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Investigation of Ovine Theileriosis in Animal Production Research Stations (APRS) in the Sudan

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Abstract:
A cross sectional study was conducted in May (summer) and December (winter) 2016 to investigate the prevalence of ovine theileriosis and its association with the presence of the vector (ticks) in Elhuda and Elnuhud research stations in the Sudan. A total of 400 blood samples were collected, 200 samples from each station and age, sex, ecotype and body condition score of these animals were recorded. In Elhuda station, the positive results of microscopic examination for *Theieria* spp were 15.3% (31 out of 202) while in Elnuhud were 11.6% (23 out of 198) with an overall prevalence 13.4%. Fluorescent antibody test (IFAT) showed that, 27.7% of samples (56 out of 202) and 16.2% of samples (32 out of 198) were positive for *Theileria lestoquardi* antibodies in Elhuda and Elnuhud respectively, with an overall prevalence 22.0%. The main prevalent Ticks in Elhuda were *Rhipicephalus evertsi evertsi* and *Hyalomma anatolicum* while in Elnuhud in addition to these two species *H. dromedarii* and *H. impeltatum*. Were present the results showed significant association between *T. lestoquardi* infection and following factors under significant level of P-value ≤ 0.005; including ecotype (p-value = 0.005), Body Condition Score (p-value =0.003), Month (p-value =0.000) and location (P-value = 0.005).
Keywords: Theileria spp, sheep, Ticks, IFAT.

Introduction:

Piroplasmosis caused by different tick-borne haemoprotozoan parasites is a major constraint of small ruminant production in Asia, Africa and southern Europe (Mehlhorn and Schein, 1984). The infection, caused by pathogenic *Babesia* and *Theileria* species is associated with a high degree of morbidity and mortality particularly in exotic animals (Mehlhorn *et al.,* 1994). Piroplasmosis caused by different tick-borne haemoprotozoan parasites is a major constraint of small ruminant.

Although cattle theileriosis has been extensively investigated, little is known about sheep theileriosis (Gao *et al*., 2002). Later interest has arisen in sheep theileriosis (Altay *et al*., 2005).*Theileria lestoquardi,* previously known as *T. hirci,* is an important tick-borne protozoan parasite of sheep in the world, It occurs in south-eastern Europe, northern Africa, western and central Asia and in India (Uilenberg, 1981).It was assumed that is pathogenic for small ruminants (Luo and Yin 1997).It causes malignant ovine theileriosis (MOT), also known as Malignant Small Ruminant theileriosis, which causes acute, subacute or chronic forms of the disease leading to high mortality rates among sheep. The pavasites is mainly transmitted by *Hyalomma anatolicum* (Soulsby, 1982).This species is considered the only

one that is highly pathogenic compared with other species (*T. lestoquardi, T.ovis* and *T. seperata*) of ovine theileriosis (Uilenberg, 1981) are less pathogenic and have lower importance (Soulsby, 1982).

In order to improve the control measures against tick-borne disease, including MOT, future research on these diseases are needed. Additionally, it's necessary to define the prevalence of tick-borne pathogens in the target populations, moreover the accurate identification of these organisms is essential to understand their epidemiology to update their geographical distribution and to develop new tools for diagnosis (Oura *et al*., 2004).

Referring to all above, the aim of the present study was to performed to dedect *T. lestoquardi* involved in ovine theileriosis in Elhuda and Elnuhud research Stations.

Material and Methods:

Study area:

1- El-Huda Research Station:

El-Huda stationis located at approximately 14°15'N latitude and 320°50'E longitude, at an altitude of about 250 m, about 90 km north-west of Wad Medani and about 150 km south of Khartoum, concerns with three subtypes of Sudanese desert sheep (Shugor, Dubasi and Watish) and comprises a number of 250 heads (APRC, 2016) (Figure:1).

2- **El-Nuhud Research Station:**

El-Nuhud station is located in West Kordofan State, latitude and longitude are 12°42'N, 28°25

59.99'E, which found in savannah geographical zone and concerns with two subtypes of Sudanese desert sheep (Hamari and Kabashi) with a number of 300 head (APRC, 2016)

(Figure: 1).

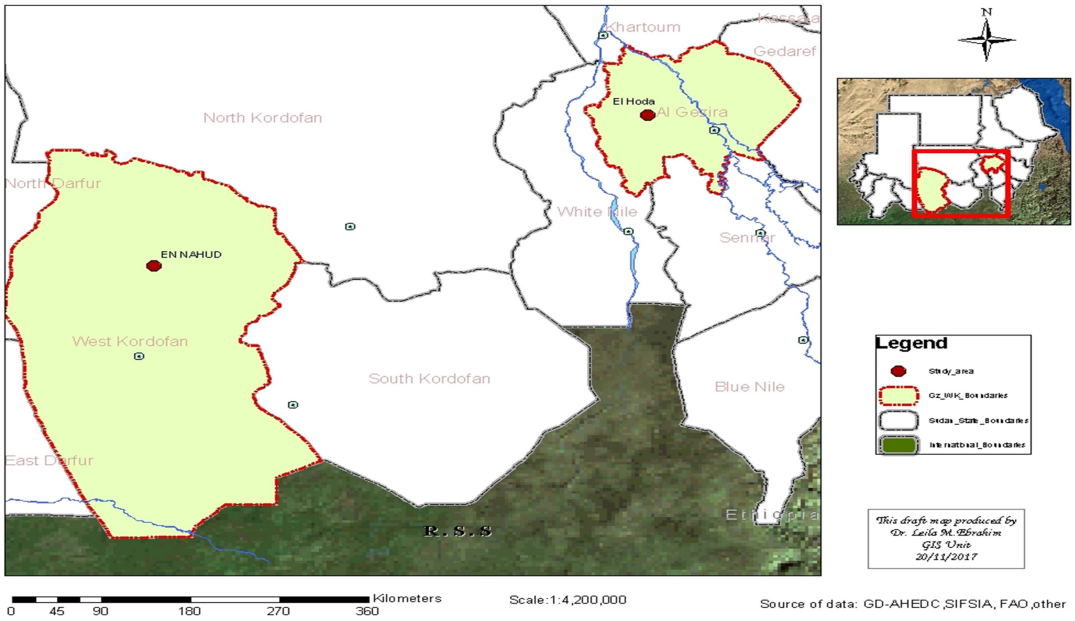

Figure: 1: Study area map

Sample collection and study design:

202 sheep were randomly chosen from Elhuda and 198 sheep from Elnuhud research station each samples collected at two months (May and December 2016). The blood was collected from the jugular vein and transferred into tubes containing Ethylene Diamine Tetra-acetic

Acid (EDTA) and immediately used for blood smears preparation. .For sera preparation, blood was collected in plain vaccutainers and left at room temperature until serum separation. The sera were then collected in Ependorf tubes and stored at – 20 0C till used.

Information regarding age, sex, ecotype and body condition score of the animals were recorded during samples collection. The ages of the animals were determined and they were conveniently classified into three groups, animals over three years, animals between one and three years and animals under one year old. The body condition scores of animals were evaluated and classified as poor, moderate and good. The ecotype of sheep were classified into Shugor, Dubasi, Watish, Hamari and Kabashi.

Blood Smears:

Thin blood smears were prepared and fixed in methanol for 5 min and stained with 10% dilute Giemsa's solution for 45 min. Blood smears were examined for intraerythrocytic forms of *Theileria* spp. piroplasm under ×100 objective magnification. More than 50 microscopic fields per slide were observed.

Antigen preparation:

The schizont antigen was prepared according to a published protocol (FAO, 1984). Briefly, the antigen was prepared from a local *T. lestoquardi* cell line at low passage (<20 passage) in12-well Teflon-coated multispot slides (Highveld Biological, USA). Antigen-coated slides were individually wrapped in tissue paper and then packed in aluminum foil with five slides in each packet. The slide packets were labeled and stored in air tight, waterproof plastic containers at −20 ◦C until used.

Conjugate:

Rabbit anti-sheep immune gamma globulin (IgG) conjugated to fluorescein isothiocyanate (FITC) were obtained from Nordic Immunological laboratory, Netherland.

Control sera:

A positive control serum (C+ve) was obtained from Razzi institute, Iran while the negative control sera (C-ve) was obtained from BDSL, UK. Both control sera(C+ve and C-ve) were diluted directly to 1\80.

Indirect fluorescent Antibody (IFA test):

Materials preparation and running the test were performed as described by Burridge *et al.*, (1974) and FAO, (1984). The examination was carried out using Olympus vanox incident-light excitation fluorescent microscope (Japan). The slides were examined under 40X objective using diluted glycerol (nine part of glycerol and one part PBS).

Data analysis:

The collected data entered into an Excel spreadsheet (Microsoft Excel, 2007). Statistical analysis was performed using statistical package for the social sciences (SPSS), version 16 software. Percentage was used to calculate prevalence. Data were statistically analyzed using Chi-squared test to calculate degree of association between risk factors and prevalence of *Theileria lestoquardi* infection, 95% confidence interval (CI) and $p \leq 0.005$ was considered for statistically significant difference.

Results:

Theileria spp were detected in 31 out of 202 (15.3%) animals by microscopic examination in Elhuda, while 23 out of 198 (11.6%) animals were detected in Elnuhud, with an overall prevalence of (13.4%) (Table: 1; Fig: 2).

On the other hand, IFAT results showed that *T. lestoquardi* antibodies were detected in 56 out of 202 (27.7%) in Elhuda and 32 out of 198 (16.2%) in Elnuhud Station with an overall prevalence of (22.0%) (Table: 2; Fig: 3).

Risk factors analysis:

Concerning the ecotype in Elhuda station, microscopic examination results showed that the *Theileria* spp infection rates were 14.7%, 17.5% and 15% in Shugor, Dubasi and Watish respectively (Table: 3). IFAT results showed that the *Theileria lestoquardi* were 35.3% in Shugor, 18.8% in Dubasi and 25% in Watish (Table: 4). While in Elnuhud station, the infection rates of Kabashi were 10%, 16.7%, and in Hamari 11.6%, 15.9% by microscopic examination and IFAT respectively (Table: 3,4). There was no significant by microscopic examination association between infection with *Theileria* spp and the ecotype of sheep (X^2=2.320: p-value=0.677). (Table: 3), but by IFAT there was significant association between infection with *T. lestoquardi* and the ecotype of sheep (X^2=15.048: p-value=0.005) (Table: 4).

In relation to age groups of sheep, 9 out of 71 (12.7%) of sheep over three years , 38 out of 291 (13.1%) of sheep between one and three and 7 out of 38 (18.4%) of sheep under one year old were infected with *Theileria* spp (Table: 3). However, 9 out of 71 (21.1%) of sheep over three years , 64 out of 291 (22%) of sheep between one and three and 15 out of 38 (23.7%) of sheep under one year old were infected with *T. lestoquardi* (Table: 4).

There was no significant association was observed between infection with *Theileria* spp or *T. lestoquardi* and the age of animal (Table. 3, 4),

Although, the highest infection rate was observed in male compared with female as shown in table 3 and 4. But no significant association was observed between infection with *Theileria* spp or *T. lestoquardi* and sex of animal (x^2= 1.133; P =0.287) (x^2= 2.414; P =0 .120).

The rate of the infection based on the Body Condition Score (BCS) was 15.3% in good, 10.9% in moderate and 33.3% in poor score, there was Significant relationship between BCS and *Theileria* spp infection (Table: 3). Significant relationship (Table: 4) was observed between body condition and *T. lestoquardi* infection.

The same infection rate was observed by blood examination during winter (14%) compared with summer (13%), there was no significant association (Table: 3) between the infection rate and the season (x2= 0.086: P=0.770). But the highest infection rate was observed by IFAT during May (29.5%) compared with December (14.5%). The analyses of the data revealed significant association between the infection rate and the season (x2= 13.112) (P=0.000) (Table: 4).

Concerning stations of examined animals, the rate of infection at Elhuda (15.5%), and Elnuhud (11.5%). The analysis showed significant association (Table: 3) between the infection with *Theileria* spp and the station of the examined sheep (x^2= 1.370; P = 0.242). Whilst the rate of infection at Elhuda and Elnuhud Station was 28% and 16%, respectively. The analysis showed significant association (Table: 4) between the infection with *T. lestoquardi* and the station of the examined sheep (x^2= 8.392; P = 0.004).

Table(1) Detection of *Theileria* species piroplasm in sheep (n≡400) in Elhuda and Elnuhud research stations by Blood Smear (B.S):

Station	Blood Examined	Positive	Prevalence (%)
El-huda	202	31	15.3%
El-nuhud	198	23	11.6%
Total	400	54	13.4%

Table (2) Detection of *T. lestoquardi* antibodies in sheep (n≡400) in Elhuda and Elnuhud research stations by indirect immunofluorescent Test (IFAT):

Station	Serum Examined	Positive	Prevalence (%)

El-huda	202	56	27.7%
El-nuhud	198	32	16.2%
Total	400	88	22.0%

Table(3)Summary of statistical analysis for risk factors associated with *Theileria* spp infection (n≡400), using the Chi-squared test pertains to blood smear results in Elhuda and Elnuhud research stations, Sudan:

Risk factor	No. tested	No. positive (%)	Df	X^2	p- value
Ecotype					
Shugor	102	15 (14.7%)			
Dubasi	80	14 (17.5%)	4	2.320	0.677
Watish	20	3 (15.0%)			
Hamari	138	16 (11.6%			
Kabashi	60	6 (10.0%)			
Age					
Less than ≤1	38	7 (18.4%)			
Between 1-3	291	38 (13.1%)	2	0.878	0.645
Above >3	71	9 (12.7%)			
Sex					
Female	351	45(12.8%)	1	1.133	0.287
Male	49	9 (18.4%)			
BCS					
Poor	21	21 (33.3%)	2	8.874	0.012*
Moderate	248	27 (10.9%)			
Good	131	20 (15.3%)			
Month					
May 2016	200	26 (13.0%)	1	0.086	0.770
December 2016	200	28 (14.0%)			
Location					
Elhuda	202	31 (15.3%)	1	1.192	0.275
Elnuhud	198	23 (11.6%)			

*= means significant association at p≤ 0.05

Table(4) Summary of statistical analysis for risk factors associated with *T. lestoquardi* infection (n≡400), using the Chi-squared test pertains to IFAT results in Elhuda and Elnuhud research stations, Sudan:

Risk factor	No. tested	No. positive (%)	Df	X^2	p- value
Ecotype					
Shugor	102	36 (35.3%)			
Dubasi	80	15 (18.8%)	4	15.048	0.005*
Watish	20	5 (25.0%)			
Hamari	138	22 (15.9%)			
Kabashi	60	10 (16.7%)			
Age					
Less than ≤1	38	15 (23.7%			
Between 1-3	291	64 (22.0%)	2	4.765	0.092
Above >3	71	9 (21.1%)			
Sex					
Female	351	73 (20.8)	1	2.414	0.120
Male	49	15 (30.6)			
BCS					
Poor	21	11 (52.4%)	2	11.931	0.003*

Moderate	248	50 (20.2%)			
Good	131	27 (20.6%)			
Month					
May 2016	200	29 (14.5%)	1	13.112	0.000*
December 2016	200	59 (29.5%)			
Location					
Elhuda	202	56 (27.7%)	1	7.788	0.005*
Elnuhud	198	32 (16.2%)			

*= means significant association at p≤ 0.05

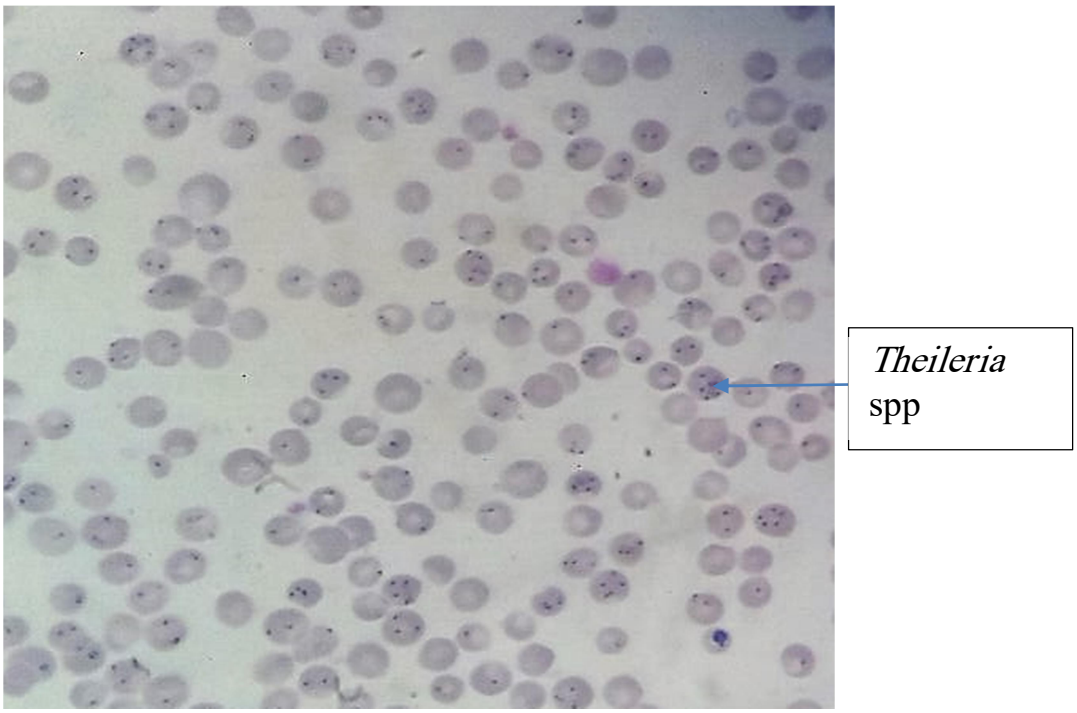

Figure: 2: *Theileria* spp infection in erythrocyte.

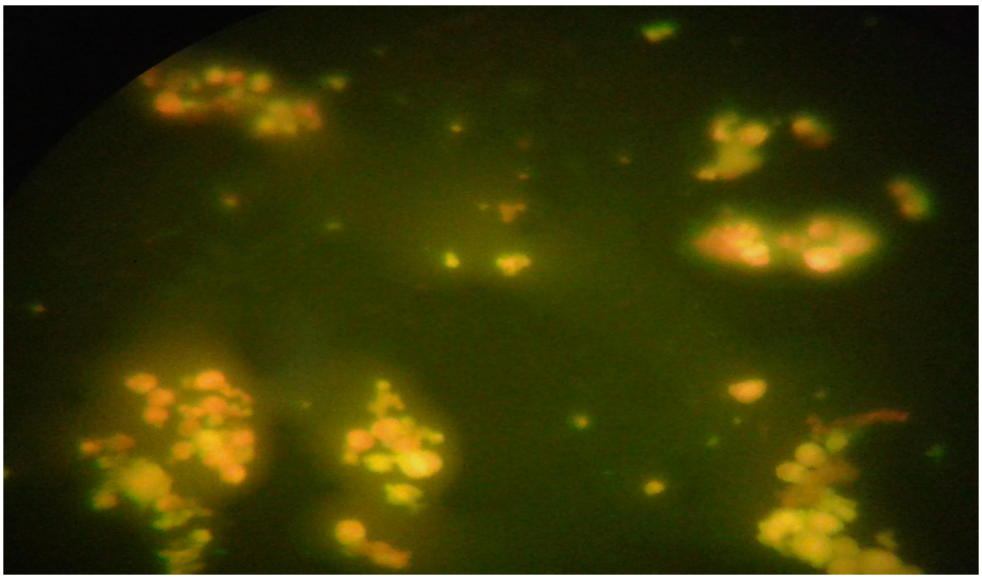

Figure: 3: Detection of *Theileria lestoquardi* Antibodies using schizont at antigen in IFAT.

Discussion:

Ovine theileriosis is a significant disease of small ruminants in tropics and subtropics region (Criado *et al.,* 2009). *T. lestoquardi* is the most pathogenic among the various species causing malignant theileriosis in sheep; it's a severe lymphoproliferative disease with high morbidity and mortality rate (Naz *et al.,* 2012). In the current study, the prevalence rate of *Theileria* spp was 13.4% and of *T. lestoquardi* antibodies was 22.0% as detected by IFA test.

Regarding to blood examination, in this study our results is similar to Aktas *et al.* (2005) who detected 15.5% prevalence rate of *Theileria* spp and contradicted with Yaghfoori *et al.* (2013) in I ran, who reported 46% of *Theileria* infection by blood smears, Durrani *et al.* (2011) in Bakestan, recorded 22% and Ahmed *et al.* (2003) who recorded the prevalence rate of *Theileria* infection in resident animals 22.1% and from pre slaughtered animals 17.8% in River Nile State in the Sudan. These variations in the prevalence rate may be attributed to the locations differences and sample size or to agro climate condition which affect victors of population dynamics.

In this study the prevalence rate of *T. lestoquardi* was(22.0%) by IFAT, this prevalence coincide with similar results by Hassan *et al.* (2018) who reported that the prevalence rate of *T. lestoquardi as* 20.6% in sheep in Khartoum state in the Sudan.

Statistical significant was observed between animal ecotype and *T. lestoquardi* infection, the highest prevalence was in Shugor (35.3%), Dubasi (18.8%), Watish (25%), Hamari (15.9%) and Kabashi (16.7%). In previous study conducted by Hassan *et al.* (2018) they recorded the highest prevalence rate of *T. lestoquardi* in Dubasi followed by Kabashi, Baladi and Hamari ecotype, this is could be due to genetic variation between breeds and the differences in sample size.

The results of age factor disclosed that the Sheep under one year old were more prone to *T. lestoquardi* infection (23.7%), followed by those between one and three years (22%) and finally sheep over three years old (21.1%), this is disagreed with Hassan *et al.* (2018) who found that 93% of seropositive animals belonged to older one because young animal had less exposure time to ticks infestation.

No-significant association was observed relating to age. A similar observation was reported previously by Naz *et al.* (2012); Durrani *et al. (*2012) during studies in small ruminants from Multan and Lahore districts in Pakistan respectively.

The study revealed that, males were more likely to have *T. lestoquardi* infection (30.6%) comparing to females (20.8%), but statistically this difference was not –significant. Our results is in line with Rehman *et al.* (2010) and Naz *et al.* (2012) who reported that gender does not affect the incidence of ovine theileriosis and Bell-Sakyi *et al.* (2004) in Ghana and Dhaim and A'aiz (2014) in Iraq showed that, *Theileria* infection did not affected with animal gender .

Various body condition score of sheep was recorded in this study (Table 3). Strong association between the BCS and *T. lestoquardi* infection, the rate of infection is higher in sheep with poor body condition. This might be due to fact that animals of poor BCS were of low immunity.

Two stations have been scrutinized for the prevalence of *T. lestoquardi* infection. The highest prevalence of infection was recorded in Elhuda station (28%) and the lowest one recorded in Elnuhud (16%). Significant association between *T. lestoquardi* infection and district have been investigated. This finding could be attributed to the fact that: most of ecotype exist in Elhuda station were obtained from resident animals, from around markets , where these ecotype are mainly raised along the Nile, therefore, favorable microhabitat for survival and reproduction of the dominant tick vector *Hyalomma anatolicum* exists (Ahmed, 1999) .

Observations of two seasons (summer & winter) showed significance effect on the *T. lestoquardi* infection rate. Our results is disagreed with Asmaa *et al.* (2016) in Egypt who reported the fact that, the vector is found to be active throughout most of the year even in small number in the study area.

When Ticks data was analyzed separately it was seen that the main prevalent ticks in Elhuda was *R. e. evertsi* and *H. anatolicum* like in Elnuhud. These results confirmed that ticks in particular *H. anatolicum* act as a vector for transmission of *T. lestoquardi*. Similar trend of tick infestation and its association with theileriosis has previously been reported by Hayati (2015).

The presence of carrier animals, susceptible animals and the vector (*H. anatolicum)* may lead to sporadic cases or outbreak of MOT in the two stations.

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