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Tsetse Fly Infection Dynamicsand its Implications with Control of Trypanosomiasis in Kajo-Keji County, South Sudan

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Abstract

A two-year study was conducted in Kajo-Keji County South Sudan to evaluate tsetse fly infection dynamics which implicate the control of trypanosomiasis in the study area.Infection dynamics ofthe flieswas assessed and monitored using biconical traps.Captured flies were identified, segregated into sexes and age examined using wing fray and ovarian techniques for males and females, respectively. RIME-LAMP test was used to detect Trypanosome species in the midguts of wild tsetse flies.The non-parametric Wilcoxon Signed Rank Test was used to assess the difference in the number of the infected flies between the dry and the wet season counts.The mean infected *G.f. fuscipes*were: male 6 ±1.4, female 3 ± 1.0 and male 10 ± 2.0, female 3 ± 0.7 in the dry and wet seasons of the year 2011; male,6 ± 1.4,female 6 ±1.4 and male,10 ± 2, female3 ± 0.8 in the dry and wet seasons of 2012.Infection showed significant differences (Z=-2.03, P = 0.04) with both seasons in 2011 and no significant differences (Z=-1.41, P = 0.16) in 2012. Number of infected male and female flies was positively correlated with the fly age in the dry (Male, R^2=0.94; female, R^2=0.86) and wet (Male, R^2=0.97; female, R^2=1) seasons in 2011 and (Male, R^2=0.90; female, R^2=0.94) in the dry and wet (Male, R^2= 0.97 female, R^2=1) seasons in 2012.These results showed that*G.f.fuscipes*were infected with *T.brucei gambiense* and they were proved to be potential vectors for HAT in the study area. Hence, the implications of Tsetse fly infection dynamics in the control of trypanosomiasis need development of further control strategiesfor sustainable development of livestock and human resources in Kajo-Keji County.

Keywords:*Glossina f.fuscipes*; Infection Dynamics; Trypanosomiasis;Kajo-Keji County; South Sudan.

1. Introduction

Tsetse flies arebiological vectors of protozoan parasites of the Genus *Trypanosoma*, the pathogenic agents of Human African Trypanosomiasis (HAT), commonly known as sleeping sickness in humans, and African Trypanosomiasis (AAT) or Nagana in cattle (Vreysen et al., 2013; Ciosi et al., 2014).In sub-Saharan Africa Glossina species infest a total area of 7 million Km^2 of whicha territory of 250,000km² of mostly fertile and potentially agricultural land being infested in South Sudan.

Tsetse and trypanosomiasis lies at the heart of Africa's struggle against poverty affecting 38 countries in sub-Saharan Africa where the disease is endemic (PAAT, 2008). It is one of the important neglected diseases, posing impact on the socio-economic development of the poor rural inhabitants (Okoh et al., 2011).

Seven species of tsetse exist in the Sudan including Glossina morsitans submorsitans, G. pallidipes, G. fuscipes fuscipes, *G. tachinoides, G. fusca fusca, G. fuscipleuris* and *G. longipennis*(Mohammed, 2004).The area infested by tsetse covers most of southern Sudan, although flies are absent from the Sudd and the flood plains of the greater Upper Nile State. Recently the presence of *G. f.fuscipes*has been reported in some streams located in Kajo-Keji County (KKC), South Sudan (Lukaw et al., 2014).

Streams of KKC play a pivotal role in sustaining livelihoods of the indigenous inhabitants in the study area.Firstly, they are sources of potable water for both the inhabitants and their farm animals;secondly, the streams are sites of crops and

vegetables cultivation and thirdly, they providesites along the banks where the native people bath and soak their harvested cassava for further processing before it is finally used as flour.

No study has been conducted on tsetse dynamics infection and its implication with the control of trypanosomiasis in the study area. Knowledge of the dynamics of tsetse flies is imperativefor understanding the relationship between tsetse and hosts, which in turn clarifies the role that tsetse play in disease transmission, which may be useful for determination of trypanosomiasis hot spots (Malele et al., 2007). Tsetse population dynamics may therefore be important in setting appropriate control strategy (Torr et al., 2005).

The purpose of this study was to provideinformationand data on the dynamics of *G. f.fuscipes*which implicate control of trypanosomiasis in KKC, South Sudan.

2. Materials and Methods

2.1 Description of the Study Area

The study was conducted over two years from January 2011 to December 2012 in KKC, Central Equatoria State (CES), South Sudan. KKC is located between geographical latitudes 3.67203- 4.13238 ˚N and longitudes 31.1004 -31.8172 ˚E. It covers an area of approximately 113,000 km² bordering Uganda in the South, Yei River County in the West, Juba County in the North and the River Nile in the East. KKC is an area of the tropical rainforest with moderate soil fertility and the climate is marked by minimal variations in seasonal temperatures **(Figure 1)**.The annual rainfall ranges between 1200 and 2000 mm for about 8 months from March to October. The dry season starts from November to March and the wet season starts from April to October.KKC is endowed with a number of streams. The banks of these streams are inhabited with various types of vegetation, trees and tsetse flies. The habitats on the bank of each stream were classified into single forest gallery, double forest gallery and peri-domesticated forest gallery based on its vegetation covers, trees and other ecological attributes.

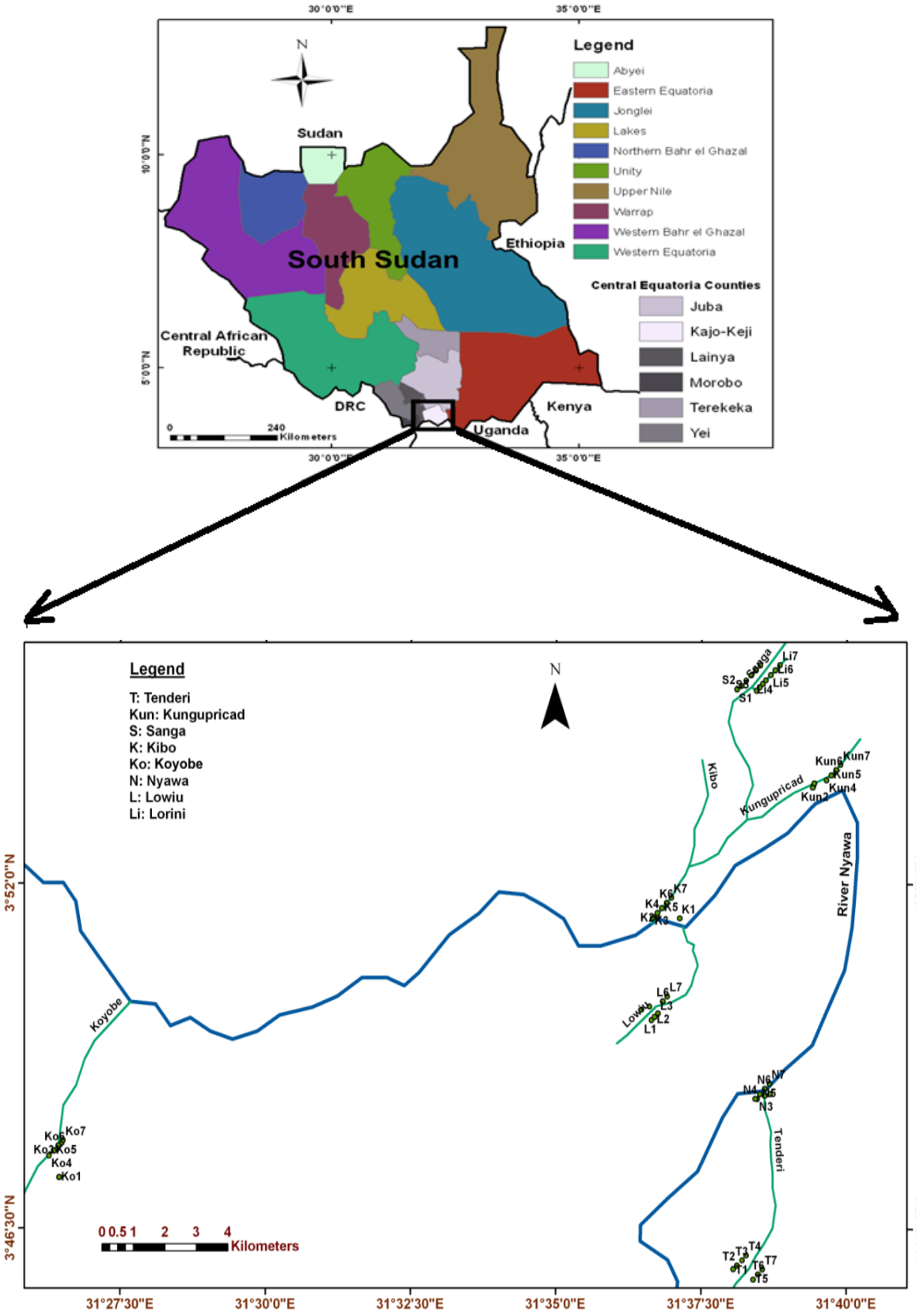

Figure 1: Map of Kajo-Keji County, Central Equatoria State South Sudan

2.2Entomological Survey

Tsetse field surveys conducted in the study area for two years as from January 2011 to December 2012. Sampling of flies was conducted for five consecutive days in each month as from 8:00 a.m to 4:00 p.m for 24 months during the wet and the dry seasons. Tsetse samples were taken from eight streams which include Lorini, Kungupiri and Sanga in Lire Payam; Tenderi in Kangapo I Payam; Kibo, Lowiyu and Nyawa in Kangapo II Payam as well as Koyibo in Liwolo Payam. The sample size of tsetse was determined by 95% confidence interval at a desired level of 5% (Thrusfield, 1995) and the stratified random sampling method was used for monitoring the prevalence of tsetse and assessing species diversity and distribution. Unbaited biconical traps were deployed in seven different sites along the banks of the eight streams (Challier et al., 1977).These traps were deployed 150 m apart and 5 m distant from the streams (Mohamed-Ahmad and Wynholds, 1997) once every week during both the wet and the dry seasons. Captured flies were collected every 24 hours, counted, stored in cool boxes, sorted into males and females and identified based on morphological characteristics (Ohaeri and Eluwa, 2007). Age (days) for both male and female flies were estimated as indicated in the FAO Tsetse Training Manual (1982). Moreover, estimation of male and female fly ages was based on the wing fray analysis and the configuration of the ovary, respectively. FAO Tsetse Training Manual (1982) shows that the trailing end of the fly wing is examined with a dissecting microscope to assess the degree of wing fray and then assigned to six standards. The mean wing fray value is then obtained by multiplying the number of flies in each category by a factor of 1, 2, 3, 4.4, 5.5 and 6.9, respectively. The sum of the product is divided by the total number of wings examined; the mean value is calculated and crosschecked with the standard to obtain the estimated average age in days; the female flies age was assessed by the Ovarian method and the uterine contents including egg, 1^{st} , 2^{nd} , 3^{rd}instar larvae (Saunders, 1962).

2.3Morphological Identification and Determination of Infection Rate (IR%) inTsetse

Dissection was conducted to determine the infection in wild tsetse fly midguts as described in the FAO Tsetse Training Manual (1982). This method makes use of trypanosome developmental sites in the tsetse flies since it is difficult to identify trypanosomes morphologically. Live flies were dissected under dissection microscope as described by Lloyd and Johnson (1924).The midguts were removed and placed onto a clean glass slide containing one drop of phosphate buffered saline (PBS) adjusted to pH of 7.6. Each midgut was crashed to expose the trypanosomes under a dissecting microscope. The presence of trypanosomes in the midgut was indicated by their motility. The motility was confirmed by examining under stereo microscope and re-examined under higher magnification. Parasites detected in midguts were recorded as Nannomonas. From each dissection step to another, dissection sets were washed with 3-5% Sodium hypochlorite and dipped into distilled or deionized water.

$$\text{The IR\% was determined as } \frac{\text{Number of the infected flies}}{\text{Total number of dissected flies}} = \quad \text{X100}$$

The trypanosome species in the tsetse infected midgut was identified using RIME-LAMP assay as described in Lukaw et al.(2015).

2.4. Statistical Analysis

The difference in the number of the infected flies between the dry and the wet seasons was assessed by the Wilcoxon Signed Ranks Test and the correlation of the number of the infected flies with age was represented graphically.Statisticswas calculated using IBM SPSS-21 compatible with Windows.

3. Results

3.1. Trypanosome Species Identified Using RIME-LAMP.

The visual appearance of RIME-LAMP products from *G.f .fuscipes*midgut samples using SYBR Green-I (Invitrogen) is shown in **(figure 2)**. Template in tube1 is a positive control; tube 2, negative control; tube 3, negative result and tube 4 is a positive result. A positive result indicates the presence of the TgSGP.

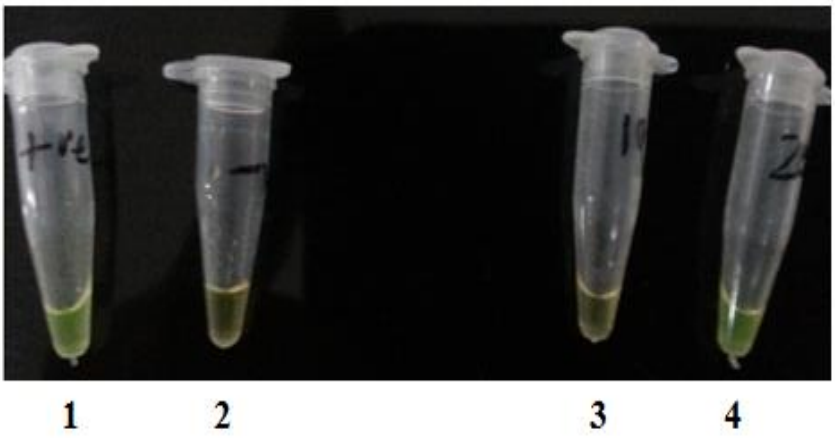

Figure 2: The visual appearance of repetitive insertion mobile element/ loop- mediated isothermal amplification (RIME-LAMP) products.

3.2. Infection Rate% Dynamics in the Dry and Wet Seasons during the Study Period (Jan 2011-Dec 2012)

The mean number of the infected flies were in the order: 6 ±1.4 male, 3 ± 1 female and 10 ± 2male,3 ± 0.7 female in the dry and wet seasons, respectively in the year 2011 ; 6 ± 1.4 male,6 ±1.4female and 10 ± 2male,3 ± 0.8 female in the dry and the wet seasons, respectively in 2012. The numbers of infected flies in the wet season were significantly higher (Z=-2.03, P = 0.04) than the dry season of 2011. However, no significant difference (Z=-1.41, P = 0.16) in the number of the infected flies between the dry and the wet seasons of 2012found as shown in **(table 1)**.

Table 1. Infection rate dynamics of *Glossina fuscipes fuscipes* in the dry and wet seasons in Kajo-Keji County, South Sudan.

	Year 2011				**Year 2012**			
	Dry season		Wet season		Dry season		Wet season	
	Male	female	Male	female	Male	female	Male	female
Min	0	0	0	0	0	0	0	0
M ± SEM	6±1.4	3±1	10±2	3±0.7	6±1.4	6±1.4	10±2	3±0.8
Max	9	6	14	5	9	9	14	5
Wilcoxon Signed Rank Test	Z=-2.03, P = 0.04*				Z=-1.41, P = 0.16NS			

* Significant, P<0.05; NS not significant, P>0.05; Min, minimum; M, mean; SE, standard error of the mean; max, maximum

3.2. Correlation of Infection Rate (%) with the Fly Ages

The number of the infected male and female flies was positively correlated with the fly age in the dry and the wet seasons in both years (2011 and 2012).The number of the infected male and female flies was positively correlated with the fly age(male, R^2 = 0.94 and female, R^2 =0.84) and (male, R^2 = 0.97 and female, R^2 =1) in the dry and the wet seasons respectively of 2011 **(Figures 3and 4)**; (male, R^2 = 0.90 and female, R^2 =0.94) and (male, R^2 = 0.97 and female, R^2 =1) in 2012 **(Figures 5and 6)**.

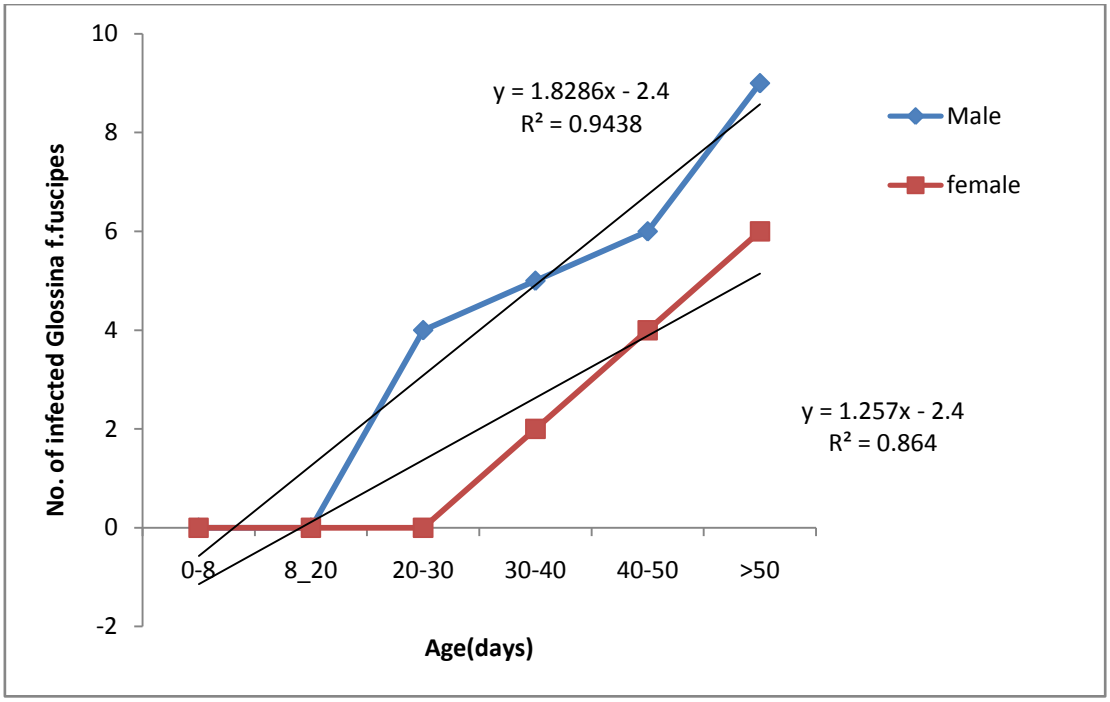

Figure 3: Correlation of Glossina F.Fuscipesinfection rate with agesinthe dry season of the year 2011 in Kajo-Keji County, South Sudan

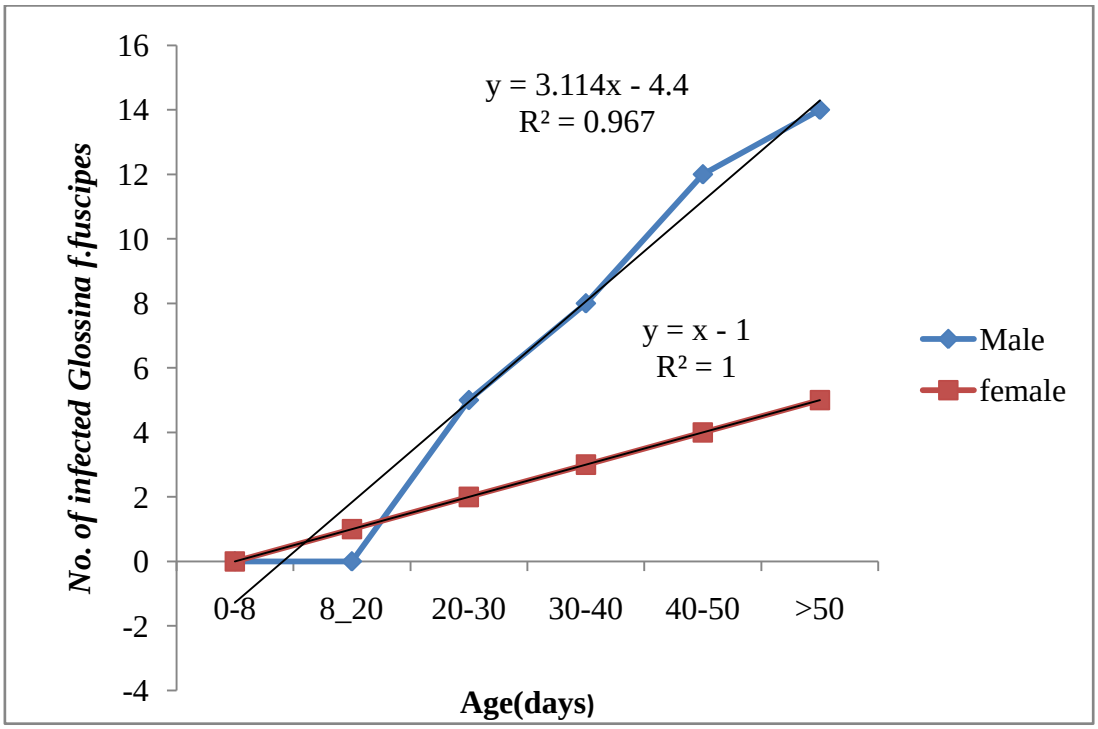

Figure 4: Correlation of Glossina f.fuscipes infection rate with ages in the wet season in the year 2011 in Kajo-Keji County, South Sudan

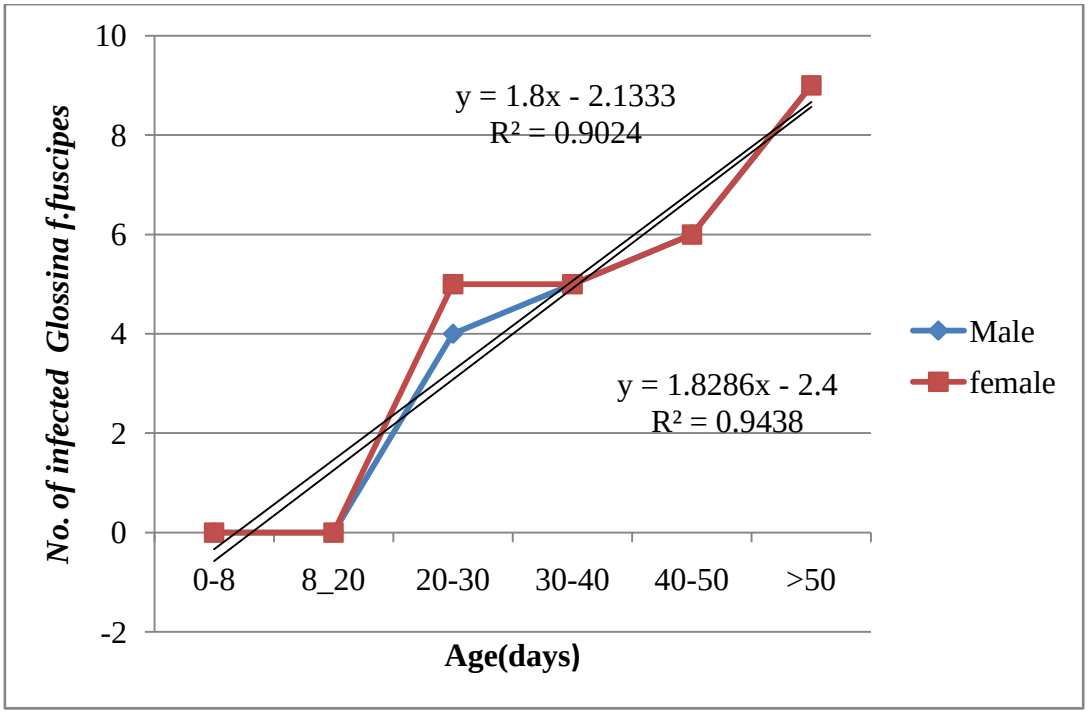

Figure5: Correlation of Glossina F.Fuscipes infection rate with ages in the dry season in the year 2012 in Kajo-Keji County, South Sudan

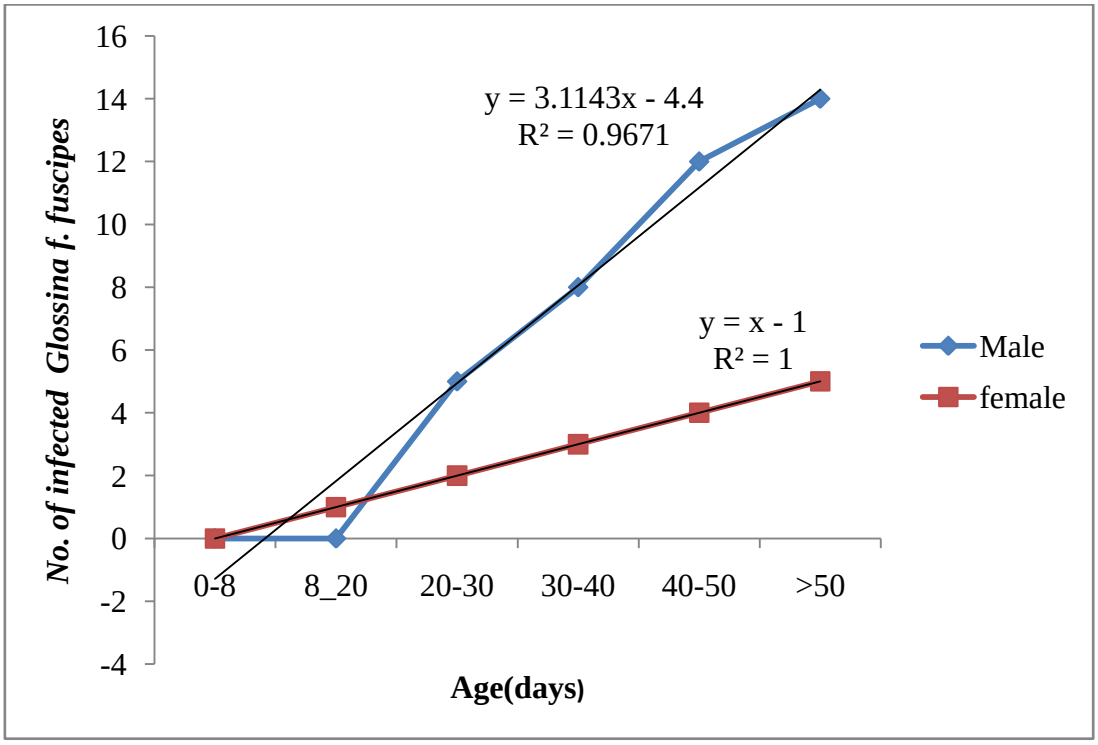

Figure6: Correlation of Glossina F.Fuscipes infection rate with ages in the wet season in theyear 2012 in Kajo-Keji County, South Sudan

Discussion

As reported earlier that *G.f.fuscipes*was the only vector of HAT found in the study area (Mohammed et al., 2008; Lukaw et al., 2014). This is evidenced by the fact that *G.f.fuscipes*are confined to gallery forests which allow such flies to penetrate into dry savanna areas. Such gallery forests provide shade and maintain an appropriate microclimate for tsetse as well as habitats for their vertebrate hosts (Bouyer et al.,2010). Moreover, it seems that the presence of *G.f.fuscipes*in the study area is justifiable in their ability to easily adapt themselves to peridomestic situations and their linear habitat that allows them to easily disperse between favorable riverine forests acting as genetic corridors (Ahmed, 2004). The finding that *G.f.fuscipes*was the only species of *Glossina* encountered in the study area might also be justified in the sense thatunbaitedbiconical traps used for capturing tsetse were inefficient for capturing the forest and savannah species of tsetse flies (Leak, 1998). Secondly the traps were deployed along water courses away from the forests. This might have excluded the possibility of capturing the non-riverine tsetse flies.

The fly infection rates with the trypanosomes are influenced by season, age and fly habitat and as such infection rates are variables across the seasons (Desta et al., 2013). Rainfall (wet season) seems to act as the trigger for movement between subpopulations (Brightwell et al., 1997) allowing the fly to widen their range as environmental conditions are much more favourable for the flies than in the dry seasons. Therefore flies have high probability of encountering their hosts as they increase the frequencies of their movements over a wider area. This might explain that the wet season catches in one of the years of sampling had more infected flies than the dry season**(Table 1)**.

In this study there was significant difference between infected male and female *Glossina f.fuscipes*in the dry and the wet seasons during the study period; male flies were relatively more infected than the female. Trypanosomes infection rate was reported to vary with sex, age, species of trypanosome and the tsetse fly, hunger state of the fly and season (Okoh etal., 2012). Moreover, an increased male susceptibility to trypanosome infection has been reported (Reinfenberg et al.,1997; Samdi et al., 2011; Peacock et al., 2012).The higher infection rates observed in the male flies in this study might be attributed to the fact that the male flies were more susceptible to trypanosome infection as they are the most active segments of the population and so expend more energy for flight action which may result in frequent feeding and thus predispose the fly to trypanosome infection (Okoh et al., 2012). Further studies attributed the higher infection in the males to the reason that male tsetse flies produce significantly more mature trypanosome infections than do females and the underlying mechanism of these sex differences seems to be the operation of a product(s) of an X-linked gene that kills or prevents migrating parasites from maturing(Welburn and Maudlin,1999). But,other studiesasserted that due to the natural longevity of females to cope with reproductive efficiency and production of much more larvae for biotic survival of the flies that female flies should have higher infection rates than males (Kohagne etal.,2010; Desta et al.,2013).However, Bitew etal.(2011) and Nthiwa et al.(2015) reported equal proportion of both infected male and female of G.F.Fuscipesflies.

It isapparent in this study that higher infection rates were observed in older flies than in the young ones andinfection seemed to be positively correlated with the age of the fly. This is evidenced by the finding that infection rates are influenced by season age and fly habitat and as such older flies are more likely tomature with trypanosome infection than younger, this is because an older fly will have more chance to become infected and an older fly will have more time for its infection to become mature (Desta et al., 2013) while infection takes between 5-53 days to develop to maturity. It was observed that in the field, the proportion of infected flies will increase with increasing age (Kubi etal., 2005) with overall result that the number of infected older flies becomes apparently dominating the sample.

Glossina f. fuscipesis one of the most important trypanosomiasisvectors in the East Africa (Malele et al., 2013; Mohammed et al., 2013), and therefore, it is to be noted here that regardless of whether males are more infected than females or vice versa or both sexes are equally infected, tsetse flies are vectors of HAT and both sexes feed on blood and can equally transmit the disease.

The occurrence of HAT in an area is acutely determined by the presence of three factors: the parasite (Trypanosoma), the vector (*Glossina* or tsetse fly) and the human host (Kohagne et al., 2010). Vector-mediated parasite transmission studies have historically proved critical to the understanding of certain aspects of infectious disease processes, and the link between the tsetse fly and HAT was confirmed between 1895 and 1903 by Dr. Gordon Bruce who conclusively demonstrated that flies transmit the disease from sick to healthy hosts (Ndungu et al., 2013). Attempts to control African trypanosomiasis have a long history, dating from the colonial period when European powers were concerned by epidemics of the human disease and the chronic loss of livestock impeding both transport and agriculture and their attempts were mainly based on active detection and treatment of sleeping sickness cases, combined with major programmes to control tsetse by bush-clearance to eliminate tsetse resting sites, wild game culling to reduce the parasite reservoirs and host availability for tsetse, and insecticidal spraying of tsetse resting sites(Schofield and Kabayo,2008). Other techniques including traps, insecticide-impregnated targets, live-baits, sequential aerial spraying, and sterile male release are also used (Bouyer et al., 2003). Community participation in controlling the disease might be effective through awareness campaigns that have to teach methods of sleeping sickness transmission, signs and symptoms as well as treatment, prevention, and control to communities surrounding tsetse-infected streams of KKC. Such attempts to involve

local communities in the fight against HAT have never been implemented in the study area, but it was introduced in Western Equatoria State,South Sudan in 2011 under Pan African Tsetse and Trypanosomiasis Control Campaign (PATTEC) that carried preliminary studies on population density of tsetse fly. A number of native people were trained on tsetse fly identification and sex segregation; biconical trap making, trap deployment and tsetse fly harvesting and collection methods. The training had an impact on reducing the population density of the fly for the subsequent years. Studies on trypanosomiasis risk assessment and blood meal analysis need to be carried out to collect and establish information on the epidemiology of the disease and on the feeding preferences of G.F.Fuscipesin order to establish the role played by preferred hosts in disease transmission in KKC.

Conclusion

*G.f.fuscipes*was infected by human trypanosomes and seems to be the main vector of HAT in the study area.G. F. Fuscipes infection rate was positively correlated with the fly age and older flies showed higher infection rate than young ones. Seasons seemed to show significant effect on the infection rate of *G.f.fuscipes*in the study area. Further studies on HAT risk assessment and identification of other possible animal hosts and feeding preferences of *G.f.fuscipes*are needed to establish data and information on the epidemiology of the disease and the role other unidentified hosts play in disease transmission.

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Conflict of Interests

The authors declare no conflict of interests.

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