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Relative abundance of tsetse fly species and their infection rates in Simanjiro, Northern Tanzania

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Abstract

This study was conducted to determine the prevalence of trypanosomes, infective to cattle and humans in tsetse flies caught in Simanjiro district, which borders with Tarangire National park in northern Tanzania. A total of 1000 tsetse flies were caught during the semi dry season in July and August 2014 using F 3 and Epsilon traps. Results revealed *Glossina swynertonni* as the most abundant species (72%) followed by *Glossina morsitans morsitans* (21%) and *Glossina pallidipes* (7%) in the study area. Whole fly DNA was extracted from all the 1000 flies caught in the study area. Analysis of fly DNA for detection of trypanosome species showed a 3% (30/1000) overall infection rate of which 13.3% (4/30) flies were co-infected with *Trypanosoma brucei* and *Trypanosoma vivax*, while 3.3% (1/30) were co-infected with *Trypanosoma congolense* and *Tripanosoma vivax*. Majority of flies (83.3%, 25/30) were infected with *T. vivax*. All three trypanosome species were detected in *G.swynertonni* flies but *G.m. morsitans* carried *T. vivax* and *T. brucei*. No parasites were detected in *G. pallidipes* flies. Further, no human infective trypanosomes were detected when *T. brucei* positive flies were analysed with specific primers for SRA-PCR. The study confirms presence of cattle infective trypanosomes and absence of human-infective trypanosomes in tsetse flies caught in the study area.

Keywords: *prevalence, traps, trypanosomes, wildlife*

Introduction

An understanding of tsetse population distribution and dynamics is essential for understanding the epidemiology of human and animal trypanosomiasis (Aksoy 2003; Hao et al 2001). There are about 30 known species and subspecies of tsetse flies belonging to the genus Glossina, which are divided into three distinct groups or subgenera: Austenia (G. fusca group), Nemorhina (G.

palpalis group) and Glossina (G. morsitans group). These species vary in distribution and capacity to transmit trypanosomiasis (Hao et al 2001, Jamonneau et al 2004, Walshe et al 2009). *G. pallidipes, G. brevipalpis, G. m. moristans* and *G. swynertoni* have been reported to be of importance in transmitting trypanosomiasis in both cattle and humans (Auty et al 2012, Malele et al 2003, Sindato et al 2007) in Northern parts of Tanzania. Distribution of tsetse flies is determined by climate and influenced by altitude, vegetation and availability of host. Movement of tsetse flies, on the other hand, is affected by factors like humidity, availability of shades, host density and odour plumes.

Several species of trypanosomes are known to infect cattle including *T. congolense, T. brucei and T. vivax* (WHO, media centre, 2012). These cause Animal African Trypanosomiasis (AAT) or Nagana in cattle which results into animal loss and reduction in productivity. *T. brucei rhodensience* and *T. brucei gambiense* are known to infect humans causing Human African Trypanosomiasis (HAT) or Sleeping sickness. *T. brucei rhodensience* is common in East Africa, while *T. b. gambiense* is common in West Africa. These are known to cause acute and chronic form of the disease, respectively (Aksoy 2003, Jamonneauet al 2004, Roditi and Lehane 2008). Trypanosome species present in tsetse flies vary according to local fauna and feeding preferences of flies (Kuboki et al 2003).

The internal transcribed spacer (ITS) region is a conserved region of rDNA with variable sizes in all species of trypanosomes, which can be used for diagnostic purposes. The ITS has been proven to be of high sensitivity in discriminating all species of trypanosomes based on amplicon size (Njiru et al 2005). Presence of a gene (SRA) that confers resistance to survive in human serum has been used as a diagnostic method for differentiating between human infective and non human infective trypanosomes (Radwanska et al 2002).

Trypanosomiasis has been a challenge in pastoral communities due to its effect to livestock and human health. Dynamics of human activity including agro-pastoralism, livestock-wildlife interface and the impeding socio-ecological-environmental drivers have been major reasons for increase of Trypanosomiasis in some societies. Maasai agro-pastoralists in northern Tanzania have been forced into new areas searching for good pasture and water for their cattle as well as areas for farming. Some of the new areas in the Maasai steppe are tsetse infested with an interface of wildlife, livestock and humans, and may therefore expose people and their livestock to increased risk of Trypanosomiasis. The aim of this study was to determine the relative abundance of tsetse fly species and their infection rates with human and livestock trypanosomes in Simanjiro district, bordering with Tarangire National park.

Methodology

Study site

Tsetse flies were collected from Simanjiro district, Northern Tanzania which lies between $3^0$52’ and $4^0$24’ south and $36^0$05’and $36^0$39’ east. The district borders with Tarangire National park on the eastern side. The study was conducted in July and August 2014. Annual rainfall in Simanjiro district is 650 mm per annum and temperature varies between 18-30°C. The population according

to 2012 census was 178,693 and change of population was found to be +2. 37% per year. Simanjiro covers 19928.1 km^2 and most of it is covered with open woodland, bushy forests and grassland vegetation. Parts of the district are an important wildlife dispersal area, which brings humans and wildlife into contact and sometimes conflict. Wild animals found in the interface area include baboon, buffalo, cheetah, elephant, eland, giraffe, grant gazelle, greater kudu, impala, lesser kudu, lions, spotted hyena, velvet monkey, warthogs, wildebeest, wild dog and zebra. Main economic activities in the district are livestock keeping and farming.

Sampling

Epsilon and F3 Traps, previously proven to be most effective for catching tsetse flies in Savannah vegetation in East Africa including the Northern part of Tanzania were used. Geo-references of sites and traps location were taken by Global Positioning System. Combinations of the following attractants, phenol, octanol and acetone were used to increase tsetse fly catches. Acetone and octanol were kept in plastic bottles with a hole of almost 4 mm in the top of the lid of plastic containers while phenol in small sachets was kept in small pockets in the front side of respective trap. Sampling areas were categorized as being either, near residence, animal grazing areas or near Tarangire National park. In each sampling area category, 3 sites were identified and 4 traps were deployed in each site in the in the ratio 3:1 epsilon and f3, respectively.

Stratified sampling was used to allocate sites in different types of vegetations in each particular category. Traps were located 200m apart in nine sites in the three identified categories.To allow maximum capture during day time, traps were located under trees. Trapped tsetse were harvested after every 24 hours, counted and sorted by species and sex. A total of 1000 flies were harvested and preserved singly in eppendorf tubes containing absolute ethanol until laboratory analysis.

DNA extraction

DNA was extracted from individual flies. Tsetse flies were disrupted by grinding with hand pestel and DNA was extracted using Ammonium Acetate Precipitation protocol for DNA extraction based on protocol described by Bruford et al (1998). DNA samples were stored at -20^0C until further analysis.

Identification of Trypanosomes by PCR

Polymerase chain reaction (PCR) for identification of trypanosome species was done on fly-specific pools, whereby each pool was constituted by mixing 10 individual DNA samples in equal volumes. Individual DNA samples of any positive pool was further analysed in order to establish prevalence of trypanosome species. The PCR amplifications targeting ITS1 gene were performed in a total volume of 15 µl containing 7. 5µl Dream Taq master mix, 200nM of forward and reverse primers and 3. 9µl of nuclease free water. The PCR products were separated on 2 % gel green stained agarose gels and positive results were identified based on PCR products sizes corresponding to 300bp for *T. vivax*, 400bp for *T. brucei* and 700bp for *T. congolense savannah*. *T. brucei* positive samples were further tested using SRA gene amplification primers in order to identify human infective trypanosomes. Primer sequences and cycling conditions are shown in Table 1.

Table 1: Primers sequences and their cycling conditions

Target	Sequences	Authors
ITS 1	CF 5'-CCG GAA GTT CAC CGA TAT TG-3' BR 5'-TTG CTG CGT TCT TCA ACG AA-3' Cycling conditions: 94 C for 3min followed by 30 cycles of 94 C for 30sec, 55 C for 30sec, 70 C for 30sec and lastly at 72 C for 10 min.	Njiru et al *2005*
SRA	For 5'-ATAGTGACAAGATGCGTACTCAACGC-3' Rev5'- ATGTGTTCGAGTACTTCGGTCACGCT3' Cycling conditions: 94 C for 10min, 35cycles of 94 C for 1min, 68 C for 1min and 72C for 60sec and lastly by 72 C for 10 min	Radwanska et al 2002

Results

Abundance of tsetse fly species

Three fly species were found to occur in the study area with *G. swynertonni* being the most abundant (72%) followed by *G. morsitans morsitans* (21%) and *G. pallidipes* (7%). A total of 79.9% of caught flies were non teneral while 20.1% were teneral flies (Table 3). Also 53.1% of all flies were females and 46.9% were males. The apparent density of flies caught in traps was generally 50% lower than total flies observed around the traps. Flies which landed on the traps but did not fall into catching bottles were not recorded or collected for this study. Few flies were caught in sites located near human activities while higher tsetse fly density was observed in sites located in an animal grazing zone and near Tarangire National Park (Table 2). Large groups of cattle were found feeding in sites near human residence and farmers reported the application of acaricides on livestock to prevent tsetse bites. Moreover, various species of game animals were found in high concentration in sites located in grazing the zone as well as near Tarangire National Park.

Table 2: Apparent density of tsetse flies in different sites of the study area

Area category	Sites	Apparent Density
Near humans residence/activities	A	0.05
	B	0.07
	C	0.25
	Total	**0.37**
Grazing zone	D	0.57
	E	8
	F	5.46
	Total	**14.03**
Near Tarangire National Park	H	13.57
	I	2.02
	G	0.79
	Total	**16.38**

Although trap preference was indicated for the three fly species but association between type of trap and fly species was not significant. Thus, *G. m. morsitans* showed preference to Epsilon traps although some catches were also found in F3 traps. *G. m. morsitans* showed least

preference to moving objects. *G. pallidipes* preferred moving objects followed by F3 and Epsilon. *G. swynertonni* catches were mostly found in F3 followed by Epsilon, while moving objects were the least preferred trap by *G. swynertonni* (Figure 1).

Table 3: Trap preference of Tsetse flies

Fly Species	Type of traps			
	Epsilon	**F3**	**Moving object**	**Total (N, %)**
G. m. morsitans	166	48	0	214 (21. 4)
G. palidippes	38	13	16	67 (6. 7)
G. swynertonni	478	170	71	719 (71. 9)
Total*	682	231	87	1000 (100)

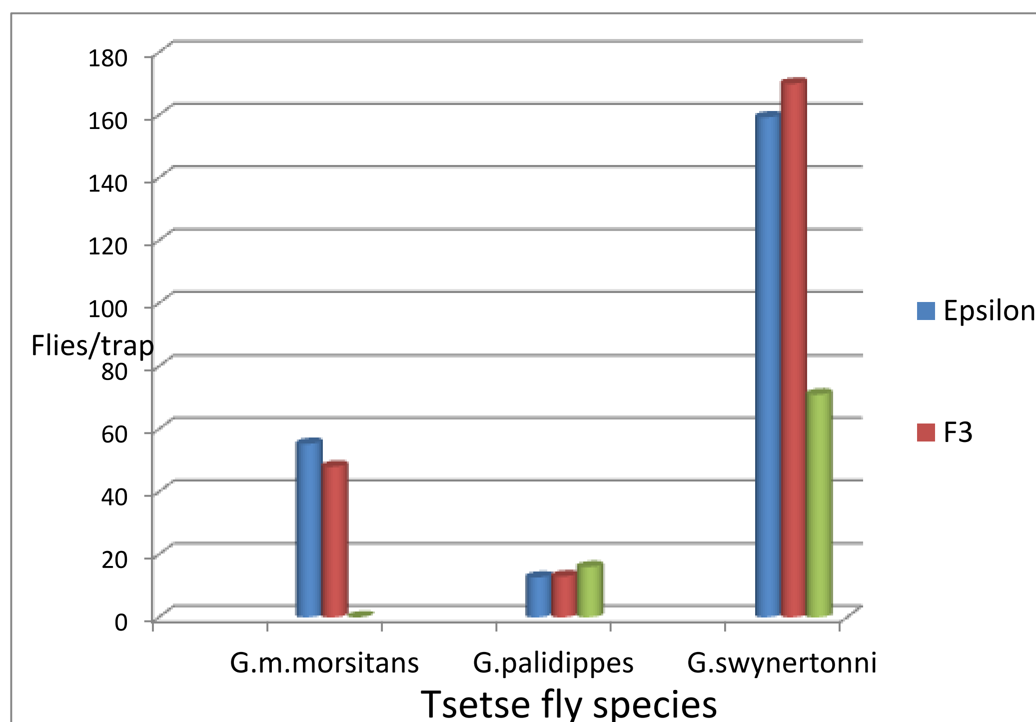

Figure 1: Traps preference by different tsetse fly species

Prevalence of Trypanosome species in tsetse flies

Nine out of 100 DNA pools constituted from 1000 flies contained positive PCR signal for presence of typanosomes. Further analysis of individual fly DNA samples revealed 30 flies being positive for Trypanosome species. It was found that all positive flies were caught in livestock grazing areas and in close proximity to the Tarangire National park, where type of vegetation was either wooded grassland or grassland. The frequency of the trypanosome species was 100% (30/30) for *T. vivax*, 13% (4/30) for *T. brucei* and 3% (1/30) for *T. congolense*. While all the three trypanosome species were found in *G. swynertonni,* only *T. vivax* and *T. brucei* were detected in *G. m. morsitans*. Furthermore, *T. brucei* and *T. congolense* were only detected as co-

infection with *T. vivax* giving a prevalence of 16% co-infection in tsetse flies. There was no significant association between species of flies and trypanosomes found in them (Table 4). When the four *T. brucei* positive samples were further tested for *T. brucei rhodensiense* using SRA - PCR test no positive results were indicated.

Table 4: Infection rates of Tsetse flies with different Trypanosome species

Fly species	Trypanosomes species				
	T. v	*T. v/T. b*	*T. v/T. c*	Negative	Total
G. m. morsitans	9	2	0	203	214
G. palidippes	0	0	0	67	67
G. swynertonni	21	2	1	695	719
Total	30	4	1	965	1000

Discussion

Tsetse flies were less abundant in areas closer to human activity, such as livestock grazing areas, farming areas as well as human residence. This finding has shown that human activities reported in this study have a significant influence on abundance of tsetse flies. The low abundance of tsetse flies closer to human activities as well as in areas where groups of cattle were found is attributed to tsetse control efforts by farmers through use of insecticides on cattle. Most of the farmers interviewed in the study area reported to dip or spray their livestock to prevent their cattle from tsetse fly bites. Our results confirm previous findings by (Malele, 2011) and (Munang'andu 2012) that anthropogenic activities pose a significant threat of reducing tsetse habitat.

Increase of tsetse fly catches observed closer to the Tarangire National park affirms the relationship between density of wild animals and abundance of tsetse flies. In areas where cattle are absent or acaricides are used on cattle, wildlife is the only source of blood meal for tsetse flies. In our study majority of flies (79. 9%) were non-teneral, and these were caught in traps placed closer to wildlife. These findings verify that ecological factors, which support survival of wildlife species also provide adequate habitat for vector tsetse flies, what was also reported by Van den Bossche et al (2010) and Malele et al (2011).

In the present study trap preference varied between tsetse fly species. Relative to stationary traps, *G. swynnertonni* flies were attracted to moving objects more than the other two fly species detected in this study. Moreover, it was observed that despite the large number of flies around traps, fewer than 50% of the flies entered the trap. Accordingly the apparent density of flies measured by number of caught flies was lower compared to the actual number of flies in the study area. Tsetse reluctance to fall into traps is an apparently common phenomenon and our findings are in agreement with a previous study of Morsitans tsetse flies (Malele 2012, Shaw et al 2007), where only 37-40% of flies approaching a trap landed on it.

The three tsetse species, *G. swynnertonni*, *G. m. morsitans* and *G. pallidipes* are among common fly species reported to occur in the Savannah vegetations, to which Simanjiro district is part of. The abundance of these species was also previously reported in Northern Tanzania around Tarangire National park (Adams et al 2008, Malele et al 2007; Sindato et al 2012). Two

of fly species found in this study, *G. swynnertonni* and *G. m. morsitans* have particular importance in the epidemiology of animal trypanosomiasis. Firstly, these flies were caught at interface areas where both livestock and wildlife graze. Secondly, the two fly species were all infected by trypanosome species circulating in the study area. Moreover, *G. swynnertonni* has shown significant epidemiological importance due to being infected with all 3 trypanosome species, *T. vivax*, *T. brucei* and *T. congolense* whereas *G. m. morsitans* was infected by only *T. vivax*, *T. brucei.* Our data further showed co-infection in 5 out of 30 tsetse flies. This finding is particularly important and is supported by prevailing ecological factors as well presence of wild animals such as lions, spotted hyena, zebra, waterbuck and giraffe known to be reservoirs for Trypanosome species (Auty et al 2012; Ashcroft 1959).

The most prevalent trypanosome species was found to be *T. vivax*. This is a predominant species in vertebrate hosts. Accordingly, its prevalence would be expected to be high in wildlife as in well cattle which graze in interface areas. Moreover, all infected flies were caught from grazing areas and near the Tarangire National park but not near human residence. When the *T. brucei* positive flies were tested for presence of human infective trypanosomes, they were all negative for *T. brucei rhodensiense,* implying absence of risk to Human African Trypanosomiasis in the study area.

Conclusions

- We have confirmed the presence of animal trypanosomes but absence of human-infective trypanosomes in the study area.
- Abundance of tsetse flies as well as their infection with trypanosomes have been shown to be associated with anthropological activities.
- Distance to the Tarangire National park seemed to be a factor that determines abundance of tsetse flies.
- Our study therefore points to the importance of strategic vector control regimes in livestock-wildlife interface areas so as to reduce risk and transmission of both animal and human trypanosomiasis posed by wildlife.

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