*	*R. e. evertsi*	*R. sanguineus*	Total
Sites											
El Jabalain	40	5.78±1.0a	1.05±0.31b	0.5±0.3b	0.1±0.1b	0.4±0.2a	0.3±0.1a	0.75±0.2a	2.3±.4b	0.7±0.3a	11.9±1.3a
Kenana	40	1.85±0.36b	1.0±0.3 b	0.05±0.03b	0.4±0.1 b	0.2±0.1a	0.2±0.1b	0.1±0.06b	1.75±.3b	0.25±0.1 b	5.75±0.5b
Rabak	40	1.5±0.4b	1.2±0.35b	2.45±0.6a	1.1±0.3 b	0.2±0.1a	0.2±0.1b	0.2±0.1b	3.05±1a	0.08±0.04b	10.0±1.3a
Kosti	40	4.95±0.8a	2.8±0.5a	0.5±0.5b	0.4±0.2 b	0.6±0.3a	0.2±0.1b	0.1±0.06b	1.5±.4b	0.1±0.05b	11.0±1.1a
El Gezira Aba	40	5.4±0.7a	1.4±1.4b	0.0±0.0b	0.13±0.08b	0.3±0.1a	0.3±0.1b	0.2±0.1b	2.0 ±0.1b	0.4±0.2 b	10.0±1.3a
El Getaina	25	0.83±0.30b	0.8±0. 2b	0.85±0.31b	0.5±0.2 b	0.05±0.03	0.1±0.1b	0.05±0.03b	0.85±0.2b	0.0±0.0 b	4.1 ±0.4b
El Dowaim	40	0.0±0.0 b	0.7±0.2 b	0.6±0.3b	7.0±1.0 a	0.2±0.1a	0.0±0.0a	0.0±0.0 b	0.44±0.2c	0.0±0.0 b	5.75±0.5b
Animal breed											
Butana	14	4±1.6 a	1.1±0.3b	1.0±0.5b	0.6±0.3 b	0.07±0.07a	0.0±0.0b	0.5±0.3a	2.6±1.5a	0.9±9 a	10.8±2.2a
Kenana	217	3.4±0.3 a	1.4±0.2a	0.4±0.1b	0.5±0.1 b	0.3±0.1a	0.2±0.1b	0.2±0.1 a	1.6±0.2a	0.2±0.1 b	8.3±0.4a
Cross	34	0.5±0.4 b	0.6±0.2a	2.7±0.6a	4.9±1.0 b	0.3±0.2a	0.03±0.03b	0.2±0.1a	2.4±1 a	0.0±0.0 b	11.6±1.0b
Coat colour											
White	124	3.8±0.5 a	1.4±0.3a	0.4±0.1b	0.5±0.1 b	0.3±0.1a	0.3±0.1b	0.2±0.1b	2.0±0.3a	0.1±0.05b	9.0±1.0b
Brown	18	3.1±1.3 a	1.1±0.3a	1.4±0.1a	1.8±0.1 a	0.06±0.06a	0.0±0.0b	0.6±0.2a	2.8±0.8a	0.7±0.6 a	11.6±2.0a
Gray	123	2.3±0.1 a	1.2±0.2a	1.0±0.2b	1.5±0.3 a	0.3±0.2a	0.1±0.1a	0.2±0.1b	1.5±0.3a	0.2±0.1 b	8.25±0.5b

Means (±SE) followed by the same letter (a, b) in each column for each parameter are not significantly different at 5% level according to REGWQ range test.

Out of 82 cattle, 50 (61%) were seropositive to *T. annulata* infection. The highest seroprevalence rate (100%) was in El Getaina and the lowest (30.8%) was in Kosti while the seroprevalence at the rest sites were El Jabalain (53.3%), Kenana (66.7%), Rabak (71.43%), El Gezira Aba (37.5%) and El Dowaim (64.3%) (**Table 3**). The seroprevalence of *T. annulata* infection in zebu cattle (Kenana and Butana) and their cross-bred lines with Friesian cattle were 17.51%, 14.29% and 29.41%, respectively. The seropositivity of *T. annulata* according to coat color of cattle was white (17.89%), brown (22.22%) and gray (19.35%).

Table 2: Ticks on cattle from different sites in White Nile State during March to June, 2012.

Site	*Amblyomma variegatum*		*Hyalomma dromedarii*	
	Male	Female	Male	Female
El Jabalain	2	0	1	0
Kenana	0	0	0	2
Rabak	0	0	0	0
Kosti	2	3	4	3
El Gezira Aba	0	0	0	0
EL Dowaim	0	0	7	2
El Getaina	0	0	6	1
Total	4	3	18	8

Table 3: Seroprevalence of *T. annulata* in cattle in White Nile State using ELISA during March to June, 2012.

Site	No. of animals examined	Number positive	Prevalence (%)
El Jabalain	15	8	53.3
Kenana	6	4	66.7
Rabak	14	10	71.4
Kosti	13	4	30.8
El Gezira Aba	8	3	37.5
El Dowaim	14	9	64.3
El Getaina	12	12	100
Total	82	50	61

The overall prevalence rate of *Theileria* spp. piroplasms was 6.7% (7/105). The highest prevalence rate (20%) was reported in Kosti, in Rabak (13.3%) and the lowest (6.7%) was encountered in El Jabalain and El Gezira Aba. There were no *Theileria* spp. piroplasms detected from the rest of the sites (**Table 4**).

Table 4: Prevalence of *Theileria* spp. piroplasms in cattle using blood smear test in White Nile State during March to June, 2012.

Site	No. of cattle examined	No. positive	Prevalence (%)
El Jabalain	15	1	6.7
Kenana	15	0	0
Rabak	15	2	13.3
Kosti	15	3	20
El Gezira Aba	15	1	6.7
El Dowaim	15	0	0
El Getaina	15	0	0
Total	105	7	6.7%

The distribution of ticks in White Nile State is affected by the establishment of irrigated agricultural schemes, altitude, rainfall, ambient temperature and free animal movement where there are six borders and the longest one with the Republic of South Sudan. The free movement of tick infested -hosts rendered the distribution of many species to be wild. For instance, *H. anatolicum* the main vector of tropical theileriosis, is widely distributed in White Nile State, *A. lepidum* which was previously uncommon west of the River Nile is currently widespread in Kordofan and Darfur (Salih, 2003). Several species of ticks were encountered in White Nile State that may be due to the extensive animal movement, high animal population density and to the establishment of large irrigated agricultural schemes. Tick species identified in the current study are comparable with reported by other investigators in other regions of Sudan (El Imam, 1999; El Hussein et al., 2012). Most cattle surveyed were kept under zero grazing system in which local materials are used for sheds and fences building. Hence, muddy walls with crevices and troughs of water and feed serve as tick shelters allowing them to reproduce and survive under any adverse environmental conditions. *R.* (*B.*) *decoloratus* and *R.* (*B.*) *annulatus* were identified in all sites except El Getaina. Salih (2003) found a few numbers of *R.* (*B.*)

decoloratus on cattle in Rabak, but he did not find them from the northern parts of White Nile State. This indicates that these two tick species are still restricted to the southern parts of central Sudan. *R. e. evertsi* and *R. sanguineus* group were present in most sites investigated in the present study.

The absence of *A. lepidum* and *A. variegatum* in the northern parts of the state was not surprising because these tick species are known to survive in humid areas. Although had not been reported from El dowaim (Salih, 2003), however, it was identified in the current study in the same area but at low burden of infestation. Movement of tick–infested cattle between South Kordofan and Upper Nile States, where this tick species exists as well as migration of birds may have introduced *A. lepidum* in El dowaim. A very few numbers of *A. variegatum* is reported in the present study. This may be attributed to its seasonal activity, as this survey was conducted during March to June, 2012. El Imam (1999) found *A. variegatum* and *H. anatolicum* for the first time in Kosti. He reported these two tick species at very low abundance. He, also, identified *H. excavatum* in the same area which was not found in the current study. Local White Nile State farmers control ticks using indiscriminately chemical acaricides which may have caused fluctuation in tick population. The absence of any tick species in a given period does not mean its absence from the area, as it can establish itself when the optimum environmental conditions prevail.

The low burden of *H. impeltatum* and *H. dromedarii* encountered in this study may be due to the fact that cattle do not frequently come in contact with camels in these areas. *H. dromedarii* and *H. impeltatum* are well known as camel-infesting ticks. Hence, their absence in El Geizra Aba, Rabak and El Dowaim could be due to the scarcity of camels in these sites. In White Nile State, camels represent the lowest animal population a fact which helps explain the low number of these tick species. Ahmed (2012) stated that *H. dromedarii*, *H. impeltatum* and *H. rufipes* were the most abundant tick species in North Kordofan State where cattle co-exist the same habitats with camel in grazing areas and watering points. El Ghali and Hassan (2009) found that *H. dromedarii* represented the main tick species infesting camels followed by *H. impeltatum* and *H. anatolicum* in Northern Sudan. *Hyalomma rufipes*, *H. truncatum*, *R. e. evertsi* and *R. sanguineus* group were not affected by the geographic variations of the sites except the absence of *R. sanguineus* in El Dowaim and El Getaina which can be interpreted for absence of dogs in farms or around pens. The dogs are regarded the main hosts of *R. sanguineus*.

The presence of *H. anatolicum* in El Jabalain (13˚ - 12˚ 30′ N, 32˚48′ - 33˚15′ E) in the southern parts of White Nile State that was used to be free from this tick species is an alarming situation. However, since a few numbers of males and females were recovered may indicate that this tick species was accidentally or incidentally introduced but its establishment in El Jabalain should not be ruled out. If it has established in El Jabalain, this fact implies that *H. anatolicum* is constantly moving southwards. Salih (2003) found many of this tick species of both sexes in Um Benin. This finding indicates that the limit of *H. anatolicum* has moved southwards to latitude 12˚ 30′ N. This may be attributed to the movement of cattle from north Sudan to South Sudan through this site in search of pasture and watering points. Ahmed (2012) did not find *H. anatolicum* in North Kordofan State between 11˚15′ - 16˚45′ N, 27˚5′ - 32˚15′ E.

In the current study, cattle with brown coat color carried significantly more ticks than cattle with gray coat color and cattle with white coat color. This required further investigation and large sample size in different seasons. Tick burdens were correlated with host body coat color. It was reported that cattle with white coat carried significantly more ticks than brown hosts and black coat cattle carried the lowest number of ticks.

The prevalence of eleven tick species in this study may indicate the prevalence of tick-borne diseases (TBDs) in the area particularly tropical theileriosis, heart water and babesiosis. The presence of *H. anatolicum* in the northern and central parts of the state implies that tropical theileriosis is likely to be enzootic where this tick has been established. This is supported by the high seroprevalence rates of tropical theileriosis in El Getaina, El Dowaim and Rabak where most cross bred cattle are kept and the abundance of the vector (*H. anatolicum*) was high. Seroprevalence of *T. annulata* was recorded at all sites during the current study. Salih (2003) reported 34.05% of *T. annulata* antibodies in Rabak and 30.8% in Kosti. In the present study, using ELISA, 61% were seropositive for *T. annulata* whereas 6.7% showed *Theileria* spp. piroplasms using blood smear test. The fact that ELISA detected *T. annulata* antibodies in cattle at all sites as far south as El Jabalain for the first time implies that tropical theileriosis could have become enzootic in the whole state. This fact coupled with the prevalence of the vector *H. anatolicum* in El Jabalain is a serious problem in animal health and production. It could be predicted that tropical theileriosis may cross the borders to South Sudan if tick control measures are not put into place. In Rabak and El Getaina, cross-bred cattle were more seropositive to *T. annulata* than native

cattle. This supports the suggestion that cross bred cattle are more susceptible to tropical theileriosis than native cattle which have the ability to limit the macroschizont index (Bakheit and Latif, 2002). *Theileria* spp. piroplasms detected by the blood smear examination may not necessarily indicate that cattle are suffering from clinical tropical theileriosis. Their presence may be an indication of infection with non-pathogenic *Theileria* species. It could, also be, an indication of a sub-clinical tropical theileriosis securing an enzootic stable situation.

If routine serological and parasitological surveys of cattle for tick-borne diseases combined with tick surveys and tick infection rates, then, prediction of *Theileria* species could be made in control programs.

CONCLUSION

The current study revealed that seroprevalence of *T. annulata* was widespread in the White Nile State. It has also, revealed that indirect ELISA assay is a useful technique in determine the seroprevalence of *T. annulata* infection. Further studies are warranted or periodical surveys including large number of animals at the state level to avail the prevalence of ticks infesting cattle and to determine the seroprevalence of tropical theileriosis. It is, also, recommended that to solicit allocated funds for projects in vaccination against ticks and tropical theileriosis and an extension programs for the cattle owners to better understanding of control of TBDs in White Nile State.

ACKNOWLEDGEMENT

The authors wish to thank the Director and the staffs of RabaK Regional Veterinary Research Laboratory (RRVRL) for helping in sample collection. We are grateful to Dr. Khitma Hassan El Malik, Dr. Magdi Badawi and Prof. Ahmed El Ghali for their support during the study.

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