Parasite Epidemiology and Control 10 (2020) e00148

Contents lists available at ScienceDirect

Parasite Epidemiology and Control

journal homepage: www.elsevier.com/locate/parepi

Prevalence of ticks (Acari: Ixodidae) and *Theileria annulata* infection of cattle in Gezira State, Sudan

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ARTICLE INFO

Article history:
Received 7 December 2018
Received in revised form 25 September 2019
Accepted 6 April 2020

Keywords:
Gezira State
IFA test
Ticks
Theileria annulata
Sudan

This study was conducted in Gezira State, central Sudan during January, May and August 2014 to determine species of ticks infesting cattle and prevalence of *Theileria annulata* infection and antibodies. A total of 200 head of cattle were sampled for ticks, blood smears, lymph node biopsy smears and sera from five study areas. Zebu (Butana and Kenana) and cross-bred (Zebu X Friesian) cattle of both sexes were sampled. Cattle age groups were younger than one year, one year to younger than four years and four years and older. Coat colour of cattle was recorded. The results revealed that cattle were infested with *Rhipicephalus evertsi evertsi* (n = 562, 51.6%), *Hyalomma anatolicum* (n = 289, 26.5%), *H. rufipes* (n = 70, 6.4%), *R. sanguineus sanguineus* (n = 59, 5.4%), *R. decoloratus* (n = 39, 3.6%), *Amblyomma lepidum* (n = 35, 3.2%), *H. impeltatum* (n = 26, 2.4%) and *H. dromedarii* (n = 9, 0.8%). Out of 200 blood and biopsy smears, 33 (16.5%) showed piroplasms for *Theileria* spp., while 29 (14.5%) were positive for *Theileria* spp. schizonts. Seropositivity for *T. annulata* using indirect fluorescent antibody (IFA) test was 94 (47%). It is concluded that there are three genera of hard ticks and eight species in Gezira State and tropical theileriosis is endemic in the state. These findings are the first records in Gezira State, Central Sudan and it is highly recommended to consider the endemicity of tropical theileriosis particularly in cattle upgrading programmes.

1. Introduction

The Sudan is one of the largest African countries with livestock estimated to be about 104 million heads with 29.6 million cattle, 39.3 million sheep, 30.4 million goats, 4.7 million camels and 0.5 million horses (Anon, 2013). Tick species infesting livestock in the Sudan are *Hyalomma anatolicum*, *H. impressum*, *H. dromedarii*, *H. impeltatum*, *H. rufipes*, *H. truncatum*, *Rhipicephalus evertsi evertsi*, *R. sanguineus*, *R. praetextatus*, *R. annulatus*, *R. decoloratus*, *Amblyomma lepidum* and *A. variegatum* (Hoogstraal, 1956; Karrar et al., 1963; Osman et al., 1982). Salih et al. (2004) reported 11 species of ticks infesting cattle, among which was *H. anatolicum* the known vector of tropical theileriosis, in different localities in central, western, eastern and northern Sudan. Guma et al. (2015) recorded *H. anatolicum* in El Jabalain in the southern parts of White Nile State that had been free of this

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https://doi.org/10.1016/j.parepi.2020.e00148

tick species. They concluded that this is an alarming situation since this fact implies that *H. anatolicum* is constantly moving southwards.

Theileria annulata which causes tropical theileriosis and is transmitted by *H. anatolicum* is the most important *Theileria* species in cattle and buffalo (Robinson, 1982; El Hussein et al., 2002). The latter authors considered this disease the most important tick-borne disease in the Sudan. Latif (1994) reported that 85% of some farms investigated in Khartoum State experienced clinical theileriosis and mortality of 22% and 30% in young calves and heifers, respectively. He estimated the annual losses in Khartoum State as US$ 4–6 million per year. In north Sudan, the economic losses due to tropical theileriosis were estimated at 30% of expected profitability (Gamal and El Hussein, 2003).

Although investigations on ticks infesting cattle and serosurveillance on *Theileria annulata* have been conducted along the White Nile (Guma et al., 2015) and Blue Nile (FAO, 1983a,b), there are no documented studies hitherto in the central Gezira ecosystem although it is widely considered that central Gezira is an endemic area of tropical theileriosis. Hence, the objectives of this study were to conduct parasitological investigations on *Theileria* spp. infection of cattle and to determine prevalence of *Theileria annulata* antibodies of cattle in central Gezira, Sudan.

2. Materials and methods

2.1. Study area

This study was carried out in Gezira State which is located in the eastern region of the central Sudan and lies between latitudes 13° 32′ - 15° 30′ N and longitudes 32° 22′ - 34° 20′ E. It is bordered by Khartoum State to the north, Sinnar State to the south, Gedarif State to the east and White Nile State to the west (Fig. 1). It has a total area of about 27,549 km^2 and a human population of 3,529,992 that subsists mainly on agriculture and livestock rearing (Sudan census, April 2008). Livestock population is about 9,824,922 heads including cattle (3,618,418), sheep (3,780,015), goats (2,317,881) and camels (108,608) (Ministry of Agriculture and Animal Resources, Gezira State, 2013). The mean daily maximum temperature is 42 °C in May and the mean daily minimum temperature is 14 °C in January. The rainy season is from June and October, the peak is in July and August. The mean relative humidity ranges from 24% in May and 80% in August (Anon, 2013). Five areas along the west and south-west region of the state were selected to conduct this investigation. These were Abugota in the north, El Mihaireba, El Hoda, El Managil and Aburuwaishid in the far south (Fig. 1).

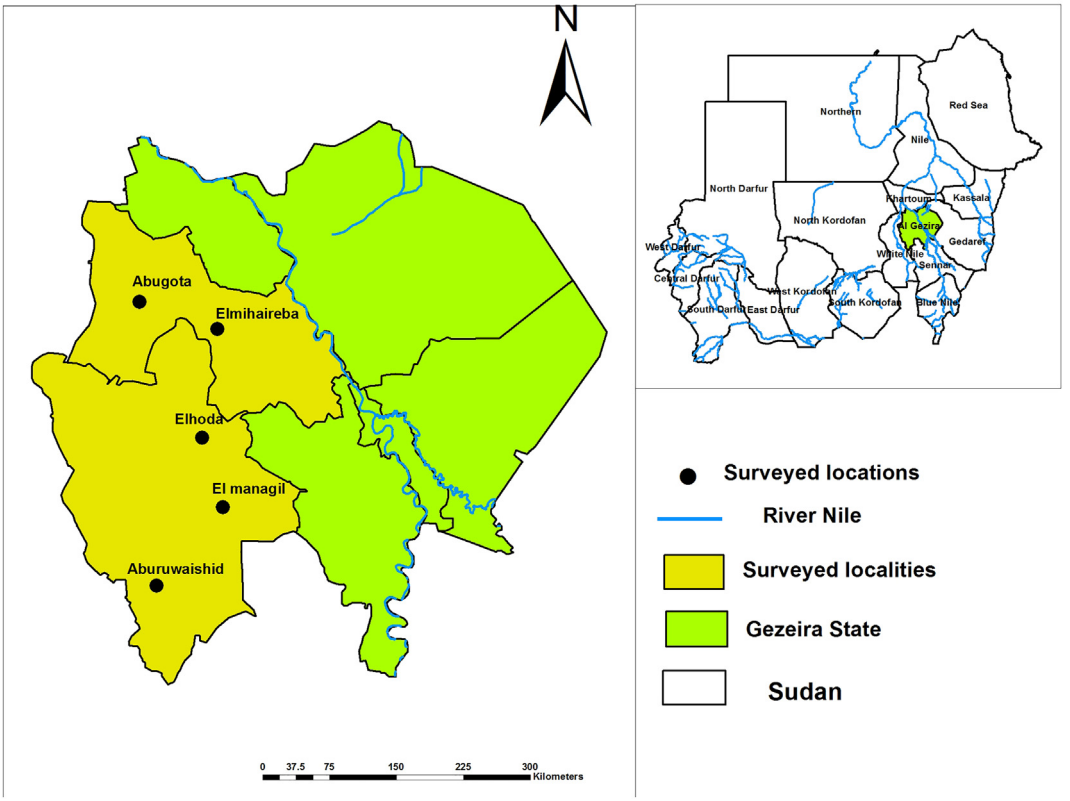

Fig. 1. Map of Sudan showing Gezira State and the locations where samples were collected.

2.2. Collection and examination of samples

Sample collection was carried out in January, May and August 2014. Five farms of cattle that set apart in each area were selected and on each farm eight cattle of different breed, age group, sex and coat colour were selected. The breeds were Zebu (Butana and Kenana) and cross-bred (Zebu X Friesian). Age groups were one-year old calves, more than one to less than four-year-old heifers or steers and four years and older cows or bulls. Coat colours were white, brown, black and white and black. Ticks, blood smears, lymph node biopsy smears and whole blood were collected. All visible attached adult ticks were collected using a pair of blunt metal forceps from the predilection sites of body of 200 head of cattle, 40 from each study area. The ticks of each animal were separately preserved in vials containing 70% ethanol and labeled indicating animal number, area, age, sex, coat colour and date. The ticks were identified according to the methods described by Hoogstraal (1956), Walker et al. (2000) and Walker et al. (2003). Thin blood smears were prepared from the same 200 heads of cattle. They were prepared, fixed by absolute methanol and stained with 10% Giemsa's stain and thoroughly examined for the presence of *Theileria* spp. piroplasms. Biopsy smears were prepared from the prescapular lymph nodes using a syringe with needle, air-dried and fixed by absolute methanol and stained by 10% Giemsa's stain. The smears were thoroughly examined searching for *Theileria* spp. schizonts.

Whole blood was collected from the same 200 heads of cattle. Five milliliters of blood were collected from the jugular vein using plain vacutainer tubes. The blood was allowed to clot overnight and then centrifuged at 1500 rpm for 10 min. Serum was, then, removed by a Pasteur pipette for each sample and placed in eppendorf tubes and labeled prior to storing at −20 °C until used. Indirect fluorescent antibody (IFA) test was carried out for detection of *T. annulata* antibodies. Techniques used were essentially described elsewhere (Burridge and Kimber, 1972; FAO, 1984). Positive and negative *T. annulata* control sera were kindly provided by the Cell Culture Unit, Central Veterinary Research Laboratories, Khartoum, Sudan.

2.3. Statistical analyses

Data collected on ticks were subjected to an appropriate general linear model (GLM) procedure of the statistical analysis system (SAS) package. The SAS was used to perform analysis of variance (ANOVA). Mean separations were performed using Rayan-Einot-Gabriel-Welsch (REGW) multiple range test (Day and Quinn, 1989). To determine the level of agreement between the three diagnostic tests the results were subjected to Kappa coefficient analysis using Stata (Stata, 2000). Cross tabulation analysis of the agreement between blood smear test and indirect fluorescent antibody test and between blood smear test and biopsy smear test was carried out.

3. Results

The tick species identified were *Rhipicephalus evertsi evertsi* (n = 562, 51.6%), *Hyalomma anatolicum* (n = 289, 26.5%), *H. rufipes* (n = 70, 6.4%), *R. sanguineus sanguineus* (n = 59, 5.4%), *R. decoloratus* (n = 39, 3.6%), *Amblyomma lepidum* (n = 35,

Table 1
Means (±SE) of ticks collected from cattle in Central Gezira State during January, May and August 2014.

Parameters	Animals	*A. lepidum*	*H. anatolicum*	*H. rufipes*	*H. impeltatum*	*R. e. evertsi*	*R. sanguineus*	*R. decoloratus*	Total
Localities									
Abugota	40	0.43 ± 0.21 a	2.05 ± 0.40a	0.50 ± 0.27a	0.05 ± 0.05b	2.63 ± 0.43ab	0.50 ± 0.2ab	0.35 ± 0.18a	6.50 ± 0.37a
Mihaireba	40	0.35 ± 0.12ab	1.63 ± 0.30b	0.03 ± 0.03a	0.55 ± 0.26a	4.00 ± 0.45a	0.98 ± 0.32a	0.00 ± 0a	7.53 ± 0.56a
El Managil	40	0.10 ± 0.06ab	0.45 ± 0.12b	0.45 ± 0.13a	0.05 ± 0.03b	3.00 ± 0.35a	0.00 ± 0b	0.00 ± 0a	4.05 ± 0.3ab
El Hoda	40	0.0 ± 0b	2.60 ± 0.52a	0.23 ± 0.16a	0.0 ± 0b	1.45 ± 0.32b	0.00 ± 0.0b	0.15 ± 0.10a	4.43 ± 0.54b
Aburuashid	40	0.0 ± 0b	0.55 ± 0.18b	0.55 ± 0.21a	0.0 ± 0b	2.85 ± 0.4ab	0.00 ± 0b	0.55 ± 0.25a	4.50 ± 0.31b
Animal sex									
Bulls	56	0.07 ± 0.04a	1.59 ± 0.31a	0.13 ± 0.07a	0.07 ± 0.07a	2.75 ± 0.4a	0.07 ± 0.06a	0.20 ± 0.1a	4.88 ± 0.4a
Cows	144	0.22 ± 0.07a	1.40 ± 0.19a	0.44 ± 0.11a	0.15 ± 0.07a	2.80 ± 0.2a	0.38 ± 0.11a	0.22 ± 0.08a	5.60 ± 0.3a
Animal Breed									
Cross	126	0.25 ± 0.08a	1.45 ± 0.20b	0.21 ± 0.07a	0.10 ± 0.05b	2.95 ± 0.2a	0.37 ± 0.12a	0.18 ± 0.07a	5.52 ± 0.3b
Butana	19	0.16 ± 0.12a	2.53 ± 0.76a	0.53 ± 0.47a	0.58 ± 0.47a	3.21 ± 0.6a	0.42 ± 0.30a	0.0 ± 0a	7.42 ± 1.2a
Kenana	55	0.02 ± 0.02a	1.09 ± 0.25b	0.62 ± 0.19a	0.04 ± 0.03b	2.25 ± 0.3a	0.07 ± 0.07a	0.35 ± 0.17a	4.44 ± 0.3b
Animal age (years)									
<1	68	0.01 ± 0.01a	1.25 ± 0.24a	0.10 ± 0.04a	0.06 ± 0.06a	2.90 ± 0.3a	0.06 ± 0.05b	0.18 ± 0.10a	4.59 ± 0.3b
1 < 4	44	0.23 ± 0.09a	1.98 ± 0.42a	0.34 ± 0.19a	0.25 ± 0.21a	2.34 ± 0.4a	0.89 ± 0.29a	0.16 ± 0.11a	6.18 ± 0.6a
>4	88	0.27 ± 0.10a	1.35 ± 0.23a	0.55 ± 0.15a	0.13 ± 0.06a	2.92 ± 0.3a	0.18 ± 0.10b	0.26 ± 0.12a	5.66 ± 0.3a
Coat colour									
Black	57	0.07 ± 0.04a	1.60 ± 0.29b	0.30 ± 0.13a	0.09 ± 0.06b	2.72 ± 0.3a	0.26 ± 0.12a	0.11 ± 0.07a	5.14 ± 0.3b
White	62	0.19 ± 0.12a	1.03 ± 0.22b	0.42 ± 0.14a	0.03 ± 0.02b	2.48 ± 0.4a	0.18 ± 0.13a	0.37 ± 0.17a	4.71 ± 0.3b
Brown	23	0.13 ± 0.10a	2.83 ± 0.77a	0.61 ± 0.42a	0.48 ± 0.39a	3.13 ± 0.6a	0.35 ± 0.25a	0.00 ± 0a	7.52 ± 1.0a
B & W	58	0.28 ± 0.10a	1.22 ± 0.25b	0.22 ± 0.10a	0.14 ± 0.1ab	3.03 ± 0.3a	0.43 ± 0.20a	0.22 ± 0.12a	5.55 ± 0.4b

Means (±SE) followed by the same letter in each column for each parameter are not significantly different at 5% according to REGW range test. B & W = Black and White.

3.2%), *H. impeltatum* (n = 26, 2.4%) and *H. dromedarii* (n = 9, 0.8%). For the majority of parameters investigated, no significant difference was found given the low number of ticks collected per animal. El Mihaireba recorded the highest mean number of ticks followed by Abugota, Aburuwaishid, El Hoda and El Managil (Table 1). The highest mean of *H. anatolicum* was recorded in El Hoda and the lowest in El Managil. According to the sex of cattle, the highest total mean of ticks was recorded on female cattle (Table 1). The highest total mean of ticks was on Butana type followed by cross-bred, then Kenana. The highest mean total of ticks was recorded from age group 1 < 4 years followed by >4 years whereas calves of <1 year carried the lowest tick means. According to the coat colour of the cattle, the highest mean of tick infestation was carried by brown ones, followed by black and white coat colour, black coat colour and white coat colour cattle.

The highest prevalence of *Theileria* spp. piroplasms was in Abugota followed by El Hoda, El Mihaireba, El Managil and the lowest was in Aburuwaishid (Table 2). The prevalence was higher in males than in females. The prevalence was the higher among cross-bred (Friesian x Zebu) than Zebu breeds (Table 2). Out of 200 samples of lymph node biopsy smears, 29 cattle (14.5%) were positive for *Theileria* spp. macroschizonts. The highest prevalence was in El Mihaireba followed by Abugota, El Hoda, El Managil and the lowest was in Aburuwaishid (Table 2). The prevalence was higher in females and in cross-bred (Friesian x Zebu) and then Zebu breeds and highest among cattle from one year to less than four years old heifers and steers.

The overall prevalence of *Theileria annulata* antibodies was 94/200 (47%). The highest prevalence was in Abugota followed by El Mihaireba, El Huda, El Managil and the lowest was in Aburuwaishid. Higher prevalence was among cross-bred (Friesian x Zebu) followed by Zebu breeds and among calves up to one year old followed by cattle less than four years old heifers and steers and the lowest prevalence in cattle older than four years.

The level of agreement between blood smear (BS) and lymph node smear (LNS) techniques was very high (88%) with kappa coefficient value of 0.5422 (Table 3). The level of agreement between BS technique and indirect fluorescent antibody (IFA) test result was (68.50%) with kappa coefficient value of 0.3436 (Table 3). The same result was obtained for the level of agreement between LNS and IFA test to be (66.50%) with kappa coefficient value of 0.3002.

The cross tabulation between BS and LNS revealed that 19 positive *T. annulata* samples agreed between the two tests. Likewise, the two tests agreed on 157 samples as designated negative. On the other hand, 10 samples were positive by LNS but negative by BS. The same was reported for 14 samples positive by BS but negative by LNS. The cross tabulation between BS and IFA test showed that the two tests agreed on 32 positive samples, and 105 negative samples. The two tests disagreed on one sample which was positive by BS but negative by IFA test. Also, there were 62 samples positive by IFA test but negative by BS.

Table 2
Prevalence of *Theileria* spp. piroplasms determined by blood smears (BS), *Theileria* spp. schizonts determined by lymph nodes smears (LNS) and *T. annulata* antibodies using indirect fluorescent antibody (IFA) test among cattle in Central Gezira State in January, May and August 2014.

Parameters	Animals examined	BS	LNS	IFA
		Positive *Theileria* spp. piroplasms (%)	Positive *Theileria* spp. schizonts (%)	Positive *T. annulata* antibodies (%)
Localities				
Aburuwaishid	40	2 (5)	3 (7.5)	14 (35)
El Managil	40	3 (7.5)	5 (12.5)	17 (42.5)
El Huda	40	8 (20)	6 (15)	19 (47.5)
El Mihaireba	40	7 (17.5)	8 (20)	20 (50)
Abugota	40	13 (32.5)	7 (17.5)	24 (60)
Total	200	33 (16.5)	29 (14.5)	94 (47)
Animal sex				
Bulls	56	17 (30.4)	8 (14.3)	39 (69.6)
Cows	144	16 (11.1)	21 (14.6)	55 (38.2)
Total	200	33 (16.5)	29 (14.5)	94 (47)
Animal breed				
Zebu	74	8 (10.8)	7 (9.5)	29 (39.2)
Cross	126	25 (19.8)	22 (17.5)	65 (51.6)
Total	200	33 (16.5)	29 (14.5)	94 (47)
Animal age (years)				
<1	68	15 (22.1)	9 (13.2)	41 (60.3)
>1 < 4	44	10 (22.7)	7 (15.9)	25 (56.8)
≥4	88	8 (9.1)	13 (14.8)	28 (31.8)
Total	200	33 (16.5)	29 (14.5)	94 (47)
Animal coat colour				
White	62	4 (6.5)	5 (8.1)	25 (40.3)
Black	57	12 (21.1)	12 (21.1)	27 (47.4)
Brown	23	4 (17.4)	5 (21.7)	11 (47.8)
Black & white	58	13 (22.4)	7 (12.1)	31 (53.4)
Total	200	33 (16.5)	29 (14.5)	94 (47)

Table 3
Level of agreement between blood smears (BS), lymph nodes smears (LNS) and indirect fluorescent antibody (IFA) test for detection of *Theileria* spp. piroplasms, macroschizonts and *T. annulata* antibodies respectively, by using Sigma Stata for windows version 2.0 (Kappa Coefficient).

	Agreement	Expected agreement	Kappa	Z	Pr > Z
BS x LNS	88.00%	73.87%	0.5422	7.69	0.0000
BS x IFA test	68.50%	52.01%	0.3436	6.29	0.0000
LNS x IFA test	66.50%	52.13%	0.3002	5.78	0.0000

4. Discussion

The cattle population has increased rapidly in Gezira State due to the increase in number of agricultural farms and availability of agricultural byproducts of sorghum, cotton plant, groundnut and wheat plant (Anon, 2013). This cross-sectional survey was conducted to provide an understanding on the prevalence of ticks and tropical theileriosis in Central Gezira State. High prevalence of *T. annulata* antibodies in Abugota 60%, El Mihaireba 50% and El Hoda 47.5% were reported where most of cross-bred cattle are kept. *Rhipicephalus evertsi evertsi* was recorded in all localities with the highest mean in El Mihaireba. Balfour (1911, cited in Hoogstraal, 1956), mentioned that *R. evertsi evertsi* was introduced to Khartoum on cattle from South Sudan (Malakal). This study revealed that the tick species of *R. evertsi evertsi* predominate compared to all other tick species (51.6%) and it is wide spread in the state and infested all the breed of cattle and in all age groups which may indicate that this tick species is tolerant to the environment in the area. Its role in transmission of *Theileria*, *Babesia* and *Anaplasma* spp. should be investigated. *Rhipicephalus decoloratus* that was found in three localities is possibly attributed to the wet and dry period during collection (Osman et al., 1982). Salih et al. (2004) reported that few numbers of *R. decoloratus* on cattle in Madani, as they are restricted to the southern parts of the central Sudan (FAO, 1983a). *Rhipicephalus s. sanguineus* was reported in El Mihaireba and Abugota only in the northern parts of the state. The absence of this species from other localities can be attributed to the absence of dogs from farms where the dogs are regarded as the main host of this tick species beside sheep, goat, birds, horses and donkeys (Hoogstraal, 1956).

Amblyomma lepidum was recorded in Abugota, El Mihaireba and El Managil in low abundance which could coincide with the spread of heartwater disease, while *A. variegatum* was not recorded in this study. Salih et al. (2004) reported that *A. variegatum* was not reported in Madani. The low abundance of the camel ticks *H. impeltatum* and *H. dromedarii* in this study could be due to the fact that cattle do not frequently come in contact with camels in these areas. The current distribution of ticks in Gezira State is affected by; the cross breeding programmes in cattle that occurred in the last 30 years, movement of animals across the state, dense vegetation in irrigating schemes and small bushes in other lands, rainfall in July, August and September and type of the soil which in most areas are clay. The free movement of cattle from the Butana region of Eastern Sudan into the Gezira scheme after harvesting the crops allows dispersal of many tick species. Some environmental factors were observed that could possibly be responsible for the low tick abundance. These factors include deforestation, misuse of land that established large mechanized schemes and animal movement due to lack of feed in dry season (Hassan and Salih, 2013). Other factors such as, application of acaricides and biological means of control through introduction of chickens (Hassan et al., 1991, 1992) as practiced in many cattle farms might have resulted in reducing tick numbers as well as regular removal of manure for manufacturing building bricks. Cattle owners control ticks by using acaricides randomly which may have caused fluctuation in tick population (FAO, 2004). The disappearance of any tick species in any given period does not mean its absence from the area, as it can reappear when the optimum climatic conditions prevail (Osman et al., 1982).

The results showed that calves younger than one year old carried low numbers of tick species compared to other age groups except for *R. e. evertsi*. This finding is in line with El Imam (2003). Young calves are kept in fences with less contact with tick sources like plants and grasses in the grazing field. Hassan (1997), also, found less ticks on calves and he attributed that to maternal immunity and small size of calves. However, Latif et al. (1991) found no differences between numbers of ticks carried by calves 12–18 months old and older cattle. The finding that Butana and cross-bred types carried more ticks than Kenana type could be attributed to the fact that Kenana type is more resistant to ticks infestation than other cattle types (Latif, 1984). However, these results are essentially observational because not equal numbers of each category have been sampled.

Cattle of brown coat colour carried significantly more ticks than cattle of white coat colour. On the contrary, Hassan (1997) found that tick burdens were correlated with host coat colour and cattle of white coat colour carried significantly more ticks than brown host, while black cattle carried the least number of ticks. He suggested that ticks picked by animal with black or brown coat colour die or leave before the attachment, due to the relatively raised temperature in the host skin microenvironment generated by the dark coat colour. Female cattle carried more ticks than males except for *H. anatolicum* which infested males more than females. The number of ticks collected per animal was so low that no meaningful conclusion could be made regarding the parameters investigated and tick number. In order to construct a tick prevalence and distribution map in Gezira State, large scale and systematic survey is recommended including collection of ticks from bovine, ovine, caprine and equine species from different localities of the state throughout the year to cover all seasons.

In Gezira State, Giemsa stained blood and lymph smears are the only available methods for field veterinarians to detect piroplasms and schizonts. Some of piroplasms detected in blood smears could be *T. mutans* or *T. velifera* which are not pathogenic to cattle. It may, also, be an indication of a subclinical case of *T. annulata* infection creating an enzootic stable situation. *T. annulata* infection was found to be widespread in all localities investigated during this study. The sero-prevalence of *T. annulata* and the

proportion of positive sera, was well correlated with the prevalence of the tick vector *H. anatolicum*, hence the values of *T. annulata* antibodies observed were in Abugota 60%, El Mihaireba 50%, El Hoda 47.5%, El Managil 42.5% and Aburuwaishid 35%. FAO (1983b) recorded 76% in Nisheishiba and 3% in Um Benin in Eastern parts of Gezira. The current result in comparison with those of FAO indicates that the infection rate of tropical theileriosis decreases in a southwards direction and is correlated with level of infestation by *H. anatolicum*. Kenana type cattle which were found in the southern parts of the state were resistant to ticks (Latif, 1984). The result of cross tabulation between BS test and IFA test revealed that 62 cattle were seropositive, but no parasite was seen by BS test. This result indicated that seropositive cattle carried antibodies in their blood for a long period post infection or the parasitaemia was below the limit of detection of blood smear. It has to be stated that the serology (IFAT test) measures a different parameter from the smear tests, ideally the most sensitive, specific test probably PCR should be used for this type of comparison.

Theileria spp. piroplasms detected in the blood smears should not be considered as tropical theileriosis infection unless confirmed by macroschizonts detection in the lymph node biopsy smears. This method was not considered confirmatory compared to other methods (Shayan and Rahbari, 2005). In the current study, *Theileria* spp. piroplasms were detected in 16.5% of BS similar to that of El Ghali and El Hussein (1995) who diagnosed 14.8% and 18.0% incidence of clinical theileriosis during the years 91/92 and 92/93, respectively among cattle admitted to Atbara Veterinary Hospital, Northern State using BS test. In the current study, 16.5% was detected by BS and 14.5% by lymph node biopsy smears for *Theileria* spp. schizonts, in cases among apparently healthy cattle.

Prevalence of *Theileria* spp. schizonts detected by blood and lymph node biopsy smears was slightly higher in female cows than in males. This may be attributed to stress factors like pregnancy and lactation which affect the immunity status of the female cows. This finding agrees with Darghouth et al. (1996) who reported that older cattle in Tunisia had a lower immunity against *Theileria* spp. due to the fact that pregnancy and lactation act as stress factors. However, these results are essentially observational because not equal numbers of each category have been sampled. On the other hand, the current study revealed that cattle of four years and older had a higher *Theileria* spp. schizonts than young calves. This finding could be attributed to the high infestation of *H. anatolicum* in older cattle and it may indicate that group age is a risk factor. Prevalence of *Theileria* spp. schizonts was more prevalent among cross-bred than in Zebu breeds, 17.5% and 9.5%, respectively. This supports the suggestion that the cross-bred cattle are more sensitive to theileriosis than the local breeds which have the ability to limit the macroschizonts index as reported by Bakheit and Latif (2002).

In conclusion, this is the first study of this kind to be conducted in that region of Gezira State. The study revealed that *Hyalomma anatolicum* is widespread in Gezira State and coinciding with incidence of tropical theileriosis evidenced by seropositivity of 47%, *Theileria* spp. piroplasms 16.5% and *Theileria* spp. schizonts 14.5%. It has to be kept in mind that, in spite of high seropositivity of infection, the number of ticks on animals is low. This would indicate past infection more than clinical cases. The study showed that *R. evertsi evertsi* are the predominant all tick species and its role in *Theileria*, *Babesia* and *Anaplasma* spp. transmission need to be investigated in the future. It is recommended to conduct more research including more numbers of animals to study the prevalence and population dynamics of ticks throughout the year to determine seasonality and prevalence of *Theileria annulata* infection using enzyme linked immunosorbent assay and molecular characterization using polymerase chain reaction.

It has to be emphasis that the differences reported herein are observational/preliminary and need a larger study with good design to remove confounding factors before they can be confirmed.

Acknowledgements

The authors would like to express their thanks to the staff of Animal Resources Administration, Hassahessa Locality, Gezira State for their help, encouragement and support. Our thanks are also extended to the cattle owners and pastoralists for their permission to take samples from their animals. This study was supported by Deutsche Forschungsgemeinschaft (DFG) project 'Molecular epidemiology network for promotion and support of delivery of live vaccines against *T. parva* and *T. annulata* infection in Eastern and Northern Africa' (AH 41/7-1).

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