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Diverse Techniques in Detecting the Tropical Theileriosis Among Cattle in Blue Nile State, Sudan

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ABSTRACT

Tropical theileriosis, which is caused by *Theileria annulata*, is considered as a serious illness that impairs animal fertility and production in the world's tropical and subtropical regions. This study investigates bovine tropical theileriosis in Blue Nile State, Sudan. A total of 472 blood samples were collected from six localities namely Al Damazine, Geissan, Alrosires, Altadamon, Baw, and Wadalmahi from Kenana and Umbararo cattle breeds of different age groups: calves under two years old, heifers and steers between two and four years old, and cows and bulls above four years old. Out of 472 peripheral blood smears, 20 (4.2%, 95% CI: 2.6-6.5%) were *Theileria* piroplasm's positive with the highest prevalence in Al Damazine and the lowest prevalence in Altadamon. The prevalence reported by PCR was 8.2% (4/49 blood spots, 95% CI: 2.3-19.6%), where heifers and steers showed a higher prevalence rate compared with other age categories. Microscopic analysis and PCR revealed that Kenana cattle had a high prevalence when compared to Umbararo animals. *T. annulata* seroprevalence was 42.8% (202/472, 95% CI: 38.3-47.4%) by immunofluorescence antibody test (IFAT). Alrosires had the highest seroprevalence, and Baw had the lowest. Heifers and steers had a significantly high association with the *T. annulata* seroprevalence compared to other age groups. The odds ratio of *T. annulata* seropositive in Kenana cattle was 1.6 times more likely than Umbararo cattle. In Blue Nile State, where the management program needs to be strengthened, *T. annulata* is rapidly expanding and poses a threat to the health of cattle.

Key words: Tropical theileriosis; Risk factors; Cattle; Immunofluorescence antibody test; *Theileria annulata*; Sudan.

INTRODUCTION

Tropical theileriosis is a tick-borne disease (TBD) of cows and water buffaloes caused by *Theileria annulata* and transmitted by ticks of the genus *Hyalomma*. The severity of tropical theileriosis varies greatly depending on the breed of cattle; foreign breeds are very sensitive, whilst native breeds are resistant to the infection (Larcombe et al. 2022). The disease poses a threat to about 250 million cattle worldwide (Erdemir et al. 2012). Tropical theileriosis is becoming more prevalent in a number of European, Asian, and African countries; Sudan is one of the countries where this disease has a particularly negative impact on animal productivity (Gharbi et al. 2022). European cattle imported to endemic areas may have 20–90% mortalities, tropical cattle breeds are relatively immune to the disease. While 10-20% of calves may die in endemic areas with frequent tick challenges, infection typically results in a mild illness (Flach and Ouhelli 1992). Although significant mortality rates in cattle of all ages were noted in endemic regions and during outbreaks as a result of sporadic tick infestation, animals that recovered from the acute infection may become long-term persistent and increase the circulation of the parasite in the region (Brown 1990). Despite the fact that carrier animals remained unnoticed, piroplasm infections are frequently detected by peripheral thin blood smears. In order to accurately diagnose tropical theileriosis, particularly in latently infected cattle, sensitive techniques such as PCR and IFAT were needed (Dumanli et al. 2005). The three-host tick *Hyalomma anatolicum* and the two-host tick *H. detritum* with their capacity of transstadial transmission, are the most significant vectors for *T. annulate*.

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In addition to the pathogen and the breed of cattle, other risk factors including tick density and animal age may affect the severity of the disease (Li et al.2020; Gharbi et al. 2022; Kozhabaev et al. 2023). Tropical theileriosis is the most widely distributed TBDs affecting cows in Sudan (Walker et al. 1983; El Hussein et al. 2012). Antibodies of *T. annulate* were detected in South Darfur State (Abdalla and Hassan 2010; Abaker et al. 2017), North Kurdofan (Mohammed-Ahmed et al. 2018), and White Nile State (Guma et al. 2015). The growing number of cattle, the nomadism of cattle owners, and the emergence of parasite vectors in the research region might all have a substantial impact on the disease spreading. Since the disease has been noted to exist in Blue Nile State, the current study was designed to look at the prevalence of tropical theileriosis in cattle there.

MATERIALS AND METHODS

Study Area

The present study was carried out in the Blue Nile State, which is located in the southeast of Sudan. It extends from latitudes 12˚ 40″ to 9˚ 20″, and longitudes 35˚10″ to 33˚ 30″. The climate is rich savannah with heavy rains, ranging between 40 to 700mm. Administratively, Blue Nile State is divided into seven localities: Al Damazine, Kurmuk, Alrosires, Altadamon, Baw, Wadalmahi and Geissan. The total livestock is estimated at 15.28 million out of which 5.7, 4.3 8, and 0.15 million cattle, goats, sheep, and camel, respectively. The three types of husbandries are seminomadic, nomadic, and settle.

Animal Welfare and Consent of Cattle Owners

Before beginning the sampling process on the sampling day, the research's goals and the consent of the livestock owner were secured. The jugular vein was venipunctured by a qualified veterinarian or veterinary technologist in accordance with the protocols for collecting blood from cattle. According to the convenience of the animal owner to participate in the study, samples were collected after obtaining the owner's agreement. During the sample collection, a questionnaire was simultaneously gathered for having information about farms and animals. Since non-invasive methods or hazardous materials were utilized, the ethics committee of the Central Veterinary Research Laboratory declined to grant ethical permission.

Sample Collection

A total of 472 blood samples of apparently healthy cattle were collected from six localities (Al Damazine, Geissan, Alrosires, Altadamon, Baw, and Wadalmahi) from March to December 2016 (Table 1). Samples were collected from cattle of both sexes at different age groups; calves under two years old, heifers and steers between two and four years old, and cows and bulls above four years old. Three ml of whole blood was collected from the jugular vein via venipuncture in sterile EDTA vacutainer tubes. Blood spots on filter paper of 1.5cm^2 area were prepared, air-dried, labeled indicating animal number, and stored at -20°C until tested. Additionally, five ml of whole blood was collected in plain vacutainer tubes. The serum was extracted from the blood after it had been allowed to clot at room temperature, kept in Eppendorf tubes, labeled, and maintained at -20°C until it was analyzed.

Peripheral Thin Blood Film

Peripheral thin blood smears were prepared, air-dried, and fixed in absolute methanol for a few minutes, stained by 10% Giemsa's stain for 45 minutes, and examined under a light microscope using oil immersion at 100X lens (Olympus CX21).

Indirect Fluorescent Antibody Test (IFAT)

The procedure was conducted as described by Burridge and Kimber (1972). The conjugate was used in dilution 1/20 and Evans blue at a concentration of 0.01% was added to the conjugate as a counter stain. Test sera were diluted in phosphate buffer saline (PBS) 1/80 for screening the antibodies against *Theileria annulate* according to FAO (1984). Positive control and negative sera that diluted at 1/80 were obtained from the protozoology unit in Central Veterinary Research Laboratories, Sudan. The examination was carried out using Olympus Vanox incident-light excitation fluorescent microscope (Japan), the slides were examined under 40X objective using nine parts of glycerol and one part of PBS.

Deoxyribonucleic Acid (DNA) Extraction and Molecular Detection

DNA was extracted from the blood spots on filter paper using the phenol-chloroform-isomylethanol protocol. For the amplification of the 30Kda (*T*ams 1), the forward primer used was N516 5' GTAACCTTTAAAAACGT 3' and the reverse primer was N517 5' GTTACGAACATGGGTT 3' (*T. annulata* specific) giving a band size of 721bp (d´Oliveira et al. 1995). Thermocycler condition was as follows; 94^o C for 5min, and then 30 cycles consisting of 94^o C for 1min, 55^{o}C for 1min, 72^{o}C for 1min, and the final extension step at 72^{o}C for 7min, and hold on 4^{o}C. In each run positive control (DNA extracted from *T. annulata* cell line and negative control (distilled water) were included. Amplified templates were separated by 1.5% agarose gel electrophoresis. A volume of 5µL of 100bp DNA-ladder was loaded and the gel was run at 120V for 40min and transferred to a UV illuminator for examination and documentation.

Data Analysis

Statistical analysis was performed using 'Statistical Package for Social Science' (SPSS), version 20 (IBM). In contrast, univariate logistic regression was used for risk factors with more than two levels, such as age and sample location, while the Pearson Chi-square or Fisher Exact test was used to assess risk factors with two levels, such as gender and breed. Statistical significance was considered when the probability is below 0.05 with a 95% confidence interval.

RESULTS

Analysis of Sample Population

A total of 472 heads of cattle were sampled in the present study from six localities namely Al Damazine, Geissan, Alrosires, Altadamon, Baw, and Wadalmahi in the Blue Nile State. According to the animal's maturity, the studied cattle's age was divided into three groups; calves under two years old, heifers and steers 2-4 years old and cows and bulls above four years old. Female cattle were

Table 1: Frequency of cattle sampled in each group of variables in Blue Nile State, Sudan

Variables	Number	%
Age		
Calf	150	31.8
Heifer (2-4 year's old)	101	21.4
Cow	221	46.8
Gender		
Female	343	72.7
Male	129	27.3
Breed		
Kenana	232	49.2
Umbararo	240	50.8
Location		
Al Damazine	82	17.4
Altadamon	90	19.1
Alrosires	100	21.2
Wadalmahi	100	21.2
Baw	50	10.6
Geissan	50	10.6
Total	472	100

Table 2: Univariate analysis for testing the association of the risk factors with *T. annulata* seroprevalence among cattle in Blue Nile State, Sudan

Risk factors	IFAT Positive No. (%)	Negative No. (%)	Chi-square values	P-value
Age				
Heifer	53 (57)	40 (43)	4.43	0.044
Calf	61 (44.9)	75 (55.1)		
Cow	88 (44.4)	110 (55.6)		
Gender				
Female	143 (46)	168 (54)	0.81	0.385
Male	59 (50.9)	57 (49.1)		
Breed				
Kenana	118 (52.9)	105 (47.1)	5.89	0.016
Umbararo	84 (41.2)	120 (58.8)		
Location				
Al Damazine	46 (58.2)	33 (41.8)	30.11	0.001
Altadamon	30 (34.9)	56 (65.1)		
Alrosires	44 (67.7)	21 (32.3)		
Wadalmahi	50 (51)	48 (49)		
Geissan	20 (40)	30 (60)		
Baw	12 (24.5)	37 (75.5)		

Table 3: Binary logistic regression and odds ratio of risk factors associated with *T. annulata* seroprevalence among cattle in Blue Nile State, Sudan

Variables	*β*	*SE β*	*P*-value	Adjusted odds ratio	95% CI lower	95% CI upper
Cattle breed						
Umbararo	0.000	-	-	1.000	-	-
Kenana	0.473	0.196	0.015	1.605	1.09	2.36
Age						
Heifers/steers	0.000	-	-	1.000	-	-
Calf	-0.488	0.271	0.072	0.614	0.36	1.05
Cows/bulls	-0.505	0.254	0.047	0.604	0.37	0.99
Location						
Baw	0.000	-	-	1.000	-	-
Al Damazine	1.458	0.403	0.001	4.298	1.95	9.50
Altadamon	0.502	0.402	0.212	1.652	0.75	3.63
Alrosires	1.866	0.425	0.001	6.460	2.81	14.86
Wadalmahi	1.167	0.389	0.003	3.212	1.50	6.88
Geissan	0.721	0.440	0.102	2.056	0.87	4.87

β: logistic coefficients, SE: standard error, CI: confidence interval

most predominant in the field, comprising about 72.7% (343/472) of the total animals examined. Samples were composed of two types of cattle breeds that were reared in the area, namely Kenana and Umbararo (Table 1).

Prevalence of Infection using different Techniques

The overall prevalence based on blood smears was 4.2% (20/472, 95%CI: 2.6-6.5%). DNA of *T. annulata* was determined using the polymerase chain reaction (PCR). Out of 49 DNA samples examined, four animals were positive for *T. annulata* (8.2%, 95% CI: 2.3-19.6%). When *T. annulata* schizont antigen was employed in IFAT to identify *T. annulata* antibodies, the overall seroprevalence was 42.8% (202/472, CI: 38.3-47.4%).

Single-factor Analysis of the Potential Risk Factors

The possible risk factors such as age, gender, breed, and location were examined for association with *T. annulata* antibodies. Location, age, and cattle breed were factors significantly associated with *T. annulata* seroprevalence ($P<0.05$) whereas gender was not ($P>0.05$) (Table 2). The highest prevalence rate was 44/65 (67.7%) in Alrosires, whereas the lowest was recorded in Baw (12/49, 24.5%).

The odds ratio of the seropositive samples from Wadalmahi, Al Damazine, and Alrosires localities were about 3.2, 4.3, and 6.5 times more likely than seropositive samples from Baw locality, respectively. The heifers/steers seropositive cattle were 1.7 times more likely to have circulating antibodies against *T. annulata* than the cows/bulls, whereas Kenana breed was 1.6 times more likely to be *T. annulata* seropositive than Umbararo breed (Table 3).

DISCUSSION

In Sudan, ticks and pathogens are considered as devastating problem that jeopardize livestock. The most vital TBDs of cows in Sudan is the tropical theileriosis that caused by *Theileria annulata,* which leads to massive economic losses in production and reproduction of cattle in the country (El Hussein et al. 2004; Gharbi et al. 2022). No data is available regarding the diversity of *Theileria* parasite and the suitability of PCR in detecting *T. annulata* infection under field conditions in Blue Nile state. Therefore, the present study provides an epidemiological view on *T. annulata* infection of cattle in Blue Nile state using different diagnostic techniques.

This cross-sectional study revealed a prevalence of *Theileria* spp. piroplasm at 4.2% (20/472) using blood smear technique, while 202 samples out of 472 (42.8%) were positive for *T. annulata* antibodies using IFAT and 4 out of 49 (8.2%) were positive for *T. annulata* DNA using PCR. When compared to serological testing, peripheral thin blood film had a lower rate of *T. annulata* detection. This finding is similar to previous studies of advantage of IFAT over blood smears (Ali et al. 2006; El Hussein et al. 2012; Abaker et al. 2017; Mohammed-Ahmed et al. 2018). *T. annulata* infection is not always indicated by the presence of piroplasms; instead, *T. mutans* piroplasms, which are non-pathogenic and rarely cause disease in cattle, may also be present. *T. mutans* is anticipated to be widespread in the study area given the presence of its vector (*Amblyomma lepidum*). Microscopic examination using blood smears cannot differentiate between the *T.*

annulata and *T. mutans*. Hence, the combination of clinical symptoms, smears, and the presence of *H. anatolicum* ticks could improve diagnosis of tropical theileriosis (El Hussein et al. 1991). In addition, the low sensitivity and specificity of thin blood smears could be because of artifacts and low parasitemia in the host, as observed in carrier animals. Therefore, sensitive techniques, such as PCR could enhance the detection accuracy of *T. annulata* (Ullah et al. 2021).

T. annulata's 30-kDa major merozoite surface antigen is encoded by a gene, and a primer for this gene has been developed (d'Oliveira et al. 1995). Polymerase chain reaction reported *T. annulata* DNA at 8.2% in the current investigation, despite the small sample size employed for logistical reasons. It was demonstrated that PCR is more capable of detecting *T. annulata* compared to peripheral thin blood smears. This result is in line with Ali et al. (2006) in Khartoum, Mohammed-Ahmed et al. (2018) in North Kordofan and Abaker et al. (2017) in Nyala who noted that when compared to PCR, blood smears had the lowest sensitivity in identifying *T. annulata* in carrier cattle. Epidemiological studies on *T. annulata* using RLB technique showed that, the prevalence of *T. annulata* DNA decreased from 73.1% in northern Sudan in River Nile State (Taha et al. 2022) to 48.1% Khartoum State (Ali et al. 2006) to 8.2% prevalence rate as reported in the current study. Whereas it was 11.4% in North Kordofan (Mohammed-Ahmed et al. 2018) and 39% in Nyala, western Sudan. This is due to the fact that, the abundance of *H. anatolicum* in northern Sudan is dominant and the ticks are prevalent throughout the year. It may also be related to the introduction of cattle with foreign blood into endemic regions that suffered from intensified breaking of enzootic stability.

The seropositivity for *T. annulata* antibodies was 42.8% (202/472 serum samples) using IFA test. This result is consistent with earlier research by Elhaj (2010), who used the same methodology to report a prevalence of 55% in dairy cattle in Khartoum State. However, it was relatively higher than the seroprevalence recorded in Blue Nile and El Hussein et al. (2012) who found circulation of *T. annulata* antibodies in cattle at 19% using the same technique. The relatively high seroprevalence observed in this study could be attributed to environmental conditions, which may enhance the distribution, establishment, survival, and reproduction of *T. annulata* vector (*H. anatolicum*) in the Blue Nile State over the few last years and thus increased the frequency of the disease in dairy farms in the area. The development of anti-theilerial drug resistance, inadequate acaricide recovery, dynamic of tick survival, and host factors might all contribute to the disease's expansion outside the recognized endemic areas despite the fact that the *T. annulata* vaccine is available in Sudan (Gharbi et al. 2022).

Alrosires, Al Damazine and Wadalmahi localities had a significantly high seroprevalence, according to this study. The seropositivity could be developed when the cattle were grazing freely through neighboring countries like the Republic of South Sudan and Ethiopia, or it may be as a result of serological cross-reaction with *T. mutans* (Mohammed-Ahmed et al. 2018). According to this study, heifers and steers from two to four years old had more *T. annulata* antibodies than animals of other ages. This result is consistent with previous authors' findings that the age of the host may be a potential risk factor for tropical theileriosis (Abaker et al. 2017; Ullah et al. 2021; El Damaty et al. 2022). The dominance of endemic stability in Sudan could possibly emphasize why tropical theileriosis highly affected heifers and to some extend calves (Gharbi et al. 2022).

The seroprevalence rate of *T. annulata* infection in Kenana cattle was higher than the Umbararo cattle using *T. annulata* antibodies IFA test. The Kenana cattle were 1.6 more likely to have circulating antibodies of *T. annulata* than Umbararo cattle. Although owners frequently sprayed acaricide to prevent ticks on Kenana cattle rather than Umbararo cattle, the former was raised in favorable environmental circumstances. The Kenana calves could tolerate *T. annulata* infection better than Friesian calves because they have a lower ability for schizont proliferation according to Bakheit and Latif (2002). However, given that both cattle breeds are thought to be indigenous, our findings call for more research to understand how Umbararo cattle are able to overcome *T. annulata* infection.

Conclusion

It is concluded that tropical theileriosis is widespread and constitutes a hazard to cattle health in Blue Nile State. It is strongly recommended not to introduce livestock from Central Sudan into state without applying strict tick control measures. Molecular characterization and sequencing of *T. annulata* are recommended to be applied in Blue Nile State to conduct an accurate mapping of the disease spreading in the area.

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Author Contributions

EKH and BEM designed the study, and SDA secured the research fund. BEM, SDA and SAA executed the experiment. EEI performed the statistical analysis and interpreted the results. BEM drafted the manuscript, EEI and SDA critically revised the manuscript. All authors have read and approved the manuscript.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

REFERENCES

Abaker IA, Salih DA, Haj LME, Ahmed RE, Osman MM and Ali AM, 2017. Prevalence of *Theileria annulata* in dairy cattle

in Nyala, South Darfur State, Sudan. Veterinary World 10(12): 1475-1480. https://doi.org/10.14202/vetworld.2017.1475-1480

Abdalla MM and Hassan SM, 2010. Current status of distribution of ticks (Acari: Ixodidae) infesting cattle in South Darfur State, Sudan. University of Khartoum. Journal of Veterinary and Animal production 1(2): 76–97.

Ali AM, Bakheit MA, Mukhtar MM, Hassan SM, Ahmed JS and Seitzar U, 2006. Epidemiology of *Theileria annulata* infection of dairy cattle in Sudan using molecular techniques. Annals of the New York Academy of Sciences 1081: 471-472. https://doi.org/10.1196/annals.1373.068

Bakheit MA and Latif AA, 2002. The innate resistance of Kenana cattle to tropical theileriosis (*Theileria annulata* infection) in the Sudan. Annals of the New York Academy of Sciences 969: 159-163. https://doi.org/10.1111/j.1749-6632.2002.tb04370.x

Brown CGD, 1990. Control of tropical theileriosis (*Theileria annulata* infection of cattle). Parasitologia 32: 23–31.

Burridge MJ and Kimber, CD, 1972. The indirect fluorescent antibody test for experimental East Coast Fever (*Theileria parva* infection of cattle). Evaluation of a cell culture schizont antigen. Research in Veterinary Science 13: 451-455. https://doi.org/10.1016/S0034-5288(18)33992-4

d´Oliveira C, Weide MVD, Habela MA, Jacquiet P and Jongejan F, 1995. Detection of *Theileria annulata* in blood samples of carrier cattle by PCR. Journal of Clinical Microbiology 33: 2665-2669. https://doi.org/10.1128/jcm.33.10.2665-2669.1995

Dumanli N, Aktas M, Cetinkaya B, Cakmak A, Koroglu E, Saki CE, Erdogmus Z, Nalbantoglu S, Ongor H, Simşek S, Karahan M and Altay K, 2005. Prevalence and distribution of tropical theileriosis in eastern Turkey. Veterinary Parasitology 127(1): 9-15. https://doi.org/10.1016/j.vetpar.2004.08.006

El Damaty HM, Yousef SG, El-Balkemy FA, Nekouei O, Mahmmod YS and Elsohaby I, 2022. Seroprevalence and risk factors of tropical theileriosis in smallholder asymptomatic large ruminants in Egypt. Frontiers in Veterinary Science 9: 1004378. https://doi.org/10.3389/fvets.2022.1004378

El Hussein AM, Hassan SM and Salih DA, 2012. Current situation of tropical theileriosis in the Sudan. Parasitology Research 111: 503-508. https://doi.org/10.1007/s00436-012-2951-5

El Hussein AM, Majid AA and Hassan SM, 2004. The present status of tick-borne diseases in the Sudan. Archives de L'institut Pasteur de Tunis 81(1-4): 31-34.

El Hussein AM, Mohammed SA, Osman AK and Osman OM, 1991. A preliminary survey of blood parasites and brucellosis in dairy cattle in Northern State. The Sudan Journal of Veterinary Research 10: 51-56.

Elhaj LM, 2010. Studies on the epidemiology and control of *Theileria annulata* in Khartoum State using macroschizont-infected attenuated cell culture vaccine. Ph.D. Thesis, Sudan Academy of Sciences.

Erdemir A, Aktas M and Dumanli N, 2012. Isolation, cloning and sequence analysis of the lactate dehydrogenase gene from *Theileria annulata* may lead to design of new antitheilerial drugs. Veterinarni Medicina 57(10): 559-567. https://doi.org/10.17221/6368-VETMED

FAO 1984. Tick and tick- borne disease control. A practical field manual. Volume 1 and 2. Food and Agricultural Organization of the United Nations, Rome.

Flach EJ and Ouhelli H, 1992. The epidemiology of tropical theileriosis (*Theileria annulata* infection of cattle) in an endemic area of Morocco. Veterinary Parasitology 44: 51-65. https://doi.org/10.1016/0304-4017(92)90143-W

Gharbi M, Darghouth MA, Elati K, Al-Hosary AAT, Ayadi O, Salih DA, El Hussein AM, Mhadhbi M, Khamassi Khbou M, Hassan SM, Obara I, Ahmed LS and Ahmed J, 2022. Current status of tropical theileriosis in Northern Africa: A review of recent epidemiological investigations and implications for control. Transboundary and Emerging Diseases 67 (S1): 8-25. https://doi.org/10.1111/tbed.13312

Guma EI, Hussien MO, Salih DA, Salim B and Hassan SM, 2015. Prevalence of ticks (Acari: Ixodidae) and *Theileria annulata* antibodies in White Nile State, Sudan. Journal of Advanced Veterinary and Animal Research 2(1): 69–73. https://doi.org/10.5455/javar.2015.b53

Kozhabaev M, Kuzerbaeva A, Baizhanov K, Tulemetova S and Nurkhodzhaev N, 2023. Study of the dynamics of distribution, seasonality, and degree of infection with bovine theileriosis in the Territory of Turkestan Region. International Journal of Veterinary Science 12(3): 382-388. https://doi.org/10.47278/journal.ijvs/2022.203

Larcombe SD, Capewell P, Jensen K, Weir W, Kinnaird J, Glass EJ and Shiels BR, 2022. Susceptibility to disease (tropical theileriosis) is associated with differential expression of host genes that possess motifs recognised by a pathogen DNA binding protein. PLOS ONE 17(1): e0262051. https://doi.org/10.1371/journal.pone.0262051

Li K, Luo H, Mehmood K, Shahzad M and Li J, 2020. Exploring the potential parasitic pathogens causing diarrheal death to yak calves with bloody excrement through high-throughput sequencing. Agrobiological Records 1: 1-5. https://doi.org/10.47278/journal.abr/2020.001

Mohammed-Ahmed GM, Hassan SM, El Hussein AM and Salih DA, 2018. Molecular, serological and parasitological survey of *Theileria annulata* in North Kurdofan State, Sudan. Veterinary Parasitology, Regional Study Reports 13: 24-29. https://doi.org/10.1016/j.vprsr.2018.03.006

Taha KM, Mohammad AA, Ebrahim NM, El Hag LM, Elsheikh FM, Hussien MO and Mohammed SB, 2022. Cross-infectivity of *Theileria annulata* and *Theileria lestoquardi* between cattle and sheep in the field in Sudan. Veterinary Research Notes 2(5): 43–47. https://doi.org/10.5455/vrn.2022.b12

Ullah R, Shams S, Khan MA, Ayaz S, Akbar Nu, Din Qu, Khan A, Leon R and Zeb J, 2021. Epidemiology and molecular characterization of *Theileria annulata* in cattle from central Khyber Pakhtunkhwa, Pakistan. PLOS ONE 16(9): e0249417. https://doi.org/10.1371/journal.pone.0249417

Walker AR, Latif AA, Morzaria SP and Jongejan F, 1983. Natural infection rates of *Hyalomma anatolicum* with *Theileria* in Sudan. Research in Veterinary Science 35: 87-90. https://doi.org/10.1016/S0034-5288(18)32209-4