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Full Length Research Paper

Factors influencing the sero-prevalence of *Trypanosoma brucei gambiense* sleeping sickness in Juba District, Central Equatoria State, Southern Sudan

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A surveillance of the Gambian type of Human African Trypanosomosis (HAT) conducted in Juba area using the Card Agglutination Test for Trypanosomosis (CATT) showed that 257 (11.1%) out of 2322 individuals were sero-positive. The sero-positive rate in the pooled adults was higher but not significanty different from that of the pooled children. The adult females sero- prevalence rate was significantly higher than those of adult males, male or female children. There were no significant differences in the sero-prevalence rates between adult men and male or female children. The internally-displaced group and the military personnel showed statistically higher sero-positive rates than the resident groups regardless of the presence or absence of the only extant tsetse species, *Glossina fuscipes fuscipes*. The proportions of sero-positives differed significantly between locations in the study area. The respondents examined along the riverine vegetation had a statistically higher encounter with the disease than those in the derived savanna and the open savanna woodland. There was no significant correlation between the density of *G. fuscipes fuscipes* and the incidence of *Trypanosoma brucei gambiense*.

Key words: CATT, sero-prevalence, sleeping sickness, HAT, Gambian type.

INTRODUCTION

At the end of the19th century, sleeping sickness decimated the populations of vast areas in the Equatoria region of Southern Sudan (Kuzoe, 1991). The devastating epidemic in Southern Sudan was attributed to the Gambian form of the disease (Bloss, 1960; Ford, 1971) although, Sudan lies in the edge of the geographical distribution of the rhodesiense and gambiense, the two types of sleeping sickness. Additionally, at that time there were no effective diagnostic techniques, which might have lead to the suspicion that the causative agent was anything other than *Trypanosoma brucei gambiense*. The main characteristic feature of the history of Human African Trypanosomosis (HAT) in the Sudan is a cycling between periodic devastating epidemics interspersed with long periods of low-level endemicity.

The epidemics of sleeping sickness flared up mainly as a sequel to civil unrest in the country or its neighbours, the Congo and Uganda, most likely due to population dislocation and the collapse of the health system it caused (Hunt and Bloss, 1945; Bloss, 1960; Hutchinson, 1975; Snow, 1983; 1984; Kuzoe, 1993; Moore et al., 1999; Moore and Richer, 2001).

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Abbreviations: HAT, Human African trypanosomiasis; **CATT,** card agglutination test for trypanosomosis.

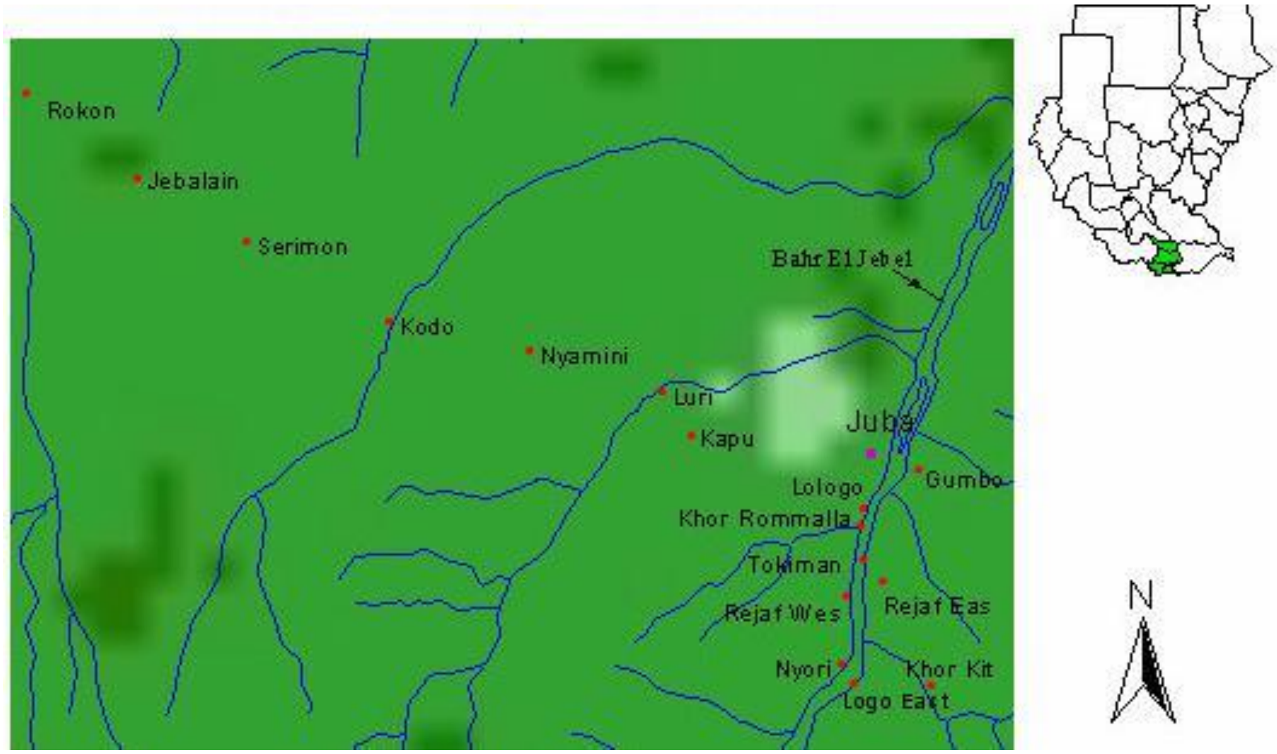

Figure 1. Study area showing the various location surveyed for tsetse and Gampian sleeping sickness in Bahr El Jebel State, Sudan.

Presently, the national efforts are expanding to address the problems posed by sleeping sickness in Southern Sudan. Yet, there are serious limiting factors that restrict and suspend these efforts. These include security and instability altogether with movement of refugees and the internally-displaced people which have led to new epidemiological patterns that are inadequately elucidated.

However, the recent introduction of Card Agglutination Trypanosomosis Test/ *T. brucei gambiense* (CATT), a serodiagnostic screening test for antibodies detection, during active surveillance has been a major breakthrough in the control of sleeping sickness (CATT/wb: Magnus et al.,1978). The present HAT surveillance was conducted using CATT in the accessible villages around metropolitan Juba, the Central Equatoria State capital, together with the accessible camps of the military and internally-dispalced people.

The main objective was to identify the factors influceing the sero- prevalence of sleeping sickness in the country in order to understand the disease epidemiology, and consequently to formulate a suitable integrated strategy for control.

MATERIALS AND METHODS

Locations surveyed

The study was conducted in Juba district (Latitude 4˚ 40'-5˚ N and Longitude 30˚ 30'-31˚ 45' E). The areas covered represented the major types of vegetation. In the derived savanna woodland west of Juba town the locations examined were: Nyamini, Koda, Serimon, El -Jebalain and Rokon, in the open savanna woodland North west of Juba, the locations were: Kapu, and Luri, in the riverine vegetation South of Juba on the western bank of River Bahr El Jebel, Lologo, Khor Rommalla, Tokiman, Rejaf West, Nyori and on the eastern bank of the river: Gumbo, Rejaf East, Khor Kit and Logo East, were screened (Figure 1).

Screening procedures for human African trypanosomosis

At each location visited, at least 95% of the consenting inhabitants were screened for *T. brucei gambiense* sleeping sickness using the card agglutination test for trypanosomosis (CATT/wb: Magnus et al., 1978). Blood was collected from the basilic veins of consenting individuals using heparinized capillary tubes. Confirmed CATT positives were recorded according to location, vegetation, residence, gender, age group and tsetse occurrence.

Tsetse survey

The biconical trap is effective for sampling *Glossina palpalis* group tsetse (Challier et al., 1977; Odulaja and Mohamed-Ahmed, 1997) and the Epsilon trap is similarly effective against *Glossina morsitans* group tsetse (Hargrove and Langley, 1990). Since the two groups of tsetse flies are thought to occur in the study area both of the latter traps were used in order to define the tsetse occurrence, their diversity and apparent densities. In each vegetation type traps were placed 200 m apart for 3 days during each of the wet and dry seasons. Captured flies (pooled males and females) were collected

Table 1. The percentage of inhabitants seropositive for human African trypanosomosis according to age groups and gender in Juba area, Central Equatoria State, Sudan.

Classification of individuals	% sero-prevalence	Chi^2 value	Probability, P
Pooled adults Pooled children	11.9 (194/1630) 9.10 (63/692)	3.6df = 1	p = 0.058
Pooled males Pooled females	9.53 (139/1459) 13.67 (118/863)	9.05df = 1	p<0.0026
Pooled adult males Pooled adult females	9.45 (104/1100) 16.98 (90/530)	18.61df = 1	p<0.00001
Pooled adult males Pooled male children	9.45 (104/1100) 9.75 (35/359)	3.80df = 1	p>0.9
Pooled adult females Pooled female children	16.98 (90/530) 8.41 (28/333)	12.02df = 1	p<0.0005
Pooled male children Pooled female children	9.75 (35/359) 8.41(28/333)	0.23df = 1	p>0.63

every 24 h, identified, sexed and counted. It is a fact that the transmission of *T. b. gambiense* occurs among humans is through the agency of tsetse flies, the *palpalis* group. For this reason we tried to find out the relationship between the density of tsetse (x) in each location and the seroprevalence rate (y) in the same location using the simple linear regression equation (y = a + bx) where a is the intercept or constant and (b) is the regfression coefficient or slope.

RESULTS

Human African trypanosomosis (HAT) sero-prevalence

The surveillance of HAT, covered 2322 persons. Regardless of age and sex, 257 individuals (11.07%) of the total scanned were seropositive.

Sero-prevalence of HAT based on pooled gender and age-groups

The results are presented in Table 1. The sero-prevalence rate in the pooled adults, although, are relatively higher than in the pooled children and the difference was marginally insignificant ($Chi^2 = 3.6$; $p = 0.058$). However, regression analysis showed a significant positive correlation between the square of age of respondents regardless of sex or location (x) and their HAT seroprvalence rate (y) ($Y = 1.07 + 0.039x$; $r = 0.9656$; df =169; $t = 2.082$; $p < 0.04$). On the other hand, the adult females sero-prevalence rate was significantly higher than that of the adult males, male or female children ($Chi^2 = 9.05$; df = 2, $p = 0.0026$). In contrast, there was no significant difference in the sero-prevalence rate between men and male children.

Sero-prevalence of HAT based on residence

The pooled internally-displaced group showed a statistically higher HAT seropositive rate ($p < 0.000$), when compared with the pooled resident groups where *Glossina fuscipes fuscipes* had been caught in biconical traps or the pooled resident groups where tsetse had not been seen in traps. Conversely, paired comparisons showed insignificant difference in HAT encounter between the two resident groups ($Chi^2 = 0.007$:$p > 0.93$) (Table 2).

HAT sero- prevalence based on gender and age group in relation to residence

Table 3 compares the sero-prevalence rates in the internally-displaced respondents according to gender and age group. Paired comparisons showed significant variations between pairs excluding the male children/female children. Furthermore, comparisons between residents of tsetse infested areas showed essentially similar sero-prevalence rates irrespective of sex or age (Table 4). However, for the inhabitants of tsetse free areas there were significant discrepancies in sero-prevalence rates between sexes and age groups barring adult female/female children and male children/ female children (Table 5). Subsequent regression

Table 2. The percentage of male and female inhabitants seropositive for Human African Trypanosomosis according to residence in Juba area, Central Equatoria State, Sudan.

Classification of individuals	% sero-prevalence	Chi^2 value	Probability, P
Total displaced	18.09 (131/724)	52.75	P<0.000
Total resident with tsetse	7.93 (23/290)	df = 2	
Total resident with no tsetse	7.87 (103/1308)		
Total displaced	18.09 (131/724)	15.82	P<0.0007
Total resident with tsetse	7.93 (23/290)	df = 1	
Total displaced	18.09 (131/724)	46.76	P<0.000
Total resident with no tsetse	7.87 (103/1308)	df = 1	
Total resident with tsetse	7.939 (23/290)	0.007	P>0.93
Total resident with no tsetse	7.87 (103/1308)	df = 1	

Table 3. The percentage of internally- displaced people seropositive for human African trypanosomosis according to gender and age-groups in Juba area, Central Equatoria State, Sudan.

Classification of individuals	% sero-prevalence	Chi^2 value	Probability, P
Pooled adults	26.93 (108/401)	28.46 df = 1	p<0.000
Pooled children	9.16 (23/251)		
Pooled adult males	21.97 (49/223)	5.724 df = 1	p<0.0167
Pooled adult females	49.58 (59/178)		
Pooled adult males	21.97 (49/223)	8.167 df = 1	p<0.0042
Pooled male children	9.09 (11/121)		
Pooled adult females	49.58 (59/178)	22.89 df = 1	p<0.000
Pooled female children	9.23 (12/130)		
Pooled male children	9.09 (11/121)	0.0325 df = 1	p>0.857
Pooled female children	9.23 (12/130)		

Table 4. The percentage of human African trypanosomosis seropositive residents of tsetse infested areas according to gender and age-groups in Juba area, Central Equatoria State, Sudan.

Classification of individuals	% sero-prevalence	Chi^2 value	Probability, P
Pooled adults	9.05 (20/221)	1.01 df = 1	p>0.314
Pooled children	4.35 (3/69)		
Pooled adult males	9.30 (16/172)	1.371 df = 1	p>0.971
Pooled adult females	8.16 (4/49)		
Pooled adult males	9.30 (16/172)	0.793 df = 1	p>0.373
Pooled male children	2.94 (1/34)		
Pooled adult females	8.16 (4/49)	0.0 df = 1	p = 1
Pooled female children	5.71 (2/35)		
Pooled male children	2.49 (1/34)	0.0007 df = 1	p>0.979
Pooled female children	5.71 (2/35)		

Table 5. The percentage of human African trypanosomosis seropositive inhabitants of tsetse free areas according to gender and age-groups in Juba area, Central Equatoria State, Sudan.

Classification of individuals	% sero-prevalence	Chi^2 value	Probability, P
Pooled adults Pooled children	7.05 (66/936) 15.55 (37/275)	10.39 df = 1	p<0.00126
Pooled adult males Pooled adult females	5.53 (39/705) 11.69 (27/231)	9.144 df = 1	p<0.00249
Pooled adult males Pooled male children	5.53 (39/705) 11.27 (23/204)	7.331 df = 1	p<0.0067
Pooled adult females Pooled female children	11.69 (27/231) 8.33 (14/71)	2.339 df = 1	p>0.126
Pooled male children Pooled female children	11.27 (23/204) 8.33 (14/71)	2.54 df = 1	p>0.11

analysis showed a significant positive correlation between the square of age of the internally-displaced female respondents (x) and their HAT sero-prevalence (y) (Y = 3.457 + 0.0002x; r + 0.9418; df =28 ; t = 14.077; p < 0.000001). No similar siignificant correlations could be established between age and HAT seroprvalence in any other group, irrespective of type of residence (p > 0.2 - 0.9).

Variation of human African trypanosomosis sero-prevalence with vegetation type

The results shown in Table 6 reveal that the respondents examined along the riverine vegetation had a statistically higher encounter with the disease compared with those in the derived savanna woodland or the open savanna woodland.

Sero-prevalence of human African trypanosomosis based on location of respondents

The pooled sero-prevalence of the disease was relatively high in Lologo, Khor Rommalla, Tokiman, Gumbo and Kapu locations where the internally-displaced people were being settled at the time of the survey. Analysis of sero-prevalence data obtained on the displaced people showed a significant difference between settlers locations, probably highlighting their encounter with varing trypanosomosis challenge either at their abandoned original homes or enroute to these camps. Similar treatment of data, pertaining to military personnel (all adult males) who were prepetually (and are) on the move showed a relatively high sero- prevalence rate. As with the displaced people, there was also significant variation between locations. In contrast, Table 7 clearly suggests that the encounter of the diseases among residents in established villages and hamlets was relatively low with insignificant difference in sero-prevalence rates between these villages.

Relationship between human African trypanosomosis sero-prevalence and density of *G. fuscipes fuscipes*

Regression analysis (Table 8) showed no significant correlation between the density of *G. f. fuscipes* and the sero-prevalence of HAT in Southern Sudan (r + 0.698; p = 0.191; df = 8).

DISCUSSION

An active surveillance was conducted in reachable villages and camps around Juba, the Central Equatoria State capital. Under civil conflict circumstances time and efficiency are vital factors. For these reasons the card-agglutination test for trypanosomosis (CATT), is a serologic technique, highly sensitive and efficient for diagnosis of *T. b. gambiense* sleeping sickness (Magnus et al., 1978) and recommended for active surveillance to estimate the disease sero-prevelance rate was adopted (W H O, 1998). Moreover, the test is quick, easy to perform and permits the examination of hundreds of people each day (Chappuis et al., 2002, 2004).

In the present study some 2322 consenting individuals in Juba area were screened for *gambiense* HAT. Of these 257 (11.1%) were seropositive. This level of sero-prevalence is almost identical to those levels regarded as epidemics in Western Equatoria (Moore et al., 1999;

Table 6. Comparisons between human African trypanosomosis seropositive individuals in open savanna woodland, derived savanna woodland and riverine vegetation, Juba area, Central Equatoria State, Sudan.

Vegetation	% sero-prevalence	Chi^2 value	Probability, P
Open savanna inhabitants	4.93 (17/345)	82.13 df = 2	p<0.000
Derived savanna inhabitants	7.04 (78/1108)		
Riverine vegetation inhabitants	18.64 (162/869)		
Open savanna inhabitants	4.93 (17/345	1.59 df = 1	p>0.207
Derived savanna inhabitants	7.04 (78/1108)		
Open savanna inhabitants	4.93 (17/345)	35.87 df = 1	p<0.000
Riverine vegetation inhabitants	18.64 (162/869)		
Derived savanna inhabitants	7.04 (78/1108)	60.39 df = 1	p<0.000
Riverine vegetation inhabitants	18.64 (162/869)		

Table 7. The perecntage of human African trypanosomosis seropositives according to locations and residence in Juba area, Central Equatoria State, Sudan.

Residence	Village	% sero-prevalence	Chi^2 value	Probability, P
Displaced camps	Lologo	38.03 (81/213)	93.92 df = 4	p <0.000
	Khor Rommalla	21.36 (22/103)		
	Tokiman	14.81 (4/27)		
	Gumbo	7.04 (7/94)		
	Kabu	5.92 (17/287)		
	Sub-total	18.09 (131/724)		
Permanent resident	Rejaf East	12.5 (25/200)	1.698 df =5	p>0.79
	Kit	7.69 (1/13)		
	Logo East	8.22 (6/73)		
	Rejaf West	12.5 (11/88)		
	Nyori	8.62 (5/58)		
	Luri	0 (0/58)		
	Sub-total	9.8 (48/490)		
Mobile military camps	Nyamini	15.14 (28/185)	32.68 df = 4	p <0.000
	Koda	8.48 (15/177)		
	Serimon	1.75 (5/285)		
	El Jebalain	4.76 (8/168)		
	Rokon	7.51 (22/293)		
	Sub-total	7.04 (78/1108)		

Moore and Richer, 2001). It is suggested that the break down of the health service and the displacement of the population as a consequence of the civil conflict has aggravated the situation of sleeping sickness in the area. During the last decade there has been a similar strong resurgence of human African trypanosomosis in several countries including Angola and the Democratic Republic of Congo (Van Nieuwenhove et al., 2001; Chappuis et al., 2002). The major cause of these flare-ups is the collapse of sleeping sickness control programmes as a result of strife in these countries (Ekwanzala et al., 1996; Chappuis et al., 2002).

In the surveillance higher sero-prevalence rates were recorded in adults rather than in children. Similar results were obtained during a *T*. *b*. *gambiense* sleeping sickness surveillance in the Ivory Coast (Stanghellini and Duvallat, 1981). The high sero-prevalence of the disease in adults might be attributed to the community social behaviour and daily activities which take the adult into close and more frequent contact with the vector *G.*

Table 8. The sero-prevalence rate of human African trypanosomosis in relation to *G. fuscipes fuscipes* apparet density in Central Equatoria State, Sudan.

Location	Fly apparent density	Sero-prevalence rate
Rejaf East	0.0	12.50 (25/200)
Kit	3.5±0.65	07.69 (01/13)
Logo Eat	4.0±1.15	08.22 (06/73)
Rejaf West	1.3±0.88	12.50 (11/88)
Nyori	5.0±1.15	08.22 (05/58)
Luri	0.0±0.30	00.00 (0.0/58)

Y = 2.826 + 1.62x; r + 0.696; df + 4; t + 1.681; P>0.191
Fly apparent density = (male +female tsetse/trap/day)
Sero-prevalence rate =(total number of seropositives divided by total examined at a location, between parentheses)

fuscipes fuscipes in watering sites. In general terms, the frequency of human/fly contact decides the disease incidence rate (Gouteux, 1985).

In the present work, the sero-prevalence rates were found to be significantly higher in adult females and children compared with that of adult men ($p < 0.0005$; $Chi^2 = 9$). It has been observed that there are substantial variations between men and women in the type and duration of their daily activities within the local tsetse biotopes where the disease might be transmitted, possibly resulting in profound differences in exposure between men and women and the children who, usually pursue their mothers. The adult men are mainly traders, agropastoralist and junior workers in government offices thus they have lower intensity of fly-contact compared with women. Recently, there has been considerable interest towards the influence of sex or gender on tropical diseases, sex referring to a biological characteristic while gender denotes socially constructed behaviour, expectations and roles that derive from, but may not depend on sex (Vlassoff and Manderson, 1998). It has been perceived that women had higher apparent incidence of Gambian trypanosomosis than men due to their selective activities which take them into contact with tsetse e.g. water collection, washing, firewood cutting and bathing. Children, especially girls, usually follow their mothers (Pepin et al., 2002). Dissimilarity in incidence of African trypanosomosis between genders was reported in many countreies. For instance in Fankana-Kalakitini, Kwamouth and Nioki foci of the Congo women had higher incidence of trypanosomosis than men (Henry et al., 1982; Pepin et al., 2002). Conversely, in other African countries, the incidence was higher in males in some foci. The differences in population structures of endemic villages as well as the differences in exposure to infected tsetse between men and women probably explain most of the gender variation in the incidence of HAT (Pepin and Meda, 2001).

In the present work the HAT sero-prevalence rate of the internally-displaced people and military was signi-ficantly higher than that scored for the resident population. This might be due to the fact that the internally-displaced people and the military had probably come from highly endemic foci of sleeping sickness in Western Equatoria where they might have harboured the infection before they migrated or operated as a result of the civil unrest. The role of the population sectors who are perpetually on the move in the spread of HAT is well established in the literature and had already been discussed (Adekolu-John, 1978). In our study, the high prevalence of the disease recorded in certain locations and its absence or low prevalence in others, might be attributed to the presence of the internally-displaced people –including the government soldiers, the presence or absence of tsetse in the location and the daily activity of residents in endemic areas. Thus, the differences in the sero-prevalence rates of *T. b. gambiense* in Juba area is in tandem with patterns of the disease in Central Africa including Uganda and the Kenya shores of lake Victoria (Hide, 1999; Van Nieuwenhove et al., 2001).

Significantly, higher prevalence of HAT was detected in residents who dwell close to or visit rivers and water-courses where *G .f. fuscipes* exists: in riverine galleries, thickets and farm hedges. Thus, people living or visiting these fly haunts are exposed to higher frequency of tsetse bites and hence, are at a higher risk of contracting sleeping sickness (Mulligan, 1970; Gouteux, 1985). Moreover, the war has forced the residents to flee their villages to hide in the riverine vegetation where *G. f. fuscipes* occurs.

It is well known that the *palpalis* group tsetse are more dependent on riverside vegetation (De La Rocque et al., 2001) and much less affected by human settlement. They are also better able to adapt to transformed environments as long as the vegetation cover provides them with appropriate habitats. Indeed *G. f. fuscipes* , a riverine tsetse, was reported to adjust itself in changing habitats (Okoth,1982). Due to their opportunistic feeding habits, the *palpalis* tsetse are also able to adapt from a preference for feeding on wild animals to feeding mainly on reptiles, humans and domestic animals (Cuisance et al., 1973; Jordan, 1989; Mohamed-Ahmed and Odulaja, 1997; Clausen et al.,1998). They are also more capable of adapting to peri-domestic habitats (Toure, 1974; Kuzoe et al., 1985). Adaptation to peri- domestic habitats together with the opportunistic feeding habits enable *G. f. fuscipes* to survive and to tolerate the adverse effect of game animals rarity (Weitz, 1963, 1970; Boyt et al., 1978; Okoth, 1986). Thus, creation of peri-domestic environments suitable for the fly might result in new HAT epidemiological systems closly linked to humans (De La Rocque et al., 1998). Indeed, due to the civil strife in southern Sudan people were settling in hamlets along the watercourses concealed in the riverine vegetation for security reasons. This may explain why the sero-prevalence rate was higher in riverine vegetations rather than savanna areas or inside villages.

Correlation analysis showed no significant association between HAT sero-prevalence rate in a location and the density of *G. f. fuscipes* in that location (Table 8). This finding agrees with most reports from sleeping sickness foci elsewhere in Africa where no significant relationship was demonstrated between the incidence of Gambian sleeping sickness in inhabitants and the density of species of riverine tsetse (Gouteux et al., 1993). Conversely, in the Central African Republic the rate of *T*. *b*. *gambiense* transmission was found to be significantly correlated with the density of the vector fly (Leak, 1999). The above results confirm the presence of *T. b. gambiense* sleeping sickness in Juba area but do not eliminate the possibility of infection with *Trypanosoma brucei rohdesiense* , as CATT appears to be sensitive for the former only. The Sudan lies in the interface of the geographical location of the two types of sleeping sickness (Baker et al., 1970). Considering the instability of people and their livestock due to the war there is surely a high likelihood of the spread and overlap of the two types of sleeping sicknesses. Furthermore, some strain of *T. b gambiense* lack the specific *gene* coding for CATT-antigen (Penchenier et al., 2003), consequently, the level of the seropositive obtained here almost cer-tainly underestimated the true prevalence of infection. For these reasons the use of other diagnostic devices and procedures that might help to detect the true infection with both *T. b. gambiense* and *T. b. rohdesiense* are demanded.

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