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ORIGINAL ARTICLE

Prevalence of ticks (Acari: Ixodidae) and *Theileria lestoquardi* in sheep at El Huda and El Nuhud animals production research stations, Sudan

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Abstract A cross-sectional study was performed 200 blood samples and 314 tick samples in El Huda and El Nuhud animals production research stations, Sudan, in May (summer) and December (winter) in 2016, to determine the prevalence of *Theileria lestoquardi* in sheep and the potential risk factors associated with the infection. A total of 200 blood samples and 314 tick samples were collected from El Huda (n = 103 blood, 97 tick) and El Nuhud (n = 97 blood, 217 tick) stations. Data on the risk factors, such as age, sex, ecotype of sheep, body condition score and seasons were recorded. The overall prevalence of *Theileria lestoquardi* was 13% (26/200) using PCR. A significant variation in the prevalence of *Theileria lestoquardi* was observed between the stations and the ecotype of sheep ($p \leq 0.05$), whereas the highest prevalence was recorded in El-Huda station (19.4%) as well as in Shugor (22.8%). Other risk factors, like age, sex, body condition, and seasons were not found to be significantly associated with infection. However, the highest prevalence rate was recorded in old animals (21.6%) than the other, in males (17.9%) than females (12.2%), in animals with poor body condition (26.1%) than the other, and in winter (16%) than summer (10%). Four tick species i.e. *Rhipicephalus evertsi evertsi* (63.1%), *Hyalomma anatolicum* (13.8%), *Hyalomma dromedarii* (8.8%), and *Hyalomma impeltatum* (14.3%) were recorded in El Nuhud station. While in El Huda station, only *Rhipicephalus evertsi evertsi* (79.4%), *Hyalomma anatolicum* (20.6%) were recorded. This study revealed that 13% of sheep were suffering from *Theileria lestoquardi* which is a considerable number at the stations. Therefore, further epidemiological investigations on disease throughout the year are required in order to set a well-coordinated control program.

Keywords *Theileria lestoquardi* · Sheep · Ticks · PCR · Sudan

Introduction

Tick-borne diseases (TBDs) are present throughout the world, particularly in the tropical and subtropical regions. These diseases are considered a significant threat to global food security (Jabbar et al. 2015). Ovine theileriosis is a tick-borne disease caused by *Theileria* spp, which is an apicomplexan protozoan parasite that exists in a wide zone of northern Africa, south-eastern Europe, central and western Asia and in India (Uilenberg 1981). Of the *Theileria* species affecting sheep and goats, *T. lestoquardi* is considered the major pathogenic species that causes malignant ovine theileriosis (Luo and Yin 1997; Mehlhorn et al. 1994). On the other hand, *T. separata* and *T. ovis* cause either low or non-pathogenic theileriosis in goats and sheep (Hassan et al. 2015).

Theileria lestoquardi is mainly transmitted by *Hyalomma anatolicum* (Taha and El Hussein 2010). The typical clinical symptoms of *T. lestoquardi* infections are high fever, enlargement of the lymph nodes, emaciation, anorexia, intermittent diarrhoea or constipation and weight

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loss (Tageldin et al. 2005). In Sudan, malignant ovine theileriosis was reported by El Ghali and El Hussein (1995) and Ahmed (1999), who illustrated that the disease caused significant losses among sheep.

The diagnosis of theileriosis is mainly based on the blood smears examination. However, this method is unable to differentiate between the species due to morphological similarity (Salih et al. 2015). PCR has become a favored method of diagnosis in epidemiological studies as this method, which allows the detection of parasite at the low level of parasitaemia and differentiation between *Theileria* species (Tuli et al. 2015; Altay et al. 2005).

El Huda and El Nuhud animal production research stations are considered the most important research centers in Sudan that constructed for many purposes such as production and distribution of improved animals for breeding purposes and research on various aspects of animal production (FAO 2005). In order to improve the control measures against the tick-borne diseases, including malignant ovine theileriosis. It is necessary to known the prevalence of the disease in the target populations. Moreover, the detection of these organisms is necessary for a better understanding of their epidemiology (Oura et al. 2004).

Referring to all above, this study was carried out to determine the prevalence of *Theileria lestoquardi* in sheep and the potential risk factors associated with the infection in El Huda and El Nuhud Animal Production Research Stations.

Materials and methods

Study areas

EI Huda station is located in Al Gezira State at approximately 14°55′N latitude and 32°91′E longitude, about 150 km south of Khartoum (capital city of Sudan) and about 90 km north-west of Wad Medani (Fig. 1). The animal herd consists of three ecotypes of Sudanese desert sheep namely Shugor, Dubasi and Watish (Ahmed et al. 2018).

El Nuhud station is located in West Kordofan State at latitude 12°42′ N and longitude 28°25′ E, which is found in savannah zone (Fig. 1). The animal herd consists of two ecotypes of Sudanese desert sheep namely Hamari and Kabashi (APRC 2016).

Collection of samples and study design

Blood samples

A total number of 103 and 97 sheep were randomly selected from El Huda station and El Nuhud station, respectively, in May and December 2016. Blood samples were collected directly from the Jugular veins into EDTA containing tubes, which preserved in transport cooler box with thermometer. Meanwhile, individual animal data including age, sex, ecotype and body condition score were recorded. The age of the animal was estimated by the method explained by De-Lahunta and Habel (1986) and classified into three groups; animals over 3 years (old animal), animals between 1 and 3 years (middle age animal) and animals under 1 year old (young animal) (Table 1). The body condition scores of animals were evaluated and classified into three groups; poor, moderate and good body condition. The ecotypes of sheep were classified into; Shugor, Dubasi, Watish, Hamari and Kabashi (Table 1).

Tick samples

Ticks were collected from their predilection sites from 200 sheep and from the ground of both stations by using a pair of blunt metal forceps. The ticks were individually stored in a labelled tube containing 70% ethanol. Under a dissecting microscope, ticks were identified based on morphological characteristics using the key identification guide (Walker et al. 2003).

Molecular detection

DNA extraction

DNA was extracted from the whole blood samples using the phenol–chloroform extraction method following the protocol described by Sambrook et al. (1989). The isolated DNA stored at -20 °C until used. For quality assessment, 2 µl of extracted DNA were analyzed on 1.5% agarose gel.

Polymerase chain reaction (PCR)

Two primer pairs [Forward 5′-GTGCCGCAAGTGAGTCA-3′ and Reverse 5′-GGACTGATGAGAAGACGATGAG-3′] were used to amplify a 730 bp fragment of the 18S rRNA gene of *T. lestoquardi* according to the method described by Taha et al. (2011). The positive control was prepared from *T. lestoquardi* culture (Central Laboratory, Sudan), while PCR mixture was used without DNA template as a negative control.

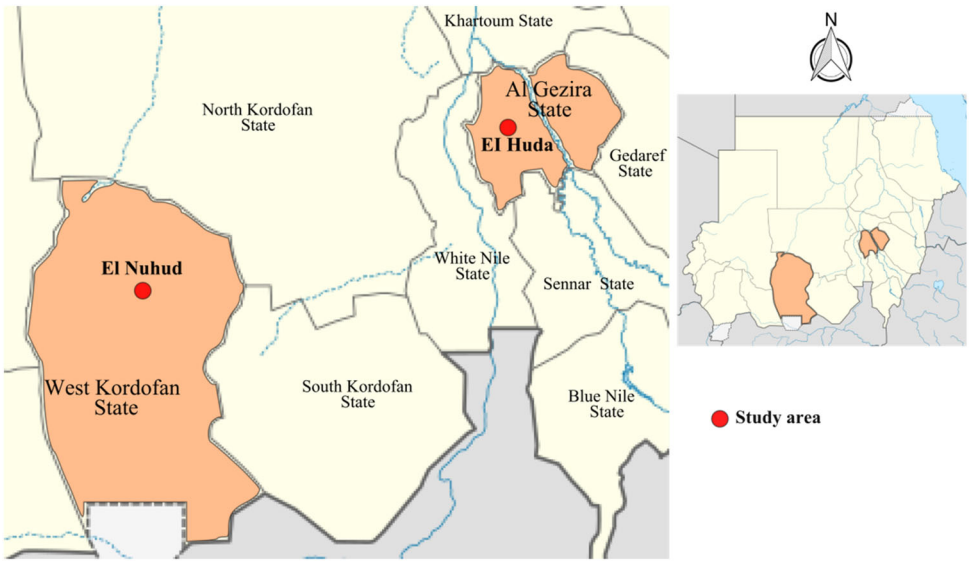

Fig. 1 Study areas (https://ar.wikipedia.org/wiki/%D9%85%D9%84%D9%81:Sudan_location_map.svg)

Table 1 Number of blood samples based on the ecotypes and age in each station

Station	Ecotype					Age		
	Shugor	Dubasi	Watish	Hamari	Kabashi	Young animal	Middle age animal	Old animal
El Huda	57	35	11	–	–	3	65	35
						Shugor (0)	Shugor (42)	Shugor (15)
						Dubasi (2)	Dubasi (16)	Dubasi (17)
						Watish (1)	Watish (7)	Watish (3)
El Nuhud	–	–	–	68	29	12	83	2
						Hamari (8)	Hamari (58)	Hamari (2)
						Kabashi (4)	Kabashi (25)	Kabashi (0)
Total	57	35	11	68	29	15	148	37

PCR was performed in a final reaction volume of 20 µl containing; 6 µl of H_2O, 2 µl of each primer, 5 µl of genomic DNA and 5 µl of Maxime PCR Premix (iNtRON Biotechnology, Korea). The Maxime PCR Premix contained; 1 × reaction buffer (10 ×), 2.5 U of iTaqTM DNA Polymerase (5 U/µl), 2.5 mM of each dNTPs and 1 × Gel loading buffer. The amplification was performed with an initial denaturation at 94 °C for 3 min followed by 35 cycles of 94 °C for 1 min, 56 °C for 1 min, 72 °C for 1 min and final extension step at 72 °C for 7 min. The PCR products were visualized on 1.5% agarose gel stained with Ethidium Bromide.

Data analysis

The IBM SPSS 16 Package was utilized in the analysis. Descriptive statistics were determined for all quantitative variables. Data were analysed using Chi square test to calculate the degree of association between risk factors and the prevalence of *Theileria lestoquardi* infection. Differences were considered significant at $p \leq 0.05$.

Results

The overall prevalence

The overall prevalence of *T. lestoquardi* was 13% (26/200) using PCR (Table 2). A significant variation in the

Table 2 Prevalence of *T.lestoquardi* using PCR on the basis of the Stations, ecotype, age, sex, body condition score (BCS), and season in sheep (n = 200) in El Huda and El Nuhud stations

Risk factor	No. tested	No. positive (%)	df	X^2	*p* value
Station					
El Huda	103	20 (19.4)	1	7.733	0.005*
El Nuhud	97	6 (6.2)			
Ecotype					
Shugor	57	13 (22.8)	4	9.416	0.050*
Dubasi	35	5 (14.3)			
Watish	11	2 (18.2)			
Hamari	68	5 (7.4)			
Kabashi	29	1 (3.4)			
Age					
Young	15	0 (0.00)	2	4.765	0.092
Middle age	148	18 (12.2)			
Old	37	8 (21.6)			
Sex					
Female	172	21 (12.2)	1	0.679	0.410
Male	28	5 (17.9)			
BCS					
Poor	23	6 (26.1)	2	4.525	0.104
Moderate	112	11 (9.8)			
Good	65	9 (13.8)			
Season					
Summer (May 2016)	100	10 (10.0)	1	1.592	0.207
Winter (December 2016)	100	16 (16.0)			

*Significant association at $p \leq 0.05$

prevalence of *T. lestoquardi* was observed between the two stations (*p* value = 0.005), whereas El Huda station 19.4% (20 out of 103) having a significantly higher prevalence than El Nuhud station 6.2% (6 out of 97) (Table 2).

Prevalence of *T. lestoquardi* by stations, ecotype, age, sex, body condition scores and seasons

There was significant variation ($p \leq 0.05$) in the prevalence of *T. lestoquardi* between the ecotype of sheep (*p* value = 0.050), where the highest prevalence rate was reported in Shugor (22.8%), followed by Watish (18.2%), Dubasi (14.3%), Hamari (7.4%) and the lowest prevalence was reported in Kabashi (3.4%) (Table 2).

Risk factors like age, sex, body condition score and season were not significantly associated with the prevalence of *T. lestoquardi* in sheep ($p > 0.05$) (Table 2). Animals of the age group > 3 years old (old animal) showed the highest prevalence rate (21.6%) whereas animals of the age group between 1 and 3 years old (middle age animal) showed the lowest prevalence rate (12.2%), while no infection was observed in the animal less than 1 year old (young animal) (Table 2). The higher infection rate was observed in male (17.9%) compared with female (12.2%) as shown in Table 2.

The prevalence of the infection based on the body condition score (BCS) was (13.8%) in good body condition animals, followed by moderate body condition (9.8%) and the highest prevalence of *T. lestoquardi* was reported in animals with poor body conditions (26.1%). The higher infection rate was observed during winter (16%) compared with summer (10%) (Table 2).

Tick species

In December 2016, a total of 97 ticks belonging to 2 species were collected from El Huda station, whereas a total of 217 ticks belonging to 4 species were collected from and El Nuhud station. *Rhipicephalus evertsi evertsi* and *Hyalomma anatolicum* were the only two species of ticks were recorded in El Huda station, while in El Nuhud station in

Table 3 Number and sex of ticks recorded in El Nuhud and El Huda station in December 2016

Species	*Hyalomma impeltatum*		*Hyalomma dromedarii*		*Hyalomma anatolicum*		*Rhipicephalus evertsi evertsi*		Total (n = 314)
Gender	Male	Female	Male	Female	Male	Female	Male	Female	
El Huda station	0	0	0	0	7	13	43	34	97
Total					20 (20.6%)		77 (79.4%)		
El Nuhud station	12	19	9	10	18	12	90	47	217
Total	31(14.3%)		19 (8.8%)		30 (13.8%)		137 (63.1%)		

addition to above two species *Hyalomma dromedarii* and *Hyalomma impeltatum* were isolated as well (Table 3).

Discussion

In small ruminants, ovine theileriosis is considered as one of the most significant disease particularly in tropics and subtropics that leads to economic losses in sheep and goat (Zaeem et al. 2010). Among the various species of *Theileria*, *T. lestoquardi* is the most pathogenic species infecting sheep and goats, causing malignant ovine theileriosis, a severe lymphoproliferative disease with high morbidity and mortality rate in sheep (Naz et al. 2012).

The overall prevalence of *T. lestoquardi* was 13% as detected by PCR test. This finding was slightly lower than the previous reports, where the prevalences was 21% in district Lahore, Pakistan using PCR (Durrani et al. 2011) and 20% in Khartoum State, Sudan using indirect fluorescent antibody test (Hassan et al. 2018). The difference in the prevalence rate might be due to the variation in the geographical area and in the sensitivity of the diagnostic tests (Aziz and Al-Barwary 2019; Dharanesha et al. 2017). Moreover, the environmental conditions like temperature and rainfall impact on the vectors, which in turn affect on the transmission of the disease (Brand and Keeling 2017).

The Chi square analysis showed that the station (locality) and the ecotype were the two risk factors associated significantly with the infection with *T. lestoquardi*. *T. lestoquardi*. The highest prevalence was recorded in El Huda station (19.4%) compared with El Nuhud (6.2%). This results could be attributed to the fact that the majority of the sheep that exist in El Huda station are mainly raised along river banks, where the environmental conditions are favorable for the tick vector to survive and reproduce (Salih et al. 2003; Ahmed 1999).

Among the Sudanese sheep ecotypes, Shugor showed the highest infection rate with *T. lestoquardi* (22.8%) compared with the other ecotypes. The variation in the susceptibility among the Sudanese sheep ecotypes was also illustrated by El Imam et al. (2015), who found that desert ecotype was the most susceptible ecotype. Moreover, the effect of the host genetics on the susceptibility or resistance to parasite among sheep breeds has been demonstrated in several studies (Guo et al. 2016).

The results showed that the prevalence of *T. lestoquardi* was higher in old sheep (21.6%) compared with other ($p > 0.05$). This result is in agreement with Khan (Khan et al. 2017) who demonstrated that small ruminant above 2 years was more susceptible to infection with theileriosis than other groups of animals in Pakistan. These results could attribute to the fact that the newborn animals received antibodies against *Theileria* spp through colostrum, which in turn results in the low prevalence of theileriosis among these animals (Toye et al. 2013). In addition, the young animal had less exposure time to ticks infestation compared with old animal (Hassan et al. 2018).

Although in the current study a slightly higher prevalence rate was reported in males (17.9%) than females (12.2%), this difference was not significant. This result is in agreement with Osman (Osman et al. 2017) and Taha (Taha et al. 2015) who demonstrated the highest prevalence rate of *T. lestoquardi* in male sheep than females. In contrast, the results of this study disagree with Naz et al. (2012) and Rehman et al. (2010) who found greater positivity of *Theileria* spp. in female than male. This variation may be due to the big difference in the sample size of male and female included in our study, where the number of females was 6 times more than male. Another fact that could also explain this variation where that majority of female sheep are kept indoors for breeding (APRC 2016).

Based on the body condition, the results illustrated that the prevalence of *T. lestoquardi* was higher in animals with poor body condition (26.1%). This could explain the well-known fact that the animal with poor body condition has a weak immune system, which in turn raises the susceptibility of the animal to infectious diseases (Hamsho et al. 2015).

Although there was a variation in the prevalence of *T. lestoquardi* between summer (10%) and winter (16%). There was no significant association between the prevalence of infection and season. In many tropical and sub-

tropical countries, the transmission of tick-borne diseases may be continuous throughout the year and the vector can be active most of the year even in small number (FAO 1983).

Rhipicephalus evertsi evertsi was the predominant species of tick in El Huda station (79.4%) and El Nuhud station (63.1%). Similarly, Hayati et al. (2020) reported that 51.6% of the ticks collected from Al Gezira State was *Rhipicephalus evertsi evertsi.* Moreover, the present of this species in Kordofan, where El Nuhud station is located, was reported in several studies (El Ghali and Hassan 2012).

Based on the findings of this study, it is concluded that the infection with *T. lestoquardi* was higher in El Huda station compared with El Nuhud using PCR. On the other hand, a variable like the ecotype of sheep is significantly associated with the infection with *T. lestoquardi*. Therefore, attention should be given to prevent and control the disease, especially where the prevalence of the disease is higher.

Conclusion

The present study reflects that *T. lestoquardi* is an important tick-borne problem in sheep at El Huda and El Nuhud animals production research stations, Sudan. Adverse effects of this disease on health and production of the sheep needs further investigation on the epidemiology of the disease throughout the year using molecular detection of *T. lestoquardi* in different ticks species.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest in relation to this work.

Ethical approval All procedures performed in the study involving animals were in accordance with the ethical standards of the Institutional Ethics Committee of Sudan University of Science and Technology, Decision number DSR-IEC-01-016.

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