HEMATOPHAGOUS FLIES SPECIES DIVERSITY, EFFICIENCY OF TRAPS AND ASSOCAITED PROTOZOAN PATHOGENS IN CATTLE IN ARBA MINCH ZURIA, MIRAB ABAYA AND KUCHA DISTRICTS OF GAMO ZONE

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ABBREVIATIONS

AD -apparent density

CSA- central statstical agency

m.a.s.l. - meter above sea level

NITTCE- National Institute for Tsetse and Trypanosomosis Control and Eradication

PCV –packed cell volume

RF- Riparian forest

SAT- sequential aerial technique

SIT- sterile insect technique

ABSTRACT

Hematophagous flies like *Glossina* species, *Tabanid*, and *Stomoxys* are economically very important parasitic arthropods affecting domestic animals and humans due to their persistent and painful bites, annoyance, blood-feeding behavior and vectors of pathogens. Despite their great importance of *Tabanids* and *Stomoxys* only little information is available about these biting flies in Ethiopia. The little information from Ethiopia on these arthropods was reported by few investigators only at the genus level during their studies on *Glossina* in some areas. The objectives of the present study were to identify hematophagous fly species affecting domestic animals in the study area, to detect associated blood parasites affecting cattle and to compare catching efficiency of NGU, Biconical and Sticky types of traps in three different districts, viz, Arba Minch, Mirab Abaya and Kucha districts found in Gamo zone. For this purpose, a total of 87 traps were deployed at 29 sites by rotating NGU, Biconical, and sticky panel for sampling the flies, and a total of 409 blood samples were collected from cattle. A total of 2985 flies were collected during the study period were morphologically identified into 2536 (85%) genus *Glossina*, 234 (7.8%) *Stomoxys*, and 215 (7.2%) *Tabanid*. The result of the study demonstrated that the genus *Glossina* 85% (2536/2536) comprised the majority and the predominant proportion of the collected flies followed by 234 (7.8%) Stomoxys and 215 (7.2%) Tabanid. The results of fly identification revealed the presence of two tsetse species *G. pallidipes* 79.1% (n= 2005/2536) collected from all the three study districts, however, *G. fuscipes* 21% (n=531/2536) was collected only from Kucha district. A total of 12 species were twelve species identified were; *Ancala. Africana, Atylotus agrestis, Tbanus donaldsoni, T. gratus, T. taeniola variatus, T. taeniatus, T. par, Stomoxys. n. nigra, S. sitiens, S. indica, Glossina pallidipes* and *G. f. fuscipes*. *Haematopota* and *chrysop* were identified in to genus level. Apparent densities (AD) of *Tabanids, Stomoxys, G. f. fuscipes. Haematopota* and *chrysop* were identified in to genus level. Apparent densities (AD) of *Tabanids, Stomoxys, G. f. fuscipes*, and *G. pallidipes* collected from Arba Minch were 1.72, 2.6, 0 and 20.8, respectively. In Mirab Abaya district the apparent densities (AD) of Tabanids, *stomoxys, G. f. fuscipes*, and *G. pallidipes* were 0.18, 1.27, 0 and 14.61 whereas in Kucha district the AD of 0.18, 0.3, 8.85 and 0.08 was recorded for *Tabanid, Stomoxys, G. f.fuscipes*, and *G. pallidipes*, respectively. The overall catch of *G. pallidipes* in Arba Minch was significantly higher than the catch in Mirab Abaya (P= 0.0006) and Kucha (P=0.0000) districts. The overall catch of the sticky trap of all fly types was significantly higher than the biconical trap. The Biconical

trap showed significantly better efficiency (P= < 0.05) than NGU in the catch of *Stomoxys* and *G. f.fuscipes*. But the NGU trap showed better catch efficiency (P= < 0.0002) in *G.pallidipes* and *Tabanids* than the biconical trap. Of 409 blood samples examined by BCT, 15 were positive for Trypanosomes (n=3 *T. vivax,* n=12 *T.congolensie*) and prevalence was 3.4%. The overall mean PCV of cattle positive for trypanosomes was 20.4 which, however, the overall mean PCV of cattle negative for trypanosomes was 28.05.

Keywords: *Tabanid, Stomoxys, Glossina, Cattle, hematophagous flies, traps, blood parasit*

1. INTRODUCTION

Hematophagous flies like *Glossinidae*, *Tabanidae*, and the genus *Stomoxys* are the most important arthropods affecting domestic animals (Lehane, 2005). They are potential vectors of cyclically and mechanically transmitted pathogens including viral, bacterial, protozoal, and nematode diseases in livestock (Bitome Essono et al., 2015; Mullens, 2019). Hematophagous flies are also a serious nuisance to livestock and result in significant production losses (Dougherty et al., 1993). Biting flies are well known for their interrupted feeding which is considered as the key factor for efficient transmission of pathogens to new hosts (Kelly-hope et al., 2012; Moeti O. Taioe et al., 2017). Suitable temperature, humidity (rainfall), and altitude of an area are among the major factors for the survival and reproduction of hematophagous flies (Hurd, 2010; Langley, 1975).

Hematophagous flies impose both direct and indirect negative impacts on the productivity and health of domestic animals. The magnitude of their attack on domestic animals is determined by their host preference behavior. For instance, different Glossina species prefer different hosts on which to feed (Clausen et al., 1998). Stomoxys mainly feed on cattle followed by horses (Warnes and Finlayson, 1987) whereas Tabanid species mainly prefer horses, cattle, and pigs (Muzari et al., 2010).

Tsetse flies infest about 10 million km2 of 37 sub-Saharan countries in Africa (PATTEC, 2001) of which about 220,000 km2 of the land in Ethiopia is infested with five species of tsetse flies (Bekele et al., 2010). Based on climates, vegetation, and fauna characteristics of ecology, tsetse flies are classified into three groups as Savannah (Morsitans group), riverine (Papalisgroup), and forest (Fusca group). Woodland, Savannah, and rainforest types of agroecology provide suitable habitat for tsetse flies but there is no single type of vegetation suitable for all species of flies (Hordofa and Haile, 2017). In Ethiopia Glossina species are comparatively the most studied flies than the other types of hematophagous flies. Five species of *Glossina (G. morsitans submorsitans, G. pallidipes, G. tachinoides, G. fuscipes fuscipes,* and *G. longipennis)* have been recorded in Ethiopia but only four are widespread and of significant economic importance. Tsetse transmitted species of trypanosomes like *Trypanosoma congolense, T. vivax,* and *T. brucei* are the most important, in terms of economic loss in domestic livestock. The closely related *T. brucei* subspecies, *T. b.*

rhodesiense causes human sleeping sickness. In the regions infested with tsetse flies, chronic trypanosomosis causes a severe reduction in animal productivity reflected in poor growth, low milk yields, reduced capacity as work animals, and infertility. The annual loss in meat production alone due to trypanosomosis is valued at about US$4.75 billion (FAO, 1988). The reduced capacity for work animals is also a very important factor where 80% of the traction power in African agriculture is provided by animals.

Tabanids are robust medium-to-large (6–30 mm) biting flies commonly referred to as horse or deer flies (Service, 2012). They belong to the family Tabanidae which is further divided into four subfamilies, namely Chrysopsinae, Pangoniinae, Scepsidinae, and Tabaninae, comprising of more than 4400 species belonging to 114 genera, with a cosmopolitan distribution. However, in Africa, only members from the subfamilies Chrysopsinae, Tabaninae, and various species of the genus Philoliche from the subfamily Pangoniinae, are of economic, medical and veterinary importance (Baldacchino., *et al* 2014). Although there are many accounts of the Tabanidae of in other parts of the world, they are mostly fragmentary and cursory, particularly for Ethiopia and, as a result, the tabanid fauna of Ethiopia is poorly known. The genera of the family Tabanids reported to occur in Ethiopia are Tabanus, *Chrysops, Atylotus*, and *Haematopota* (Cherenet et al., 2004). Each of these genera has adapted to a certain locality for breeding and resting. Tabanid have different ecological habitats where they are numerous or scarce. Their catch is high around the watery area or wet habitat however, their number is few in the forest area (Sinshaw et al., 2006).

Tabanid flies are implicated to play a great role in the mechanical transmission of various pathogens of medical and veterinary importance. The mechanical model of transmission may either occur through contamination of mouthparts or regurgitation (Baldacchino., *et al* 2013). Defecation is also significant as pathogens can be ingested and deposited on food or other surfaces, however, if the pathogens do not multiply within the alimentary canal of the insect then this is defined as mechanical transmission (Foil and Gorham, 2000). Tabanid flies are vectors of most disease-causing bacteria and viruses in animals and humans such as Bacillus anthracis, *Listeria monocytogenes, Anaplasma marginale, Coxiella burnetii,* and rinderpest virus (Esterhuizen, 2006). Protozoan parasites, including the apicomplexan Besnoitia besnoiti and various trypanosome species (*Trypanosoma brucei brucei, Trypanosoma congolense, Trypanosoma evansi, Trypanosoma theileri* and *Trypanosoma vivax*), have also been reported to be amongst the haemoparasites disease agents transmitted by *tabanid* flies (Baldacchino et

al. 2014). These pathogens are mechanically transmitted to susceptible hosts during interrupted feeding by the flies. As a result, *tabanid* flies cause major economic losses to both agriculture and dairy production sectors. There is a knowledge gap in the recent abundance and species composition of *tabanid* flies that occur in Ethiopia. Additionally, there is no data on the occurrence of protozoan parasites in *tabanid* flies from Ethiopia. As a result, in the current study, we report on the characterization of *tabanid* flies in Ethiopia. Furthermore, this study has conducted to investigate protozoan parasites in the blood of cattle associated with *tabanid* flies.

Stable flies (*Stomoxys* spp.) recently surpassed horn flies (Haematobia spp.) as the most important arthropod pest of cattle production. Both males and females are blood-sucking flies that attack domestic, wild animals, and sometimes human beings across the world. They feed on blood from a wide range of hosts including mammals like rats, rabbits, monkeys, cattle, horses, and humans. The Stomoxys flies are as large as musca domestica and they are also called the biting house flies because of their morphology, color, and their living habitat associated with humans and livestock. About 18 species of the genus Stomoxys have already been reported, but only *S. calcitrans* is cosmopolitan. (other 17 are tropical species: 12 of them can be found in the Ethiopian region (Africa and neighboring Islands), namely, *S. n. niger, S. varipes, S. ochrosoma, S. inornatus, S. boueti, S. transvittatus, S. pallidus, S. luteolus, S. xanthomelas, S. omega, S. stigma,* and *S. taeniatus* (Dawit et al., 2012a; Duguma et al., 2015; Sinshaw et al., 2006). Little is known about *Stomoxys* spp. of Ethiopia. Species of the genus Stomoxys reported to occur in Ethiopia include *Stomoxys calcitrans, S.niger S. sitiens, , S. taeniatus, , S. pallida* and *S. inornatus*.

The presence of wild and domestic fauna, savanna or forest, and the nature of the vegetation cover of the ground make a variation in the catch of Stomoxys (Duvallet and Pont, 2008). Stomoxys rest in sunny sites when the air temperature is below about 20°C and in shady sites when the temperature exceeds about 30°C. Large numbers of stable flies can appear on beaches 10 or more miles from the nearest likely breeding sites (Mullen and Durden, 2019). Stomoxys spp. population peaks under relatively wet conditions with moderate temperatures (daily maxima under 30°C). Precipitation and temperature have been reported to affect Stomoxys activity (Lendzele et al., 2019). Several studies have indicated wetter or cooler than normal weather as being conducive to higher population levels.

Stable flies (Stomoxys spp.) are implicated to cause notable economic losses and several studies have attempted to estimate their economic impact on cattle production. When under the mass attack of the stable fly, significant economic losses due to the reduction of anticipated gross weight gain and a 30-40 % decrease in milk yields have been observed (Hall et al., 1982; Mullens et al., 1988). Campbell et al. (2001) reported weigh gains by grazing cattle were reduced an average of 0.20 kg per steer per day by an average of 2.79 flies per leg, representing a 19 % reduction in weight gain or approximately 7% per stable fly. Besides, stable flies have been known as a mechanical vector for several pathogens including *Anaplasma marginale* (anaplasmosis), Trypanosoma spp. (trypanosomosis) as well as different viruses, including bovine leucosis virus, lumpy skin disease virus (Mihok et al., 1995).

Despite the extensive presence of hematophagous flies in different parts of Ethiopia and huge negative impacts on domestic animals, there is very little information on these very important insects in Ethiopia. Furthermore, the available information is fragmented in scope, coverage, and quality, and is neither comprehensive nor well organized. In addition to this, NGU, biconical and sticky panel traps are widely used in Ethiopia for Glossina species survey (Abebe et al., 2017; Feldmann et al., 2005; Sinshaw et al., 2006; Zeleke, 2011) but their fly catch efficiency was not compared. To provide baseline data to develop an effective control of hematophagous flies in cattle and other domestic animals, it is necessary to determine as much as possible their biology, ecology, and species diversity of an area. Therefore, the present study was designed to investigate hematophagous flies of economic importance in domestic animals in 3 districts of Gamo zone in southern Ethiopia with main objectives:

To investigate the occurrence and species diversity of hematophagous flies in the 3 districts of the Gamo zone in southern Ethiopia.

To determine the occurrence of pathogens affecting cattle associated with hematophagous flies in the study areas.

To compare hematophagous fly catch efficiency of biconical, sticky and NGU traps in the study areas.

2. LITERATURE REVIEW

2.1. Classification of hematophagous flies

The order Diptera is divided into three suborders. The Suborder Cyclorrhapha (contains Gillosinidae and Muscidae) and Suborder Brachycera (containsTabanids) are the most important suborder containing major hematophagous flies of veterinary importance (Lehane, 2005; Radostits *et al*., 2006).

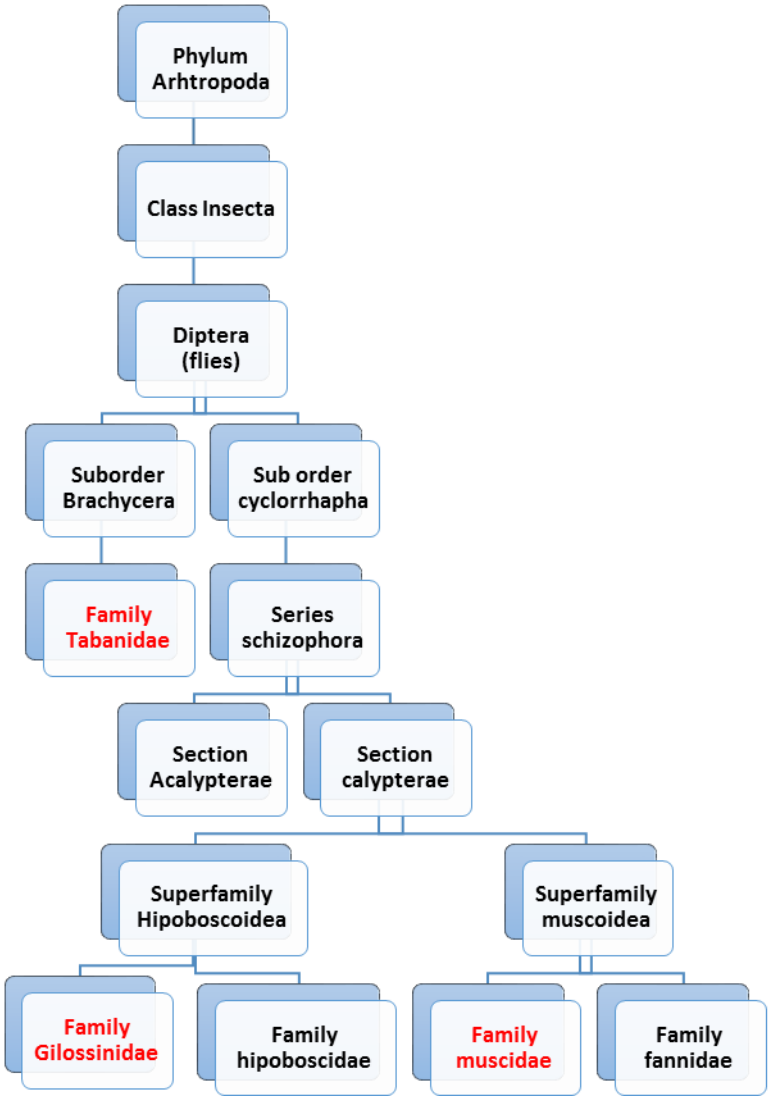

Figure 1: Fly classification

Source: Wall and Shearer (1997) pp. 151

2.2. Morphological description of haematophagous flies

Collection or sampling of any fly species is a prerequisite for identification (Table 1). Flies are collected using traps, which is made up of locally available blue and black fabrics. The color of tsetse collecting trap is important: blue attracts tsetse flies while black stimulates them to land (Reginald De Deken, 2007). There are different types of traps such as Biconical trap, NGU trap, sticky pannel traps, Vavoua trap, F3 trap, Epsilon trap, Pyramidal trap, Nzi trap and Tetra trap (Baldacchino *et al*., 2014).

For tsetse fly control programs, NGU trap is the better choice and pyramidal and biconical traps also used (Malele *et al*., 2007). The biconical trap with its later improvements has since been widely used against *Glossina* species in several countries particularly in West and Central Africa (WHO, 2004). In Ethiopia, National Institute for Tsetse fly and Trypanosomosis Control and Eradication widely uses NGU, Biconical and Sticky traps for tsetse survey and monitoring activities. The Nzi trap is a multipurpose trap which is very efficient in the catch of *Tabanid*, *Stomoxys*, and tsetse flies (Mihok, 2002). Vavoua traps are highly efficient for Stomoxyinae (Mihok, S., Kang'ethe, E.K., Kamau, 1995).

Using odour bait, such as CO_2, acetone, and aged mammalian urine, is often recommended in order to increase the efficiency of traps (WHO, 2004). These odour baits have a capacity to attract flies from a far distance to the site where traps are deployed (Green, 1986; Mihok, S., Kang'ethe, E.K., Kamau, 1995). The choice of attractant bait depends upon the type of fly species to be collected. For example, aged horse urine is effective for tabanids whereas, acetone and animal urine are effective for tsetse flies, but not effective for *Stomoxys*. However, octanol compounds are recommended attractant for *stomoxys* (Baldacchino *et al*., 2014).

Table 1. morphological identification *glossina*, *tabanid* and *stomoxys*

Flies	Identification keys for genus/species level	References
Tabanid	Antennal length and segments, wing vein, wing transparency, wing color, bands of eyes, eye colour, leg colour, abdominal pattern,	(Krcmar and Hackenberger, Davorka K. Hackenberger, 2011; Claire M. Mugasa *et al*., 2018; Snyder, 1955, 1954; Moeti O. Taioe *et al*., 2017)
Stomoxys	thoracic pattern, wings venation, dorso-abdominal pattern, legs, proboscis, Body colour, arista, molecular characterization	(Couri *et al*., 2012; Grzywacz *et al.*, 2017; Masmeatathip *et al*., 2006a; Singh and Achint, 2017)
Glossina	habitat, proboscis, leg colour, wing venation, size, posterior end of the abdomen, hypopygium (superior and inferior claspers),	(Leak *et al.*, 2008; Orji *et al.*, 2015; Pollock, 1992)

Molecular method is used for identification *Stomoxys* due to the fact that morphological identification of Stomoxyinae flies is difficult, and there is a risk of misidentification (T Changbunjong *et al*., 2016; Claire M Mugasa *et al*., 2018).

2.3. Life cycle of hematophagous flies

2.3.1. Glossina

The mating of *Glossina* probably takes place near to or on host animals (Fig. 2). Male flies settle on the back of the female, and the claspers at the posterior end of the male abdomen grip the female abdomen. Mating for more than 60 minutes is required to trigger the generation of hormones in the female that stimulate ovulation (Lehane, 2005). Female *Glossina* is mated before or after the 1stblood meal. Female *Glossina* mate only once in her life but sometimes may mate more than once; male *Glossina* can mate several times and older are better able to mate than young ones. The sperm is put there in a spermatophore and sperms travel their way up from the spermatophore into the spermathecal ducts and, then

spermathecae. The females use to fertilize each egg with one sperm from the storage, the spermathecae, for the rest of life.

The egg is fertilized in the uterus by sperm from the spermathecae the fertilized egg remains lay in the uterus for about four days, while the development of the first instars larva takes place inside. The 1st, 2nd, and 3rd instar larva development take place in *Glossina* uterus and the fully grown larva is dropped by the female fly (Leak, 1999).

Figure 2: *Glossina* mating, depositing larva and pupa
Source: Setegn, (2010)

The fully grown larva has a pair of large black swellings at the posterior end. These are the polypneustic lobes, which carry many small holes through which the larva breathes. The polypneustic lobes are at first white, becoming black later (Kebede *et al*., 2015; M.A. Taylor, R.L. Coop, 2016). All the food for the three stages of larva comes from the milk gland of the mother fly. The milky secretion of this gland is poured out of the duct of the gland at the head end of the larva. The larva sucks up this secretion and passes it straight to the midgut (Leak, 1999). When discharged the larva, it burrows into the ground and becomes barrel-shaped, darkens and then be called a pupa. There is no feeding by the larva after it is dropped by the female.

The pupal stage usually lasts about four to five weeks before it emerges as an adult fly.in suitable temperature and humidity. From the time of emergence of the fly to the time to take its first blood meal, the young fly is called a teneral fly. At suitable condition female fly produce a mature larva every 9–10 days, except for the first one which may take 18–20 days from the time of emergence of the fly from the puparium (Setegn, 2010).

2.3.2. Tabanids

Tabanid mate in flight especially in the morning and mating in the laboratory has never been induced, which is a great barrier of colony development in the laboratory (Baldacchino *et al*., 2014). Their eggs are deposited in layers on vegetation or other objects hanging over the water. After oviposition, the female will deposit a waterproof secretion that covers the eggs to prevent desiccation and parasitizim by wasps. Egg hatching occurs more quickly when relative humidity and temperatures are high (Mullen and Durden, 2019). Once the larvae have dropped into the water after hatching, they burrow in the river substrate or the ground along the river bank. The larval stage usually lasts from one to three years, depending on the species to complete the 6 to 13 stages of development (known as 'instars') that are typical in the *Tabanid* life cycle (Fig. 3). The larva of tabanids feeds on organic debris and small invertebrates in a variety of aquatic to semi-aquatic habitats (Mullen and Durden, 2019; Sinshaw *et al*., 2006). Most species of horse and deer flies have only one generation per year (Gillott, 2005).

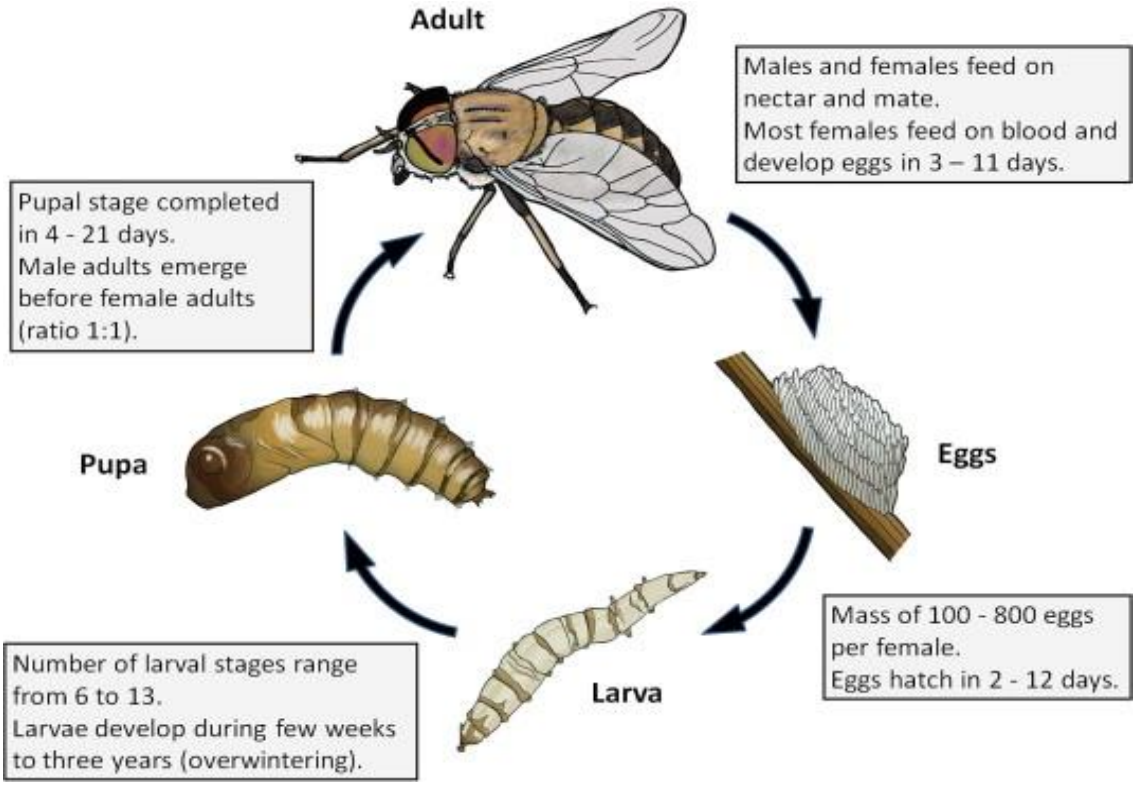

Figure 3. Life cycle of Tabanid flies

Source: Baldacchino *et al (2014)*

Larvae prey on aquatic and semi-aquatic invertebrates and they are also cannibalistic (Capinera, 2008; Gillott, 2005; Mullen and Durden, 2019). Adult females seek blood meal

especially large mammals after mating but the males do not feed on blood ((Muzari *et al*., 2010). Most temperate species have one generation per year (univoltine) but some tropical species have two or three generations per year (bi- or tri-voltine). Large temperate species may spend two or three years as larvae. The longevity of adults is about two or 3 weeks; this is very short relative to the larval stage.

2.3.3. Stomoxys

The female lays batches of 25-50 yellowish-white eggs in manure and moist decaying vegetable matter, such as hay and straw contaminated with urine. Each female fly may lay up to 800 eggs in her lifetime, with each batch requiring a separate blood meal. The complete life cycle from egg to adult fly may take 12–60 days depending mainly on temperature. After emergence, the adult females require several blood meals before the ovaries mature and egg-laying can start (Kaufman and Weeks, 2019; Taylor *et al*., 2016).

Figure 4. Eggs, larvae, pupae, and adult of *Stomoxys calcitrans*
Source: Kaufman and Weeks (2019)

Eggs are small, approximately 1 mm in length, white, and sausage-shaped. They are smooth and curved on one side and straight with a longitudinal groove on the other side. A larva grows from the translucent first instar of about 1.25 mm to 11-12 mm third instar larva that is pale yellow to creamy-white with a mouth hook and two posterior spiracles. The third instar larval skin hardens to form a puparium that is reddish-brown and capsule-like. The larva then

forms a pupa inside the puparium. The puparium is 4.5-6 mm in length and wider at the head end (Fig. 4).

2.4. Host location of hematophagus flies

Glossina, *Tabanid* and *Stomoxys* locate their host for blood meal by the vision and olfactory stimuli (Baldacchino *et al*., 2014; Gatehouse and Lewis, 1973). The vision and olfactory stimuli principle are exploited in the design of traps which mimic key features of host animals, attracting the *Glossina* in such a way that they are then captured or killed. In the case of *Glossina* search for blood meals or resting places partly or wholly by sight, and are attracted by large objects that move or contrast with the landscape (Leak, 1999; Reginald De Deken, 2007). Nevertheless, the host or objects on which they rest may vary depending upon the group of tsetse. For instance, the savannah tsetse mainly feeds on large animals, while the riverine type feeds primarily on reptiles and ungulates (Tikubet and Gemetchu, 1984). Stable flies feed mainly on large ungulates such as cattle and horses (Warnes and Finlayson, 1987) whereas Tabanid species mainly prefer horses followed by cattle and pigs.

2.5. Seasonal abundance of hematophagus flies

Each of the countries in Africa has unique climatic conditions and temperatures which vary greatly throughout the continent. Generally, counties like Ethiopia have two seasons, wet and dry ((Fazzini *et al*., 2015)). The activity of biting flies during the wet season is increased (Table 2) (Oku *et al*., 2011; Sinshaw *et al*., 2006). Seasonal trend of abundance in the *Glossina*, Tabanids, and *Stomoxys* are linked with the seasonal epidemiology and occurrence of vector-borne diseases (Cherenet *et al*., 2004; Sinshaw *et al*., 2006)

Table 2. Seasonality of hematophaus flies

Fly species / genera	Fly catch or apparent density		Place	Reference
	dry	wet		
Glossina	8.3 (AD)	5 (AD)	South Sudan	(Cherenet *et al.*, 2004)
G. morsitans	Sig. Low	Sig. High	Southwest Eth.	(Negash *et al*., 2004)
Tabanid	Low	High	Brazil	(Kirstein *et al*., 2013)
S. calcitrans	Sign. Low	Sign. High	New Zealand	(Heath, 2002)
G. morsitans	Low	peak	Cameroon	((Sevidzem *et al*., 2015)
G. tachnoides	peak	Low	Cameroon	
Tabanid, Stomoxys	low	peak		
Stomoxys	Low	Peak	Central Eth.	((Dawit *et al.*, 2012b)
Tabanus, Hematopota Chrysops, Stomoxys spp.	Low	High	Amhara region	(Sinshaw *et al.*, 2006)

Note: sign – significantly

During the dry season, the savanna tsetse concentrate near denser vegetation along drainage lines to have the humid condition, while during the rainy season they spread out in the wooded savanna. During the dry season, animals travel long distances to reach watering points and grassland but during the rainy season cattle are walking shorter distances but flies are more dispersed and contact them (Reginald De Deken, 2007). Tsetse catch during the dry season is higher than in the wet season (Lukaw *et al*., 2016; Zekarias *et al*., 2014). Vegetation coverage and humidity are very important particularly for developing pupae and resting adults (Hordofa and Haile, 2017; Rogers *et al.,* 2016). *Tabanids* and *Stomoxys* flies are abundant during the rainy season while their population decline during the dry season. The seasonality of hematophagous flies is shown on Table 2.

2.6. Distribution and their present status in Ethiopia

2.6.1. Glossina

In Africa the tsetse fly belt covers about 10 million km^2 and it is spread over 37 sub-Saharan countries and comprises over thirty species and sub-species of tsetse flies that inhabit different habitats or ecological zones (PATTEC, 2001). Tsetse flies are found only in a belt of African countries extending from southern Sahara (Lat.15°N) in the north to Zimbabwe and Mozambique in the south (Lat.20-30°S). This region provides favorable biotic and abiotic environmental factors for their breeding activities. The most important factors that affect the distribution of tsetse flies are temperature, rainfall, and vegetation type.

The range of tsetse flies does not extend into areas with very high or low temperatures. Their geographical range is limited by the excessive drought conditions in the North, the cold temperature in the South and by high altitude regions (Maudlin, 2006; Rogers and Hendrickx, 1994). The average annual temperature ranging between 20°C and 25°C is the optimal temperature for their survival tsetse fly (Radostits *et al.*, 2006). The mortality rate is very high at temperatures exceeding 30° to 32°C (Hordofa and Haile, 2017; Leak, 1999). Favorable humidity and temperature of tsetse flies in the rearing center is 75-80 % and 23-24°C, respectively (Assefa, 2012). Tsetse passes most of their time at rest in shaded areas around lower woody parts of vegetation and most of them hide in the trunks holes and between roots.

Tsetse flies are closely related to the vegetation that protects them from solar radiation and wind. The type of land that are suitable habitat for tsetse flies are woodland, savannah and rainforest. However, the type of vegetation requirement varies depending up on the species of flies (Hordofa and Haile, 2017). Based on their habitat tsetse flies are divided into three groups as *morsitans, palpalis*, and f*usca* group, found in savannah, riverine and forest areas respectively (IRLI, 2011).

In Ethiopia, the savannah type *Glossina pallidipes* and *Glossina submorsitans*, the riverine type *G. fuscipes fuscepes* and *G. tachnoides*, and the forest type *G. longipennis are* present (Aksoy *et al*., 2003; Hordofa and Haile, 2017) but *longipannis* has no recent published information in Ethiopia. Tsetse flies are confined to the longitude between 33°E and 38°E longitude and latitude of 5° and 12°N in Ethiopia (Meyer *et al*., 2016). The area coverage of tsetse in Ethiopia is about 1.1 million km^2 with 220,000 km^2 particularly in the western and

southern lowlands of fertile areas under threat of tsetse-transmitted trypanosomosis. Particularly western and southern lowlands are infested and preventing agricultural activities (Duguma *et al*., 2015).

2.6.2. Tabanid

Tabanid species are divided into 30 genera, which have about 4000 species, seasonally present in all kinds of landscapes, latitudes, and altitudes (Baldacchino *et al*., 2014). Three genera of major veterinary importance of the Tabanid include; genus *Tabanus*, *Haematopota*, and *Chrysops* (Radostits *et al*., 2006). They occur in temperate, subtropica land tropical locations, but *Haematopota* is absent from Australia and South America (Wall and Shearer, 1997). Tabanids mostly occur in warm areas with suitable moist locations for breeding, but also occupy a wide range of habitats from deserts to alpine meadows.

Suitable breeding sites for *Tabanid* flies are aquatic, semi-aquatic and wet ground habitats such as damp areas around lakes, ponds, streams, shallow lakes, and along the water's edge (Mullen and Durden, 2019). *Tabanus*, *Haematopota* and *Chrysops* are reported to be found in different parts of Ethiopia (Lelisa *et al.*, 2014; Sinshaw *et al*., 2006; Tafese *et al*., 2012; Zeleke, 2011). They are found from sea level to at least 3,300m or more (Middlekauff and Lane, 2015). Tabanids can be separated into three morpho-ecological groups: (i) rheophilous and subrheophilous species found in streams, (ii) hydrobionts and hemihydrobionts inhabiting slow-moving or stagnant water bodies, or littoral areas (banks), (iii) and edaphobionts living in drier soil such as under forest litter, usually far from water bodies.

The available information indicates that (Table 3) recent reports from Ethiopia showed that major hematophagous flies affecting domestic animals are *Glossina, Tabanids,* and *Stomoxys*. Information on distribution of these flies in Ethiopia usually available alongside tsetse data in which the major objective is to conduct study on tsetse flies (Abebe *et al*., 2017b; Cherenet *et al*., 2004; Kitila *et al*., 2017; Sinshaw *et al.*, 2006). However, information obtained from tsetse infested and non-infested areas showed that these flies are widely distributed in several locations in Ethiopia. Tabanid species reported from Amhara region, Ethiopia include *Atylotus agrestis, Chrysops streptobalia, Haematopota lasiops,* and *Tabanus atrimanus*. Reports from other regions of Ethiopia are available only at genus level.

2.6.3. Stomoxys

There are 18 known species in the genus *Stomoxys,* of which 17 of them are distributed in the tropical region of the world. *Stomoxys calcitrans* is the only species that is present worldwide. Their breeding is continuous in tropical and subtropical climates. Suitable temperature and humidity are important their breeding. When the temperature is below about 20°C the fly rest in a sunny site and when temperatures exceed about 30°C they rest in shady sites. Suitable moisture levels are usually 30% to 75%. In temperate regions, the species is thought to overwinter as immatures wherever larval substrates do not freeze (Mullen and Durden, 2019). Sinshaw *et al.* (2006) reported 7 species *Stomoxys* from Amhara region including *S. calcitrans, S. niger, S. pulla, S. sitiens, S. uruma, S. pal* and *S. pallida* and also Dawit *et al* (2012) reported *S. calcitrans, S. n. niger, S. sitiens, S. taeniata, S. inornatus* and *S. ochrosoma* from Oromia regional State in central Ethiopia. However, Duvallet and Pont (2008) questioned the validity of the species of *Stomoxys* previously reported by Sinshaw *et al.* (2006) and suggested the presence of misidentification by the authors.

Table 3. Distribution of Glossina, Tabanids, Stomoxys in Ethiopia

Zone	Distr	*Stomoxys*	*Tabanid*	*Glossina*	Reference
South omo		St	Tab	G.p	(Senait *et al.*, 2016)
W. Wollega	Gimb	St	Tab	G.m.m, G.t	(Batu *et al.*, 2017)
Ilu Aba Bora	Dari mu	St	Tab	G.m.m, G.p	(Habte, 2015)
Upper Didessa valley		St	Tab, Ha	G.m.m	(Desta, 2018)
Amhara region (Lake Tana)	S.c, S.n, S.p, S.s, S.u, S.pal		A.a, C.s, H.l		(Sinshaw *et al.*, 2006)
W & E Gojjam, Awi		S.c	C.s, T.a,	G.m.m, G.t	(Cherenet*et al., 2006)*
Wolaita		St	Tab	G.p	(Gona *et al.*, 2016)
Omo-Gibe		St	Tab	G.f.f, G.p	(Abebe *et al.*, 2017)
Dawurro		St	Tab	G.f.f, G.p	(Barata and Eshetu, 2017)
STEP area		St	Tab	G.p	(Zekarias *et al.*, 2014)
Benshangul	Mandura	St	Tab, Ha		(Lelisa *et al.*, 2015)
South Ethiopia	Amaro	St	Tab, Ha	G.p	(Desta, Beyene and Haile, 2013)
Ilu Aba Bora	Yayo	St	Tab, Ha	G.f.f G.p	(Kitila *et al.*, 2017)
South western Ethiopia				G.f.f, G.m.m, G.t, G.p	(Duguma *et al.*, 2015)
Central Ethiopia	(Dawit *et al.*, 2012)				
Ethiopia	S.c, S.n, S.v, S.t				
Southern and central Eth., Tigray, E.wollega, Gondar, Bahir dar, North shewa					(Mulugeta *et al.*, 2010; Amuamuta *et al.*, 2012; Gemeda *et al.*, 2014; Teshome, 2016; Shibeshi *et al.*, 2018)

Notes: A.a: Atylotus agrestis ,**T.a:**Tabanus atrimanus, **C.s:**Chrysops streptobalia, **H.l**: Haematopota lasiops, **H.m**: Haematopota maculosifacies, **S.c**: Stomoxys calcitrans, **S.n**: S. nigra, **S.p**: S. pulla, **S.pal**: S. pallida, **S.s**: S. sitiens, **S.t**: S. taeniata, **S.u**: S. uruma, **S.i**: S. inornatus, **S.o**: S. ochrosoma, **Tab**: Tabanus, **St**: stomoxys, **Ha**: Haematopota, **G.m.m**: G. m. sub morsitans, **G.p**: G. pallidipes, **G.t**: G. tachnoides, **G.f.f**: G. f. fuscipes , **M.o**: Melophagous ovinus

2.7. Pathogen Transmission

Once the flies have landed on a host, they must probe the skin to find a blood source. Probing might be interrupted if no blood vessel is pierced, or if the host defends the vector, or if the blood flow dries up. The persistent insect will then attempt to feed again by probing in a different place or by moving to a new host. The vectored parasite will be advantaged if the vector needs several attempts to obtain blood meals (H. Hurd, 2010; Langley, 1975). The two main ways of pathogen transmission are mechanical transmission without developmental change of the pathogen in the arthropods and biological transmission in which the pathogen must undergo some type of biological development in the body of the vector arthropod to complete its life cycle (M.A. Taylor, R.L. Coop, 2016). *Tabanids* have been described as the mechanical vectors of over 35 pathogenic agents of livestock (Foil and Hogsette, 1994).

Stomoxys and *Tabanids* attack their hosts several times to get sufficient blood meal to produce their eggs. This activity makes them one of the highly efficient mechanical vectors of several blood borne pathogens (Lehane, 2005; Mullen and Durden, 2019). In the absence of tsetse flies, Trypanosomes can be transmitted mechanically by *Stomoxinae* and *Tabanid* flies (Mihok *et al*., 1995).

Tsetse flies include all the species in the genus *Glossina* and are important vectors of human and animal African trypanosomosis (Rogers and Hendrickx, 1994). Since they are obligate parasites that live by feeding only on the blood of animals, they suck blood infected with the *Trypanosoma* species and transmit this disease to previously uninfected animals (Reginald De Deken, 2007). Infections are present as Duttonella, Nannomonas, and Trypanozoon (or as *T. vivax*, *T. congolense* and *T. brucei*. The sites of development of trypanosomes in tsetse: *T.vivax*in *the* proboscis, *T. congolencei* in midgut and proboscis and *T. brucei*in *the* midgut and salivary glands (Cecchi *et al*., 2015).

Table 4. Pathogens identified from hematophagus *flies*

Fly type	Pathogens identified	Country	Reference
Tananid (Chrysops)	Filarial nematodes such as *Loa Loa, Dirofilaria repens*(*biological transmission)*	Democratic Republic of Congo(DRC)	(Kelly-hope *et al.*, 2012)
Ancala,, Atylotus, Haematopota,, Philoliche, Tabanus	*T. congolense, T. theileri, T, vivax*	South Africa	(Desquesnes and Lamine, 2003; Moeti O. Taioe *et al*., 2017)
Tabanids	Besnoitia species, *B. bigemina, T. parva, T. evansi*	Zambia	(Taioe *et al.*, 2017)
H. pluvialis, T.distinguendusT. bromius	*Anaplasma phagocytophilum*	Poland	(Werszko *et al*., 2019)
Stomoxys	T. b. rhodesiense, T. b. brucei	southern Sudan	(Mohammed *et al.*, 2010)
Stomoxys	Rift valley fever (mechanical vect)	USA	(Turell *et al*., 2010)

2.7. Detection of pathogens in hematophagous flies and affected animals

Infection rate is the number of vectors infected over numbers examined. Collection of infected flies to identify potential vectors of diseases of vertebrates is one of the primary aims to undertake the infection rate of potential vectors. The microscopic examination of dissected flies (dissection method) remains the most used method to detect trypanosomes and other pathogenic parasites inside their vector flies (Abdi *et al.,* 2017). Molecular detection of the dissected proboscis, med-gut, and salivary gland is also possible. The infection rate of *Glossina* with trypanosomes is usually low, ranging from 1 to 20% of the flies, each is infected for life and the infection rate is determined by the parasite, the vector, the host and the environment (Krafsur, 2009; Taylor *et al*., 2016). The number is commonly expressed per 1,000 vectors tested, because of the typically low positivity rate (Mullens, 2019).

In the case of tsetse flies, trypanosome infections were identified by using a compound microscope at a magnification of ×40 objective, using the methods of parasites found in the midgut, salivary glands, and mouthparts were regarded as *Trypanozoon; T. brucei*-type (Abbelle *et al.,* 1999) those located in the mouthparts and midguts were *Nanomonas; T. congolense*-type (RISTIC and McINTYRE, 1981), while those found in the mouthparts only was put in the group of *Duttonella*; *T.vivax*-type infection, immature infections, when only the midgut was found infected. In Ethiopia, the infection rate of trypanosome in *Glossina* species is about 7% (Desta, 2018; Desta *et al*., 2013). Tabanids and Stomoxys are potential vectors

for trypanosomes infection and this can be indicated in the laboratory (Mihok *et al*., 1995; SUMBA *et al*., 1998).

Babesia, Theileria, Trypanoma and *Microfilaria* are also detected morphologically from stable flies by Giemsa stain (Fig. 5-11). The developmental stages of *Babesia* in vector are sporogony and gametogony which have different forms such as sporokines, zygote, gamete, and gamont (Ristic, 2018). *Babesia* identified from fly gut is annular, irregular and pear shaped (Fig 11). Multiple *Theileria* species are seen within hemolytic erythrocytes in midgut of the flies. The *Microfilaria* in midgut of stable fly is slender, undulation motile, with long pointed tail, measuring 218 - 300µ in length, 5.6 – 6.9 µ in width, milky or colorless (don't stained with Giemsa stain), surrounded by aggregation of RBCs and WBCs (Hadi and Al-Amery, 2012).

Trypanosome, Babesia, Theileria, Trypanoma and *Microfilaria* detected from blood by preparing thin smear by fixing methanol and flooding with Giemsa working solution. The body length and width, the distance from posterior end of the body to kinetoplast and the shape of the posterior end of the body were given more weight for species identification of trypanosome parasites. For instance, *T. congolosie* identified by absence of free flagellum, marginal kinetoplast, and pointed posterior end and *T. Vivax* identified by presence of free flagellum, large and terminal kinetoplast and rounded posterior end (Wanger *et al*., 1992). Microscopic examination of babesia by giemsa stained smear is often more problematic due to low circulating parasitaemia for limited periods of time (Wanger *et al*., 1992). Smears stained Giemsa also used to detect Theileria parasites based on their morphological features.

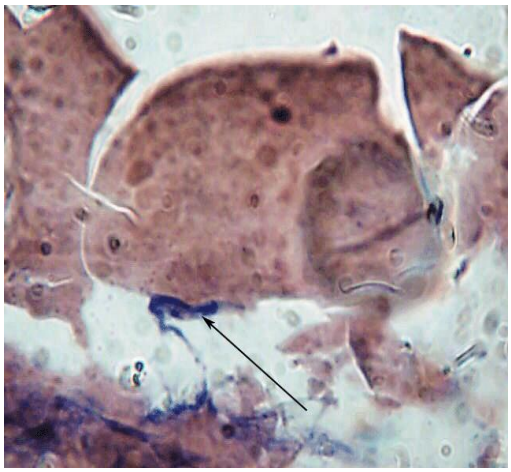

Figure 5. Trypanosoma spp. In stable fly

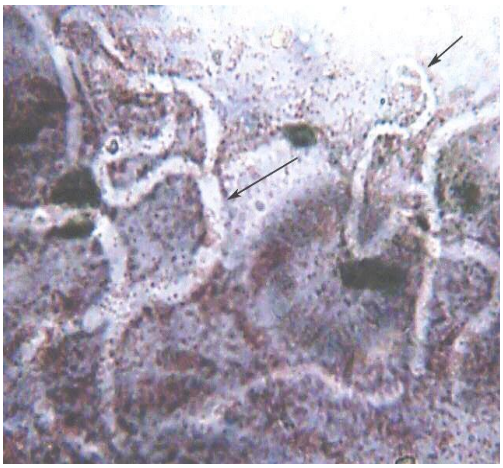

Figure 6. Microfilaria in stable fly

Source: Hadi and Al-Amery (2012)

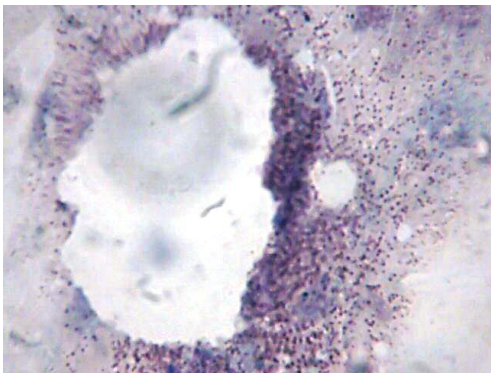

Figure 7. Multiple Theileria spp. hemolytic RBC in stable fly

Source: Hadi and Al-Amery (2012

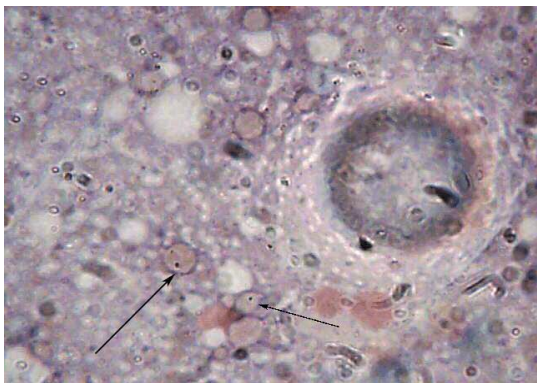

Figure 8. Ring form of *Theileria sp* in stable fly

Source: Hadi and Al-Amery (2012)

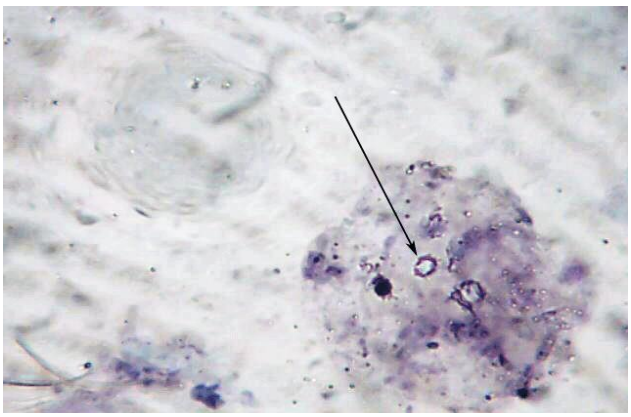

Figure 9. Annular Form of *Babesia* Sp. in Stable Fly

Source: Hadi and Al-Amery (2012)

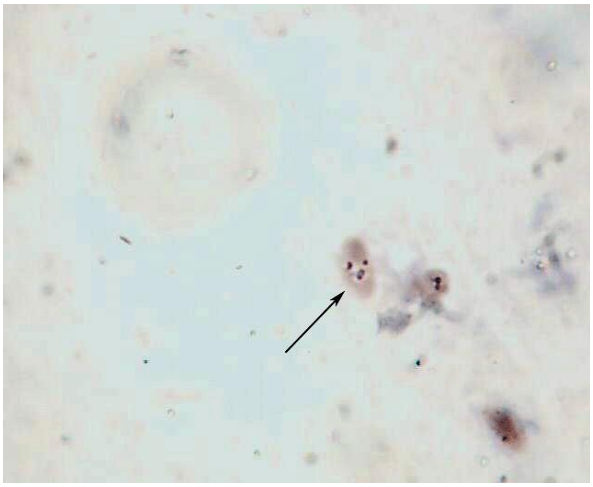

Figure 10. Irregular form of *Babesia* sp. in stable fly

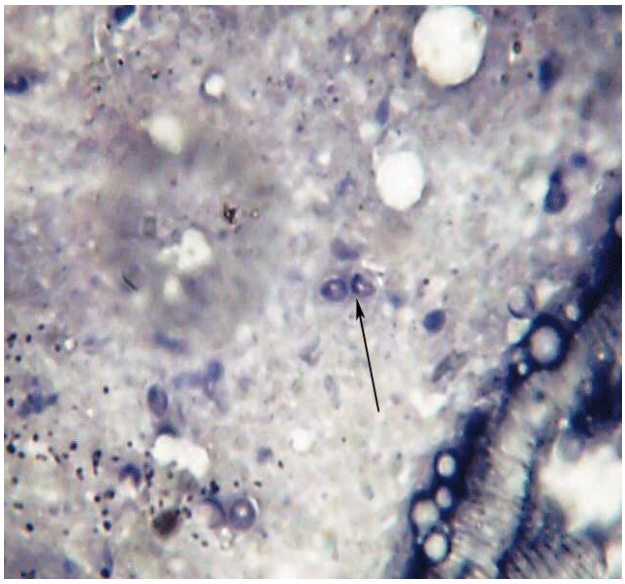

Figure 11. Pear – shaped of *Babesia* sp. in stable fly

Source: Hadi and Al-Amery (2012

2.8. Economic impact of hematophagus flies

Tabanid and *Stomoxys* have a great economic impact on livestock production and human health due to pathogen transmission and nuisance whereas tsetse is the main cyclical and mechanical vector for livestock and humans. *Hippoboscids* are also vectors for pathogens and they attack the host directly by sucking blood and leading loss of condition and anemia. In addition to this, *Hippoboscids* such as *Melophagus ovinus* leads to pruritus and rubbing, wool loss (Wall and Shearer, 1997).

Tsetse-transmitted animal trypanosomosis is one of the most significant and costly diseases in the country where tsetse flies are highly distributed. Cost of tsetse control and treatment wastes a huge amount of money. Generally, tsetse transmitted trypanosomiasis reduces livestock productivity by 20 to 40 percent, which results in $4.5 billion losses each year in Africa. The health and economic implications of trypanosomiasis thus make the tsetse fly a critical socioeconomic threat to sub-Saharan Africa (McCord *et al*., 2013). The pathogen status of haematophagous flies is not studied in Ethiopia. But they are an important vector for pathogens such as trypanosomes in different parts of the country.

2.8.1. Fly annoyance

Tabanid flies are a serious nuisance to livestock and even moderate numbers of flies feeding on livestock can result in significant production losses. The annoyance level of tabanids is closely related to fly abundance; large numbers of bites can induce stress and reduce the quantity and quality of food intake, thus lowers the yields of milk, meat, and manure production in cattle. An average of 66–90 horse flies per animal per day resulted in a weight loss of 0.08–0.10 kg per animal per day (Foil and Hogsette, 1994). Riding activity is impossible during the tabanid season due to the violent reaction of horses to Tabanid bites.

Stable flies irritate and annoy cattle and may cause foot-stomping, throwing of the head toward the front legs, ear movement, skin twitching, tail swishing, and wound licking (Dougherty *et al*., 1993) (Fig. 12). In addition to this, they induce the gathering of animals for mutual protection; meanwhile, they favor the development of pathogens in the hosts and their transmission. This results in a reduction of the time spent feeding and the total feed intake. In the USA the estimated annual cost of *S.calcitrans* control was US$9.80 per head for the cattle industry. When the causes of weight loss were evaluated, it was found that approximately 72% was due to bunching of animals and the resulting heat stress, and the remaining 28% was due to actual stable fly feeding and the energy used to fight the flies. Although more limited in distribution than *S. calcitrans, S. niger* is a serious and often underrated pest of livestock, particularly cattle, in Africa (Foil and Hogsette, 1994).

Figure 12. Holstein cattle bunching in response to attack by flies or fly worry

Source: Photograph by Roger D. Moon Gary R. and Lance A., (2002).

2.9. Control

2.9.1. Glossina control

In Ethiopia, a total area infested by tsetse flies is estimated at about 21.7% of the area of the country. Ethiopian Government and non-governmental organizations struggle to control tsetse by different methods (African Development Bank, 2013; Hordofa and Haile, 2017) (Table5).

Table 5. Tsetse control techniques used in Ethiopia

Tsetse control techniques	Spraying in insecticides on the environment	Ground spray
		Aerial spray (SAT)
	bait technology	Insecticides treated cattle (Deltamethrin 1%)
		Insecticides impregnated target and trap (Deltamethrin 0.4%)
	reduce the birth rate	SIT

Collar repellents from un-preferred hosts or ‘cows in waterbuck clothing’ method are practiced in Kenya, argued to have some potential method for preventing the contact between host and vector at tsetse infested area (Saini *et al*., 2017) (Fig. 13).

Figure 13. Collar repellent method for tsetse control
Source: Saini *et al* (2017)

2.9.2. Tabanid and Stomoxys control

Tabanid control is difficult to achieve. Currently, there are no effective long-term method to control tabanids. Control of tabanids has to be based on protective measures against adults rather than larvae; effective reduction of populations remains a challenge due to multiple factors associated with the life cycle (Foil and Hogsette, 1994). These factors include (i) female adults spend only a few minutes feeding on a host to obtain enough blood to produce eggs (Mullen and Durden, 2019), (ii) they can feed on domestic hosts, but also on wild hosts which are sufficient blood sources to maintain populations, and (iii) they lay their eggs in a wide variety of sites and the larvae are ubiquitous throughout the environment (Baldacchino *et al*., 2014; Capinera, 2008).
Use of insecticides for control of larvae or pupae, which are typically inaccessible in soil, is generally ineffective and can result in environmental damage but the use of baited trap with better attractants (CO_2) provides another potential control technique (Shapiro and Patricia, 2010).

Methods suggested for integrated Tabanid control strategies are i) Selective animal grazing in large open areas, well away from wooded habitats and/or at high elevation levels ii) Livestock enclosures and smoke repellent iii) applying insecticide on livestock iv) Repellents which is

detected by female tabanids olfactory stimuli but no long-lasting products are readily-available to test now, v) use of attractants such as Carbon dioxide (CO2), octenol and phenols, aged mammalian urine, insecticide-impregnated blue-black targets can be used in control trap (Baldacchino *et al*., 2014).

Another control technique is against nuisance and biting flies on cattle kept under zero-grazing system (Fig. 14), which practiced in Tanzania. Using an insecticide incorporated screen or ZeroFly Screen to control *Glossina*, stomoxynae, Tabanidae, and muscinae have indicated effective result in a field experiment (Nagagi *et al*., 2017).

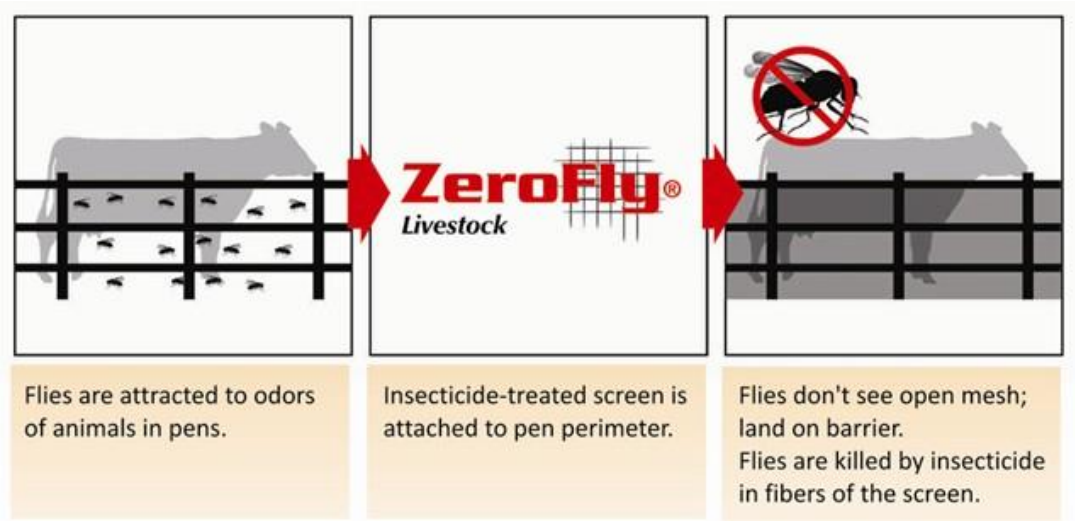

Figure 14. Zero grazing method

Source: https://slideplayer.com/slide/3992791/

Control techniques of *Stomoxys* include disrupting their breeding site and applying pesticides around it, using traps, insecticide-impregnated ear tags, applying parasitoid wasps attacking pupae and applying insecticides on cattle. Efficacy of the entomopathogenic fungi, Metarhiziumanisopliae, in the control of infestation by stable flies *Stomoxys calcitrans* under natural infestation conditions, shows effectiveness to control the natural infestation in Mexico (Cruz-vazquez *et al.*, 2015). Tsetse traps, such as Vavoua trap, are effective tools for investigating the biology of *Stomoxys* and have obvious potential for stable fly control.

In Ethiopia, there are no control strategies regarding *Tabanid* and *stomoxys* but chemicals applied onto cattle during a tsetse/trypanosomes control program in Ethiopia resulted in a reduction of tabanids and other biting flies (Leak, 1999).

3. MATERIALS AND METHODS

3.1. Study Area

The study was carried out in 3 districts of Gamo zone situated in SNNP regional state. Gamo zone is located in the south west part of the east African rift valley and contains two rift valley lakes and Nech sar national park. The zone constitutes 14 districts and 4 town administrations including Arba Minch town. The study districts are located between the latitudes of 6.728997 N and 5.677812 S and between the longitudes of 38.413847 E to 36.573365 W. The districts are selected purposely based on lack of information on diversity of hematophagous fly in genus and species level except for *Glossina* and transport accessibility. The fly and blood samples were collected from lowland areas of the districts where the national institute for tsetse and trypanosomosis control and eradication (NITTCE) has worked. According to CSA (Central Statistical Agency, 2017) the estimated livestock population of the zone were; 1, 301, 056 cattle; 476, 329 sheep; 392, 380 goats, 50, 296 horses; 15, 244 mules; 65, 441 donkeys. The study was carried out in Arba Minch Zuria, Mirab Abaya and Kucha districts of Gamo zone situated in SNNP regional state about 510, 463 and 400 km respectively South of Addis Ababa, the capital city of Ethiopia (Fig. 1).

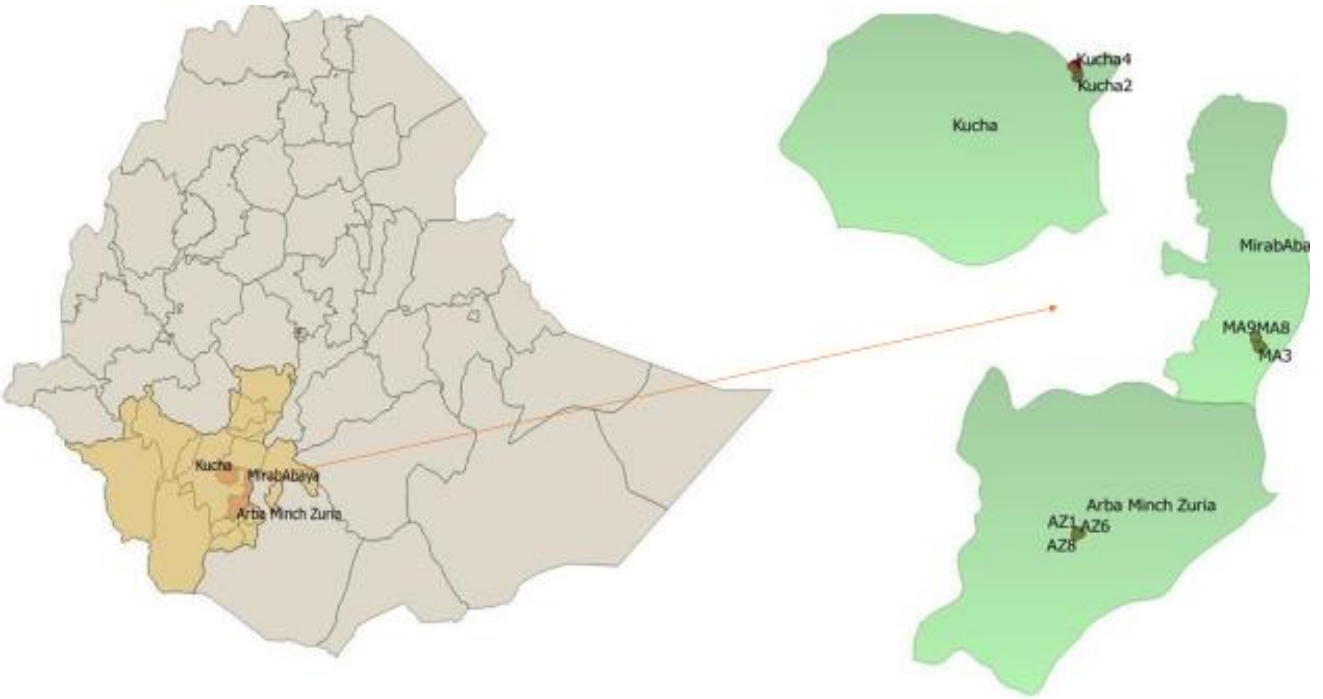

Figure 15. Map of Ethiopia to show the study area

Arba Minch Zuria district, the 1st study area (Fig. 15), is located about 510 km south of Addis Ababa, the capital city of Ethiopia (Fig. 15). The dominant vegetation around grazing land are wooded grass lands in the district (Zeryehun and Abraham, 2012). The altitude of the district lies between 1150 and 3300 masl. Rainfall pattern is bimodal, erratic and unreliable, with the mean annual precipitation ranging from 750 mm– 1300 mm depending on altitude and moisture bearing seasonal air current (Gochera et al 2020**).** The average minimum and maximum temperature of the Arba Minch zuria district is ranging from 17.3°C and 30.6°C. Generally, about 50.8% is lowland, 10.8% is wet/moist highland, and 38.2% is midland. In 2014 the number of livestock existing in the district was 26.987 oxen, 32,056 cows, 30,714 heifers and calves, 35,226 sheep, 20,894 goats, 1871 donkeys, 16,091 horses, and 933 mules.

Mirab Abaya district the 2nd study area, is located about 379 km south of Addis Ababa, and west of Lake Abaya. The district is divided into 24 Kebeles,3 one urban and 23 rural. It has three major agro-ecologies: dega (high land), woina dega (mid-altitude) and kolla (low land). Out of the 24 Kebeles, 16 are in the kolla agro-ecology, six are in the dega agro-ecology and the other two are in the woina dega agro-ecology. The average annual rainfall in the dega and woina dega agro-ecologies is 580mm. In the kola, average annual rainfall ranges from 1,000-1,100mm and the mean temperature is 23^{o}C (Deneke and Habtamu, 2008).. The agro-ecological zones coverage of high land, mid land and low land are 11%, 27% and 62% of the total area respectively of the district (Tefera, 2008).

Kucha district, the 3rd study area and the district center is located 450 kms south of Addis Ababa. The district has sub-humid climate with moderately hot temperature and the mean annual rainfall varies between 1100 mm - 1600 mm whereas the mean monthly temperature ranges between 20°C-25°C. The overall altitude of the Woreda varies between 1000-2250 meters above sea level. Bush land, wooded grass land (WGL) especially along the side of grazing area draining lines and a high gallery forest along the rivers and Shrub land are the dominant vegetation cover. Livestock existing in the district are 120,464 Cattle, 24,404 Sheep, 3,880 Goats, 38 Horses, 1598 Donkeys, 332 Mules and 65,539 Poultry.The district is known for hilly and undulating midland. According to the information obtained from the district Office of Agricultural Development, 20% of the topography is flat whereas 35% is steep to gentle slope (Abayneh and Tadesse, 2019; Getachew and Tadele, 2015). The total area is 1391.90 km which is divided into two traditional agro-climatic zones namely: mid highland

(Gezze) and lowland *(Gara)* accounting for 50.6% and 49.4% of the total area respectively. Most of the area is found in the Omo Gibe drainage basin (Ayza, 2012).

3.2. Data collection and study design

3.2.1. Trapping and fly collection

Fly survey was undertaken by employing cross-sectional study design. A total of 87 NGU, biconical and sticky traps baited with aged cow urine were deployed from December 2019 to March 2020. In Arba Minch zuria, Mirab Abaya and Kucha districts of 27, 30 and 30 traps were deployed respectively at selected sites by rotating NGU, biconical and sticky panel traps at the same Geo-referenced position by GPS (Table 6). The importance of rotating traps was to compare trap efficiency. Traps, which are used in Ethiopia especially by NITTCE (NGU, biconical and sticky traps (Fig. 16&17) were deployed on selected sites at distance of 150 to 200 meters (Vale, 1979; WHO, 2004). Trapping sites were selected to represent the habitats that could be related to fly multiplication, behavior, feeding, and other related aspects. Hence grazing area, watering points, riverbank on the vegetation of riverine forest, wooded grass land and bushlands were included. Materials used to deploy NGU and biconical traps are traps, pole, cone, cage, mesh, plastic band, grease, bottle, aged cow urine and GPS. The traps were deployed by clearings 3-4 m radius to facilitate the visibility of the traps (Leak *et al.*, 2008). Sticky trap covered with transparent plastic was deployed and painted by Temo-O-Cid glue using brush (Fig.16).

Table 6. Descriptions of fly collection sites

Trap ID	District	Latitude	Longitude	Altitude	Vegetatn
AZ1	Arba Minch Zuria (near to lake Chamo)	5.94087	37.53688	1126	WGL
AZ2		5.93827	37.5369	1084	WGL
AZ3		5.93765	37.53673	1117	WGL
AZ3		5.93765	37.53673	1117	WGL
AZ4		5.93749	37.53736	1108	WGL
AZ5		5.93893	37.53965	1107	WGL
AZ6		5.93805	37.54113	1114	RF
AZ8		5.93545	37.5354	1119	RF
AZ9		5.93608	37.53649	1110	WGL
Kucha1	Kucha (around Deme river which is Omo river tributary)	6.60275	37.53683	1279	WGL
Kucha2		6.60637	37.53748	1278	WGL
Kucha3		6.61071	37.53746	1220	WGL
Kucha4		6.61561	37.5358	1180	WGL
Kucha5		6.6185	37.53296	1182	WGL
Kucha6		6.62233	37.53533	1180	RF
Kucha7		6.62142	37.53433	1175	RF
Kucha8		6.6203	37.53335	1172	RF
Kucha9		6.62008	37.53184	1175	RF
Kucha10		6.61924	37.53068	1170	RF
MA1	Mirab Abaya (around Lake Abaya)	6.20957	37.78838	1188	Bush
MA2		6.21209	37.78622	1189	Bush
MA3		6.30865	37.78352	1185	Bush
MA4		6.21048	37.78395	1190	Bush
MA5		6.21429	37.78412	1188	Bush
MA6		6.21932	37.78026	1198	Bush
MA7		6.2219	37.78022	1198	Bush
MA8		6.2235	37.78054	1208	Bush
MA9		6.22451	37.78001	1204	Bush
MA10		6.22618	37.77945	1205	Cul.

Note: coordinates (Map datum - WGS 84, FRMT- hdddd.dddd)

Figure 16. Sticky panel trap used for collection of flies
Photo: Birhanu Haile

Figure 17. NGU and Biconical traps used for collection of flies
(Photo by Birhanu Haile)

Fly samples were collected after every 48 hours of traps deployment and the traps were redeployed by rotating traps. The catchments of each trap in cage were labeled and kept in moist or cooled containers with cage for transportation to working site where the fly samples were identified by using stereo microscope at species. Fly identification was carried out in

Arba Minch Tsetse and Trypanosomosis Control and Eradication centers and Veterinary Parasitology Laboratory of College of Veterinary Medicine and Agriculture (CVMA)of Addis Ababa University.

3.2.2. Morphological identification of haematophagous flies

Morphological identification of haematophagous flies was carried out using identification keys of Claire Mugasa *et al* (2018), Pollck (1992), Taioe *et al* (2017) and Zumpt (1973 under microscope and the taxonomic keys of identification used for fly is provided as follows:

Ancala africana Gray, 1922

This fly is 17 mm in length and the eyes were greenish without bands in living specimens and black on dried specimens (Fig.19A and B). The callus was dark brown and had a quadrate shape. The facial area was yellow covered with golden hairs. The antennae were blackish in pigmentation. It had an orange yellowish thorax with no patterns. The dorsum of abdomen was orange with black hair patches on the margins of all tergites ventrally and the last segment was completely black with patches of white markings. The wing membrane had broad transverse brown band shading crossing the distal cell but not reaching the hind margin and extends to the tips of the wing veins R2+3 and R4 (Moeti O Taioe *et al.*, 2017).

Atylotus agrestis Wiedemann, 1828

It was identified by characteristics described by Moeti O. Taioe *et al.* (2017) and Veer (2002). The adult fly was 13–15 mm long. There was a single purple band on its eye in dried specimen and reddish black in living specimen (Fig. 20). Eye colour in live had shining dark spots. The frons was grey and yellow towards the vertex. The upper and lower calli were circular and shining brown in colour. The scutellum was uniformly brownish in colour. The abdomen was blackish with two orange sublateral stripes and yellow ground colour (Fig. 20).

Tabanus donaldsoni Carter, 1912

Head wider than thorax. Eyes dark green in live but turn black after storage. Scape with white hair at base with black hair anteriorly. Small pedicel with short white hair all over and black hair only at anterior border. Thorax blackish brown with white and black hair. Median stripe

not seen, sub-lateral stripes light brown and indistinct (only seen up to middle of thorax). Wing clear and R4 on the wing has prominent distinct appendix (Fig. 21). Golden-brown dorsal and ventral surfaces of abdomen without patterns but have black and white recumbent hair.

Tabanus gratus Loew, 1858

The specimen was identified by characteristics described by Moeti O. Taioe *et al* (2018) and Taioe *et al* (2017). The body length of this specimen was about 12 mm. Head was wider than thorax and eyes were green with three crimson bands in both living and dry specimens (Fig. 22). Basal callus dark brown, large and as wide as frons, extending upwards in slender projection connecting with smaller upper tear-drop shaped callus or oval. Scape large, light brown with white hair except at tip of the segment with black hair. Pedicel small and brown, more or less covered (obscured) between scape and flagellum, black hair at anterior end. The first two segments of the antennae were whitish and the rest orange. The thorax had dark brown fine hairs with white median and sublateral stripes. The dorsum of the abdomen was dark brown with three clearly defined white stripes. The median stripe was narrow on the second segment, becoming broadest on the hind margin of the forth segment and narrowing again towards the posterior. The wings were clear with yellow veins.

Tabanus taeniatus Macquart, 1834

The specimen was identified by morphological characteristics described by Moeti O. Taioe *et al* (2017). The eyes were green violet without bands on living specimens but turned brownish black on dried specimens (Fig. 23). The frons was honey-brown with black hairs and yellow hairs towards the margins of the eyes. The upper and lower calli were irregular and black in colour, and not connected. The proboscis was blackish with orange yellowish palpi. The thorax had ashy black hairs and a faint narrow brown median line. The scutellum was blackish brown with patches of grey on the sides. The dorsum of the abdomen was ashy black with three whitish grey longitudinal stripes. The two sublateral stripes ended on the seventh tergite which was completely greyish. The hairs were black on the dark areas of the abdomen and whitish yellow on the grey stripes. The wings were clear with pale yellow veins.

Tabanus taeniola variatus Oldroyd, 1954

The sample was identified by morphological characteristics described by Mugasa *et al* (2018). Head was wide and posterior vertex slightly concave. Eyes black without bands, separated by greyish frons with white hair and black hair posteriorly. Bell-shaped callus; dark brown basal callus joined to upper callus by narrow constriction (Fig. 24). Antennae with two shades of brown; scape is light brown with white hair at base and black hair at anterior. Anterior-most point of scapes has brownish orange colour. Pedicel also light brown, small, appears engulfed between scape and flagellum segment with black hair anteriorly that appear as thorn-like projection. Flagellum light brown at base up to projection, but darkens anteriorly to dark brown; annulations black. Second segment of palpus dull white with white hair ventrally, dorsally few black hairs mixed among the white. Thorax greyish brown with long whitish grey hair mixed with black hair. Median stripe distinctly whitish grey reaching posterior thorax. Sublateral stripes greyish white and less distinct, but nevertheless reach the posterior thorax. Dark brown scutellum with recumbent grey hair, especially at margins. Tufts of white hair at postalar callus wing base. Halteres whitish to golden yellow. Wing clear and R4 without appendix. Middle leg same as hind leg. Distal third (1/3) of fore tibia of foreleg is black with black hair; the rest of tibia light brown with white hair interspersed with few black hairs. Brownish yellow hind tibia with white hair interspersed with few black hair, hind femur ashy grey with long white hair. Tarsus light brown with black hair (Suppl. material 2: Figure S5E) and each leg has two tarsal claws.

Abdomen golden brown with black and white hair, with dorso-medial black band on first two segments, broad on first segment and narrower on second. Band does not completely traverse second segment. Segments with distinct triangular pattern medially light brown anteriorly and fades into greyish brown posteriorly. Triangular patterns have black hair mixed with white hair. Abdomen with black hair laterally; medial abdomen greyish brown with white and black hair. Ventrally brown surface with whitish hair except for last two black segments

Tabanus par Walker, 1858

The specimen was identified by characteristics described by Oldroyd (1954). *Tabanus par* is easily recognized by the complete yellow appearance of the species, only shared by *Tabanus donaldsonii* (18-19 mm) *T. par* is small with a body length of this species between 10-12 mm.

The eyes were green and without bands on living and black dry specimens (Fig. 25). The tomentum of the frons was golden yellow with black hairs. The calli are yellowish to a brown, united into an elongated shape. The thorax was without any patterns and scutellum was black in ground colour. The dorsum of the abdomen was orange without any patterns and clothed with a mixture of black and golden yellow fine hairs. The wings were clear with yellow veins (Mazibuko, 2018; Moeti O. Taioe *et al*., 2017).

Stomoxys

Stomoxys samples were identified to species level using the identification key of Zumpt (1973) by morphological characteristics such as abdominal pattern, wing-venation, head, thorax, legs, arista (Zumpt, 1973).

S. sitiens

Abdomen with sublateral spots of middle segments wider and more oval (Fig. 26). Frons narrower, in male about twice as wide and in female about three times as wide as antenna seen in profile. Thorax and abdomen dense grayish or brownish, not shiny. First posterior cell of wing (*R5*) at apex less than one half as wide as at widest point, media more strongly curved upward and sinuous terminally. Legs dark colored, tibiae more or less extensively yellow with dark-brown or blackish tarsi. Hind femur with relatively short ventral hairs. Antennae dark-brown to blackish, third antennal segment about 3 times as long as the second, arista with long dorsal hairs. Palpi yellow, proboscis dark reddish-brown. Thorax black, with a dense grey and olive pollinosity (any fine dusting over a surface regardless of its color) (Stone, 1938). Mesonotum with four longitudinal black stripes (Crosskey, 1993; Masmeatathip *et al*., 2006; Zumpt, 1973).

S. indica

Abdomen grey to olive-brown with distinct spots or transverse stretch bands Fig. 26). Width of frons at the narrowest point ¼ or less of eye length. First posterior cell of wing (*R5*) at apex one half as wide as at widest point with slightly curved upward media. Legs yellow-brown to dark-brown, fore-metatarsus simple, without rows of erect hairs. Third antennal segment light color. Fore metatarsus simple without rows of erect hairs. 1st posterior cell of wing R5 at the

apex ½ as wide as at widest point, media slightly curved upward. Legs with tibia and tarsi yellow brown. Femora mostly darkened but sometimes pale like tibia. Densely grey and olive brown pollinose. Mesonotal and abdominal pattern similar to that of *S. uruma* (Tanasak Changbunjong *et al*., 2016).

S. n. niger

Abdomen with broad transverse bands which however may be reduced to separate spots (Fig. 26). Hairs on mesonotum relatively short and not so dense. Bristles shorter and thicker. Nominate form with a banded abdomen and dark legs of which only the knees are yellow brown and hind femur with short ventral hairs. Abdomen with upper surface of first segment uniformly grey or yellowish grey (not darkened along hind margin); middle segments (T3 and T4) each with a pair of widely separated sublateral brown spots near hind margin. Abdomen with upper surface of first segment margined by obvious black-brown band; middle segments without spots but with whole of hind margins black- brown and shiny (Crosskey, 1993; Zumpt, 1973).

Genus *Chrysops*

Generally, members of this genus are fragile black flies smaller than all the previously described genera. Members have a characteristic black band that transverses the wing from anterior to posterior margin (Fig. 31). The sample was identified by morphological characteristics described by Service (2012). *Chrysops* species are medium-sized flies about 6–12mm long and most of them have iridescent eyes, commonly with spots of red, green or purple. The wings are held partially over the abdomen in an open scissor like fashion, and usually have one or more brownish or black transverse bands from anterior to posterior margin. In many species the abdomen is blackish with orange or yellow patches or bands. The most reliable method of distinguishing Chrysops from Tabanus and Haematopota is by their antennae (Fig. 27). In Chrysops the second segment of the antenna is long and cylindrical and lacks any projection while the third segment has four small subdivisions. The hind tibiae have apical spurs which are absent in Tabanus and Haematopota.

Genus *Haematopota* identification

Genus *Haematopota* (clegs) *are* distinguished by wing colouration (mottled) and the shape of their antennae (Mullen and Durden, 2019). This sample was identified by keys of morphological features described by Service (2012) and Mullen and Durden (2019). Haematopota species are dark grey medium sized flies, which are easily distinguished from Tabanus and Chrysops because wings are dusty grey and dotted pattern or mottled. The eyes have zigzag bands of iridescent colors. Antennae are rather similar to those of Tabanus but are usually a little longer. The third segment is straight, not curved as in Tabanus, has only three, not four, small subdivisions and does not bear a dorsal projection.

Glossina species identification

Morphological identification of *Glossina* was made depending on keys of Pollock (1992) to determine the species, the number of fly catch per trap per day (the apparent density), and the sex of the fly. In general, the adult Glossina are narrow, yellow to dark brown and have a long, rigid and forward projecting proboscis, and the wings are held over the abdomen like a closed pair of scissors when at rest. They are easily distinguished from all other flies by the characteristic hatchet cell in the wing (Leak *et al.*, 2008). The sex identification was done by examining the posterior end of the abdomen. The male fly has a lump or knob like structure on the ventral side of abdomen (hypopygium) at the posterior end but not in females (Mullen and Durden, 2019). The abdomen has seven visible segments and each segment of the back of the abdomen has a harder cuticle forming a plate or tergite, unlike the elastic ventral surface referred to earlier. The colouring and markings of the tergites are useful for species identification.

Glossina pallidipes Austen, 1903

G. pallidipes was identified by isentification key Pollock (1992). Front and middle tarsi uniformly yellowish brown. The free end of the antenna (third antennal segment) projects forwards in G. pallidipes much more than it does in G. morsitans. The hairy covering (antennal fringe) to the front of the third antennal segment is much longer in G. pallidipes than it is in G. morsitans. So that Fringe of long hairs on the antennal 3rd segment, visible with x10 magnification The hairs in the other species are much more difficult to see with a

hand lens. The scutellum carries a pair of bristles (strong hairs) near the center, and these project back over the abdomen. In female G. morsitans and G. swynnertoni a pair of bristles are very short but in female G. longipalpis and G. pallidipes they are about as long as in the male. All tarsal segments of the front leg are pale. On the front leg the tarsal segment before the last is black in G. morsitans and G. swynnertoni. Last two tarsal segments of the hind leg dark colored. Forward projection at end of third antennal segment long and pointed (Pollock, 1992) (Fig.28).

G. fuscipes fuscipes

G. fuscipes fuscipes was identified morphologically by their dark color of most of the tarsal segments of hind leg and by their very narrow pale area across each abdominal segment. In addition to this, *G.fuscipes* is very dark brown, almost black in color. In the female, the median scutellar bristles is long (Pollock, 1992).

3.2.3. Blood sample collection and identification

Systematic random sampling method was applied to collect blood samples while cattle were coming out of their crush in their order. Total of 409 samples blood parasites were collected where the haematophagous flies were collected. Materials for parasitological sample collection are 70% alcohol, cotton, sterile lancet, sealer, heparin coated capillary tubes, microhematocrit centrifuge, microhematocrit reader, ice box, slide, cover slip, methanol, Giemsa working solution, oil immersion and microscope. Blood sample was collected for both BCT and Giemsa stain from marginal ear vein of cattle with two heparinized capillary tubes per animal and the animal information was recorded on prepared sheet. After filling of capillary tubes until its ¾ of its length, one end of capillary tube was sealed by sealer and placed on capillary holder on sealer by matching sample number with sealer number. In addition, thin smears fixed with methanol were stored in slide boxes and transported to the laboratory for staining and examination for possible presence of other haemoparasites.

Then the samples were labeled and placed in ice box and the parasitological process was carried out immediately. Packed cell volume (PCV) was measured by hematocrit reader method and trypanosomes motility were detected by BCT (Buffy Coat Technique). PCV was used to identify anaemic animals i.e. PCV less than 25% (Alemu *et al.,* 2007). Giemsa stain

was used to identify morphology of blood parasites such as *Babesia, Theileria, Anaplasma*, and trypanosomes.

3.3. Data Management and analysis

The flies collected from each trap site and the data of blood sample examined by BCT and Giemsa stain were inserted in to MS Excel spread sheet to create database and transferred to R-software program of the computer to the statistical analysis. Then, to compare fly catch efficiency of each traps and districts Poisson regression were used, and to compare mean catch of t-.test was applied. Flies apparent density analysis was used to calculate an apparent fly catch per trap per day *(*F/T/D) analysis were used.

3.4. Ethical consideration

Approval and ethical clearance was obtained from Research Ethics Review Committee of Addis Ababa University (Ref. No: VM/ERC/24/01/12/2020). The purpose of study was explained to the livestock owners and verbal consent was obtained. Blood sample was taken from the ear vein of the animals and they were not injured severely. The sampling sites were thoroughly disinfected before and after sampling.

4. RESULTS

4.1. Entomological survey

The collected haematophagous flies were morphologically identified to the level of species or genus level as much as possible. Accordingly, a total of 2985 flies were collected during the study period were morphologically identified into 2536 (85%) genus Glossina, 215 (7.2%) *Tabanid* and 234 (7.8%) *Stomoxys* (Table 7). The result of the study demonstrated that the genus Glossina 85% (2536/2536) comprised the majority and the predominant proportion of the collected flies followed by *Tabanid* and 234 (7.8%) *Stomoxys* (Table 7).

The results of fly identification revealed the presence of two tsetse species *G. pallidipes* 79.1% (n= 2005/2536) collected from all the three study districts, however *G. fuscipes* 21% (n=531/2536) was collected only from Kucha district (Fig. 19 and 20). An overall apparent density of 14.6 flies/traps/day was recorded for *Glossina* species (Table 7).

Collected *Tabanids* were morphologically classified into five genera (*Ancala*, *Atylotus* and *Tabanus*) and *Haematopota* and *Chrysops*. During the study period male *Tabanid* were not collected. Few *Tabanids* and *Stomoxys* were not identified into species level because of their distorted structure. An overall apparent density of 1.2 and 1.3 were recorded for *Tabanid* and *Stomoxys* respectively. Haematophagous flies collected from the 3 study districts and identified into species in order of abundance and prevalence are *Glossina pallidipes, G. fuscipes fuscipes, T. taeniola variatus, An. Africana Ancala Africana, Atylotus agrestis, Tabanus donaldsoni, T. gratus, T. taeniola variatus, T. taeniatus, T. par, Haematopota spp, chrysop spp, stomoxys n. nigra, S. sitiens, S. indica,* (Table 7, Fig. 19-28).

Table 7. Overall species and genera of haematophagous flies identified from the 3 districts during the study period

District	Glossina spp.85% (2536/2985)		*Tabanid* spp.7.2% (215/2985)									*Stomoxys* spp. 7.8%(2342985)			
	G.ff	**G. pal**	**T. gratus**	**T.t.v.**	**T. par**	**T.don**	**At. agrestis**	**Chr**	**Haem**	**An. Af**	Un-i-**tabs**	**S. sit**	**S. n.n.**	**S. ind**	**Unid_ stom**
A.M.	**0**	**1123**	0	27	0	0	39	0	2	18	9	**15**	**47**	**26**	52
Kucha	531	5	2	5	0	0	0	0	2	2	0	3	12	0	3
M.A.	0	877	6	15	67	3	1	2	0	7	8	5	36	11	24
Total	531	2005	8	47	67	3	40	2	4	27	17	23	95	37	79
% of catch	17.8	67.2	0.3	1.6	2.2	0.1	1.3	0.1	0.1	1.0	0.4	0.8	3.2	1.2	5.3

Note: A.M – Arba Minch Zuria. M.A- Mirab Abaya, G.ff- G. f.fuscipes, G.pal- G. pallidipes, T.t.v.- Tabanus taeniola variatus, T. don- T. donaldisoni, Chr- Chrysops species, Haem- Haematopota species, An. Af- Ancala Africana, Un-i-tabs- unidentified tabanids, S. sit- Stomoxys sitiens, S.n.n- S. niger niger, S. ind- S. indicus, Unid-stom- not identied stomoxys

Table 8. Haematophagous flies genera captured by the 3 types of traps from the 3 districts

Fly types	**Arba Minch Zuria**			**Mirab Abaya**			**Kucha**			**Catch in %**
	NGU	Sticky	Biconical	NGU	Sticky	Biconical	NGU	Sticky	Biconical	
G. fuscip	0	0	0	0	0	0	45	415	71	531 (17.7)
G. pallidi	517	354	252	334	480	63	2	3	0	2005 (67.2)
Tabanids	48	27	18	22	66	20	4	10	0	215 (7.2)
Stomoxys	14	58	68	8	60	8	2	15	1	234 (7.8)
Total	579	439	338	364	606	91	53	443	72	2985

4.2. Trap comparison

The total catch of NGU trap of *Glossina pallidipes, G. fusc.fuscipes, Tabanids,* and *stomoxys* are 853, 45, 79 and 24 respectively (Table 9). The respective apparent density of 14.7, 0.8, 1.4

and 0.4 for *G. pallidipes, G. fusc. fuscipes, Tabanids,* and *stomoxys* was recorded by using NGU trap. The total number of *G. pallidipes, G. f.fuscipes, Tabanids,* and *stomoxys* captured by sticky trap are 837, 415, 98 and 133 respectively. The apparent density of flies per trap per day using sticky panel trap was 14.4, 7.2, 1.7 and 2.3 for *G. pallidipes, G. fusc. fuscipes, Tabanids,* and *stomoxys* respectively. The biconical trap captured total count of *Glossina pallidipes, G. fusc. fuscipes, Tabanids,* and *stomoxys* are 315, 71, 38 and 77, respectively (Table 9).and the apparent density of the catch of *G. pallidipes, G. fusc. fuscipes, Tabanids,* and *stomoxys* was 5.4, 1.2, 0.7 and 1.3, respectively (Table 8).

Results of the present study revealed catch of sticky trap in all fly type significantly higher than biconical trap and biconical trap showed significantly better efficiency (P= < 0.05) than NGU in catch of *Stomoxys and G. f.fuscipes.* But NGU trap showed better catch efficiency (P= < 0.0002) in *G. pallidipes and Tabanids* than biconical trap. In addition, the catch of *G. pallidipes* by biconical trap in Arba Minch district was higher than its catch in Mirab Abaya (Table 8).

Table 9. Overall haematophagous fly species captured by the 3 types of traps

Trap type	G.f.f	G. pall	T.grat	T.t.v.	T.par	T.don	T.taen	At.agr	Chr	Haem	An. Af	Un-Tab	S.sit	S.n.n.	S.ind	Un-stom
NGU	45	853	0	20	16	**0**	**2**	**18**	**0**	**2**	**19**	**4**	**5**	**15**	1	3
Bicon	71	315	0	11	**11**	2	0	10	0	**0**	**4**	**0**	7	30	14	26
Sticky	415	837	8	16	**40**	**1**	2	12	2	**2**	**8**	9	11	50	22	50
Total	531	2005	8	45	65	**3**	4	40	2	4	**31**	13	23	95	37	79

4.3. Site comparisons

The catch of *G. pallidipes* in Arba Minch Zuria district was significantly higher (1123) than Mirab Abaya (877) and Kucha (P< 0.0006). In Arba Minch Zuria more than 4 species of *Tabanids* were identified and *At. agrestis* were the dominant species followed by *T. taeniola variatus* and *A. Africana.* Low number of genus *Haematopota* was caught in the district.

In Mirab Abaya district more than 7 species of *Tabanids* were identified and the dominant species in the area was *T. par* followed by *T. taeniola variatus* and *A. africana*. There is no

significant difference in catch of *Tabanids* in Arba Minch and Mirab Abaya districts (P= 0.8) but in Kucha its catch was very low compared with Arba Minch and Mirab Abaya districts (P= 0.0000). Mirab Abaya (P= 0.0002) and Kucha (P= 0.0000) districts had low number of *Stomoxys* catch compared to Arba Minch.

The number of haematophagous flies in Kucha district is very low except for *G. f. fuscipes* which was dominant in the district (Table 8) and not found in Mirab Abaya and Arba Minch Zuria. The traps deployed very near to the river caught significantly (P= 0.0000) higher number of *G. f.fuscipes* (n=531) than those traps deployed in wooded grass lands and the traps deployed at the distance of 250m and far from the river line of Deme, the tributary of Omo were were no catch of *G. fuscipes* (Table 8). Catch of *Stomoxys* (n=18), *G. pallidipes* (n=5) and *Tabanid* (n=11) in Kucka was significantly lower than to the catch in Mirab Abaya and Arba Minch zuria but 4 species of *Tabanids* and more than 3 species of *Stomoxys* were identified from the district (Fig. 18).

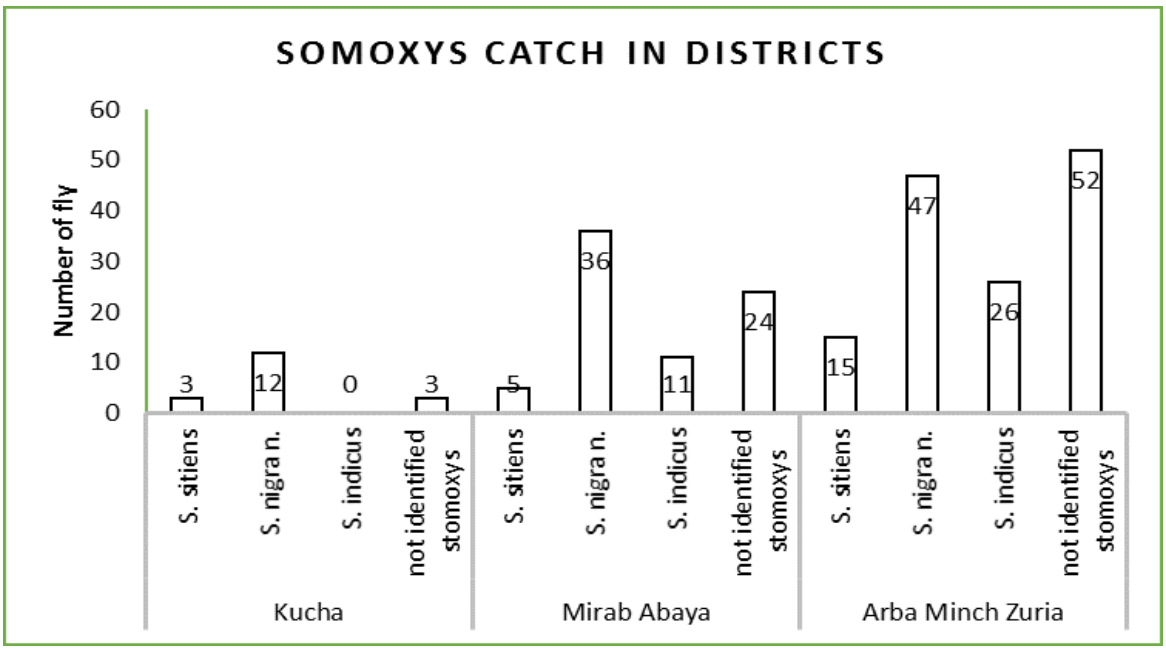

Figure 18. Catch of Stomoxys in each districts

Figure 19. An. Africana

(Photos: Birhanu Haile)

Figure 20. *At. agrestis*

Photos: Birhanu Haile

Figure 21. *T. donaldssoni*

Photos: Birhanu Haile

Figure 22. *T. gratus*

Photos: Birhanu Haile

Figure 23. *T. taeniatus*

(Photos Birhanu Haile)

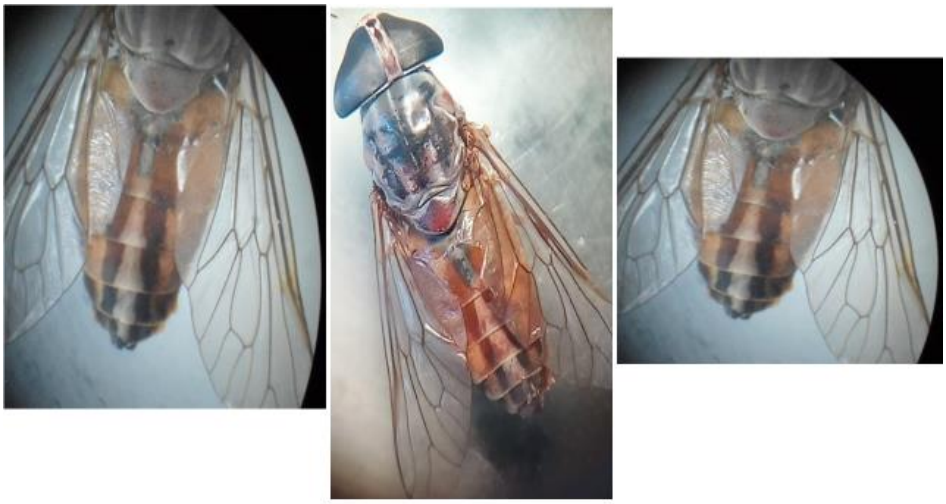

Figure 24. *T. taeniola variatus*

(Photos Birhanu Haile)

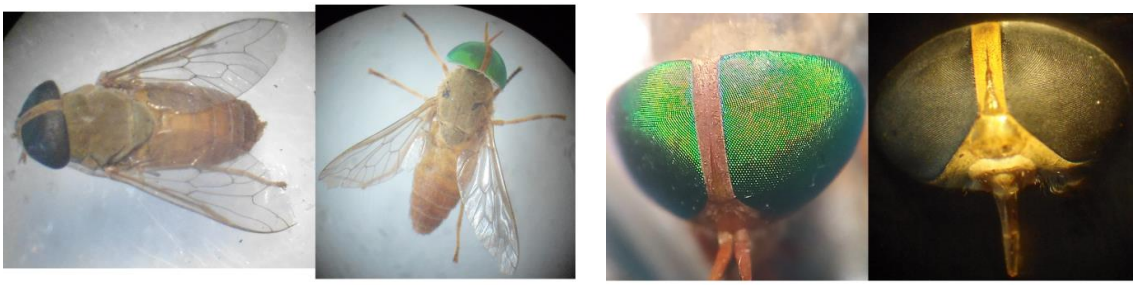

Figure 25. *T. par*

(Photos Birhanu Haile)

Figure 26. *Stomoxys species*

(Photos Birhanu Haile)

Figure 27. *Chrysops species*

(Photos Birhanu Haile)

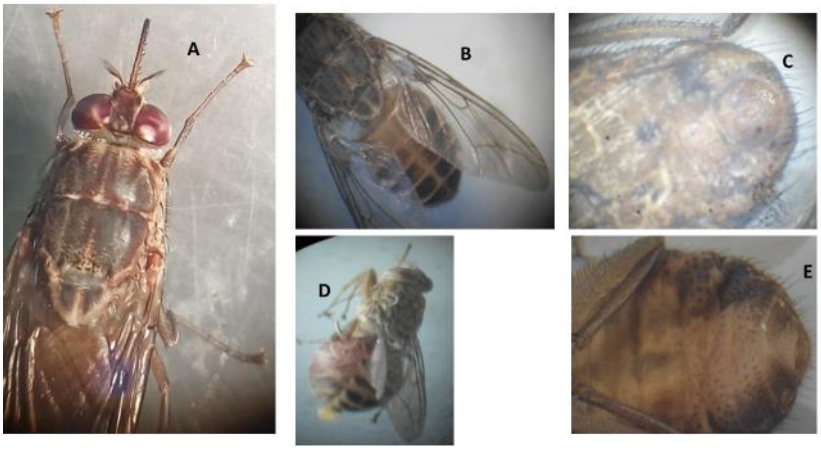

Figure 28. *G. pallidipes*
(Photos Birhanu Haile)

4.4. *Trypanosoma* species and haematological findings

Of the total of 409 blood samples examined by BCT 15 were positive for *trypanosomes* (n=3 *T. vivax,* n=12 *T. congolensie)* and prevalence was 3.4%. Out of the total of 15 samples positive for *Trypanosome* using the BCT, 12 were positive by Giemsa stain examination and other blood parasites were not detected The overall mean PCV of cattle positive for trypanosomes was 20.4 which was categorized as considered anemic as it is less than the cutoff value of 25% PCV value. However, the overall mean PCV of cattle negative for trypanosomes was 28.05 (Table 10).

Table 10. Prevalence of *Trypanosoma* species in cattle in by risk factors in the study area

Risk factors		No of samples	Trypanosomosis						Prevalence
			T. congolense			*T. vivax*			
			No posit.	X^2	p-value	No posit.	X^2	p-value	
BCS	Good	121	2	0.99	0.61	1	1.34	0.51	2.5
	Medium	118	4			0			0
	Poor	170	6			2			4.7
Sex	Male	170	5	0	0.99	1	0.084	0.77	3.5
	Female	239	7			2			3.8
District	Arba Minch	169	3			0	5	0.08	1.8
	Mirab Abaya	86	5			0			5.8
	Kucha	154	4			3			4.5
Age	1-2year	89	1	1.6	0.448	0	2.84	0.24	1.1
	>2-6year	213	8			3			5.2
	>6 year	107	3			0			2.8
PCV	Anaemic	204	11	t=4.19	0.000	2	t=-0.02	0.98	6.9
	Non-anaemic	205	1			1			1
Total		409	12			3			3.7

5. DISCUSSION

Tabanid, and *Stomoxys* are economically very important arthropods affecting domestic and wild animals and humans especially in tropical countries including Ethiopia. The present study is believed to provide additional information on these very important arthropods. During the study period, a total of 2985 hematophagous flies were captured using 3 different types of traps in 3 districts in Mirab Abaya, Arba Minch Zuria, and Kucha districts. The Glossinidae 2536 *(G. pallidipes* 2005 and *G. f.fuscipes* 531) was the dominant family captured and represented 85% of the trapped individuals whereas the Stomoxys and the Tabanidae represent 7.2% and 7.8%, respectively in the 3 districts during the present study. This finding is consistent with previous studies in Ethiopia and other African countries. However, it is very hard to assess whether this apparent heterogeneity reflects the real relative abundance of the different taxonomic groups. Indeed, a possible bias could result from the differential attractiveness of the trap used toward the different groups in interaction with the environmental parameters (for example, light intensity) as has been argued before (Mugasa et al., 2018).

This is also most probably attributed to the fact that these traps were developed and hence more suitable for tsetse flies and it is difficult to get traps specifically developed for biting flies. The low relative frequency *of tabanids* captured in our study could result either from the lower density of this taxonomic group in the biotopes sampled or from the low attractiveness of our traps. Some genus-specific trends are known to be associated with the various traps that have been designed for the capture of hematophagous flies including *tabanids* (Mugasa et al., 2018).

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The observation of *G. pallidipes* 79.1% (n= 2005/2536) in all the three study districts, however, *G. fuscipes* 21% (n=531/2536) was collected only from Kucha district in the present study. This is consistent with the previous reports by Sheferaw *et al* (2016), Zekarias (2014) and Gechere *et a*l (2012) reported that *G. pallidipes* from Southern Rift Valley area of Arba Minch and around lake Abaya *G. f.fuscipes* from Kucha district. This is most probably attributed to the fact that tsetse fly distribution is determined by ecological specialization in many species, some being adapted to forest habitats, whereas other species are more dependent on riverine or savannah habitats. However, most of the Glossina species tend to

avoid disturbed and open areas and show a discontinuous distribution determined by environmental criteria that include abiotic factors (density of the vegetation, rainfall, temperature, and saturation deficit) and host blood meal source.

During the present study, a great attempt was done to identify to species level the collected 215 Tabanids and 234 Stomoxys flies. Accordingly, *Tabanids* were identified into An. *Africana, T. gratus, T.taeniola variatus, T. par, T. donaldsoni, At. agrestis, Chrysops spp,* and *Haematopota* species while the Stomoxys species were identified into *S. sitiens, S. niger niger* and *S. indicus*. Even though there are scanty reference materials and expertise on taxonomic keys for the identification of these flies, in the present study the effort to identify these flies could be considered as the first of its kind in Ethiopia and is believed to provide additional knowledge on Tabanid and stomoxys species. This is because in Ethiopia, though there has not been a specific study on biting flies in alienation from the usual tsetse fly studies; few authors have reported the name of some of the biting flies as Tabanidae and Stomoxyinae at a family level Zekarias (2014), Gechere *et al* (2012), Zeryehun and Abriham (2012). Furthermore, Baldacchino et al. (2014) stated that *tabanids* and stomoxys are the neglected subjects of research but very important vectors of disease agents.

Horseflies belonging to five different genera representing 7 species were recorded in the current study namely: *Ancala, Atylotus, Tabanus*, and *Haematopota* and *Chrysops*. The overall number of members from the genus Tabanus was greater than all other genera combined. This is most probably attributed to the fact that since *tabanids* are strong fliers and readily disperse several kilometers in short-term flights with the unchanged local population as shown previously Mullen and Durden (2019), similar species of *tabanids* may be found in the nearby districts of the study area. This is why the fly species reported by Mugasa *et al* (2018) from East Africa (Kenya, Uganda, and Tanzania) were found in this study area of Ethiopia.

The species assignment of the Stomoxys flies in the present study indicated the occurrence of 4 Stomoxys species namely *S. sitiens, S. niger niger* and *S. indicus* in Ethiopia. This report agrees with Dawit *et a*l (2012) and Sinshaw et al. (2006) who reported different species of Stomoxys of from Amhara and Oromia regions in Ethiopia, even though Duvallet and Pont (2008) questioned the validity of their report as compared to the previous report of Zumpt (1973) which describes the stomoxine biting flies of the world. The occurrence of, S. sitiens,

S. taeniatus and S. niger was reported by Sinshaw et al. (2006) in his research conducted in three districts bordering Lake Tana of Ethiopia. Kigaye and Jiffar (1991) in a survey of ectoparasite of cattle in Harar and Dire Dawa Districts, South-Eastern part of Ethiopia reported the presence of 6 species of stable flies *S. calcitrans, S. sitiens, S. niger, S. varipes, S.bilineata* and *S. brunnipes.* Hence these Stomoxys species were known to circulate and occur in the country imposing severe economic loss. The identification result of our research was not in line with Ahmed et al. (2005) who reported a similar relative abundance of S. niger in his research conducted in Southern Kaduna, Nigeria. However, the present study is not in agreement with some other studies and these differences in the findings between these studies could be attributed to the differences in the epidemiology of the study areas and the differences in the study period. Among the Stomoxys fly species trapped *S. sitiens* was confirmed to exist in Ethiopia and this finding agrees with Masmeatathip et al. (2006) who reports *S. sitiens*, from Ethiopia, Gambia, South Africa, and Egypt.

In the present study sticky panel trap showed high performance in catching of all collected species of haematophagous flies. NGU and Sticky traps were very efficient in catching of *G. pallidipes*. The sticky trap was the most efficient in the catch of G. f.fuscipes, Stomoxys, and *Tabanids* when compared with NGU and biconical. For the *G. f.fuscipes* collection, the biconical trap is better than NGU. The present study is in agreement with that of Malele *et al* (2007) and Tandese and Tsegaye (2010) who reported the NGU trap has high performance over biconical for *G. pallidipes* collection while the biconical trap was more efficient for G. f. fuscipes than NGU. This study showed that sticky panel traps were a more efficient sampling tool for *G. f. fuscipes* than biconical and NGU. Only female *Tabanids* were captured in all our traps and this is the similar observation reported by Egri *et al* (2013) vertically deployed traps catch host-seeking female *Tabanids* and horizontally deployed traps catch water-seeking male and female *Tabanids.* 45.6% of the total catch of *Tabanids* were caught by sticky trap following NGU (36.7%) and biconical traps (17.7%). From the total catch of 234 stomoxys sticky trap caught 133 (56.8%), NGU 24 (10.3%), and biconical 77 (32.9%). Slightly higher catch of *G. Pallidipes* by biconical trap and lower *G. Pallidipes* catch by sticky traps in Arba Minch Zuria and Mirab Abaya districts (P>0.05) (Table 7). This may be due to vegetation differences of deployment sites thus; Arba Minch sites were wooded grasslands and RF whereas Mirab Abaya sites were bushlands.

Several previous reports showed widely hematophagous flies have known vectors and responsible for the transmission of numerous viral, bacterial, and protozoan diseases (Kelly-hope *et al* 2012); Taioe *et al* 2017; Desquesnes and Lamine 2003; Mohammed *et al* 2010). Trypanosomosis is one of the most important diseases vectored by hematophagous flies in Ethiopia (Alemu et al., 2007). In the present study mainly T. congolense and T. vivax were identified as a cause of cattle trypanosomosis that agrees with the reports of several previous studies in Ethiopia who concluded *T. congolense* and *T.vivax* are responsible for severe diseases in cattle, sheep, and goats. T. vivax can establish itself both inside and outside the tsetse belt area whereas *T. congolense* and *T. brucei* are only found in the tsetse belt area. This suggests that both mechanical and cyclical transmission of cattle trypanosomosis occur in the study sites. This is explained by the results of the investigative work done in an attempt to identify the vectors involved in the transmission of cattle trypanosomosis.

In the present study, the overall prevalence of trypanosomes in cattle in Arba Minch Zuria, Mirab Abaya, and Kucha districts was 1.8, 5.8, and 4.5 respectively. This result is in line with the previous report by Shiferaw *et al* (2016) who reported a 5.3% prevalence of trypanosome in Kucha district. It is also in agreement with the previous report by Zekarias *et al* (2014) and Girma *et al* (2014) who reported 1.04 % and 1.56 % prevalence in Arba Minch and Mirab Abaya districts respectively. *T. congolensie* was the more dominant species and distributed in all study districts and its overall prevalence was 3%. There was no statistical variation in the prevalence rate of the disease among study districts ($p > 0.05$).

In this study the overall average PCV of parasitaemic cattle (20.4) is significantly lower than the negative animals 28.05 ($P = 0.000$) the PCV of aparastemic animals are lower than normal (PCV less than 25% are considered as anaemic and PCV ± 25 were considered as normal (Alemu et al., 2007). Even though other diseases such as helminthosis, tick-borne diseases, and nutritional imbalances contribute to the low PCV values, in the present study 80% of positive animals showed low PCV values and 51.8% of negative animals showed PCV level.

6. CONCLUSION AND RECOMMENDATIONS

The current study attempted to identify the major hematophagous fly species and their associated blood parasites affecting health and productivity of cattle and to compare NGU, Sticky and biconical traps, which are normally used by NITTCE for tsetse survey and monitoring activities, for collection of hematophagous flies (*Glossina, Tabanids*, and *stomoxys*) found in the districts of Arba Minch Zuria, Mirab Abaya and Kucha in Gamo Zone. The study revealed that demonstrated the presence of An. Africana, At. agrestis, T. donaldsoni, T. gratus, T. taeniola variatus, T. taeniatus, T. par, Haematopota spp, chrysop spp, *stomoxys n. nigra, S. sitiens, S. indica, Glossina pallidipes,* and *G. fuscipes fuscipes* in the 3 districts. *G. pallidipes* with an overall apparent density of 11.5 fly traps per day was encountered as the most dominant fly in the study districts however, *G. f.fuscipes* was identified only from Kucha District, Deme River, which is Omo River tributary. This is the first study that describes in detail the morphology of diverse hematophagous flies from 3 different districts in Ethiopia. The list of hematophagous flies presented in this MSc thesis is can't be considered as complete given the limited study sites and duration of sampling. Thus, further investigation is required to comprehend the whole fauna of hematophagous flies and their habitat. Wider surveys that integrate climatic factors, pathogens carried by the hematophagous flies, blood meal sources for the flies, as well as the effect of human activity on their distribution are necessary if the diversity, prevalence, and role of hematophagous flies in disease transmission are to be better understood. This will, in turn, enable the instigation of more efficient control measures against these neglected vectors. This study also indicated that trypanosomosis is an important disease that affects the health and productivity of cattle in districts and *T. congolense* is the dominant species followed by *T. vivax.* The findings from the hand-full of sampling sites indicate a need to further investigate neglected insects on a larger scale to establish a concrete hematophagous flies checklist in Ethiopia.

Based on the above conclusion the following remarks are forwarded:

- Appropriate control majors to suppress Glossina, *Tabanids*, and Stomoxys should be designed to solve the existing problem but also considering the mechanical transmission role of *tabanid*s and *stomoxys,* for trypanosomosis eradication is necessary.

- Effective raising of public awareness on hematophagous as mechanical and biological vectors and their effect on productivity and health of animal and control methods should be implemented.
- More detailed and updated descriptions of Tabanid and Stomoxys studies must be required to look into entire hematophagous flies in the study area

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8. ANNEXES

Annex 1: Parasitological data collection sheet

Zone _____ Latitude _____

District _____ Longitude_____

PA _____ Altitude _____

Date _____

Number	Animal_ID	Spp	Breed	Color	Sex	Age	BCS	PCV	BCT_result	Gimsa
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
21										

Annex 2: Entomological data recording sheet

Zone _____ Latitude _____

District _____ Longitude_____

PA _____ Altitude _____

Date _____

S.N	Woreda	Kebele	Trap_type	Lat	Long	Alt	Veg.	spp1	spp2	spp3
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
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22										