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Full Length Research Paper

A survey of ticks and East Coast fever among cattle in Fangak County, Jonglei State, South Sudan

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This study was carried out in Fangak County, Jonglei State, South Sudan, with aim to identify the main ticks species and follow north limit of the theileriosis in Jonglei. Three localities namely; Hai, Toggar and Bichoul kun village in Phoum payam were selected as suspected area for East Coast fever (ECF), (Group A) and Kuer kan in Manjang payams were selected as non suspected area for ECF (Group B). These groups A and B were based on animal movement, trade business and intermarriages (animals paid for dowry). A total of 120 sera were collected from cattle of different age groups. The serum samples were tested using indirect polymorphic immunodominant molecule (PIM) ELISA to detect *Theileria parva* antibodies. The results indicated that 5/44 (11.4%) samples from non suspected area (group B) and 48/76 (63.2%) samples from suspected area (Group A) revealed antibodies. The overall positivity was 53/120 (44.17%) which was highly significant (P < 0.001) according to the locations. Three tick genera were recorded, *Amblyomma*, *Hyalomma* and *Rhipicephalus*. The species were *A. variegatum*, *A. lepidum, H. rufipes, R.* (*B.*) *decoloratus, R.* (*B.*) *annulatus, R. e. evertsi* and *R. sanguineus*. The most abundant tick species was *A. variegatum,* constituting 62%, while the lowest tick recorded was *R. e. evertsi* with prevalence rate of 2%. No *R. appendiculatus* tick was seen in Fangak area; while *T. parva* antibodies were detected. Regarding the fact that *T. parva* antibodies were present in the area with 44.17% prevalence, more efforts are needed to determine the extension of ECF and its vector *R. appendiculatus* to the northern parts of Jonglei State and this result of ECF antibodies could be an alarm to migrate cattle owners from South Sudan.

Key words: Ticks, east coast fever (ECF), ELISA, South Sudan.

INTRODUCTION

South Sudan is known to be most populated with livestock in Africa, on other hand only equatorial area is known to be endemic while two region of Upper Nile and Bahr El Ghazal is known to be free from diseases except some pockets in Bor Jonglei State and Awerial in Lake State (Kavaria et al., 2012). Ticks and tick-borne diseases (TBDs) are the major problems according to the studies carried out in the area (FAO, 1983; Julla, 1994

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and Marcellino et al., 2011b). The first assessment for ticks was carried out by Hoogstraal (1956) who found that there were 39 species of ticks in South Sudan in 150 localities. He found that *Rhipicephalus* (*B*) species, *Margaropus* spp and *Hyalomma* spp. were the main prevalent ticks. *R. appendiculatus*, the vector of East Coast Fever (ECF) caused by the *Theileria parva* is mainly distributed in areas of high rainfall and moderate temperature, such as the district of Kajo Kaji, Yei, Ngangala, Torit and Katire. In addition, these tick species was identified in Chukudum, Aswa River, Palotaka, Nimule and Juba (Morzaria et al., 1981 and Julla 1985, 1994). Korok (2005) reported the presence of eight species of ticks in Pibor area of Jonglei State while Salih et al., (2008) found ten species and three genera including the ECF vector in Central Equatoria State. Also, Marcellino et al., (2011b) reported the presence of seven species of ticks in Central Equatoria State among them was *R. appendiculatus*. On the other hand, Kivaria et al., (2012) conducted a survey in five states, and showed the presence of three genera and six species among which was the vector of ECF and presence of other main TBDs in those states.

In South Sudan, theileriosis was reported for the first time in 1950 by Hoogstraal (1956), the region then became endemic (Julla, 1994; Salih et al., 2007b and Marcellino, 2008). Then, ECF extended north direction and reached Bor area (Ochi et al., 2009; Kivaria et al., 2012). Recently the disease was reported by Malak et al., (2012) in Kajo kaji and Yei counties of central Equatoria. The impact of disease reported by Marcellino et al., (2011a) stated the losses (mortality) due to ECF was around 134325$ in two cattle camps where outbreaks for the diseases were reported. The study area is one of the active route for transporting animals from Equatoria and Bor areas, known endemic areas to ECF, to other states and north Sudan. The present study aimed at determining prevalence of ticks and ECF in Jonglei State with emphasis on ECF and determination of north limits with objectives, firstly to identify the main species of ticks prevalent in the area with special reference to the main vector of ECF, and secondly, to examine animals to follow the northward spread of ECF in Jonglei State.

MATERIALS AND METHODS

Study area

Fangak County in Jonglei State, located at 9°04′10 to 9°06 94°N, 30°53′03 to 30°88′41E is bordering Panyikang county of Upper Nile State in the North East, Ayod County to the South, Piji County to the east. Phoum al Zeraf town, the county headquarter, lies where the Bahr el Zeraf joins the White Nile from the east (Figure 1 and 2).

Vegetation cover

The area around the study region in Jonglei State is characterized by acacia tall trees forest and swampy grassland, seasonally inundated land or known locally as toich (swampy) alongside the Bhar jabel (White nile ,Kiir) and Bhar El Zaraf (Phow) with vast grazing area for cattle and wildlife reserve in the area. The annual rainfall ranges between 750 to 900 mm with the rain starting in mid April declining in October, while the mean ambient temperature is between 23 to 41°C in dry season (March, early April) and 19 to 34°C in wet season (June to August). On the other hand, elatives humidity average is between 47% in dry season and 89% in wet season.

Sampling

A convenience sampling method was applied (Smith, 2005). Thus, the target animals were the indigenous Nilotic type (*Bos indicus*). One hundred and twenty cattle was sampled. The animals were sampled from an area categorized into A, being suspected area and B, being non-suspected area for East Coast fever (ECF). Categorization into A and B areas was based on cattle movement and trading routes. Two payams (Administration Unit), Phoum in the mainland and Manjang on the island were selected as the study areas. The animals selected were 44 from Kure kan within Manjang Payam, Hai Toggar with 42 animals and Bichoul kun village with 34 animals, the males were 40 animals and females were 80 animals. Also, animals were categorized to three age groups; 1 year to less than 2 years, 2 years to less than 4 years and 4 years and above.

Serum sample

Animals were restrained and blood was collected from the jugular vein, in sterile vaccutainers without EDTA, then labeled and left overnight (12 h) to clot at room temperature (25°C). The serum was then separated and put in serum tubes, labeled and stored at -20°C until used for serological tests. The procedure of PIM ELISA was carried out as described by Katende et al. (1998).

Blood smears

One hundred and twenty blood smears were taken from the animals. The blood drop was taken from the ear vein of each animal on a clean slide, then spread with another clean slide and air dried, then fixed with absolute methanol labeled according to sample number and place of collection. The blood smears were stained using 10% Giemsa's stain before microscope examination under oil immersion (100x) was carried out.

Tick collection

Total body collection of ticks was carried out with care not to lose the mouth parts and preserved in vials containing 70% alcohol and labeled accordingly to correspond with the labelled serum samples.

Tick identification

Ticks were identified under a stereoscopic dissecting microscope, with key guidance for taxonomy as described by Hoogstraal (1956).

Statistical analysis

Ticks collected from animals were subjected to an appropriate general liner model (GLM) of statistical analysis system (SAS (Version 9) package. The SAS was used to perform analysis of variance (ANOVA) and mean separations were performed using

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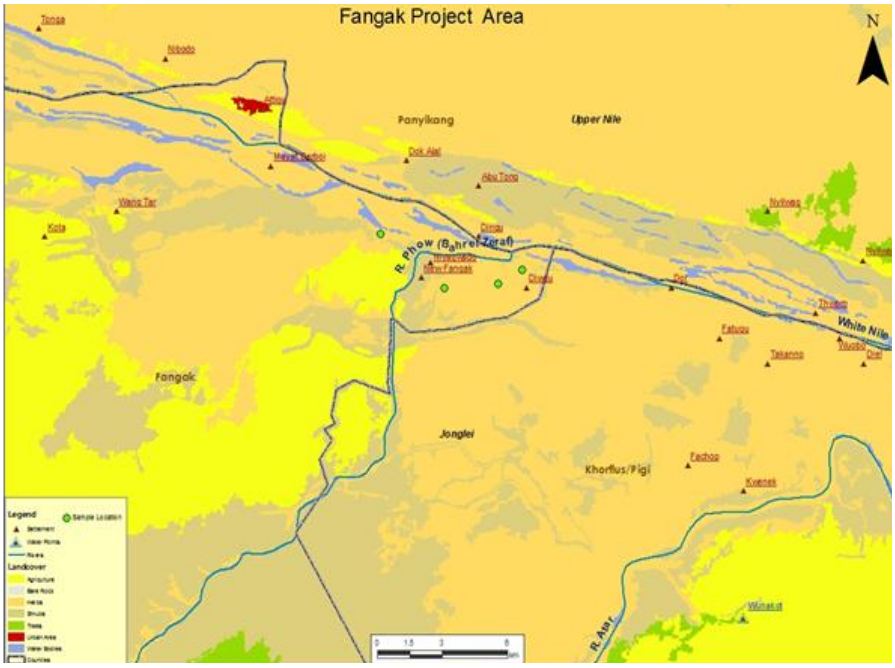

Figure 1. The map of Fangak county showing the sampling sites.

Rayan- Einot- Gebrial–Welsch multiple range test (REGWQ) (Day and Quinn, 1989).

RESULTS

Prevalence of *T. parva* antibodies in the study area

Based on the indirect PIM ELISA ,the overall prevalence of *T. parva* antibodies was 44.17% (53/120) in overall cases prevalence ranging between 4.17% (5/120), 15.83% (19/120) and 24.17% (29/120) in Kuer kan, Bichoul Kun and Hai Toggar, respectively. The prevalence for relative occurrence range was between 9.49% (5/53) in Kuer Kan, 35.85% (19/53) in Bichoul Kun and 54.72% (29/53) in Hai Toggar. While the overall prevalence cases range was between locations, showing highly significant difference ($P < 0.001$). Sero-prevalence was found to be 55.88% (19/34) in Bichoul kun, 69.05% (29/42) in Hai Toggar Phoum payam and 11.34% (5/44) in Kuer kan Manjang payam (Table 1). Concerning sex, the overall prevalence was 28.3% (34/120), while within females the prevalence was 42.50% (34/80). Meanwhile overall prevalence rate was 15.83% (19/120) for male sex. Within males the prevalence was (19/40) 47.50%. The sero-prevalence rate by sex was not significant ($P > 0.05$) (Table 2). According to the age groups, the overall prevalence rate in animals of one year to less than two years was 3.33% (4/120) and the prevalence within this age group was 44.44% (4/9). The overall prevalence rate for those between two years and less than four years was 8.33% (10/120) while the prevalence with this group was 50% (10/20). While the overall prevalence for the age group four years and above was 32.5% (39/120) and the prevalence within this group was 42.86% (39/91). The relative occurrence prevalence revealed 73.58% in 4 years and above, 18.87 and 7.55% respectively for 2 years to less than 4 years and 1 year to less than 2 years old. The sero-prevalence by age was not significant ($P > 0.05$) (Table 3).

Blood smear

The results of 120 blood smears taken from animals in the three locations revealed no piroplasms.

Tick identification results

During the study, 328 ticks were collected from 120 animals in the three locations in Phoum and Manjang

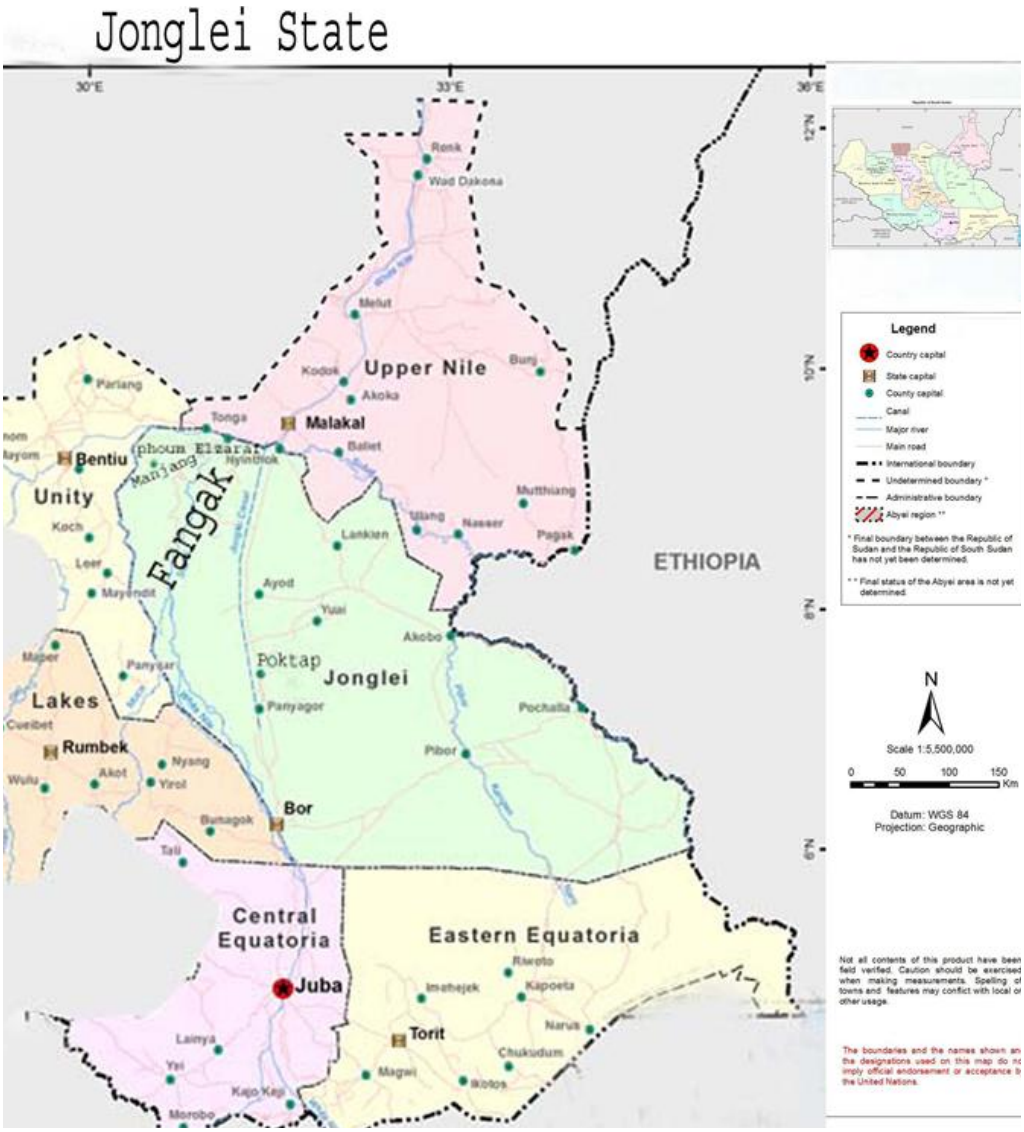

Figure 2. South Sudan Map location of Jonglei State.

Payams (administration units) (Table 4). Three tick genera and seven species were identified. Tick genera were *Amblyomma*, *Hyalomma* and *Rhipicephalus*. Tick species were *A.variegatum*, *A. lepidum*, *H. rufipes, R.* (*Boophilus*) *annulatus*, *R.* (*B.*) *decoloratus*, *R. e. evertsi* and *R. sanguineus*. The two *Amblyomma* and two *Rhipicephalus* (B) species were found throughout the study area, while *R. e. evertsi* was found only in Kuer kan village Manjang payam. Mean while *R. sanguineus* was found only in Phoum payam. The highest tick count was

Table 1. Prevalence of *Theileria parva* antibodies tested by ELISA in different locations of Fangak, Jonglei State in September 2011.

Location	No. of Animals	Results	Percentage of positive reactors		
		positive (+Ve)	Prevalence pre-cases	Overall cases prevalence	Relative occurrence prevalence
			(+Ve) %	(+Ve) %	(+Ve) %
Bichoul Kun village (Phoum payam) Suspected area	34	19	(19/34) 55.88	(19/120) 15.83	(19/53) 35.85
Kuer kan village (Manjang payam) Non suspected area	44	5	(5/44) 11.34	(5/120) 4.17	(5/53) 9.49
Hai Toggar (Phoum payam) Suspected area	42	29	(29/42) 69.05	(29/120) 24.17	(29/53) 54.72
Total	120	53	100	44.17	100

Table 2. Prevalence of *Theileria parva* antibodies tested by ELISA in different sex groups cattle, in Fangak, Jonglei state, September 2011.

Sex	No. of Animals	Results	Percentage of positive reactors		
		positive (+Ve)	Prevalence pre-cases	Overall cases prevalence	Relative occurrence prevalence
			(+Ve) %	(+Ve) %	(+Ve) %
Female	80	34	(34/80) 42.50	(34/120) 28.3	(34/53) 64.15
Male	40	19	(19/40) 47.50	(19/120) 15.83	(19/53) 35.85
Total	120	53	100	44.17	100

recorded in Phoum payam Group A. (Table 4) summarizes the frequency of adult ticks in the study area. The most abundant tick species in decreasing order were *A.variegatum* (62%). *R.* (*B.*) *decoloratus* (14%), *H. rufipes* (8%), *R.* (*B.*) *annulatus* (6%), while the least frequent ticks were *R. sanguineus* (4%), *A. lepidum* (4%), *R. e. evertsi* (2%). The male ticks represented 63.4% (208/328) outnumbering the females 36.6% (120/328), in all locations.

The geographic distribution and population density of tick species in two proposed groups

The mean tick load per location was the highest in Kuer kan village with mean load (2.8 ± 0.15), while the lowest mean load was recorded in Bichoul kun and in Hai Toggar (2.3 ± 0.14). Both bulls and cows ((2.6 ± 0.14 and 2.6 ± 09 respectively), carried the same load. The animals between one and two years were found to carry more tick load mean (2.7 ± 0.33), while animals of four years and more showed a mean of 2.6 ± 0.09. The lowest tick load was realized in animals of two years and less than four years (2.4 ± 0.16). Animals with brown coat revealed the highest mean of tick load (2.7 ± 0.12) followed by white coat animals (2.6 ± 0.12) while the lowest infested animals were of black coat with a mean of (2.3 ± 0.14) (Table 5) with no significant difference ($P > 0.05$).

Discussion

Ticks and tick-borne diseases (TBDs) are one of the most important causes of poor animal production and productivity around the world (FAO, 1983; Singla et al., 2007 and Salih et al., 2015). In the current study no parasites were seen in blood smears despite the detection of antibodies to ECF in serum sample with 44.17% positive cases. This is in agreement with finding of Malak et al., (2012) who reported prevalence of 35.60 and 70.60% in Yei and Kajo Kaji, respectively. Julla (1994) revealed 44.20% prevalence which is similar to this study and Morzaria et al., (1981) revealed 51.60 and 61.50% prevalence in Aswa river and 12.10% prevalence in Juba and 83.30% prevalence in Chukudum after outbreak. This study used indirect ELISA while the other three studies used IFA. These findings are in agreement with Marcellino (2008) with his finding in Terekaka County where he found *T. parva* antibodies without detection of *R. appendiculatus*. Although in Terekaka the prevalence was 71.80% while in this study it was 44.17%;

Table 3. Prevalence of *Theileria parva* antibodies tested by ELISA in different age groups of animals , in Fangak ,Jonglei State in September 2011.

Age group	No of animals	Results	Percentage of positive reactors		
		positive (+Ve)	Prevalence per-cases	Overall cases prevalence	Relative occurrence prevalence
			(+Ve) %	(+Ve) %	(+Ve) %
1 < 2 year	9	4	(4/9) 44.44	(4/120) 3.33	(4/53) 7.55
2 < 4 year	20	10	(10/20) 50.00	(10/120) 8.33	(10/53) 18.87
≤4 year	91	39	(39/91) 42.86	(39/120) 32.5	(39/53) 73.58
Total	(120)	(53) 44.17	100	44.17	100

Table 4. Tick species infesting cattle in two proposed groups (A and B) in Fangak, Jonglei State during September 2011.

Tick species	Group A(Phoum payam)		Group B (Manjang payam)		Percentage per group		Relative Occurrence (Total) 100%
	Male (%)	Female (%)	Male (%)	Female (%)	Group A (%)	Group B (%)	
Amblyomma variegatum	109 (33.23)	27 (8.23)	61 (18.59)	6 (1.82)	136 (41.46)	67 (20.42)	203 (62)
Amblyomma lepidum	5 (1.52)	3 (0.91)	5 (1.52)	0	8 (2.43)	5 (1.52)	13 (4)
Hyalomma rufipes	5 (1.52)	6 (1.82)	14 (4.26)	0	11 (3/35)	14 (4.26)	25 (8)
Rhipicephalus e. evertsi	0	0	2 (0.60)	3 (0.91)	0	5 (1.52)	5 (2)
Rhipicephalus sanguineus	7 (2.13)	7 (2.13)	0	0	14 (4.26)	0	14 (4)
Rhipicephalus(B)decoloratus	0	25 (7.62)	0	22 (6.7)	25 (7.62)	22 (6.7)	47 (14)
Rhipicephalus (B) annulatus	0	9 (2.72)	0	12 (3.65)	9 (2.72)	12 (3.65)	21 (6)
Total	126 (38.41)	77 (23.47)	82 (25.00)	43 (13.11)	203 (61.89)	125 (38.11)	328 (100)

he stated that due to seasonal migration of animals to endemic areas while here in Fangak County it is due to trade and intermarriage and rampant movement of animals between endemic and free zone with no proper control measures. Phoum payam act as a trade centre and main transporting station, despite the fact that Manjang Payam could be more suitable area for survival of vector of ECF due to vegetation cover in the area and similar environment of Bor county in south part of the state which known to be endemic area as reported by Kivaria et al., (2012). This is an indication that ECF can establish if the tick is introduced in this area. The sex of the animals does not have any epidemiological significance although female animals revealed in one occasion higher rates than males. This might be due to the fact that most of the herds comprises of females even most of animals paid for dowry were females. Age groups finding are similar to Salih et al., (2007a) who indicated that antibodies profile was found to increase significantly with advance in age. This study is also in agreement with Marcellino (2008) who found older animals from four years and above revealed higher prevalence than young animals of one year to less than four years. This study is in agreement with the study of Darghouth et al., (1996) who reported that older cattle had seropositive level to *T. annulata* antibodies more than younger ones.

This study agrees with Zessin and Baumann (1982) in Bahr Elghazal, South Sudan who detected *T. parva* using blood smears and lymph node smears. The current study detected the antibodies by ELISA with no presence of piroplasms in blood smears. No presence of the vector in the two areas might be due to the short time of study or the use of Oxyteracycline Long Acting by cattle owners that could have concealed the piroplasms. The trypanosomes were detected accidentally while carring the examination to find the piroplasms for TBDs. In addition area is endemic for trypanosomiasis with presence of mechanical vector. *Tabnus* spp observed do not have any impact for ECF unless on others TBDs. The results of ticks identified in this study are in agreement with those reported by Korok (2005). The finding that *A. variegatum* showed the highest prevalence rate which is in agreement with the finding of Ochi et al., (2009). Meanwhile, Korok (2005) reported *A. lepidum* was the highest, probably due to difference in climatic conditions. Fangak County experience frequent flooding and has more forest cover than Pibor which tends to be a semi-arid in nature. In contrast, while Korok (2005) found both sexes of *R* (*B*) *decoloratus* and *R* (*B*) *annulatus*, the current study showed the opposite, that is, females without males. Also, Korok (2005) found *R. praetextatus*, while this tick species was not reported in the current study.

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Table 5. Mean (±SE) numbers of ticks encountered in Fangak county Jonglei State according to locations , sex, age group and animals colour September 2011.

Locations	No of animals	Mean (± SE)
Bichoul kun village	41	2.6 ± 0.13[a]
Kuer kan village	45	2.8 ± 0.15[a]
Hai Toggar	42	2.3 ± 0.14[a]
Hai Moaazker foqu	22	2.9 ± 0.21[a]
	Sex	
Female	106	2.6 ± 0.09[a]
Male	44	2.6 ± 0.14[a]
	Animals age (years)	
1 < 2	11	2.7 ± 0.33[a]
2 < 4	24	2.4 ± 0.16[a]
≤ 4	115	2.6 ± 0.09[a]
	Animals colour	
Black	14	2.3 ± 0.14[a]
Brown	60	2.7 ± 0.12[a]
White	76	2.6 ± 0.12[a]

Means (± SE) followed by the same letter in each column are not significantly different at 5% level based on Rayan´s Qtest (REGWQ), (1989).

Generally, results of tick's collection were in agreement with the findings of Marcellino et al. (2011) and Julla (1994), but with the absence of *R. appendiculatus* in this study and absence of *A. lepidum* in Marcellino et al ., (2011b) but were present in Julla study (1994). This study agrees with Hoogstraal (1956) in Fangak where he encountered *H. rufipes* and *R* (*B*) *decoloralatus* and other species were reported in nearby areas like Malakal, Tonga and Atar. It could be concluded that ECF comprises a threat in the northern areas of Jonglei State if it is accidently introduced with its vector *R. appendiculatus*.

Conclusion

Regarding the fact that *T. parva* antibodies were present in the area with 44.17% prevalence, more efforts are needed to determine the extension of ECF and its vector *R. appendiculatus* to the northern parts of Jonglei State. Seasonal collection of ticks throughout the State and mapping of the distribution of *R. appendiculatus* in the State would help in formulating future plans for control of ECF in the State.

Impact

The finding of this study may address the cattle owners, stakeholders and policy maker to draw their attention to critical movement of ECF toward north part of the country and possibility of establishing itself in new areas and threaten most populated animal will impact of livelihood of those communities. Combined efforts by the Government, with different stakeholders to determine the presence of other T and TBDs in the State are required. The principal action should be developing and production of animal health policy to, among others, regulates animal movement within the State and to the adjacent States. Such a policy may also reduce incidence of cattle raiding amongst the tribes of Jonglei State. This result of ECF antibodies could be an alarm to migrate cattle owners from South Sudan.

Conflict of Interest

The authors have not declared any conflict of interest.

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