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Experimental comparison of survival and trypanosome infection rate between two tsetse fly species under different feeding conditions

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Abstract
The survival and infection rate of tsetse flies are important parameter for the success of sterile insect technique (SIT) and in vector monitoring program that allows a more precise evaluation of the risk of being infected in a particular region. The objectives of this longitudinal experimental study was to compare the survival and trypanosome infection rate of two tsetse fly species; *G. pallidipes* and *G. f. fuscipes* under experimentally trypanosomes infected calves (*in-vivo*) and using irradiated bovine blood (*in-vitro*) feeding conditions. A total of 900 (450 *G. pallidipes* and 450 *G. f. fuscipes*) male and female flies were used for this experiment. The flies were kept in a cage with a density of 25 flies per cage and divided into 9 groups each with 4 cages which fed on nine experimentally infected calves and irradiated bovine blood using a silicon membrane. The infection rate evaluation of the two species were carried out using warm slide technique through microscopy examination of the saliva of each survived flies. Accordingly, the survival rate, mean percentage of surviving flies, under calves feeding were 85.8% and 76.2% for *G. pallidipes* and *G. f. fuscipes*, respectively (p=0.001). Higher survival rate of females (84%) than males (77.9%) were observed which had statistically significant difference (p=0.019). Under *in-vitro* feeding, 88.4% and 92.2% survival percentage of *G. pallidipes* and *G. f. fuscipes* were observed, respectively (p=0.076). The survival rate of flies maintained under *in-vitro* feeding was higher than flies maintained under calves (*in-vivo*) feeding. The overall trypanosomes infection rate in the two species were 19.6% and 8.3% for *G. pallidipes* and *G. f. fuscipes*, respectively (p=0.002). There were significant difference in infection rate between females and males in both species in which females had higher infection rate ($p<0.05$). The higher survival and infection rate of *G. pallidipes* might be associated with both endogenous and exogenous factors. Hence in order to strengthen the fight against tsetse and trypanosomosis, further a detailed study on biology, survival and infection rate of major vectors like *G. pallidipes* should be carried out.

Keywords: *G. f. fuscipes, G. pallidipes*, Infection rate, sterile insect technique, survival, vector monitoring program

1. Introduction
Tsetse transmitted trypanosomosis in man and domestic animals pose a serious threat to the lives and livelihood of entire communities and constitute the greatest single constraint to livestock and crop production in sub-Saharan Africa. The limitations imposed by tsetse and trypanosomosis remain to frustrate efforts and hampers progress in crop and livestock production there by contributing to hunger, poverty and the suffering of entire communities in Africa (PATTEC, 2001) [32].

Tsetse flies (*Glossina* spp) can be ranked among the world's most destructive pests and are the vectors of the causative agents for sleeping sickness in humans and African Animal Trypanosomosis (AAT) or Nagana in livestock (Brun *et al*., 2010) [6]. Tsetse flies are taxonomically grouped under the genus *Glossina*, family Glossinidae, and order Diptera. They are categorized into three broad groups based on their habitat preferences, behavioural and morphological characteristics, namely (*i*) the savannah (morsitans), (*ii*) the riverine (palpalis) and (*iii*) the forest (fusca) group. These are further divided into 31 species and subspecies (Leak, 1999) [25].

The tsetse fly is a unique hematophagous insect as both male and female feed exclusively on blood but blood from a particular animals seems to be preferred for some reasons such as host odour. Feeding habits of tsetse flies are determined by blood meal analysis and are divided into five main patterns including (*i*) flies feeding mainly on suids such as *G. swynnertoni*, (*ii*)

flies feeding mainly on suids and bovids such as *G. m. morsitans*, (*iii*) flies feeding mainly on bovids such as *G. pallidipes*, (*iv*) flies feeding mainly on other mammals than suids and bovids such as *G. brevipalpis*, and (*v*) flies feeding on the most available host and man. Bush-bucks, oxen, and buffaloes are the main hosts for *G. f. fuscipes* (Clausen *et al*., 1998) [10].

Tsetse sex ratio on average is about 60-70% which females in excess. However, fluctuation in the sex ratio is observed as a function of tsetse species, season and location. Tsetse flies has been observed to reach an age of 226 days, but the age varies with tsetse species, season, and location. The typical life span of tsetse flies correspond to 160 days in the warm rainy season, 110 days in the cold dry season and only 50 days in the hot dry season. The hot dry season kills mainly the young fraction of the tsetse fly population (Challier, 1982) [9].

All 31 species of tsetse flies can transmit trypanosomes to a greater or lesser extent, but the infection prevalence varies among the tsetse species and decrease with age. For example the infection rate in the Fusca and Palpalis groups is usually much lower than in the Morsitans group. The rate of infection depends on a number of factors. These include: (*i*) Endogenous factors – are those associated with the fly such as age at first infective feed, sex, intra and inter species genetic differences, fly behavior, host preferences, physiological and biochemical state, concurrent infection by viruses, bacteria and fungi; (*ii*) Ecological factors – such as climate, availability of infected wild and domestic animals for subsequent feeding; and (*iii*) Parasite and host factors – which include parasite numbers available to the fly, type of parasite, its infectivity to the fly, immune status of the host species, host breed, host behaviors' and its attractiveness to the fly (Dyer *et al*., 2013) [11].

Tsetse flies infest 10 million Km^2 of sub-Saharan African, extending from Mali and Ethiopia in the North, Senegal in the West to Southern Somalia in the East and Angola and South Africa in the South (Cecchi *et al*., 2008) [8]. According to FLDP (1989), tsetse flies in Ethiopia are confined to the western and southern regions between longitude 33° and 38° E and latitude 5° and 12° N. A total area of 240,000 km^2 of fertile areas are under the threat of trypanosomosis. Tsetse infested areas lie in the lowlands and also in the river valleys of Abay (Blue Nile), Baro, Akobo, Didessa, Ghibe and Omo (Abebe *et al*., 2004) [1]. According to survey result conducted by Langridge (1976) five species of *Glossina* (*G. m. submorsitans*, *G. pallidipes*, *G. tachinoides*, *G. f. fuscipes* and *G. longipennis*) have been recorded from Ethiopia.

The sterile insect technique seems to be promising towards the eradication of tsetse flies in Africa in general and in particular in Ethiopia. SIT as a technique of choice, requires mass production of tsetse flies in the laboratories. The laboratory rearing of tsetse flies originally dependent on the availability of host animals for *in-vivo* feeding (Oladunmade *et al*., 1990) [30].

The frequent use of host animals as blood donors imposes the risk of over challenging them. For this reason it is necessary to develop effective and standardized tsetse fly feeding methods without using live animals for the daily blood uptake. So, appropriate use of the membrane (*in-vitro*) feeding technique provides a means to produce tsetse flies more economically and with less risk. *In-vitro* feeding system is recommended if reliable source of quality tested blood is available (Opiyo *et al*., 2000) [31].

Successful rearing of large number of insects for their continuous availability in the laboratory depends on the knowledge of insect biology, behavior, habitat and nutrition. Originally, living animals had to be used to provide tsetse flies with a movement. With the development of membrane feeding system, which flies accept as host skin and through which they ingest the blood, living animals are no longer required as hosts. Animal blood for tsetse rearing can be collected at a local abattoir and then treated with gamma radiation to eliminate any micro-organisms (Feldmann and Hendrichs, 2001) [15].

Understanding tsetse population parameter (growth rate, longevity, age structure) supports the efficiency of the different vector control methods (Peck, 2012) [33]. Moreover, survival of tsetse flies is very important for successful Sterile Insect Technique (SIT). The prevalence of trypanosome infection in the tsetse flies is often a neglected parameter probably due to the intensive labour required for its evaluation. Integration of this parameter in a monitoring program allows a more precise evaluation of the risk of being infected in a particular region. However, Information related to the survival and infection rate of different *Glossina* species is scarce in Ethiopia.

Therefore, the objectives of the present experimental study was to compare the survival and infection rate of the two tsetse fly species; *G. pallidipes* and *G. f. fuscipes* under different feeding conditions.

2. Materials and methods

2.1. Study Location

The study was conducted at the College of Veterinary Medicine and Agriculture of Addis Ababa University in Bishoftu from November 2015 to January 2016. Bishoftu is located about 45kms south east of Addis Ababa at 9^0N latitude and 40^0E longitudes at an altitude of 1850 meters above sea level in central high land of Ethiopia. It has an annual rainfall of 866 mm of which 84% is in the long rainy season (June to September). The dry season extends from October to February. The mean annual maximum and minimum temperature are 26^0C and 14^0C respectively, with mean relative humidity of 61.3% (ADARDO, 2007) [2].

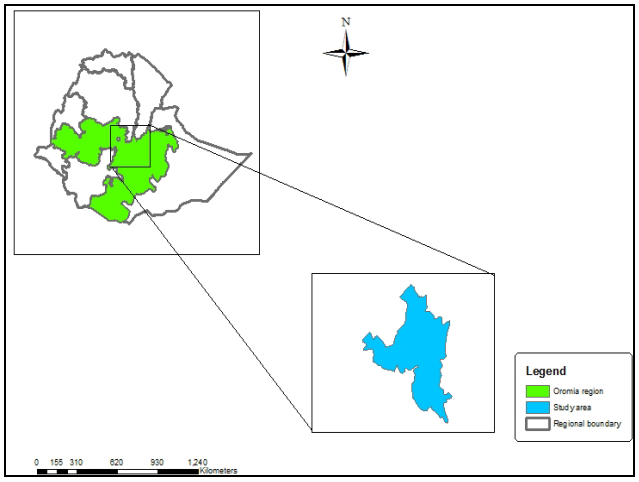

Fig 1. Map of the location of study area
Source: Design by Arc. GIS version 10.2 software

2.2. Experimental Animals

Nine young Holstein Frisians cross cattle aged 9 to 12 months

purchased from Bishoftu. On arrival at College of Veterinary Medicine and Agriculture, the animals were subjected for one month acclimatization period, during which they were ear-tagged, and screened for hemoprotozoa along with internal and external parasite. There were also 900 two different tsetse fly species namly; *G. pallidipes* and *G. f. fuscipes* that brought from National Tsetse and Trypanosome Investigation and Control Center (NTTICC) which is found at Kality within the capital, Addis Ababa Region. Flies were separated into male and female and kept in cage (diameter of 20 cm and width of 5 cm) with density of 25 flies per cage with netting on top and bottom for feeding.

Fig 2. Experimental flies in a cage

2.3. Trypanosome Inoculation and Blood Examination

After the calves had finished their acclimatization period, each calf was inoculated or infected with *T. vivax* and *T. congolense* isolate that brought from tsetse-infested area. Before inoculation the animal was injected with Dexamethasone to suppress their immune status for three consecutive days. Animals were examined daily for clinical abnormalities at their pen. Blood samples were examined for the presence of trypanosomes daily until parasites were detected in the blood using standared parasitological methods.

2.4. Materials

The list of materials used for the present experimental study were include: laboratory equipments, refrigerators, freezers, dry oven, autoclaves, infected calf, tsetse flies (*G. pallidipes and G. f. fuscipes*), holding cages (diameter of 20 cm and width of 5 cm), heating plate (with an adjustable heat source), feeding tray (anodized aluminum), silicon membrane, clips, 96% irradiated bovine blood, thermoscan, air conditioners, humidifiers. All these materials were used following the standard procedures (FAO/IAEA, 2006) [14].

2.5. Experimental Groups

Nine young aged Holstein Frisian cross calves and all 900 adult flies (*G. pallidipes* and *G. f. fuscipes*) of both female and male which brought from National Tsetse and Trypanosome Investigation and Control Centre were used for this experiments.

2.6. Study Design

Both experimental and observational study was employed beginning from November 2015 to January 2016 to compare the survival and infection rate of *G. pallidipes* and *G. f. fuscipes* tsetse fly species under different feeding conditions.

2.7. Experimental Protocol

Experiment I: Survival rate of *G. pallidipes* and *G. f. fuscipes* under *in-vivo* and *in-vitro* feeding

Evaluation of the survival rate of *G. pallidipes* and *G. f. fuscipes* under *in-vivo* and *in-vitro* feeding were carried out under infected calves and artificial membrane feeding using bovine blood. The blood was brought from Kality National Tsetse and Trypanosome Investigation and Control Center (NTTICC) that was treated with UV-gamma radiation to eliminate any micro-organisms and foreign body in the blood.

Round 1: In-vivo feeding

Prior to feeding, cages was divided into nine groups in which each groups consists of four cages based on the number and a code that tagged on calves. One animal was fed by four cages of flies (two cages for *G. pallidipes* male and female and two cages for *G. f. fuscipes* male and female). Generally there were about 36 cages for this experimental study. Feeding was carried out three days per week for three consecutive weeks. During feeding the animal was restrained in lateral recumbence and the flies let to feed for five to ten minutes. After each feeding the number of fed, unfed and dead flies were recorded.

Round 2: In-vitro feeding

All round one tsetse flies kept under live animal (*in-vivo*) feeding were transferred to *in-vitro* feeding. Silicone membrane was used for the *in-vitro* feeding of the flies (Bauer and Wetzel, 1976) [4]. During every feeding time, heating plates (mat) were disinfected with alcohol and feeding trays were placed on the heating plate for about 10-15 minutes after switched on the power supply. After heating, all fans, air conditioners and humidifiers were switched off and blood for each experimental group was pour on sterilized aluminum tray by lifting one end of the silicone membrane. The blood was hygienically spread using plastic roll bar to prevent contamination of the blood. The blood was allowed to warm up to body temperature (35-37 ^{0}C) and measured by using thermoscan before feeding to flies. Flies were picked up from the trolley, put on to the feeding tray and allowed to feed for ten minutes under off light. Then after feeding, the number of fed, unfed and dead flies were recorded. Feeding was carried out three days per week for three consecutive weeks similar to live animal (*in-vivo*) feeding. At the end of every feeding, trays and silicone membranes had thoroughly washed and rinsed by distilled water. The membrane was placed on to a feeding tray and sterilized overnight in a dry heat of 120 ^{0}C for next use (FAO/IAEA, 2006) [14].

Experiment II: Infection rate evaluation of *G. pallidipes* and *G. f. fuscipes* using warm slide technique

This was the continuous of the first survival experiment after the end of survival monitoring days that last for six weeks (eighteen days of observation). The survived flies were transferred from the circular cage into rectangular cage which is partitioned to the size of slide that accommodate only one fly per partition and code was given to each partition. Prior to transformation, the previous cage was chilled at +4 ^{0}C for 5 minute to inactivate the flies. After the inactivated flies transferred to partitioned cage, warm slide was attached to each coded partition that contain fly and code of partition was written on the slide to identify later positive flies for further species specific PCR study which was done by my advisor

Dr. Reta Duguma for his PhD fulfillment program. Accordingly, tsetse flies are allowed to salivate (probe) on warm slides, then trypanosome examinations were carried out through microscopy examination of the slide using gimsa staining (Burtt, 1946) [7].

2.8. Data Analysis

The entire data was entered to Microsoft Excel spread sheet and imported to SPSS version 20. Different descriptive statistics were used to evaluate both the survival analysis and infection rate of experimental groups. Survival rate of different *Glossina* species and sex under different feeding condition were analyzed to see their association with aforementioned factors using chi-square test. Results were presented as tables and graphs.

3. Results

3.1. Survival Rate of *G. pallidipes* and *G. f. fuscipes* Under *In-vivo* and *In-vitro* Feeding

The survival rate of the two experimental species were varied under *in-vivo* and *in-vitro* feeding. Out of the total 225 *G. pallidipes* male, 225 *G. pallidipes* female, 225 *G. f. fuscipes* male and 225 *G. f. fuscipes* female, the mean survival percentage of the flies throughout *in-vivo* feeding were 93.9%, 95.6%, 76.4% and 76%, respectively (Table 1). There were statistically significant difference in the survival rate of the two species (p=0.019) and sex (p=0.019) (Table 2). However, there was no significant difference within species sex (p>0.05). Under *in-vitro* feeding the mean survival percentage of the two species throughout the experimental observation were, *G. f. fuscipes* female (83.7%), *G. f. fuscipes* male (93.6%), *G. pallidipes* male (90%) and *G. pallidipes* female (94.2%) (Table 3). There was no statistically significant difference between the two species (p=0.076) but there was significant difference between the two sex (p=0.001) (Table 4).

Table 11. Mean survival percentage of the two species under live animal (*in-vivo*) feeding.

Species	Sex	Fed	Survived	Fed (%)	Survived (%)
G. f. fuscipes	M	157	171	92.6	76
G. f. fuscipes	F	158	172	91.9	76.4
G. pallidipes	M	159	180	93.9	80
G. pallidipes	F	197	206	95.6	91.5

Table 22. Correlation of the mean survival percentage of two species and sex under *in-vivo* feeding.

		Survived (%)	Dead (%)	X^2	P-value	S.E
Species	*G. f. fuscipes*	76.2	23.8	13.669	0.001	0.032
	G. pallidipes	85.8	14.2			
Sex	Male	77.9	22.1	5.566	0.019	0.032
	Female	84	16			

Table 33. Mean Survival percentage of the two species under *in-vitro* feeding

Species	Sex	Fed	Survived	Fed (%)	Survived (%)
G. f. fuscipes	M	137	143	96.2	83.7
G. f. fuscipes	F	160	161	99.4	93.6
G. pallidipes	M	162	162	100	90
G. pallidipes	F	194	194	94.2	94.2

Table 44. Correlation of mean survival percentage of two species and sex under *in-vitro* feeding.

		Survived (%)	Dead (%)	X^2	P-value	S.E
Species	*G .f. fuscipes*	88.4	11.6	3.150	0.076	0.036
	G. pallidipes	92.2	7.8			
Sex	Male	86.8	13.2	11.015	0.001	0.035
	Female	93.9	6.1			

Comparing the survival status of flies under calves and membrane feeding, high survival rate were observed under a membrane feeding (90.4%) than calves feeding (80.9) which was statistically significant (p<0.05) (Fig. 3).The survival rate of the two species throughout the monitoring days of experimental feeding were *G. f. fuscipes* male (63.6%) (Fig. 4), *G. f. fuscipes* female (70.5%) (Fig. 5), *G. pallidipes* male (72%) (Fig. 6) and *G. pallidipes* female (86.2%) (Fig. 7).

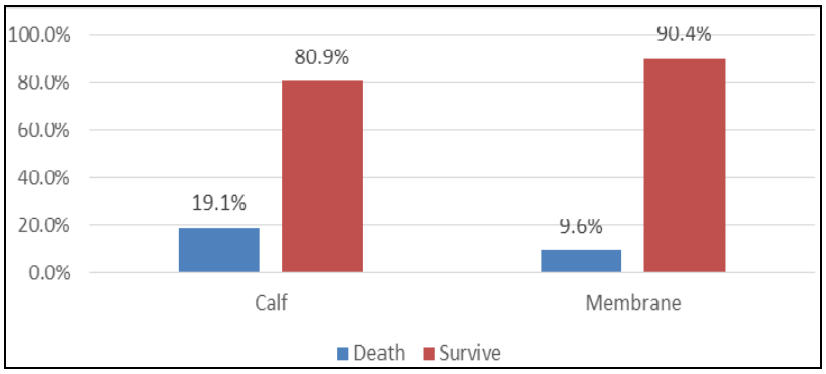

Fig 3: survival status of flies under calf and membrane feeding

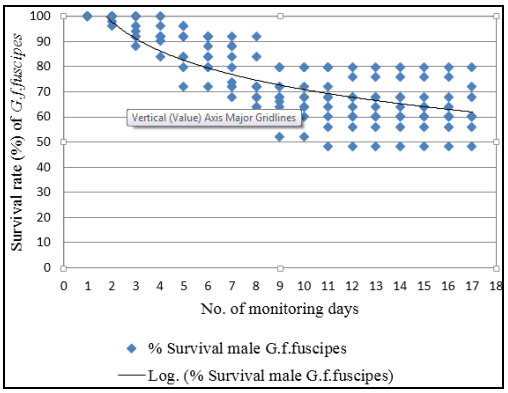

Fig 4: Survival rate (%) of male G.f. fuscipes (n=225) throughout monitoring days.

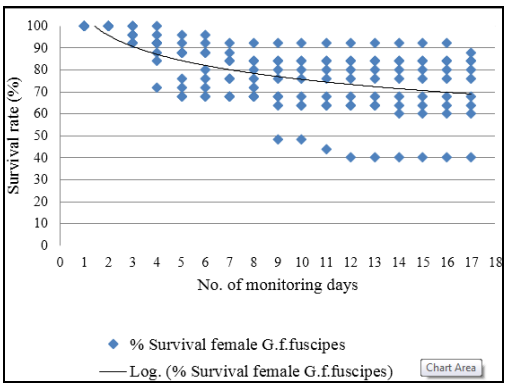

Fig 5: Survival rate (%) of female G.f. fuscipes (n=225) throughout monitoring days

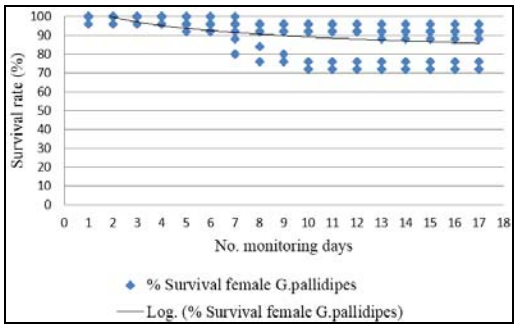

Fig 6: Survival rate (%) of female *G. pallidipes* (n=225) throughout monitoring days

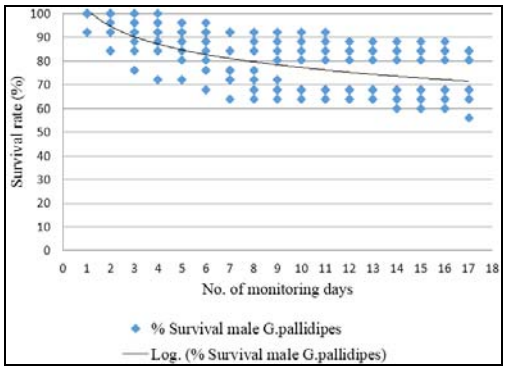

Fig 7: Survival rate (%) of male G. pallidipes (n=225) throughout monitoring days

3.2. Infection Rate of *G. pallidipes* and *G. f. fuscipes* Using Warm Slide Technique

Experimental study on infection rate of the two species were performed using warm slide technique from the survived flies that previously fed under experimentally infected calves and artificial membrane. Under microscopy examination of gimsa stained saliva of both species, high infection rate were observed in *G. pallidipes* (19.6%) than *G. f. fuscipes* (8.3%) (Table 5). The difference was statistically significant (P=0.002). Within sex of *G. pallidipes* male and female, female had high infection rate (26%) than male (13%). The difference was statistically significant (p=0.001) (Table 6).

Table 5. Comparison of the infection rate of *G. pallidipes* and *G. f. fuscipes*

Species	Mean	S.E	P-value	[95% conf. interval]
G. pallidipes	0.1961	0.27	0.002	0.14-0.25
G.f. fuscipes	0.083	0.02		0.043-0.12

Table 6. Comparison of the infection rate within sex of *G. pallidipes* species

Species	Sex	Mean	S.E	P-value	[95%conf. interval]
G. pallidipes	Male	0.13	0.03	0.015	0.07-0.19
	Female	0.26	0.44		0.17-0.35

4. Discussion

Tsetse flies (*Glossinidae*) are important vectors for transmission of the parasites trypanosome causing a disease known as Nagana among domestic animals and sleeping sickness among human (Vreysen, 2001) [39] has made them one of the most devastating insect in Africa in general and in Ethiopia, in particular.

The present study was performed to compare the survival and trypanosome infectious rate of *G. pallidipes and G. f. fuscipes* tsetse fly species that maintained under infected live animal (*in-vivo*) and *in-vitro* feeding conditions. According to the present study under *in-vivo* feeding, *G. pallidipes* showed a higher mean survival percentage than *G. f. fuscipes* (Table 2). This contradicts with the finding on *G .m. morsitans* and *G. p. palpalis* fed on cattle which was reported to be equal (Wetzel and Luger, 2003) [41]. The higher survival rate of *G. pallidipes* may be due to the difference in host specificity of *G. pallidipes* and a range of physiological adaptation to the specific host blood feeding. An important parameter that affect the survival of the tsetse population is its hunger stage. According to Moloo (1993) [27] studied on feeding habits of tsetse flies by blood meal analysis, *G. pallidipes* feed mainly on bovids than *G. f. fuscipes*. The present study was also supports Moloo blood meal analysis. According to the present study under calves (*in-vivo*) feeding, *G. pallidipes* fed more percentage than *G. f. fuscipes* (Table 1).

Another possible reason for the observed high survival rate of *G. pallidipes* may be attributed to host odours that associated with tsetse fly feeding efficiency. The naturally occurring cattle odour and related compound like acetone and phenol let the tsetse flies to sense host availability, approach, land, and probe and fed on host. For the savannah tsetse flies such as *G. morsitans* and *G. pallidipes*, host odour are the most important whereas these are less important for riverine species like *G. f. fuscipes* and *G. p. palpalis* (Gibson and Torr, 2002) [17]. The anatomical structure of the mouth parts are also very important to the life of the fly which has an effect on feeding

efficiency of the flies (FAO, 1982) [12]. *G. pallidipes* has long and pointed proboscis than *G. f. fuscipes*. This might reduce the feeding efficiency of the *G. f. fuscipes* and lead to hunger stage and cause of high mortality in the population.

Under *in-vitro* feeding on irradiated bovine blood the result showed that low difference in mean survival percentage of *G. pallidipes* (92.4%) and *G. f. fuscipes* (88.4%) which was statistically insignificant (p=0.076) (Table 4). By contrast, studies carried out in other tsetse fly species, *Stomoxys calcitrans* Linnaeus and horn flies which were also obligate blood feeders, had shown great differences in average survival rate among flies reared on cattle or other types of blood, such as pig, horse, sheep, goat, chicken or rabbit (Sutherland, 1978; Kuramochi, 2000) [37, 21]. The present study roughly agrees with the finding on *G. pallidipes* and *G. p. palpalis* using *in-vitro* feeding which was reported to be equal (Langley, 1989) [22]. This might attributed due to high feeding efficiency of both species under silicon membrane feeding due to the absence of host factor like smell and sight.

Comparing the survival status of the flies under *in-vivo* and *in-vitro* feeding, high survival rate were observed under *in-vitro* (90.4%) than *in-vivo* (80.9%) feeding. The high survival rate of flies under *in-vitro* feeding may attributed due to irradiation of blood under UV-light that reduce the bacterial and other micro-organism load in the blood that threat the life span of the flies. According to Langley and Roe (1984) [23] survival rate of tsetse flies under live animal (*in-vivo*) feeding affected by some toxic plant residues which could be present in the animal blood and with the recent treatment of the animals with drugs like ivermectin which was claimed to cause 100% mortality in *G. m. morsitans* even in low doses (>1.6 μg ml^{-1}).

The survival rate of females and males flies throughout the present experimental feeding were showed that, female flies had greater survival rate than male flies. Female *G. pallidipes* had higher mean percentage of survival (86.2%) throughout the experimental study. This was found to be almost similar with the data obtained on female *G. pallidipes* (85.8%) fed on similar bovine blood diets compare to male (FAO /IAEA, 1997) [13]. This may be associated with the longer life span of female tsetse fly than male under natural condition.

The present study revealed that a relatively higher mean survival percentage of *G. pallidipes* than *G. f. fuscipes* throughout the experimental feeding condition. This may associated with its ability to resist harsh environment like stress and climate change like temperature and humidity level together with the aforementioned factors. Many studies have demonstrated strong relationships between temperature and moisture availability, and survival of *Glossina* spp. at both coarse and fine scales (Rogers and Robinson, 2004) [35]. Study performed in South Africa on impacts of climate change on tsetse fly reported that, *G. pallidipes* showed higher resistant to temperature change than *G. palpalis* and *G. m. morsitans* (John *et al*., 2008) [19]. This both endogenous and exogenous factors might be the cause of the difference in their survival rate of the two fly species.

All 31 species of tsetse flies can transmit trypanosome to a greater or lesser extent, but the infection prevalence varies among the tsetse flies species. However, not all infectious feeds will produce mature infections in tsetse fly. Like mammalian host, trypanosome's journey, establishment, growth, survival, multiplication, and maturation in the tsetse fly is full of challenge and obstacles (Roditi and lehane, 2008; Dyer *et al*., 2013) [34, 11].

As expected, the prevalence of trypanosome infection in tsetse flies is higher in laboratory experiments than in field. Collections of tsetse flies with an overall prevalence of 25% and 10% respectively. In laboratory experiments, blood meals and external conditions are standardized and all feeding flies ingest parasites. In the present study on infection rate evaluation of the two tsetse fly species in laboratory using warm slide technique, the higher prevalence of trypanosome infection was found 19.6% within *G. pallidipes* flies. The variation may attributed due to the sensitivity of diagnostic method for detection of trypanosomes in tsetse flies.

The overall trypanosomes infection rates in *G. pallidipes* were significantly higher than in *G. f. fuscipes* in the present study (p=0.02). The result matches with the study performed in Coastal Kenya, higher infection rates were reported in *G. pallidipes* (21.7%) than in *G. f. fuscipes* (0.2%) (Njiru *et al*., 2004) [29]. The difference in infection rates between the two tsetse species could be due to variation in feeding preferences, environmental factors, and host range differences (Bouyer *et al*., 2013) [5]. The number of parasites available to infect tsetse, parasite infectivity to tsetse, and the strain or subspecies have also been found to affect infection rates in tsetse (Hu *et al*., 2008) [18]. Moreover, the nutritional status of the tsetse at the time of infective blood meal can also affect their ability to acquire trypanosome infections (Kubi *et al*., 2006) [20]. In addition, low vectorial capacity has also been reported and is attributed to higher levels of attacin expression in the proventriculus and midgut (Nayduch *et al*., 2007) [28]. This kind of trait has been reported in *G. pallidipes* and it could explain disparity of infection rates between *G. pallidipes* and *G. longipennis* (Aksoy *et al*., 2003) [3].

Within species, infection rates were also variable and this was attributed to individual host factors. For instance, the vulnerability of flies to *T. brucei* infections was shown to be due to maternally inherited features which are associated with the presence of intracellular rickettsia-like organisms (RLOs) (Wamwiri *et al*., 2013) [40]. Tsetse carrying these RLOs and other simultaneous infections such as bacteria, fungi, and virus in their midgut were more likely to be infected with trypanosomes than those without (Symula *et al*., 2013) [38].

This study revealed more infection rates in females than in males. The result agree with study performed in Nigeria, higher infection rates in female than males were reported (Samdi *et al*., 2011) [36]. Some studies suggest that female flies should have higher infection rates than males as they live longer than males and thus they have higher chances of getting infection (Mihok *et al*., 2008) [26]. This relationship has been established in the present study. In contrast, other studies explain that more males may be infected than females as they are involved in sex activities and competition than females (Zuk and McKean, 1996) [42].

5. Conclusion and Recommendations

Understanding tsetse population parameter (growth rate, longevity, age structure) supports the efficiency of the different vector control methods. Moreover, survival of tsetse flies is very important for successful Sterile Insect Technique (SIT). The prevalence of trypanosome infection in the tsetse flies is often a neglected parameter probably due to the intensive labour required for its evaluation. Integration this parameter in a monitoring program allows a more precise evaluation of the risk of being infected in a particular region.

To support the already established program in Ethiopia a research has been conducted on survival and infection rate of *G. pallidipes* and *G. f. fuscipes* maintained under different blood feeding condition.

The present study disclose a relatively higher mean survival percentage of *G. pallidipes* than *G. f. fuscipes* under both live animal (*in-vivo*) and *in-vitro* blood feeding condition. Furthermore, flies maintained under *in-vitro* feeding had higher mean percentage of survival than flies maintained under live animal (*in-vivo*) feeding. The present study also showed that female had higher survival mean percentage than male flies. According to the present study on infection rate evaluation of the two species, the higher infection rate was found in *G. pallidipes* which was higher in females than males. Therefore in order to strengthen the fight against tsetse and trypanosomosis, further a detailed study on the biology, survival and infection rate of major vectors like *G. pallidipes* should be carried out and assessed. Moreover field adaptability studies before the actual implementation of SIT is mandatory.

6. References

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