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The prevalence of *Theileria annulata* in dairy cattle in Al-Gezira State, Sudan

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Abstract:
Background*: Tropical theileriosis, a disease caused by intracellular protozoan parasites of the genus Theileria, is considered as the most important tick-borne disease in Sudan. It has been estimated that the loss due to this disease in north Sudan was approximately US$ 6000. Although several studies on the prevalence of the disease have been performed in different parts of Sudan, no study has been conducted previously in Almased County, Al- Gezira State, Sudan. This area is deemed as one of the greater communities of dairy farms in the State.*
Materials and Methods*: This study was conducted on dairy cattle in Almased County, Al-Gezira State, Sudan, from March to December 2016, to estimate the incidence of bovine tropical theileriosis. Randomly, 100 cattle were selected from three different farms. Blood samples from the ear for blood smears, blood in EDTA tubes, and serum veins were collected. The diagnosis of Theileria annulata (T.annulata) was performed using three different techniques: blood smear, indirect fluorescent antibody test (IFAT), and polymerase chain reaction (PCR).*
Results*: Out of 100 samples, 85 were positive for Theileria spp. piroplasms in the blood smears, 92 were positive for T. annulata antibodies using IFAT, and 81 were positive for T. annulata using PCR. Interestingly, the results of blood smears showed positivity higher compared with the PCR. These findings indicated that cattle were possibly infected with other Theileria species.*
Conclusion: *The study concluded that tropical theileriosis is highly prevalent among dairy cattle in the north part of Al-Gezira State. Further molecular investigations of other Theileira spp. are advised for accurate mapping of tropical theileriosis and estimating the magnitude problem of the disease in this area.*
Key Word*: Cattle, Theileria annulata, PCR, IFAT*

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I. Introduction

Sudan has one of the largest livestock populations in Africa. As estimated in 2011 by the Ministry of Animal Resources and Fisheries the total livestock population accounted for 41 million head of sheep, 32 million head of goats, 31 million head of cattle and 4.8 million head of camels. Generally, these animals provide meat and milk for local consumption and meat and live animals for export [1, 2]. The majority of these animals are owned either by traditional pastoralists or by smallholder farmers [3].

Tropical theileriosis is a disease caused by intracellular protozoan parasites of the genus *Theileria*, which is an apicomplexan protozoa parasite that exists in a wide zone of Southern Europe, Africa, and part of Asia [4]. The disease is transmitted by ixodid ticks of the genus *Hyalomma* and affects cattle and water buffalo [5]. An estimated around 250 million cattle are at risk of tropical theileriosis which acts as a major limitation on livestock production and improvement in many countries [6]. The typical clinical symptoms of Tropical theileriosis are fever, swelling of lymph nodes, anorexia, loss of conditional, lacrimation and nasal discharge. Just before death, a sharp fall in body temperature is usual. Death usually occurs between 18-24 days after infection [7]. Generally, the diagnosis of the disease is based on clinical signs and microscopic examination of blood and lymph node smears stained with Giemsa. However, the smear method has low sensitivity in diagnosing carrier cattle [8]. Additionally, several serological tests such as indirect immunofluorescent assay (IFA), enzyme-linked immunosorbent assay (ELISA) and complement fixation test (CFT) can be utilised to detect the antibodies that are circulating in the blood by using antigens that are prepared either from piroplasms or from macroschizonts [9]. Lately, PCR has been developed as a diagnostic method for accurate detection of *Theileria spp* [10].

In Sudan, Tropical theileriosis is considered the most important tick-borne disease. Latif [11] recorded that around 85% of investigated farms in Khartoum State suffered clinical theileriosis and mortalities of 30% and 22% in heifers and young calves, respectively. He calculated the losses in Khartoum State due to this disease as US$ 4–6 million per year.

Although studies on the prevalence of *T. annulata* have been carried out in various parts of Sudan [12, 13, 14], no study has been conducted previously in Almased County, Al- Gezira State, Sudan. This area is deemed as one of the greater communities of dairy farms in the State.

This study used polymerase chain reaction (PCR) to analyse the presence and prevalence of *T. annulata* infection in blood samples acquired from cattle in comparison to the smear method and indirect fluorescent antibody test (IFAT).

II. Material And Methods

Ethical approval: All animal procedures were carried out following the ethical standards established by the Institutional Ethics Committee of Sudan University of Science and Technology, Sudan (Decision No. SUST/DSR/IEC/EA/2016).

Study area: The present study was conducted during the period from March to December 2016 in Almased County, Al-Gezira State, Sudan (Fig.1). The state is located in central Sudan between Latitude 13° 20' and15° 40' north and between longitude 32° and 34° east. Generally, the state has a tropical climate characterized by a dry season from January through May followed by a rainy season from June through December. The minimum temperature ranges from 11°C to 27°C, and the maximum temperature ranges from 30°C to 43°C.

Figure 1: The study area, Almased County, Al Gezira State, Sudan. (Modify from https://greenblog.co.kr/wp-content/uploads/2021/02/sudan-contour-map-min.jpg)

Samples collection: A total of 100 cattle were selected randomly from three different dairy farms. From each animal, blood smears from the ear veins, whole blood from the jugular vein into EDTA tubes, and serum samples were collected from each animal according to the OIE technique [15].

Examination of Blood smears: The blood smears were first stained with 10% Giemsa's stain and then examined under a light microscope with a 100-oil immersion objective for the presence of *Theileria* spp. piroplasms. At least 50 tiny areas were evaluated on each slide, and the presence of one or more piroplasms was considered a positive result.

Molecular detection

DNA extraction: From the whole blood samples, DNA was extracted using the phenol-chloroform extraction method following the method illustrated by Sambrook [16]. For quality assessment, 4 µl of extracted DNA were analyzed on 1.5% agarose gel.

Polymerase chain reaction (PCR): *T. annulata* specific primers [N516: 5 GTAACCTTTAAAAACGT and N517: 5 GTTACGAACATGGGTTT] were utilized to amplify a 721 bp fragment from the gene encoding 30-KDa major *T. annulata* merozoite surface antigens (Tams 1) as described by d'Oliveira [17]. The final reaction was performed in a volume of 25µl detailed as follows: 5 µl of Maxime PCR PreMix (containing; 1x reaction buffer (10x), 2.5 U of iTaqTM DNA Polymerase (5U/ µl), 2.5 mM of each dNTPs and 1x Gel loading buffer) (iNtRON Biotechnology Korea), 12 µl of H2O, 2 µl of each primer and 4 µl of genomic DNA. The Thermocycler program was as follows: initial denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 3 min, 55°C for 1 min, 72°C for 2 min and final extension step at 72°C for 10 min. PCR products were separated on 1.5% agarose gel.

Indirect Fluorescent Antibody Test (IFAT): Detection of T. annulata antibodies in the serum samples was performed using IFAT as illustrated by FAO [18]. Positive and Negative T. annulata control sera were obtained from the Central Veterinary Research Laboratory, Soba, Sudan. The stained slides were examined under 40× objective using Olympus Vanox incident-light excitation fluorescent microscope (Japan).

III. Result

Of 100 blood smears, 85 (85%) were positive for *Theileria* spp. piroplasm (Table 1). The prevalence rate was 92%, 91% and 66.6% in farm numbers 1, 2 and 3, respectively (Table 1).

Of 100 serum samples, 92 (92%) were positive for *T. annulata* antibodies by IFAT (Table 1). Prevalence of *T. annulata* antibodies was 100%, 98% and 74% in Farm numbers 2, 1 and 3, respectively (Table 1).

A fragment of 721 bp was amplified in 81 (81%) of 100 samples (Figure-2). The prevalence of *T. annulata* was highest in farm 1 (84%), followed by farm 3 (81%), while the lowest prevalence rate was in farm 2 (73.9%).

Table 1: The prevalence of *Theileria annulata* infection in Almased County, Al-Gezira State based on microscopic examination, IFAT and PCR assay.

Number of farm	Total No. of examined animals	Diagnostic techniques		
		BF	IFAT	PCR
Farm 1	50	46 (92%)	49 (98%)	42 (84%)
Farm 2	23	21 (91%)	23 (100%)	17 (73.9%)
Farm 3	27	18 (66.6%)	20 (74%)	22 (81%)
	100	85 (85%)	92 (92%)	81(81%)

BF: Blood Smear, IFAT: Indirect Fluorescent Antibody Test, PCR: Polymerase Chain Reaction

IV. Discussion

Tropical theileriosis, caused by *T. annulata*, is one of the most important TBDs in cattle in Sudan [19]. Gamal and El Hussein [20] estimated that the loss due to an outbreak of tropical theileriosis in north Sudan was approximately US$ 6000 on one farm, and the disease had reduced the expected profitability by 30%.

Due to the progressive increase in the number of agricultural farms and the availability of farm products in Gezira State, the cattle population has risen steadily during the last years [1]. Additionally, the majority of animal owners in the State introduced exotic breeds of cattle (Friesian) and their crosses with the indigenous breeds such as Kenana or Butana to fulfil the increasing demand for milk. It is well known that this type of breed (Friesian) is very vulnerable to tropical disease such as bovine theileriosis [21].

This study has been conducted on the prevalence of tropical theileriosis in Almased County, Al Gezira State, where there is one of the larger dairy farm communities in the State. The overall prevalence of *Theileria* spp. piroplasms detected by blood smears was 85%. This result was higher compared with the result reported by Hayati [12], who detected *Theileria* spp. piroplasms in 16.5% of blood smear in the west part of Al Gezira State. Ali and Abdallah [22, 23] also documented the low prevalence of *Theileria* spp. piroplasms in Khartoum State (16.6%) and in South Darfur State (2.8%) respectively. The sensitivity of blood examination is affected by several factors such as the time of sample collection and the expertise of the hand [24, 25]. Additionally, the prevalence of tropical theileriosis is directly linked with the prevalence and existence of the infected vector [26]. Also presence of other species of *Theileria* may confuse microscopic examination. Therefore, it is expected to have a variation in the disease prevalence among the States.

In the present study, the seropositivity for *T. annulata* antibodies was 92% of 100 serum samples. It is higher than that documented by Hayati [12] who found 46.7% positive of *T. annulata* antibodies, Jaafer [27] who found 31% and Salih [28] who reported 9.8% using IFAT. Introducing Friesian cattle into central Sudan resulted in a gradual increase in disease incidence. Additionally, a lack of the regular tick eradication measure from cattle led to the spread and increase in the prevalence of the disease in the region [29].

In this study, blood smears examination showed lower results (85%) in the detection of *T. annulata* compared with IFAT (92%). This outcome is in agreement with the previous study working on *T. annulata*, in which they found that blood smears revealed 7.3% piroplasms and seropositivity of 46.7% using IFAT [13]. It is well recognized that even if the parasite has been cleared from the animal, the antibodies can remain circulating in the blood for some time after. Therefore, serological tests do not always provide information about the actual existence of the parasite. For that reason, the development of molecular tests such as PCR has made proper tools available for the direct detection of parasites [30].

In the current study, the DNA of *T. annulata* was detected in 81% of the samples using PCR. This result is lower compared with the IFAT result (92%). Taking into consideration that the IFAT is detecting the antibodies that circulation in the blood [31], while the PCR is detecting the DNA of the parasite [32]. Surprisingly, the PCR analysis revealed lower results compared with the blood smears examination. This outcome is in contrast with the previous study, which demonstrated the higher sensitivity of PCR methods compared with the

other diagnostic methods such as blood microscopic examination in the diagnosis of *T. annulata* [17]. The precise justification for this difference is the possibility of cattle getting an infection with other species of *Theileria* [33].

V. Conclusion

In conclusion, tropical theileriosis has a significant economic impact in Almased County, Al-Gezira State and the prevalence of the disease in the study area is extremely high in dairy cattle. Cattle can be infected with other species of *Theileria*, according to both blood smear and PCR data. For proper mapping of the disease's prevalence in this area, molecular characterization of other *Theileria* spp. is recommended.

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Conflict of interest

Authors have declared that no competing interests exist

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