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Full-Length Research Article

Trypanosomal Infection Rates in *Glossina pallidipes* in Bilbo village, Kamba District, Southern Ethiopia

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Abstract

This study was a cross-sectional study conducted at Bilbo village in Kamba district, Southwestern Ethiopia, from November 2018 to April 2019 with the aim of determining the infection rate of trypanosomes in *Glossina pallidipes.* A total of 384 *Glossina pallidipes* were captured using acetone and animal urine-baited NGU bi-conical and sticky traps. Their organs (proboscis, salivary gland, and midgut) were dissected and microscopically examined. About 53.39% of *Glossina pallidipes* were captured through NGU, and the remaining 29.17% and 17.45% were captured via bi-conical and sticky traps, respectively. Out of the dissected specimens, 131 (34.1%) Glossina pallidipes were found positive for trypanosomes. Of this, 109 (28.38%) and 22 (5.73%) were female and male tsetse flies, respectively. The highest proportion of the tsetse fly (19.27%) was infected by T. vivax, followed by *T. congolense,* and *T. brucei*. There was a statistically significant difference in trypanosome infection rate among the sex (P = 0.001) and age (P = 0.0024) categories of *Glossina pallidipes*. The presence of *Glossina pallidipes* positive for trypanosomes might have contributed to bovine trypanosomosis in the study area. Hence, further studies should be undertaken in order to categorically prioritize the control of tsetse flies in the study area.

Keywords*: Glossina pallidipes, T. brucei, T. congolense, T. vivax, traps, trypanosome infection rate*

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1. INTRODUCTION

Tsetse flies are the cyclic vectors of trypanosomosis, a disease occurring mostly in rural areas and affecting agro-pastoral activities in rural communities (Feldmann *et al.*, 2005). The fly has infested some 8–10 million km2 of areas in 37 sub-Saharan African countries, including Ethiopia, corresponding to approximately one-third of Africa's total landmass (Cecchi and Mattioli, 2009). In Ethiopia, about 200,000 km2 of areas in the western and southwestern parts of the country are infested with tsetse flies. As a result, African Animal Trypanosomosis (AAT) remains a serious problem in the country (Alemu et al., 2007). Tsetse flies are confined to the southern, western, and southwestern regions of Ethiopia between longitudes 33° and 38° E and latitudes 5° and 12° N. Tsetse's infested areas lie in the lowlands and also in the river valleys of Baro, Akobo, Didessa, Abay (Blue Nile), Ghibe, and Omo (Langridge, 1976). The potential productive areas in the West and southwest parts of the country are infested by tsetse flies, mainly Glossina morsitans Submorsitans, Glossina pallidipes, Glossina tachinoides, and *Glossina fuscipes fuscipes* (Leta and Frehiwot, 2010; Denu et al., 2012; Desta et al., 2013). The most important trypanosome species affecting livestock in Ethiopia are *Trypanosoma congolense, Trypanosoma vivax*, and T*rypanosoma brucei* in cattle, sheep, and goats; *Trypanosoma evansi* in camels; and *Trypanosoma equiperdium* in horses (Getachew, 2005).

Glossina pallidipes is a moristan group of flies that is widely distributed in East Africa and is a major vector of animal trypanosomosis (Ouma *et al*., 2011). It is also present in other Eastern African countries such as Uganda and Somalia (Krafsur, 2009; Cecchi *et al*., 2014).The epidemiological importance of insect vectors increases with their age because they have a higher chance of becoming infected and more time to mature the infection (Leak, 1998).

The eco-distribution of the tsetse is determined by climate, the presence of vegetation, water, and the presence of blood meals (humans and animals). According to Rogers and Robinson (2004), Moore and Messina (2010), and Kleynhans and Terblanche (2011), the tsetse fly needs a habitat that is heavily impacted by ecological and climatic characteristics, including temperature, rainfall, soil and vegetation type, and other climate variables. Tsetse lives in habitats that provide shade for developing pupae and resting and breeding sites for adults (Rogers and Robinson, 2004). Their development is constrained by temperature and humidity, just like that of many invertebrates.

Temperature extremes, above 360 °C and below 100 °C lead to adult fly mortality because of starvation and water loss. Low humidity or moisture levels (directly related to precipitation) are also involved in fly mortality (Leak, 1998; Moore and Messina, 2010). Since there was no sufficient study on the trypanosome infection rate of *Glossina pallidipe*s in this study area, this current preliminary study was aimed at obtaining baseline data on the trypanosome infection rate and associated factors influencing the occurrence of the infection of *Glossina pallidipes* in the study area.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Bilbo village, Kamba district, Gamo Zone, Southwestern Ethiopia. Kamba is bordered in the southwest by the South Omo Zone, in the West by Uba Derbretsehay, in the Northwest by Zala, in the Northeast by Deramalo, in the East by Bonke, and in the Southeast by the Dirashe Special Woreda. The climatic conditions of the study area included a short rainy season (Late April to May), a long summer rainy season (from July to October), and a long dry season (late December to April). The study area reveals annual mean minimum and maximum temperatures of 11 to 160 °C and 22 to 31.20 °C, respectively, and an annual mean rainfall range of 723 to 1182mm. The study area reveals an elevation of 1021 m.a.s.l. The study area encompassed long-grown grasses and small bushes. The dominant livestock species in the study area are cattle and goats. There are also some sheep, mules, donkeys, and chickens.

2.2 Study Population

The study was conducted on Glossina pallidipes.. A total of 384 Glossina pallidipes (266 female and 118 male tsetse flies) were captured using different types of traps. They were differentiated by sex and dissected for the determination of the trypanosome infection rate in the study area.

2.3 Study Design

A cross-sectional study was conducted to assess the trypanosome infection rate of the Glossina pallidipes collected in the study area. The study was conducted from November 2018 to April 2019.

2.4 Sample Size and Sample Size Determination

The stratified sampling technique was used for the study of trypanosome infection rates in tsetse flies. Hence, a large number of Glossina Pallidipes was captured, and at least 10% of the flies captured via traps were taken as study samples. Considering the tested population, which was over 5000, the sampling method was used with a 5% margin of error. The actual sample size has increased to 384 samples.

2.5 Materials and Collection of Tsetse Flies

The study was conducted using NGU, bi-conical, and sticky traps deployed 100 meters apart on bushland. Hence, a total of 24 traps were deployed. All the traps were baited uniformly with octenol (1-oct-3-nel), acetone, and three-week-old cow urine (Dransfield et al., 1986). All odors were placed on the ground, about 30 cm upwind of the trap. The poles of the traps were greased to prevent predators, mainly ants. Traps were allowed to stay at the site of deployment for a period of 48 hours before collection. After 48 hours of deployment, the catchments of each trap were sorted by fly species and then counted, identified, and analyzed (Leak et al., 1998).

2.5.1 Sex Determination

Tsetse flies were identified as male or female by examining the posterior end of the abdomen. The male fly has a lump on the ventral side of the abdomen (hypophgeum) at the posterior end but not in the female fly (Food and Agriculture Organization (FAO), 1992).

2.5.2 Age Determination

In male tsetse, the age estimation was done according to the degree of wear or fraying observed on the hind margin of the wing. Flies were divided into one or more of the six categories outlined by Jackson (1946) and Challier (1965) based on the degree of wear. Female flies were age graded according to the contents of the uterus and the relative development of the four ovarioles. It was feasible to age the female tsetse flies by determining their ovarian age by performing tsetse dissection and evaluating the contents of the uterus as well as the relative size of the follicles in each of the two ovarioles and in

each of the two ovules that constitute each ovary. According to Saunders (1962), each age category was divided into subgroups.

2.5.3 Determination of Trypanosome Infection Rate

Wings were removed from the flies, and the degree of wing fraying was scored on a scale of 1 to 6 (Jackson, 1946). Then, freshly killed Tsetse flies were dissected under a dissecting microscope using 0.9% saline.Then, using the techniques of Lloyd and Johnson (1924), trypanosome infections in the tsetse flies were determined using a compound microscope at a magnification of 400. A cover slip was then placed on each area of the slide where the proboscis, salivary glands, or midgut were inserted.
. Parasites found in the mid-gut, salivary glands, and mouth parts were regarded as Trypanozoon; "Trypanosoma brucei type), those located in the mouth parts and mid-guts were Nanomonas ("Trypanosoma congolense type), and those found in the mouth parts only were put in the group of Duttonella ("Trypanosoma vivax type infection). The Infection rate (IR) was calculated using the following formula:
Infection rate = (Number of tsetse flies infected)/(total number of tsetse flies dissected over a given period) X 100

2.6 Data Analysis

The data was entered into a Microsoft Excel spread sheet to create a database and transferred to the Stata 9 software before analysis. The association between trypanosome infection rate and the assumed risk factors was tested with Pearson's chi-square method. The trypanosome infection rate was calculated for all data using the number of infected tsetse flies divided by the total number of tsetse flies dissected over a given period of time and multiplied by 100.

3. RESULTS

3.1 Capturing Performance of the Traps

A total of 24 traps were deployed in the study area and 384 Glossina pallidipes were captured, of which 53.39% were caught by NGU traps, 29.17% by bi-conical traps, and 17.45% by sticky traps.

With regards to trapping performance, NGU trapped more tsetse flies than other traps in the study area.

Table 1.Glossina pallidipes capturing capacity of deployed traps in the study area

Trap type	No. of Traps	*No. of Glossina pallidipes*	Proportion (%)
NGU	8	205	53.38
Bi-conical	8	112	29.17
Sticky	8	67	17.45
Total	24	384	100

3.2 Trypanosome infection rate in body tissues of Glossina pallidipes

The greatest proportion 74 (56.4%) of infection was detected in the proboscis of the fly, whereas 34 (26%) and 23 (17.6%) of trypanosomes were in the salivary gland and mid-gut of Glossina pallidipes, respectively.

Table 2: Trypanosome infection rate in body tissues of *Glossina pallidipes* cuptured at village of Kamba district

Body tissue	**No. of examined**	**No. of positive**	**Infection rate (%)**
Proboscis	158	74	56.4%
Salivary gland	107	34	26%
Mid-gut	119	23	17.6%
Total	384	131	34.1%

3.3 Trypanosome infection rate of Glossina pallidipes

The overall infection rate of Glossina pallidipes in Bilbo village, Kamba district, was 34.11%. This accommodated T. vivax, T. congolense, and T. brucie. There was a significant difference between male and female tsetse flies (2 = 18.139; P = 0.001) in the proportion of infection with trypanosomes. Female tsetse flies revealed a higher trypanosomal infection rate (28.38%) than male tsetse flies (5.73%) in the study area. The trypanosomal infection rate was significantly higher in adult tsetse (30.73%) than young tsetse flies (3.38%) in the study area (P = 0.0024). The highest trypanosome

infection rate in the selected study area was via T. vivax, which accounted for 19.67%, followed by T. congolense and T. brucie, with an infection rate of 8.85% and 5.99%, respectively.

Table 3.The number of flies dissected and infection rate of *Glossina pallidipes* based on sex

Sex	**Number of dissected**	**Number of positive**	**No. of flies infected by trypanosome species (%)**			**Infection rate**
			T. vivax	***T.congolense***	***T. brucei***	
Female	266	109	64 (16.67)	28 (7.29)	17 (4.43)	109 (28.38)
Male	118	22	10 (2.60)	6 (1.56)	6 (1.56)	22 (5.73)
Total	384	131	74 (19.67)	34 (8.85)	23 (5.99)	131(34.11)

Table 4.Trypanosome infection rate of *Glossina pallidipes* based on sex and age at the Bilbo village

Risk factors	Category	No of examined	No of positive	Trypanosome species (%)			Infection rate	χ^2	P-value
				T. vivax	*T.congolense*	*T. brucei*			
Sex	Female	266	109	64 (16.67)	28 (7.29)	17 (4.43)	109 (28.38)	18.139	0.001
	Male	118	22	10 (2.60)	6 (1.56)	6 (1.56)	22 (5.73)		
Age	Young	70	13	6 (1.56)	4 (1.04)	3 (0.78)	13 (3.38)	9.201	0.0024
	Adult	314	118	68 (17.71)	30 (7.81)	20 (5.21)	118 (30.73)		
Total		384	131	74 (19.67)	34 (8.85)	23 (5.99)	131 (34.11)		

4. DISCUSSION

Among the traps installed in the selected geographical area, the NGU trap showed the highest capturing capacity (53.38%) of *Glossina pallidipes*, followed by the bi-conical (29.17%) and sticky type trap (17.45%). This agrees with Dransfield *et al*. (1986), who found that the NGU trap performed better in catching Glossina pallidipes than the bi-conical and sticky traps at Nguruman, Kenya.

In this study, the infection rate of *Glossina pallidipes* via the *T. vivax* group (19.67%) was found to be twice as high as that of the *T. congolense* group (8.85%). A lower rate of trypanosomal infection was recorded in the T. brucei group (5.99%). This is in agreement with the results of Bitew et al., (2011), who reported a trypanosome infection rate of 16.5% for T. vivax, 6.5% for *T. congolense*, and 0.5% for *T. brucei* in Glossina pallidipes in Gojeb Valley, southeast Ethiopia. This is also in line with

Woolhouse *et al*. (1994), who reported a trypanosome infection rate of 6.2% for *T. vivax* and 3.1% for *T. congolense* in Glossina pallidipes in the Luangwa Valley, Zambia.

In this study, the trypanosome infection rate of Glossina pallidipes in female flies (28.4%) was higher than in male flies (5.8%) and showed a statistically significant difference (P = 0.001). This is in agreement with the result of Desta *et al*., (2013) in Amaro Special District of southern Ethiopia, where the trypanosome infection rate in female Glossina pallidipes (6.43%) was higher than that of male Tsetse flies (0.49%). Studies suggest that female flies have higher infection rates than males, as they live longer than males and thus have higher chances of getting an infection (Mihok *et al*., 2008). The lower infection rate found in male flies can be explained by the low average age of trapped male flies (20 days or less). Some previous studies revealed higher infection rates in males than in females. In Nigeria, higher infection rates in males than females were reported (Samdi *et al*., 2011). However, this relationship has not been established yet. In contrast, other studies explained that more males may be infected than females as they are more involved in sex-related competition than females (Zuk and McKean, 1996).

In the current study, adult tsetse flies (30.73%) were found to have a higher chance of being infected than young tsetse flies (3.38%), which might be more likely due to the maturity of tsetse flies.

5. CONCLUSIONS AND IMPLICATIONS FOR RESEARCH

In conclusion, when compared to bi-conical and sticky traps, the NGU trap had the best ability to capture tsetse flies. As a result of the significant trypanosome infection rates found in G. pallidipe, this study also demonstrated the relevance of risk variables to the epidemiology of African Animal Trypanosomiasis (AAT). Because trypanosome infection is the main impediment to livestock production in Bilbo village, Kamba district, southwest Ethiopia, the development of Glossina pallidipes positives for trypanosome species in the research area may be a factor. Therefore, additional entomological research should be conducted in order to prioritise tsetse fly management and address the severe trypanosomosis problem in the studied area.

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CONFLICT OF INTEREST

There were, according to the authors, no conflicts of interest.

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