



Seroprevalence of *Theileria annulata* antibodies measured by indirect *Ta SP* ELISA among dairy cattle raised at Elhoush district, Gezira state, Sudan

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Abstract

In this study, enzyme-linked immunosorbent assay (ELISA) test was performed using *Theileria annulata* surface protein “*TaSP*” antigens for detection of antibodies against *T. annulata* to screen the infection within dairy cattle raised at Elhoush district, Gezira State, Sudan. Out of 474 serum samples, collected from pure local and crossed breed of both sexes and different age groups, 344 (72.6%) were found positive for *T. annulata* antibodies. The prevalence rate of *T. annulata* antibodies in female (80%) was higher than in males (20%). The highest prevalence of *T. annulata* antibodies according to age groups was (84.2%) among cattle from one to three years old followed by cattle more than three years old (75.5%) and the lowest prevalence rate (60.8%) was recorded for calves less than one year old. According to cattle breeds, the prevalence rate of *T. annulata* antibodies for Friesian was (76.0%), followed by local breeds (72.0%), Jamoos (70.4%) and Garcy (60.0%), respectively. The highest prevalence rate (79.8%) of *T. annulata* antibodies was detected in autumn, followed by winter (72.0%) and the lowest prevalence rate (68.6%) was detected in summer. The findings is a contribution to the understanding of the epidemiology of the tropical Theileriosis in such a region.

Keywords: Seroprevalence, *Theileria annulata*, cattle, Gezira, Sudan

Introduction

Tick-borne diseases (TBDs) pose a major constraint of livestock production and have considerable economic impact to rural people affecting not only their food supply, but also their daily income and other agricultural activities [1]. In 1997, the annual global losses associated to ticks and TBDs in cattle was estimated to amount between US\$ 13.9 billion and US\$ 18.7 billion [2]. Four groups of TBDs are of importance to the livestock production: theileriosis, babesiosis, anaplasmosis and heartwater (also called cowdriosis), posing major health and management problems of cattle and small ruminants in affected areas. In endemic areas, indigenous cattle have developed resistance to ticks and to tick-borne pathogens (TBPs) [1]. However, susceptibility of exotic breeds presents a major obstacle to the improvement of cattle production.

T. annulata is transmitted by ticks of the genus *Hyalomma* after cyclical development in these ticks [3]. The endemic areas of *T. annulata* extended beyond the Mediterranean regions in Southern Europe, Northern Africa including Mauritania, and Central Sudan and Himalaya [4]. Tropical theileriosis has long been recognized as a hindrance to the development of sound dairy industry in the Sudan and is a cause of major economic losses. Serological surveys indicated that *Theileria. Annulata* infection is widespread in the country but the disease mostly affects exotic dairy breeds and their crosses with indigenous breeds. Additionally, the disease has recently been identified

in Darfur and southern parts of Blue Nile State in dairy farms around large urban areas where it has never been detected before [5, 6].

T. annulata has also been reported along with *T. mutans* in the Sudan since the 1920s. Sero-epizootiological surveys conducted in Khartoum, White Nile, Gezira, and Blue Nile Provinces using immune fluorescence antibody (IFA) test revealed an overall *T. annulata* sero-prevalence rate of 38.9 % [7]. The prevalence rate declined from 90 % in Khartoum Province to less than 13 % in Blue Nile Province coinciding with the abundance of the tick vector (*Hyalomma anatolicum*) [8]. Later, by using Enzyme-linked immunosorbent assay (ELISA), antibodies against *T. annulata* were shown to be prevalent all over the Sudan ranging from 86.5 % in Khartoum to 6.3 % in Nyala [9]. Molecular diagnosis utilizing polymerase chain reaction (PCR) and reverse line blot (RLB) showed that the prevalence rates of 48.1 and 65.4 % of *T. annulata* were respectively recorded in dairy cattle in Khartoum State [10]. These studies showed that *T. annulata* infection is prevalent in all the states and in all age groups but is the highest among older animals. Age, geographic areas, and management systems are considered as the main risk factors for *T. annulata* Infection [11].

T. annulata has been reported along with *T. mutans* in the Sudan since the 1920s. Earlier surveys based on Giemsa stained blood and lymph node biopsy smears have shown high

prevalence and widespread nature of *T. annulata* infection in Northern Sudan [5, 12, 13]. These studies have also shown that a high percentage (in excess of 35 %) of the animals examined were healthy carriers of *Theileria* spp. piroplasms, and that infection was more prevalent in crossbred than in indigenous animals [5, 9]. Sero-epizootiological surveys conducted in Khartoum, White Nile, Gezira, and Blue Nile Provinces using immunofluorescence antibody (IFA) test revealed an overall *T. annulata* seroprevalence rate of 38.9 % [7]. The prevalence rate declined from 90 % in Khartoum Province to less than 13 % in Blue Nile Province coinciding with the abundance of the tick vector (*Hyalomma anatolicum*) [8]. Later, by using enzyme-linked immunosorbent assay (ELISA), antibodies against *T. annulata* were shown to be prevalent all over the Sudan ranging from 86.5 % in Khartoum to 6.3 % in Nyala [8]. Using polymerase chain reaction (PCR) and reverse line blot (RLB), the prevalence rates of 48.1 and 65.4 % of *T. annulata* were respectively recorded in dairy cattle in Khartoum State [10]. These studies showed that *T. annulata* infection is prevalent in all the states and in all age groups but the highest among older animals. The objectives of this study was to estimate the seroprevalence of *T. annulata* antibodies in cattle raised semi intensive commercial management at Elhoush district, Gezira State.

Materials and Methods

Study site

The study was carried out at Elhoush district in south Gezira State, which lines between latitudes 14° 05' 00" 30', 14° 06' 10" N and longitudes 33° 28' 35", 33° 29' 52" E (Figure 1). The area enjoys an annual rainfall of 272.1 millimeters, relative humidity is 70– 180% decrease in summer and winter (18–32%) and temperature is between 36.5°C–20.6°C.

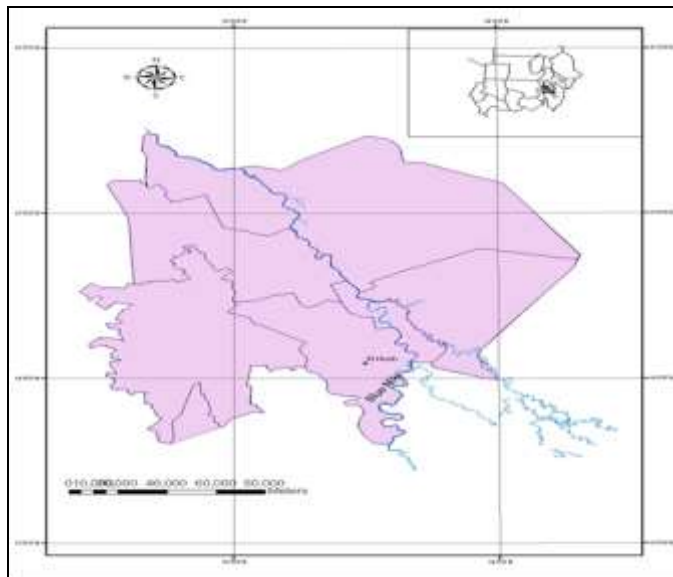


Fig 1: Map of Gezira State, indicating Elhoush District from where serum samples were collected

Sera collection

The puncture area of the jugular vein was cleaned by 70% ethanol. About 5ml of blood was collected by syringes and transferred into stopped glass test tube. Each tube was labeled

indicating, season, breeds, age, sex, and date of collection. The blood was allowed to clot at room temperature for 2-3 hours. Then the tubes were kept overnight in refrigerator at 4°C. and then centrifuged at 1500 rpm for 10 minutes. Serum was, then, removed by a separate Pasteur pipette for each sample and transferred into clean serum containers. Serum samples were then, labeled and kept at –20°C until used.

Enzyme-linked immunosorbent assay (ELISA) procedure

The recombinant *T. annulata* surface protein “*TaSP*” [14] was used in the test. The test was performed according to [15] protocol. *TaSP* antigen was diluted (1 µg/ml) in carbonate–bicarbonate coating buffer and immobilized onto 96-well ELISA plates (Nunc, Den-mark) by incubation for 1 h at 37°C. The plates were then washed and incubated for 1 h at 37°C with blocking buffer (PBS pH 7.2, supplemented with Tween-20 (0.05%) and skimmed milk (1%) (PBSTM). The plates were washed three times with PBS supplemented with Tween-20 (BPST). Sera were added at the dilution of 1:500 in PBSTM and incubated for 1 h at 37°C. All samples were tested in duplicates in the same plate. Plates were washed three times with PBST and the conjugate (rabbit-anti-bovine antibodies conjugated with horse-radish peroxidase) diluted at 1:10,000 in PBSTM was added and the plates were again incubated for 1 h at 37°C. The plates were washed three times with PBST. Thereafter, a substrate/chromogene (hydrogen peroxide (H₂O₂)/tetramethyl benzidine (TMB) solution was added to each well. Color development was allowed for 20 min after which the reaction was stopped using 1 M phosphoric acid. Absorbance was measured at 450 nm using an ELISA reader (Labsystem, Multiskan, RC). Each plate contained one positive and one negative reference serum sample, and each one was applied into four wells of the plate. The median OD values of the control sera as well as the mean OD of the duplicate test sera were calculated and the results were expressed as a percentage positive (PP) value of the reference positive control.

Statistical analysis

Data were subjected to appropriate general linear model (GLM) procedure of statistical analysis system (SAS) package. SAS was used to perform analysis of variance (ANOVA) while mean separation was performed using Ryan–Enot–Gabriel–Welsch (REGW) multiple range test. Association between antibodies prevalence among cattle breed, age, sex and seasons were assessed using the *chi*-square.

Results and Discussion

Out of 474 cattle sera collected from Elhoush district, Gezira State and tested by indirect ELISA *TaSP*, 344 (72.6 %) were found to be positive with *T. annulata* antibodies (Table 1). *T. annulata* antibodies were significantly ($P < 0.05$) prevalent among females (73.3%) than males (69.7%) (Table 1). There were significant association ($P < 0.05$) for seroprevalence of *T. annulata* antibodies among different age groups. The highest (84.2%) prevalence rate of *T. annulata* antibodies was reported in cattle from one to three years old followed by (75.5%) in cattle more than three years old and the lowest prevalence rate (60.8%) was in calves less than one year old

(Table 2). According to cattle breeds, the highest (76.0%) prevalence rate of *T. annulata* antibodies was in Friezian, followed by (72.0%) in local breeds (70.4%) in Jamoos and (60.0%) in Gearcy breed (Table 3). The antibodies persisted throughout the year at each breed, sex and age groups, and seasonality prevalence showed significant ($P < 0.05$) difference between different seasons. The highest (79.8%) prevalence rate of antibodies was detected in autumn, followed by (72.0%) in winter and the lowest (68.6%) was in summer (Table 4).

In the present investigation, Indirect ELISA for *T. annulata* antibodies was performed with TaSP protein as described [15], the overall prevalence rate of *T. annulata* among cattle at Elhoush district, south Gezira State was 72.6%. Latterly, some serological investigations have been conducted on tick-borne diseases in the Sudan. They despite that the tick-borne diseases constitute major obstacles to livestock production development in the country. The earlier investigation carried out by the Food and Agriculture Organization (FAO) group. The triangle between the Blue Nile and White Nile in Central Sudan was covered using IFAT [16]. While sero-prevalence using TaSP and IFAT for *T. annulata* antibodies were carried out in different parts of Sudan [9, 11].

The ELISA antibodies reported against *T. annulata* were prevalent all over the Sudan ranging from 86.5 % in Khartoum to 6.3 % in Nyala using IFAT [9]. Other investigations [17, 18] revealed that the overall prevalence rates of *T. annulata* were found to be 30.8%; the highest sero-prevalence for *T. annulata* (92.5%) was reported in El Damer followed by Atbara (80%). Likewise, the highest (40%) level of *T. annulata* antibodies was reported in Tokar followed by (36.7%) in Port Sudan and (16.7%) in GounbW Aoleeb [19, 20]. Using the same technique (TaSP ELISA) the antibody prevalence rates of 86.2%, 29.3%, 10%, 31.7%, and 15 % were recorded from northern Sudan, Blue Nile, eastern Sudan, White Nile, and western Sudan, respectively [21]. The highest rate (92.5 %) was recorded from

Eldamer town and the lowest (5 %) was recorded in Nyala, western Sudan. In this study there were no significant associations for the seroprevalence of *T. annulata* among different age groups. Similar result were also reported [22], they showed that there were no significant associations for the seroprevalence of *T. annulata* reported among different age groups. The current study showed that breed has no significant effect on the sero prevalence of *T. annulata* among cattle in Gezira state there were no significant associations for the seroprevalence of *T. annulata* of among different cattle breeds or sex of examined animals; this finding is strongly supported [22]. However, an outbreaks and individual clinical cases of tropical theileriosis in the Sudan are mainly seen in exotic breed animals or their crosses. The disease is rarely seen in indigenous animals except when they are under stress, e.g., during pregnancy and lactation stress, heat stress, or nutritional stress [23, 24, 13]. In this study, the sero-prevalence of *T. annulata* antibodies showed a significant difference ($P \leq 0.05$) between different age groups of cattle.

It's worth mentioning that in this study, the season as a risk factor exhibited significant difference ($P \leq 0.05$) in the seroprevalence of *T. annulata* antibodies; The high prevalence of *T. annulata* antibodies was reported in winter, this may be due to increase numbers of the vector *H. anatolicum* after rainy season in Gezira and lack of adequate feeding for infected cattle during these months, the humidity and temperature during the winter is also suitable for development of tick vector. Other studies reported that most incidences of the disease are recorded during the hot-dry summer (May-July) and cold-dry winter (November-January) with peaks in both seasons (El Ghali and ElHussein 1995; Zakia *et al.*, 2003) [24, 13]. These peaks are most probably due to stress caused by heat and lack of adequate feed during summer and change in microclimate that is conducive to high tick activity during winter months (ElGhali and ElHussein 1995; Zakia *et al.*, 2003) [24, 13].

Table 1: Seroprevalence of *Theileria annulata* by indirect Ta SP ELISA by cattle sex at Elhoush District

Sex	No. Tested	No. + ve	Prevalence (%)	95% CI	
				Lower	Upper
Female	375	275	73.3	68.82	77.78
Male	99	69	69.7	60.65	78.75
Overall	474	344	72.6	68.58	76.62

Different superscripts indicate significant difference at $P \leq 0.05$

Table 2: Seroprevalence of *Theileria annulata* using indirect TaSP ELISA by age groups (years) at Elhoush District

Age groups (years)	No. tested	No. + ve	Prevalence (%)	95% CI	
				Lower	Upper
≤1	153	93	60.8	53.06	68.54
>1 - 3	101	85	84.2	77.09	91.31
>3	220	166	75.5	69.82	81.18
Overall	474	344	72.6	68.58	76.62

Different superscripts indicate significant difference at $p \leq 0.05$

Table 3: Seroprevalence of *Theileria annulata* using indirect TaSP ELISA by season at Elhoush District during 2011

Season	No. tested	No. + ve	Prevalence (%)	95% CI	
				Lower	Upper
Autumn	104	83	79.8	72.08	87.52
Winter	186	134	72.0	65.55	78.45
Summer	185	127	68.6	61.91	75.29

Overall	474	344	72.6	68.58 - 76.62
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Table 4: Seroprevalence of *Theileria annulata* using indirect TaSP ELISA by cattle breeds at Elhoush District during 2011

Breeds	No. Tested	No. + ve	Prevalence (%)	95% CI	
				Lower	Upper
Friesian	104	79	75.6	67.79	84.21
Local	328	237	72.3	67.53	77.27
Jamoos	27	19	70.4	53.18	87.62
Gearcy	15	9	60.0	35.21	84.79
Overall	474	344	72.6	68.58	76.62

Different superscripts indicate significant difference at $p \leq 0$.

Conclusion

The findings imply that TaSP ELISA is a useful tool in the study of the epidemiology of tropical theileriosis. It is, also, concluded that the prevalence of tropical theileriosis is widely distributed in the study area. It was clearly evident from the present investigation that cattle raised at El Housh district, Gezira State are highly susceptible to tick infestations. The study revealed that tropical theileriosis exists in cattle in Gezira state as indicated by high percentage of sero prevalence for *T. annulata* among dairy cattle. The disease therefore poses a real threat for dairy and beef industries and exportation of live animals. Eradication of the vector tick (*H. anatolicum*) is only a theoretical solution of a very limited practical value. Therapeutic and/or prophylactic treatment with drugs is rather expensive and not always available. Nevertheless, immunization of cattle against *T. annulata* with a candidate vaccine based on molecular technique (DFG AH 41/7-1) would hopefully be the radical solution for this problem. The previously reported natural outbreaks were equally serious.

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