

Seroprevalence of East Coast fever in Central Equatoria State, South Sudan

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Summary

A cross-sectional survey was conducted in 2005 in different cattle camps in Juba, Mangalla and Terekeka localities of Central Equatoria State, South Sudan. Serum samples were collected from 514 cattle of different age groups. Samples were analysed using an indirect enzyme-linked immunosorbent assay (ELISA) with commercially available polymorphic immunodominant molecule (PIM) ELISA kits. The overall serological prevalence of *Theileria parva* was 70.8% (364/514). The highest rate of prevalence was observed in Mangalla (91.2%) and the lowest in Juba (61.8%), with Terekeka recording 71.8%. Regarding the age groups, older cattle (over four years of age) showed a significantly higher rate of prevalence ($p > 0.001$) than calves (below one year of age). The implications of these results in the overall epidemiology of East Coast fever in South Sudan are discussed and possible recommendations for future implementation of disease control measures are outlined.

Keywords

Central Equatoria, East Coast fever, ELISA, Enzyme-linked immunosorbent assay, Prevalence, *Theileria parva*, Serology, South Sudan.

Sieroprevalenza di febbre della costa orientale (theileriosi) nel Central Equatoria State, Sud Sudan

Riassunto

Nel 2005 è stato condotto uno studio trasversale in diversi allevamenti (campi) di bovini in località Juba, Mangalla e Terekeka nel Central Equatoria State, Sud Sudan. Sono stati prelevati campioni di siero da 514 bovini in diverse fasce di età. I campioni sono stati analizzati utilizzando un test ELISA indiretto commerciale (Polymorphic Immunodominant Molecule: PIM). La prevalenza complessiva sierologica di Theileria parva è stata del 70,8% (364/514). Il tasso di prevalenza più elevato è stato riscontrato a Mangalla (91,2%), a Terekeka il tasso è stato del 71,8%, a Juba del 61,8%. Per quanto riguarda le fasce di età, i bovini di oltre quattro anni hanno mostrato un tasso di prevalenza significativamente superiore ($p > 0,001$) rispetto a quelli di età inferiore a un anno. Nello studio si è discusso il significato epidemiologico dei risultati ottenuti, delineando le possibili linee guida per l'attuazione di misure di controllo della febbre della costa orientale (theileriosi).

Parole chiave

Central Equatoria, ELISA, Enzyme-linked immunosorbent assay, Febbre della costa orientale, Prevalenza, Sierologia, Sud Sudan, *Theileria parva*, Theileriosi.

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Introduction

East Coast fever, caused by *Theileria parva* and transmitted by *Rhipicephalus appendiculatus* (the brown ear tick), poses one of the major challenges to the development of cattle industry in many southern, Central and East African countries including the Republic of South Sudan (3). Diagnosis of *T. parva* includes the following:

- parasitological by identifying *Theileria* piroplasms and *Theileria* schizonts in blood smears or lymph nodes
- serological by the indirect fluorescent antibody (IFA) test, enzyme-linked immunosorbent assay (ELISA) and using molecular tools by conventional polymerase chain reaction (PCR) and real time PCR (13, 14).

The disease can be treated using anti-theilerial drugs (e.g. buparvaquone) if the disease is diagnosed in the early stages or, alternatively, vector control implemented by acaricide application.

The first outbreak of East Coast fever in Sudan was reported in 1950 in the Kajo Keji and Yei River districts (4). After this outbreak, the disease was not reported again until 1981 (12). In the last 28 years, Juba area has been devastated by waves of East Coast fever outbreaks (9). In 1979, additional outbreaks were recognised with the increased uncontrolled movement of cattle by Ugandan refugees (5). In 1999, there was a severe outbreak of East Coast fever in and around Juba and findings indicated that the morbidity due to East Coast fever ranged between 20%-60% and mortality reached up to 30%. In the same year, East Coast fever extended north of Juba town for the first time (9). East Coast fever has now become a problem for the agro-pastoralist communities around Juba. The cost of control is very high so that the ordinary farmer cannot afford to undertake this. This situation requires the development of effective control strategies by the veterinary authorities and an initial step towards this goal is to

establish the extent and prevalence of the disease.

The present study is aimed at establishing the seroprevalence of East Coast fever, particularly in the localities of Juba, Mangalla and Terekeka in South Sudan.

Materials and methods

Study area and husbandry practices

Central Equatoria State is situated in the centre of the Equatoria Region of South Sudan and Juba is the capital of the State as well as capital of the newly formed State of South Sudan. The climate is humid with seasonal rainfall variations usually giving two seasons: the long wet rainy season (April to November) and the short hot dry season (December to March). Total annual rainfall varies from 900 mm to 2 032 mm for 7-9 months and, in general, decreases northwards. Ambient temperature generally ranges between 20°C and 35°C. The study area is inhabited by the Bari and Mundari tribes who live along River Bahr-el Jabel. These are agro-pastoralist communities who rely heavily on agricultural activities. They keep considerable numbers of cattle, sheep and goats.

The population of livestock in Central Equatoria State is estimated at 1.5 million cattle, 1.1 million goats and 1.2 million sheep, of which 75% are found in Terekeka Province (1). All are indigenous breeds with the exception of few crossbreeds that originated from the former Juba dairy and Mafao farm projects that still exist in the Juba area. Livestock are mainly kept under a traditional local management system in which cattle are tethered during the night and released during the day for grazing. In general, livestock movements in the state are uncontrolled due to the lack of legislation.

The major cattle diseases in the area include contagious bovine pleuropneumonia, haemorrhagic septicaemia, blackwater, trypanosomosis, endo- and ectoparasites and East Coast fever which is always ranked first by livestock owners in the area.

Sample collection

A cross-sectional survey was conducted during 2005 at different cattle camps in Juba, Mangalla and Terekeka localities in South Sudan. Ten ml blood samples were collected from 514 cattle at 14 cattle camps (Table I). All sites are stocked with the indigenous breeds of cattle (Nilotic Zebu) and kept exclusively under the traditional management system. The age groups sampled were calves less than one year, yearlings 1 to 2 years, adults between 3 to 4 years and those over than 4 years. Cattle sampled were of both sexes.

Serological test

The polymorphic immunodominant molecule (PIM) recombinant antigen ELISA (Svanova®, Uppsala) was used, in accordance with the instructions of the manufacturer, to detect antibodies to *T. parva* (6) in the serum samples. The sensitivity and specificity of the PIM ELISA for *T. parva* were reported to be 99% and 98%, respectively (6). The optical density (OD) was used to compute the percent positivity (PP), using the formula mean OD (sample or negative control) divided by the mean OD value of the positive control

multiplied by 100. PP of 15% or above was considered positive (6).

Statistical analysis

The data were analysed using the Statistical Package for the Social Sciences (SPSS) (7). The *t*-test was used to assess the significance of the differences between localities, age groups and the sexes.

Results

The ELISA technique was used to detect antibodies to *T. parva* and results showed that 364 out of 514 animals tested were seropositive, indicating an overall seroprevalence of 70.8% (Table II). The overall prevalence was lowest (61.8%) in the Juba locality, intermediate (71.8%) in the Terekeka locality and highest (91.2%) in the Mangalla locality (Table II). These data showed that, in general, infection with *T. parva* is highly prevalent in all three regions.

With regard to specific locations, the prevalence rate was highest (95.9%, $p < 0.001$) in Malang (Mangalla locality) and lowest (13.6%) in Kowrigic (Juba locality) (Table II).

Table I
Cattle camps and villages from which samples were collected, showing sample size

Locality	Villages	Distance from Juba town	No. of samples
Juba	Kansuk	29 km south of Juba	24
	Longo West	22 km south of Juba	31
	Tokiman West	12 km south of Juba	22
	Tokman East	12 km south-east of Juba	27
	Gumbo	10 km east of Juba	19
	Kamiro	10 km north-west of Juba	51
	Nyambuleing	36 km north-west of Juba	52
	Kworijic	12 km north of Juba	22
Mangalla	Mangalla town	75 km north-east of Juba	53
	Malang	10 km north of Mangalla	49
Terekeka	Terekeka town	90 km north of Juba	30
	Tukoro	20 km north of Terekeka	32
	Yari	10 km north of Terekeka	53
	Lujora	10 km south of Terekeka	49
Total			514

Table II
Prevalence of *Theileria parva* antibodies in different localities in Central Equatoria State in 2005

Locality	Village	No. of animals examined	Positive		Subtotal	Percentage
			No.	Percentage		
Juba	Gumbo	19	6	31.6		
	Kamiro	52	41	78.8		
	Kansuk	24	19	79.2		
	Kworijic	22	3	13.6		
	Logo West	30	17	56.7		
	Tokiman East	27	18	66.7		
	Tokiman West	22	12	54.5		
Mangalla	Nyambulung	53	38	71.7	154/249	61.8%
	Malang	49	47	95.9*		
Terekeka	Managalla	53	46	86.8	93/102	91.2%
	Terekeka	30	17	56.7		
Tukoro	Tukoro	32	27	84.4		
	Yari	52	40	76.9		
	Lujura	49	33	67.3	117/163	71.8%
Total		514	364	70.8	364/514	70.8%

* $p \leq 0.001$

With respect to the age groups in the study area, seroprevalence was significantly higher (87.8%, $p < 0.001$) in animals over four years of age than in those of less than one year (61.4%). The prevalence was 64% in animals aged one to two years (Table III).

The prevalence of antibodies was not significantly ($p > 0.05$) different between males (65.8%) and females (73.8%) (Table IV).

Table III
Relationship between age groups and the prevalence of *Theileria parva* antibodies among cattle in Central Equatoria State in 2005

Age group (years)	No. of animals examined	Positive	
		No.	Percentage
<1	83	51	61.4
1-2	200	128	64
>3-4	108	77	71.3
>4	123	108	87.8*
Total	514	364	70.8

* $p \leq 0.001$

Table IV
Relationship between sex and prevalence of *Theileria parva* antibodies among cattle in Central Equatoria State in 2005

Sex*	No. of animals examined	Positive	
		No.	Percentage
Male	190	125	65.8
Female	324	239	73.8
Total	514	314	70.3

* $p \leq 0.001$

Discussion

There have been very few studies on the seroprevalence of East Coast fever in South Sudan. The first documented work was that of Morzaria *et al.* (12) showing that *T. parva* seroprevalence among Ugandan refugee cattle ranged between 51.6% and 61.5% in the Aswa River area. In Juba, the prevalence was only 12.1% in Nilotic cattle in a government dairy herd, but the highest rate (83.3%) was recorded at Chukudum after an outbreak of East Coast fever (12). Since these studies, Julla (5) recorded 44.2% prevalence for *T. parva*

antibodies in Juba district while Malak recorded a prevalence of 35.6% and 70.6% in Yei and Kajo Keji districts, respectively (8).

The present study showed an overall seroprevalence of 70.8% in the entire study area which is in agreement with previous findings (12) but it is higher than that reported by Julla (5). The only difference between the previous studies and the present study is application of different methodologies with the previous studies using an IFA test while the present study applied ELISA. The differences between studies could also reflect variation in vector densities from year to year due to changes in climatic conditions. In the present investigation, there was considerable variation in the seroprevalence of *T. parva* within the study regions, which ranged from 13.6% (at Kworijic, 12 km north of Juba) to 95.9% (at Malang). The low prevalence at Kworijic is in agreement with cattle owners stating that they had not experienced the disease in the last four years, which may indicate the absence of *T. parva* transmission. Further longitudinal investigation in this area is needed to clarify the situation.

The cattle from Mangalla and Terekeka showed the highest seroprevalence rates but there was a complete absence of *R. appendiculatus* in Terekeka and only one male *R. appendiculatus* was found during the survey in Mangalla (11). The absence of the vector in Terekeka may be due to overstocking and subsequent overgrazing, resulting in reduced vegetation cover (9, 10). This finding may be due to the fact that all cattle from Mangalla and Terekeka move south towards areas of northern Bari Payam, and beyond Kansuk (29 km south of Juba town) annually during the rainy season. These areas are East Coast fever-endemic zones with a sizable population of *R. appendiculatus* which is abundant during the wet season (9). Cattle can thus easily pick up the parasite during the six-month period from May to November in the East Coast fever-endemic zone. From December to April, these cattle move north to Terekeka and Mangalla where they were sampled in March. The relatively low seroprevalence of *T. parva* in the Gumbo cattle

camp (31.6%) was not surprising as most samples in that area were obtained from calves prior to the onset of rains and would thus have experienced none or a reduced tick challenge. In addition, the sampling was undertaken during a long dry season during which ticks were not expected to be active, resulting in calves having had limited contact with *R. appendiculatus*. This might be supported by the results recorded at Kamiro (78.8%) and Kansuk (79.2%) where the sampling was conducted in June and July, after the onset of the rains, thereby giving a higher tick challenge.

In this study, the seroprevalence increased with age (Table III). This finding was in agreement with those of Salih *et al.* (15) and with those of Darghouth *et al.* (2) with tropical theileriosis in Tunisia.

Although studies on other tick-borne diseases were not conducted in this survey, this area is known to harbour the tick vectors of several economically important diseases, such as babesiosis and anaplasmosis, and infections with these pathogens have recently been reported in Kajo Keji and Yei Counties by Malak *et al.* (8) and, more recently, by Salih *et al.* (16).

In conclusion, East Coast fever represents a major constraint to the livestock development in most of Central Equatoria State as shown by the high prevalence rates reported in the present study. Based on the importance of the disease, it is recommended that more research be conducted to delineate the northern limit of East Coast fever, a characterisation of its epidemiology and socio-economic impact. In addition, tick population studies, molecular detection of *T. parva* as well as other transboundary diseases and isolation and characterisation of local strains of *T. parva* should be undertaken as soon as possible to test the possibility of introducing a live vaccine using the infection and treatment method.

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