The spreading of East Coast Fever into Great Bahr- El Ghazal Region, North West of South Sudan

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SUMMARY

Theileriosis is a fatal tick-borne disease caused by *Theileria parva* (*T.parva*) and affecting cattle mainly in East and Central Africa. A cross-sectional survey was conducted during June to November 2015 in 12 localities of Great Bahr- El Ghazal Region, South Sudan to investigate the prevalence of East Coast Fever in indigenous zebu (Nilotic) cattle kept under traditional management system. A total of 600 blood and 105 lymph node smears were collected from cattle. Microscopic examination of Giemsa stained blood smears revealed prevalence of 8.7% for *Theileria spp* piroplasms. Surprisingly, all the105 lymph node smears were negative for *Theileria* schizonts. In addition, out of 599 blood samples examined, 464 (77.5%) were positive for *Theileria parva* DNA using p104 nested polymerase chain reaction (nPCR) with significant differences ($P < 0.001$) between the localities of the study areas. Out of eight pooled ticks species that were identified (*Amblyomma variegatum, Rhipicephalus evertsi evertsi, R. decoloratus, Hyalomma rufipes, R.annulatus, H. truncatum, R. sanguineus* group and *R. praetextus*), one tick species (*Amblyomma variegatum*) was positive for *Theileria parva* DNA. The study concluded that *T. parva* is endemic in the region in spite of the absence of *R. appendiculatus.* Detection of *T. parva* DNA in *A. variegatum* may indicate the role of this tick species in transmission of *T. parva* among cattle. However, a need for further investigation employing more samples and using more advanced techniques is highly recommended.

Keywords: *Epidemiology, East Coast fever; Bahr- El Ghazal; South Sudan.*

INTRODUCTION

East Coast Fever (ECF) also known as Theileriosis is a tick-borne disease of ruminants caused by the protozoan parasite *Theileria parva* (Robinson, 1982).

Like other east African countries, ECF is the most important tick borne-disease of cattle in South Sudan which is transmitted by *Rhipicephalus appendiculatus* tick (El Hussein *et al*., 2004).
It is responsible for death of thousands of cattle each year in east and central Africa (Mukhebi *et al*., 1992). The distribution of East Coast fever is strictly associated with the distribution of the vector ticks. East Coast fever in South Sudan was first reported in Kajo kaji and Yei River district boarding Uganda (Hoogstraal, 1956). ECF then extended northward and reached Jonglei and Lake States (Kivaria *et al*., 2012; Nyoap *et al*., 2015; Marcellino *et al.,* 2017).

Importantly, *T. parva* piroplasms have been detected in cattle in Baher El Gazal region in a complete absence of its known vector, *R. appendiculatus* (Zessin and Bauman, 1982). The aim of this study is to determine the prevalence of ECF in indigenous zebu (Nilotic) cattle kept under traditional management system in Great Bahr- El Ghazal states, South Sudan and to

contribute to the knowledge of ECF in cattle in the area by parasitological and molecular methods. The study is important for provision of baseline information on the distribution of the parasite and potential vectors.

MATERIALS AND METHODS

Study area

South Sudan consists of ten states and three Great Regions of Bahr- El Ghazal, Upper Nile and Equatoria. The principal species of livestock found in these regions are cattle, sheep and goats. Great Bahr- El Ghazal region is located in west and north part of South Sudan. The region consists of four states; Lakes, Warrap, Western Bahr-Ghazal and Northern Bahr- El Ghazal States. Twelve localities in three states (Three localities per state) were selected for conducting this study (Figure 1).

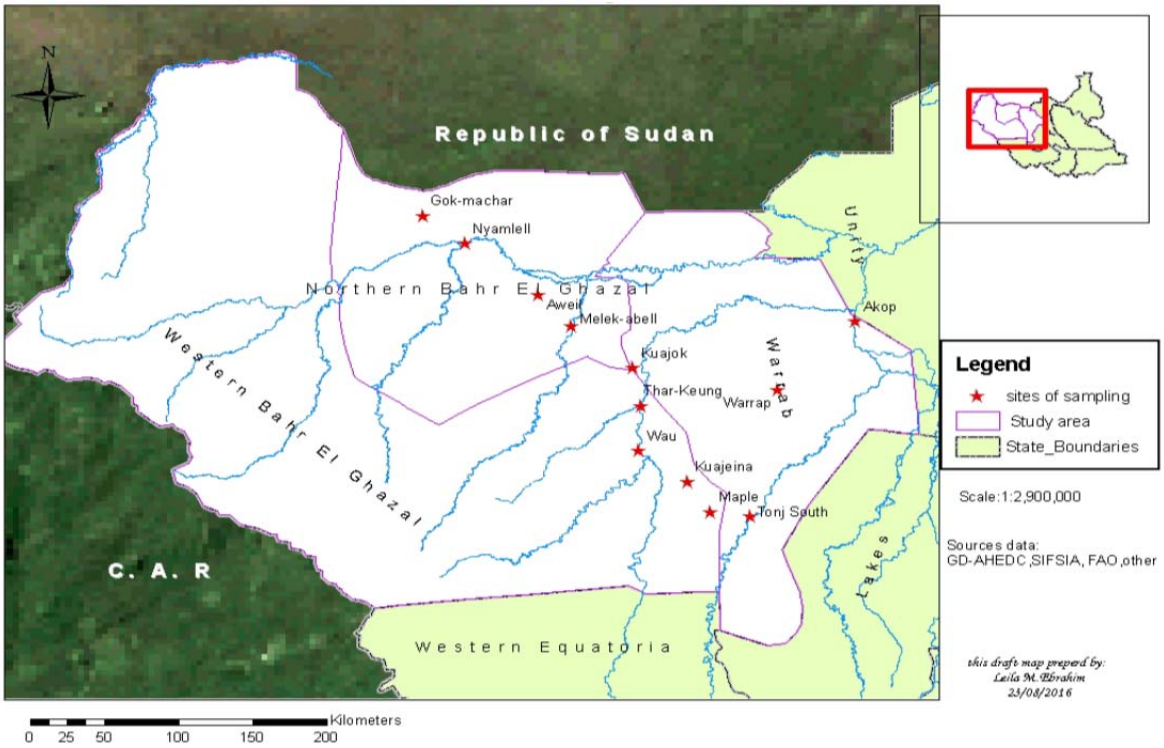

Figure 1. Map of South Sudan and Great Bahr- El Ghazal, showing the locations of samples collection (indicated with red star).

Collection of samples

In a cross-sectional survey carried out during June to November 2015, whole blood from 600 heads of cattle and lymph node biopsies from 105 heads were randomly sampled from indigenous zebu (Nilotic) cattle, which are kept under traditional management system.

Blood from the ear veins were also sampled and prepared on clean glass slides.

Fifty heads of three age groups of animals (< 1 year, 1-3 years and > 3 years) in each of the twelve localities of the study area were sampled.

Both blood and lymph node smears were air dried and fixed with absolute methyl alcohol for five minutes. The fixed slides were stained with 10% Giemsa´s stain for 45 minutes (FAO, 1984). The stained slides were examined microscopically using oil immersion lens for the presence of *Theileria* piroplasms in blood smears or *Theileria* schizonts in the lymph node smears.

Genomic DNA extraction and Polymerase Chain Reaction

The DNA was extracted from blood spots on filter paper using phenol chloroform method according to manufacturer's instructions.

DNA from eight species of ticks (*Amblyomma variegatum, R. evertsi evertsi, R.decoloratus, Hyalomma rufipes, Rhipicephalus annulatus, H. truncatum, R. sanguineus group and R. praetextus*) was extracted using five pooled ticks per species and QIAamp Kit as outlined by the manufacturer.

The p104 primers (Odongo *et al*., 2010) were used (Vivantis Technologies (South Korea) for the nested p104 polymerase chain reaction (nPCR) to screen all field samples for the presence of *T. parva*. The primary nPCR amplifications were performed using a total volume of 20 µl containing 15 µl PCR master mix and 5 µl of test DNA (Micro Amp PCR Pre-Mix-genesig® Advanced Kit, Singapore).

The PCR conditions include denaturation at 94°C and annealing at 60°C for 60 seconds each, and extension at 72°C for 60 seconds. For the secondary reaction, amount of water was 14 and 0.5µl of each forward and reverse primers and 5µl of primary PCR product was used as a template.

The PCR conditions for secondary reaction were similar to that of primary reaction except for the annealing temperature which was 55 °C for secondary reaction.

All the nPCR reactions were carried out in a thermo-cycler (Gene Amp-PCR-system, 9700, Singapore) for the amplification of 30 cycles and the products were separated on 1.5% ethidium bromide stained agarose gel before visualization with ultra violet (UV) trans-illuminator.

RESULTS

The overall prevalence of *Theileria spp*. piroplasms in indigenous zebu (Nilotic) cattle kept under traditional management system in Great Bahr- El Ghazal region, South Sudan was 8.7% (52/600) using microscopy (Table 1).

With regards to state, the highest prevalence (11.0%) was reported in Warrap State, followed by 9.5% in Western Bahr- Ghazal State, and the lowest (5.5%) was observed in Northern Bahr- El Ghazal State (Table 1).

Using blood smears, the age-specific prevalence of piroplasms was found gradually decreased with age groups being the highest in age group less than one-year old (10.8%), followed by age group 1-3 years (9.4%) and age group greater than 3 years (7.5%).

No schizonts were detected in the 105 lymph node smears examined by microscopy.

Table 1. Prevalence of *Theileria spp.* piroplasms using blood smears in Great Bahr- El Ghazal, South Sudan.

State	Locality	No. animals examined	No. positive	Prevalence of piroplasms(%)*
Western Bahr- El Ghazal (n=200)	Kuajiena	50	9	18
	Mapel	50	5	10
	Thar-kueng	50	3	6
	Wau	50	2	4
	Total	**200**	**19**	**9.5**
Northern Bahr- El Ghazal (n=200)	Aweil	50	6	12
	Gok-machar	50	4	8
	Nyamlell	50	1	2
	Malek-alell	50	0	0
	Total	**200**	**11**	**5.5**
Warrap State (n=200)	Tonj	50	7	14
	Warrap town	50	6	12
	Akop	50	5	10
	Kuajok	50	4	8
	Total	**200**	**22**	**11.0**
Total		**600**	**52**	**8.7**

*Study period: June to November, 2015

Table 2. Prevalence of *T. parva* using nPCR in different localities in Great Bahr- El Ghazal, South Sudan.

State	Locality	No. of animals	No. positive	Prevalence (%) of *T. parva**
Western Bahr- El Ghazal (n=200)	Kuajiena	50	36	72.0
	Mapel	50	37	74.0
	Thar-kueng	50	34	68.0
	Wau	50	42	84.0
	Total	**200**	**149**	**74.5**
Northern Bahr- El Ghazal (n=199)	Aweil	50	44	88.0
	Gok-machar	49	39	79.6
	Nyamlell	50	37	74.0
	Malek-alell	50	45	90.0
	Total	**199**	**165**	**82.9**
Warrap State (n=200)	Tonj	50	45	90.0
	Warrap town	50	35	70.0
	Akop	50	44	88.0
	Kuajok	50	26	52.0
	Total	**200**	**150**	**75.0**
Total		**599**	**464**	**77.5**

*Study period: June to November, 2015

Out of 599 extracted DNA samples, 464 (77.5%) were positive for *Theileria parva* DNA tested using nested polymerase chain reaction (nPCR) (Supplementary Figure 1). The age and sex-specific prevalence of *Theileria parva* using nPCR are presented in (Table 3). A significant statistical difference ($P < 0.001$) between the studied localities of the study area was observed. Furthermore, *T. parva* DNA was detected only in *A. variegatum* (Supplementary Figure 2)

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Table 3. Prevalence of *T. parva* using nPCR by age and sex

	Age/Sex	No. of animals	No. positive	Prevalence (%) of *T. parva*
Age groups	<1year	92	60	65.2
	1-3year	213	169	79.3
	>3 year	294	235	79.9
	Total	**599**	**464**	**77.5**
	Males	210	158	75.2
	Females	389	306	78.7
	Total	**599**	**464**	**77.5**

DISCUSSION

This study was initiated to investigate the prevalence of East Coast fever in indigenous zebu (Nilotic) cattle kept under traditional management system in the Great Bahr- El Ghazal region, South Sudan. Investigations directed toward determining the prevalence of *T.parva* in different regions of South Sudan and the species of tick involved, could play a magnificent role in designing strategic control of this disease and other tick-borne diseases in both North and Southern Sudan.

The overall prevalence of *Theileria spp* piroplasms in blood smears was 8.7%. This result is in agreement with Zessin and Baumann (1982) in Bahr- El Ghazal, South Sudan where they detected *T. parva* using blood and lymph node smears. The overall prevalence of *T.parva* in blood smear examination was relatively low compared to nPCR. This may be attributed to the fact that microscopic detection of piroplasms requires a high parasitaemia level.

However, carrier animals, in which low numbers of infected erythrocytes cannot be detected microscopically are important contributors to the transmission of the infection by tick bites (Skilton *et al*., 2002). The absence of schizonts in all of the 105 lymphnode smears was surprising due to the fact that *T.parva* parasites were detectable in blood smears. However the use of long acting Oxytetracycline by cattle owners could have concealed the detection of schizonts (Marcellino *et al*., 2011). On the other hand, the application of nPCR resulted in high prevalence (77.5%) of *T. parva.* The results suggest that either nPCR could detect positive samples even at conditions of low parasitemia.

This study report higher prevalence of *T.parva* compared to that reported by Nyoap *et al*. (2015) and this is attributed to the high sensitivity of the nPCR (OIE, 2014) used in this study. However, there was no recovery of *Rhipicephalus appendiculatus* in this study, the principle tick vector of *T.parva*, the finding that is similar to previous study by Marcellino (2008) and Nyoap *et al*. (2015) who detected *T. parva* antibodies without the presence of the vector *R. appendiculatus* in Central Equatoria and Jonglei States, South Sudan.

This implies that the principal vector of *T.parva* could vary with geographical locations. This theory is supported by the findings in this study where out the eight tick species examined by nPCR, *T. parva* was detected only in one species, *Amblyomma variegatum* suggesting that *A. variegatum* may play a role in transmission of *T. parva* in the absence of the principal vector *R. appendiculatus. T.parva* was detected in samples from all the regions including as far as the north

western part of South Sudan where *T.parva* is being reported for the first time.

In the affected animals, older animals showed higher prevalence (79.9%) than younger ones (65.2%) similar to the findings reported elsewhere by Salih *et al*. (2007). The cause of this difference is not clearly known. Nonetheless, the wide spread of *T.parva* implies that the parasite is now endemic in the whole of South Sudan. Based on the findings in this study, one could predict that, this disease is likely to cross the borders to the Sudan if appropriate control measures are not put in place. Lastly, the study shows that, nPCR is a useful technique for investigating the prevalence of *T. parva* where the parasites exist in low intensity and can be applied to monitor the effectiveness of *T.parva* treatment. The detection of *T. parva* DNA in *A. variegatum* indicates the role of this tick in transmission of *T.parava*. Further investigations will provide more insight on the role of this tick in *T.parva* transmission

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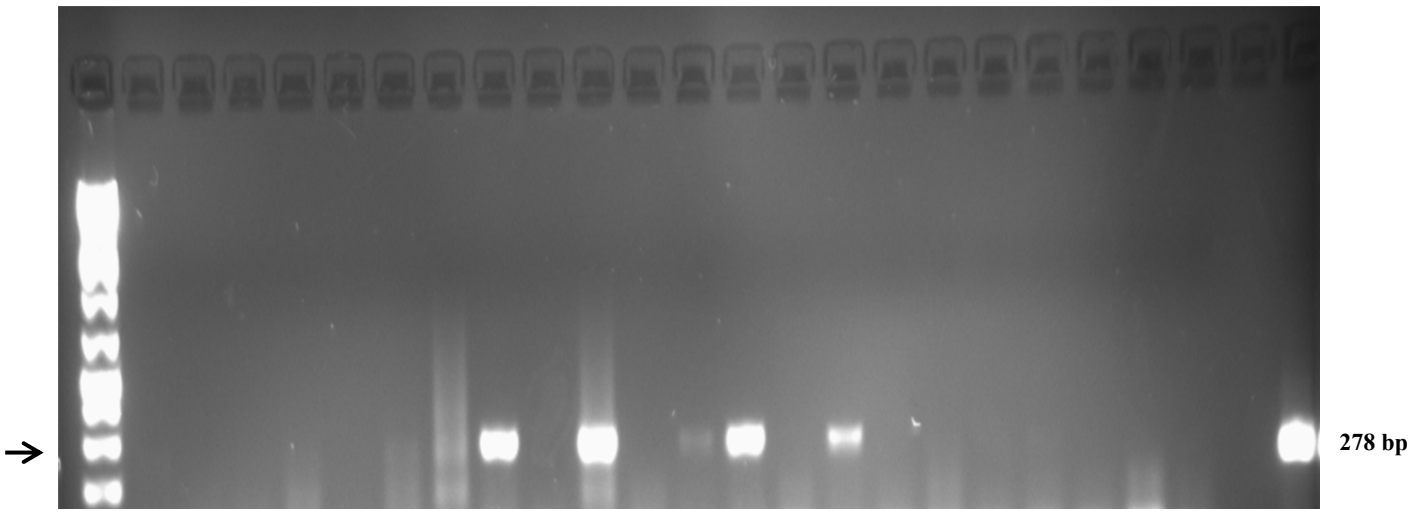
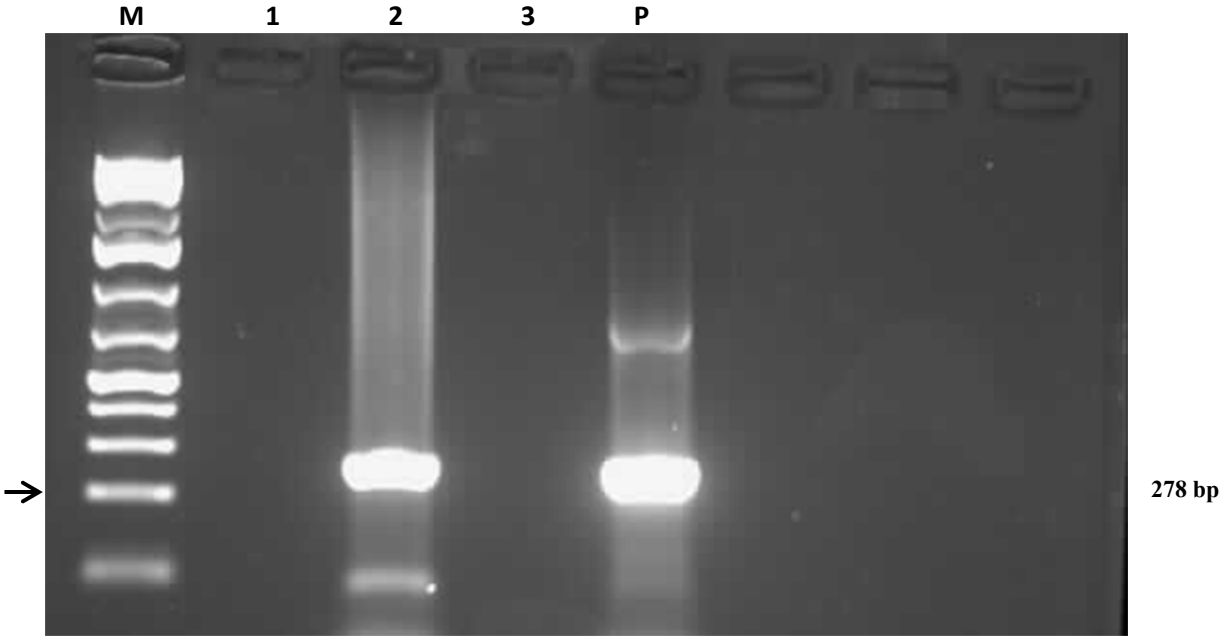


Supplementary Figure 1. 1.5% agarose gel showing *T. parva* nPCR results using p104 primers. M: 100 bp DNA-marker. Samples 8, 10, 13, and 15 positives, N: negative control, P: positive control. Region of expected band size (arrow)



Supplementary Figure 2. 1.5% agarose gel showing *T. parva* nPCR results using p104 primers. M: 100 bp DNA-marker. Lane 1 and 3: negative result, 2: *A. variegatum*, P: positive control. Region of expected band size (arrow)