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Journal of Parasitology and Vector Biology

Review

A review on epidemiological distribution, impacts and integrated control approach of tsetse fly

Kebede Shanko Hordofa* and Geremew Haile

College of Veterinary Medicine, Haramaya University, Ethiopia.

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Tsetse flies are hematophagous insects of the genus *Glossina* that belong to the family Glossinidae. They are important because of their ability to spread disease among men and among domestic animals. Tsetse flies are strictly blood feeders, and in the act of piercing the skin and sucking blood, the flies transmit blood parasite trypanosomes to previously uninfected animals or man, causing the disease nagana, which is the most important economically devastating disease in tropical countries. The tsetse transmitted trypanosomosis, hinders the effort being made for food self sufficiency. In Ethiopia, about 240,000 km^2 of the land is infested with tsetse flies and the main pathogenic trypanosomiasis that need tsetse as a biological vector are Trypanosoma congolense, Trypanosoma vivax and Trypanosoma brucei. In current Ethiopia, trypanosomosis is one of the most important diseases which contribute to direct and indirect economic losses on livestock productivity, and the extent of the disease prevalence in relation to tsetse fly control. Tsetse fly control has a great impact on economic development in terms of its cost, and some control techniques are ecologically unacceptable. Nowadays, the cheapest and quickest way of controlling trypanosomosis is reducing the number of tsetse fly vectors than treating the infected animals.

Key words: Glossina, insecticide, Nagana, sterile insect techniques, tsetse fly.

INTRODUCTION

Tsetse flies are blood sucking flies of the genus Glossina that belong to the family glossinidae (Radostitis et al., 2007). They occur only in tropical Africa, and they are important as vectors of African trypanosomosis in both animals and man. In Africa, about 10 km^2 of the land is infested by these flies, and their distribution and prevalence are most influenced by spatial factors such as climate, vegetation, rain fall and land utilization (Rogers et al., 1996).

The occurrence and impacts of African trypanosomosis on the other hand, depends on tsetse challenge, host distribution, livestock breeds, farming practice and control practices. Tsetse challenge is determined by the product of relative tsetse density, trypanosome prevalence in tsetse and the proportion of meals obtained by the tsetse from a defined host (Leak, 1999).Tsetse transmitted trypanosomosis (nagana) is one of the most ubiquitous and important constraints to agricultural development in

*Corresponding author. E-mail: Sh.kebeko@yahoo.com.

sub humid and humid zones of Africa. In Ethiopia, a total area infested by tsetse flies is estimated to be 240,000 km^2 (about 21.7% of the territory) located in the Southern, South western, Western and North western parts of the Country where, 14 million heads of cattle, equivalent number of small ruminants, nearly 7 million equines and 1.8 million camels are at risk of contracting trypanosomosis at any given time (Radostitis et al., 2007). Knowing the ecology of tsetse fly where they highly prefer for their survival and their interaction with the parasites that they transmit is crucial in the future designing and implementation of control strategies (Afewerk, 1998; Tewelde, 2001). Efforts have been made by various governmental and non-governmental organizations to control the nagana which is transmitted by the tsetse flies and the most commonly used methods to control these vectors include insecticides, sterile insect techniques, trap and targets and disruption to their ecology (Oloo et al., 2000). Therefore, the objectives of this study are:

(1) To assess some of the integrated control approach of Tsetse fly.
(2) To review Tsetse fly biology, distribution and its economic impact.

REVIEW OF LITERATURE

Biology of tsetse fly morphology

Tsetse flies are narrow bodied, yellow to dark brown and 6 to 13.5 mm long. The thorax has a dull greenish color with inconspicuous spots or stripes. The abdomen is light to dark brown with six segments that are visible from the dorsal aspects (Kahn, 2005). When resting, their wings are held over the back in a scissor like configuration with a characteristics hatched shaped cell in the center of the wings (Itard, 1989). They can be identified by their honey bee like appearance and the long proboscis with its onion shaped bulb at the base, which helps the flies to easily pierce the skin to suck blood. It is held horizontally between long pulps which are of an even thickness throughout. The proboscis is composed of a lower U-shaped labium with rasp like labella terminally and an upper narrow labrum, which together creates a food channel. Within this food channel sits the slender hypophrynx that carries saliva and anticoagulant down into the wound formed during feeding. Each antenna of Glossina has a long arista that is feathered along one edge (Urquhart et al., 1996) (Figure 1).

Life cycle

Both sexes of the tsetse are blood feeders. They depend only on vertebrate bloods for their survival (Wall and Shearer, 1997). One copulation renders a female fly fertile for her life time during which she can produce as many as 12 larvae. The females, in contrast to other muscidae, are viviparous and produce only one larva at a time, up to a total of 8 to12 larvae (Kahn, 2005).

Maturation in the uterus from fertilized egg to the mobile, 8 to 10 mm long, 3rd stage larva deposited by the adult takes approximately 10 days. The larva develops in the uterus over a period of 10 days and then deposited fully grown on moist soil or sand in shaded places, usually under bushes fallen, logs, large stones and buttress roots (Leak, 1999). Larval development is completed in the abdomen of the mother, with all three stages feeding on fluids from special uterine glands. It buries itself immediately and begins pupation within 60 to 90 min. The pupal period is relatively long taking 4 to 5 weeks, or more in cool weather. The adult flies emerge at about 22 to 60 days, depending on the temperature. Breeding generally continuous throughout the year with peak fly numbers occurring at the end of the rainy season (Symith, 1996) (Figure 2).

Epidemiological distribution

General ecology

The tsetse flies are found exclusively on the African continent, between 5°N to 20°S latitudes (Wint and Rogers, 2000). They are closely related to the vegetation which protects them from solar radiation and wind. The eco-climates generally corresponds to that of wood land areas situated in regions receiving more than 1000 mm of rain fall, but may also occur in areas with slightly lower rain fall (Meberate et al., 1999). 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).

The tsetse flies only live in regions where the average annual temperature is above 20°C of which 25°C is the optimum temperature for their survival (Radostitis et al., 2007). Tsetse flies pass most of their time at rest in shaded places in forested areas, and the preferred sites are the lower woody parts of vegetations, many of them hide in holes in the trunks of trees and between roots. They search for food only for very short periods during the day. The flies often rest close to food sources (Taylor et al., 2007). Common risk areas where animals and people are likely to be bitten by tsetse flies are on forest trails near water collection points in forest, and in vegetation close to bathing and water collection sites along the banks of rivers (Leak, 1999).

Along with the macro habitat, it is also important to know which of the microhabitats of tsetse flies are

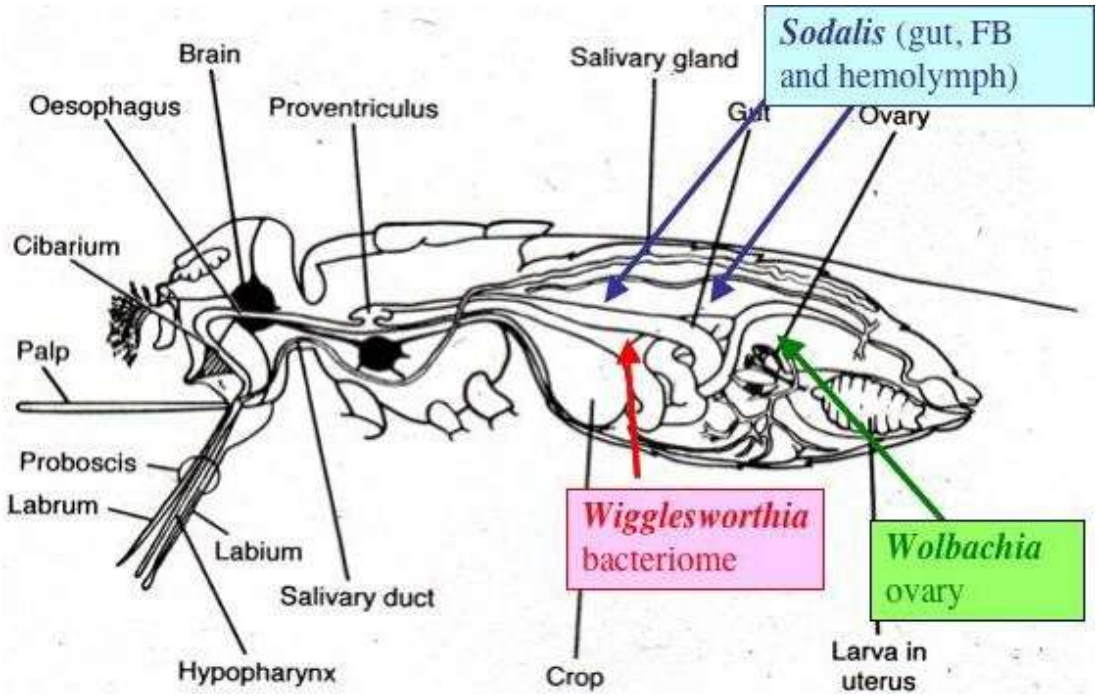

Figure 1. Anatomy and morphology of glossina (Itard, 1989).

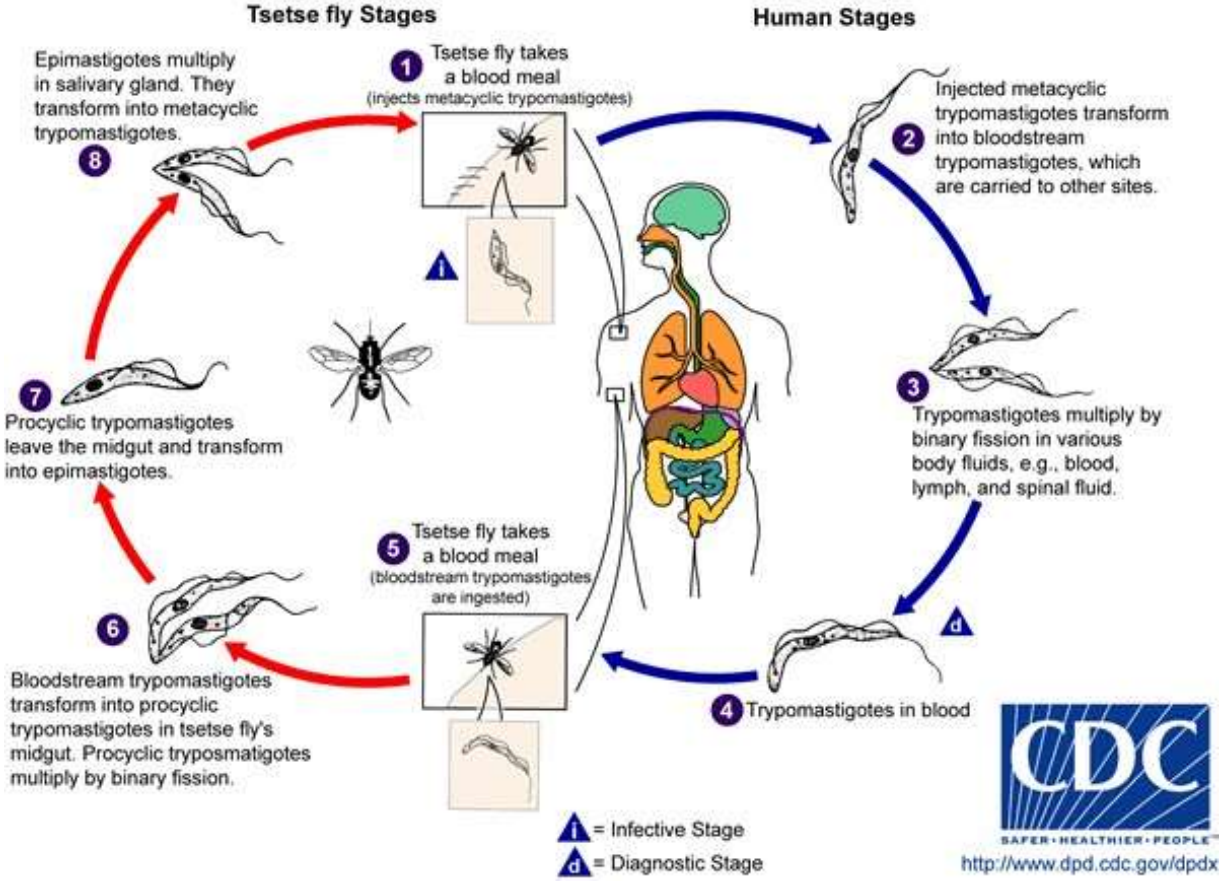

Figure 2. Life cycle of trypanosome species between tsetse fly and human (WHO, 2000).

suitable places for a species that can be depicted at a finer resolution. They can significantly differ from the surrounding areas in many ways, including the climate (Vanden et al., 2000). Suitable microhabitats for tsetse are able to provide cooler or more humid conditions, especially in particularly harsh seasons or times of the day. The fly's behavior can bring it into these places where it can survive better than if it had to suffer the general climatic conditions of the area. Vegetation is affected by temperature and humidity, the two major abiotic determinants of tsetse distribution; trees in particular provide shade for developing pupae and resting sites for adults. The analysis of the vegetation cover has often played a major role in the estimates of the tsetse distribution and in the description of their habitat (Taylor et al., 2007).

Ecological classification

Based on climates, vegetations and fauna characteristics of ecology, tsetse flies are classified into three groups (Wint and Rogers, 2000). They are savanna, riverine and forest type. The savanna tsetse flies known as *Glossina morsitans* (*G. morsitans*) concentrate in the dry season, near the source of water courses, while during the rainy season they spread out in the wooden savanna. These group feeds mainly on large animals (Ford and Katonondo, 1971). They occur mainly in Sudanese savannas with *G. submorsitans* in West and Central Africa and *G. morsitans* in East Africa. Glossina *swynnertoni* and Glossina *pallidipes* are highland species of East Africa, the first being restricted to Kenya and Tanzania, and the second species occurring from Ethiopia to Mozambique and being present in some coastal areas (Smyth, 1995).

The riverine tsetse flies (*G. palpalis*), are widely distributed near the edge of river, where the vegetation is dense, rather than at the edge of the riverine forests. They occupy the forest areas of West and Central Africa, the riverine forest penetrating into the savanna regions. These flies feed primarily on reptiles and ungulates (Tikubet and Gemechu, 1994).

The forest tsetse flies (fusca group) are densely populated where vegetations are found in transition zones between true forest and wooden lands, preferring dense shade and riverine thickets. *G. longipennis* is species of the fusca group that restricted to Kenya, Ethiopia, South-Eastern Sudan, Southern Somali, North-Western Uganda and Northern Tanzania (Aksoy et al., 2003).

Factors affecting tsetse fly distribution

There are many ecological factors which influence the distribution of tsetse flies, of which temperature, rainfall and vegetation type are the most important ones limiting their distribution (Kahn, 2005). Very cold and hot temperatures are not favorable for their activities as well as infective rates. The mortality rate is very high at temperatures exceeding 30 to 32°C (Leak, 1999).Their distribution is limited by low rain fall, and they are highly populated in the regions receiving more than 1000 mm rain fall (Ford and Katonondo, 1971). Vegetation is also another most important ecological factor. Their habitat is situated in the areas where forest is dense, bushy lands and savanna grass lands which protect them from disasters due to sun light and wind (Wint and Rogers, 2000) (Tables 1 and 2).

Impacts of tsetse flies

Disease transmission

The relationship between tsetse flies and trypanosomosis was first suspected in 1879. In 1895, in Zululand, Bruce discovered the causative agent of nagana established the role of *G. morsitans* as a vector of the disease (Itard, 1989). In 1902, Dutton discovered trypanosomes in the blood of sleeping sickness patients. In 1903, Bruce, Nabarro and Greing showed that the trypanosomes of man are transmitted by tsetse flies (Itard, 1989). Of the three groups of tsetse flies, the savanna and riverine are the most important vectors of nagana since they inhabit areas suitable for grazing and watering of livestock (Taylor et al., 2007).

Species of Glossina that are important as vectors of African trypanosomosis includes G. *morsitans*, G. *palpalis*, G. *longipalpalis*, G. *pallidipes* and G. *austeni*. Since they do not feed on any other food rather than blood, they suck blood infected with the trypanosoma species and transmit this disease to previously uninfected animals (Woolhous et al., 1994). Although, the infection rate of Glossina with trypanosomosis is usually low, ranging from 1 to 20% of the flies, each is infected for life, and their presence in any number makes the rearing of livestock extremely difficult (Krafsur, 2009). These infection rates are determined by the parasite, the vector, the host and the environment (Msang, 1999).

In general, tsetse flies are important vectors of trypanosome species including *T. vivax*, *T. brucei* and *T. congolencei,* and transmit the disease nagana among animals and among men which can be fatal if not treated (FAO, 2000). These species of trypanosomes undergo a cyclic development and multiplication in the fly until the infective metacyclic trypanosomes are produced. The sites of the three trypanosomes species found in Ethiopia takes place in the fly as described as follows: The development of T. *vivax* is confined to the proboscis. The complete cycle of development takes 12 to 13 days at 22°C and 5 days at 29°C. The development *T. congolence* commences in the mid gut and complete in

Table 1. The distribution and habitat of tsetse flies in Africa (Vanden Bosche and Vale, 2000).

Group	Specie	Habitat
The fusca group	*G. nigrofusca*	Lowland rain forest and forested areas outside of it in central and West Africa
	G. haningtoni	
	G. nashi	
	G. tabani formis	Low land rain forest in central and West Africa
	G. severini	
	G. vanhoofi	
	G. fusca	Forested areas outside the lowland rain forest in central and West Africa
	G. frezili	
	G. medicorum	
	G. schwetzi	
	G. fusciplerius	
The palpalis group	*G. brevipalpis*	Islands of in East Africa including Ethiopia, often associated with water courses
	G. longipennis	Arid habitat in East Africa
	G. palpalis	Lowland rain forest and extends into drier savanna zones along riparian vegetation of West Africa
	G. fuscipes	Lowland rain forest and extends into drier savanna zones along riparian vegetation of central Africa and also occurs in western Ethiopia and in lacustrine vegetation of lake Victoria and Tanganyika
	G. pallicera	Restricted to rain forest of West Africa
	G. caligenea	Coastal mangrove and rain forest of west Africa
	G. techinoides	Mainly along the rivers and streams in the savanna of west Africa with isolated pockets in similar vegetation in western Ethiopia
The morsitans group	*G. m. morsitans*	Savanna wood lands in Mozambique and Zimbabwe in the South to southern Tanzania
	G. m. centralis	Savanna wood lands in Botswana, Namibia and Angola into southern Uganda
	G. m. submorsitans	Savanna wood lands in Ethiopia and Uganda in the East to Senegal. It occurs in moist vegetation of southern Guinea savanna and in the drier vegetation of the Sudan zone in the North where it is seasonally concentrated along water courses
	G. swennertoni	Restricted to acacia commiphora vegetation in northern Zanzibar and extends into south Kenya
	G. longi palpis	Occurs in thickets, riparian vegetation and forest edge vegetation in west Africa
	G. pallidipes	Occurs in Eastern Africa from Ethiopia to Mozambique in thicket from dry thorn scrub through every type of bush land to light rain forest or even to the margin of rain forest
	G. austeni	Occupies scrub thicket and islands of forest along the East Africa coast from Somalia to Mozambique

the hypophrynx (proboscis). The entire cycle of development takes 19 to 53 days. The development of *T. brucei* starts in the mid gut, pass through the esophagus and pharynx into the mouth parts, enter the hypophrynx at its open anterior end, and finally pass along the salivary ducts into the salivary glands where the final stage of development takes place. The entire cycle of development takes 17 to 45 days and

Table 2. Tsetse fly species found in Ethiopia (Leak, 1999).

Tsetse fly species	Marking of the back of the abdominal segment	Coloration of tarsal segments	Size of the fly (mm)	Others
G. pallidipes	The middle of the first segments yellowish	The last two segments of the tarsus of the front leg are not black	8.5-11	The thorax is wider than other species Upper claspers in the male are short, black and flat
G. morsitans	The middle of the first segment is yellowish. Other segments are black	The last two segments of the tarsus of the hind leg are black. The last segments of the front tarsus is black	8-11	Upper claspers in the male are short, black and flat
G. fuscipes	The middle of the first segment is gray or yellow. All other segments are black	All segments of the hind tarsus are black	8-11	Mating scar in the female. Upper claspers in the male are long, black and tapered
G. tachinoides	The middle of the first segment is yellowish square in shape. Black bands running running across the middle	All segments of the hind tarsus are black	6.5-6.9	Mating scar in the female. Upper claspers in the male are long, black and tapered
G. longipennis	Pale reddish brown and no black bands on the back of the abdomen	Stocking reach from the foot to the knee. Socks are short	11.5-13.5	The bulb of the proboscis is dark colored

even longer (Aksoy et al., 2003).

Economic importance

Among the factors that limit the expected out come from animal production in tropical Africa is an animal disease (Radostits et al., 2007). Tsetse flies occur in 36 countries of 10 million squarekilometer (km^2) of Africa. The risk of trypanosomosis in much of these areas precludes farmers from keeping cattle, and small ruminants. This fact largely accounts for Africa's low livestock productivity. The animal protein produce per hectare on the continent is only one seventieth of that produced in Europe (Brown and Gilfoyle, 2010).

African trypanosomosis has both direct and indirect effects on the economic development of the tropical countries. Direct effect is that the infected livestock may have high mortality rate if not treated. Indirect effect is due to that nagana is a wasting disease and the affected animals are chronically unproductive in terms of milk, meat, manure and traction (FAO, 2000).

Tsetse transmitted animal trypanosomosis is one of the most significant and costly disease in the country where tsetse flies are highly distributed, hindering the effort being made for food self-sufficiency. In Ethiopia, about 240,000 km^2 of the land is infested with tsetse flies and preclude farmers from rearing livestock. Disease could affect development through its historical effect on shaping institutions and/or through contemporaneous impacts on health (Bourn and Scott, 1978). Another indirect effect on the economic development of the country is the costs of drug to treat the disease and control of the tsetse flies. The added risk of human infections is due to sleeping sickness, the most fatal trypanosome disease transmitted by tsetse fly has also greatly affected social, economic and agricultural of the rural communities (WHO, 2000).

Control methods

Chemical control methods

Currently, most anti-tsetse measures relay on the use of insecticides applied from the ground or by air craft. Insecticidal control is the only proven method available for large-scale use at the present time. Various methods of application are given and, of these, selective residual ground spraying and the aerosol aerial technique are more fully assessed (Vale et al., 1999).

Control by traps insecticide impregnated

Traps are devices made up of a piece of blue and black fabrics with white netting on the top creating a sharp corner, and act as an effective means of tsetse control. They are used to catch flies both for control and monitoring purpose (Vale et al., 1999). Since tsetses

have a high metabolic rate and feed exclusively on vertebrate blood, their survival depends on detecting and encountering suitable hosts on which to feed. This principle can be exploited in the design of traps which mimic key features of host animals, attracting the tsetse in such a way that they are then captured or killed. The flies 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). Certain colors especially blue attract many tsetse flies. The blue screens of the traps are consternated with black screens to make flies settle. The flies subsequently move towards the upper parts of the trap in the direction of the light (Vanden-Bosche et al., 2001).

Effective traps attract all the flies from a distance of approximately 50 m. Flies that enter the trap may die because of exposure to an insecticide impregnated in the trap material or because they are exposed to the sun (Terblanche et al., 2008). Impregnated traps have the extra advantage of flies settling on the outside, but not entering are also killed. Attractive odours are available for the control of the flies that transmit animal trypanosomosis. These attractants includes cow urine, acetone octenol and phenols. They are non-pollutant, and relatively cost effective (Robertson, 1991).

The basic design of traps are applicable in all areas of Africa with tsetse flies, but some modifications maybe needed to make them more effective under local condition. The efficiency of the traps, however, varies for different species of tsetse flies. Some of the traps available and currently used for tsetse flies in the target area include monoconical, biconical, epsilon, pyramidal and vavoua (NTTICC 1996).

Targets (insecticide treated cloth)

Targets are pieces of insecticide treated cloth measuring about 1.15 m^2 which are deployed in tsetse habitat in a similar way as traps (Hao et al., 2001). They are supported with either thin steel or wooded poles (Welburn and Maudlin, 1999). The color of the target is either black or a combination of blue black and deployed either hanged on the branches of a short tree, fixed to supporting poles or fixed to a thin stem of a plant.

Preferably, only the black portion of the target should be painted with 0.4% of deltamethrin solution and when the flies come into the direct contact with the targets, they pick a sufficient amount of insecticide enough to knock down or kill the flies within few seconds to minutes. The technique is quite simple, effective, non-pollutant, cost effective used for barrier establishment, integrated with other techniques and requires less frequent maintenance, but needs the use of insecticides and sometimes damaged bush fire, animals and people (Vande and Vale, 2000).

Insecticides aerosols (SAT)

Aerial applications of insecticides to control tsetse is based on the sequential aerosol technique where by the areas where tsetse live are sprayed with non-residual insecticide at interval designed to kill all adults initially, and then subsequently to kill young adults after they emerge (Bourn and Scott, 1998). Insecticides aerosols have a very short residual effect and kill tsetse flies. It is essential that the area to be sprayed has economic potential and also negative impacts on the environment (Hao et al., 2001).

Some species of Glossina such as G. morsitans, *G. sywnertoni* and *G. pallidipes* in East Africa occur over wide area of country, often many hundreds of square miles in extent, and for the control of such species, the use of aircraft for the application of insecticide has obvious advantages, the chief of which is their ability to cover large areas quickly (Afewerk, 1998). An application from the air has been found to be uneconomical even where it is possible against species living in high forest such as *G. palpalis* (Rogers et al., 1994). It is less adequate than ground application against riverine species like *G. palpalis*, and only small amount of insecticides reaches each individual fly; gravid females are less susceptible to insecticides than males (Leak, 1995).

Ground spray

Residual treatment of tsetse fly resting-places must be lethal if or the fly on short contact has a longer period than the maximum duration of the pupal life. In such conditions, only one spraying may be sufficient to control the species, and perhaps eradicate it in an isolated area. The first residual applications have been done against riverine species, like *G. palpalis* and *G. fuscipes*, with habitats restricted to water edge. In larger gallery forests, it is sometimes possible to open paths in the forest, which will be extensively used by moving flies, and to treat then for controlling flies. DDT suspensions and emulsions, which have been used in the first experiments, have usually been replaced by dieldrin emulsions, which are assumed to be efficient almost one year, and sometimes more than one year if applied at 4% (Kernaghan, 1996). Tsetse fly control by residual insecticides has not been carried out against high forest species and is only promising when the fly habitats are restricted (Terblanche et al., 2008).

Live bait techniques

This involves treating livestock with appropriate insecticide formulations, mostly Deltamethrin 1% usually by means of dips, or as pour-on, spot-on or spray-on

veterinary formulations along the back of the animal (Leak et al., 1995). The spraying solution of this deltamethrin is prepared by adding, for example, 50 ml of the concentration to every 10 L of water in the knapsack sprayer and sprayed on the entire body of the animal. The insecticide treated animals are said to be mobile targets and are more attractive than the stationary targets and traps. These are highly effective against tsetse flies, and have the additional advantage of controlling other flies and cattle ticks (Aksoy et al., 2001).

Non chemical control methods

Control by sterile insect techniques (SIT)

The fact that all species of tsetse normally only mate once during their life, the sperm being stored in the spermathaecae of the female, gives rise to the possibility that sterilization of the males could result in a population decrease and eventual eradication. Several investigations and field trials into the practical feasibility of utilizing this technique have been conducted in recent years (Krafsur, 2009). Normally, the policy has been the rearing of large tsetse populations in the laboratory followed by sterilization of the pupae obtained, usually by the use of radioactivity, and the dissemination of these into the field where the sterile males produced compete with wild flies for the fertilization of females. The rearing of large numbers of flies under laboratory conditions, in order to obtain sufficient numbers of pupae and the dissemination of the pupae obtained over large areas have remained insurmountable problems (Hawse, 2005). Of the species of tsetse that exist, only a few have been laboratory bred in large numbers. In addition, the advanced technology and expertise required to successfully rear large colonies is not always available in many African countries. The recurring costs of pupae production is high (currently approximately 0.25 US dollars each) and, in order to successfully compete with wild males, the numbers of sterilized pupae released must also be of a magnitude several times greater than the natural population (Mehta and Parker, 2006).

SIT relies on rearing large numbers of insects in purpose built “fly factories” sterilizing the males with carefully controlled doses of gamma radiation and finally releasing them over the targeted area (Hawse, 2005). The radiation induces sterility, but treated male flies can still fly and mate with the females. Mating between sterile released males and forest female tsetse flies produces no offspring. When sufficient sterile males are released over a long enough period, fertile mating does not occur and the pupation is eliminated (Mehta and Parker, 2006). The technique is a safe and environmentally sound method that employs a nuclear technology as a form of insect control. Sterilization can be induced by chemicals or ionizing radiation. Chemical sterilization was used in early works, but because of the hazard of environmental contamination, it is replaced by irradiation (Dame and Schmidt, 1998). When biological material is irradiated, free radicals are formed, and breaks are created in the chromosomes of the germ line and this leads to the formation of dominant lethal mutations in eggs and sperm cells. Radiation is simple process with easy and reliable quality control procedures (Dwight and Bowman, 2003). If the control of tsetse by sterilisation is to be pursued, then, in view of recent advances made in the identification of tsetse attractive odours and the development of more efficient trapping systems, consideration should be given to the capture, chemo sterilisation and release of the natural population (Brightwell et al., 1997).

Control by bush clearing

Early studies into the behavior and ecology of tsetse flies indicated that the various species showed specific preferences for certain types of habitat related to the vegetation available. This indicated the possibility of achieving eradication by the destruction of this natural vegetation and its replacement by planned land resettlement (Hawse, 2005). This method, aimed at both control and eradication, was used more extensively in the early years, but has since been abandoned mainly for two reasons: first, the implementation of planned land resettlement and soil conservation was very seldom practiced thus leading to poor utilization and consequent soil erosion. Second, in many cases, where selective destruction of the habitat was carried out rather than sheer clearing, the tsetse demonstrated the ability to adapt to the new conditions (Itard, 1989).

The method has also proved to be expensive to implement, requiring the use of heavy mechanical equipment. Nevertheless, it is still considered to be of use in reducing man/fly or cattle/fly contact in specific areas such as at water points and around small agricultural settlements. As human populations in African countries increase, the demand for land results in the destruction of tsetse habitat as an incidental achievement and in this way large area have been cleared of fly in Nigeria and more recently in Ethiopia (NTTICC, 1996). With the high rates of population increase being experienced and the consequent accelerated demand for land it may not be impractical to assume that, with the passage of time, tsetse may be eradicated from large areas of Africa without the implementation of deliberate control measures (Urquhart et al., 1996).

Control by game destruction

Tsetse flies are haematophagous insects and depend on a regular blood meal for all their nutritional requirements and to provide energy for the development of their young

ones. During the transitional phase from larva to adult, insufficient host animals should be available to ensure an adequate and regular food supply, and then the tsetse population would experience conditions of stress resulting in a population decline and subsequent control (Robertson, 1991). This fact is the basis used for the destruction of wild animal species which have been identified as preferred tsetse hosts. The method was used extensively in the past, particularly in Uganda and Zimbabwe, with reasonable control being achieved but there are several limitations. When denied a preferred host species, tsetse fly are able to adapt to feeding off other animals thus increasing the number of species that have to be destroyed (Robertson, 1991).

Obviously, this is not a method that could be implemented in many African game parks and nature reserves nor could it be applied to areas where settlement with domestic stock occurs. There are very few records of eradication having been achieved by game destruction although satisfactory control of fly populations and the prevention of their re-invasion into reclaimed areas have been reported. This method has very limited practical application in modern times except in those areas where game and cattle free hunting barriers can be maintained between tsetse belts and settled areas (Feyesa, 2004).

CONCLUSION AND RECOMMENDATIONS

Tsetse flies are hematophagous insects of the family glossinidae and are biological vectors of African trypanosomosis in both animals and man. Their distribution and prevalence are most influenced by special factors such as climate, vegetation and land utilization.

African trypanosomiasis continues to have a profound effect on sustainable development in rural sub-Saharan Africa as it affects not only the well-being of the poor and compromises their ability to produce food efficiently, but also their livestock on which their livelihoods are heavily dependent. Tsetse flies, through the cyclical transmission of trypanosomosis to both humans and their animals, greatly influence food production, natural-resource utilization and the pattern of human settlement throughout much of sub-Saharan Africa. Knowing the vector parasite interaction and having a full understanding of the complex relationships between tsetse flies and.

There are various types of techniques that have been used to control tsetse flies in integrated ways, but all have their advantages and disadvantages. Based on the conclusion, the following recommendations are made:

1. All victimed communities, veterinary authorities and government should participate in control strategies of these vectors of African trypanosomosis.
2. Educating animal owners on the problems of trypanosoma infection and on its control measure is more essential.
3. In addition to livestock health, tsetse have also public health impacts therefore, veterinarians should create awareness in communities about these vectors in order to help them protect themselves as well as their livestock.
4. Strategic tsetse fly control is very important in order to avoid economic impacts due to nagana where tsetse flies are densely populated.
5. When planning to control tsetse flies in order to control tsetse-transmitted trypanosomosis, one should consider cost-benefit analysis and environmental impacts of the techniques of control.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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Full Length Research Paper

Prevalence of bovine trypanosomiasis in Dara District Sidama Zone, Southern Ethiopia

Migbaru Keffale Bezabih[1]*, Zerihun Shabula[2] and Nateneal Tamerat Beyene[2]

[1]Department of Biomedical Science, College of Veterinary Medicine, Haramaya University, P.O. Box 301, Haramaya, Ethiopia.
[2]Southern Nations Nationalities and Peoples Region, Sidama zone, Dara District Agricultural Office, Ethiopia.

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A cross sectional study was conducted in five selected peasant Associations of Dara Woreda of Southern Ethiopia from October, 2013 to June, 2014 to estimate the prevalence of bovine trypanosomiasis and to identify the prevalent trypanosome species, and the risk factors of the disease. Blood samples were collected from the ear vein of randomly selected 384 cattle. Thin blood smear and buffy coat techniques are employed to detect the presence of the parasite and the PCV was measured to evaluate the anemic condition of the animals. Out of the total number of cattle examined, 57 were found to be positive for Trypanosomiasis giving the overall prevalence of 14.8%, out of which 47.6% *Trypanosoma congolense*, 33.3% *Trypanosoma vivax*, 9.5% *Trypanosoma brucei* and 9.5% mixed infection (*Trypanosoma congolense* and *Trypanosoma vivax*) were identified. The maximum prevalence 28.4% was observed in Safa followed by Adame, Odola, Machisho and Kumato with the prevalence of 19.8, 11.5, 10.1 and 4.9%, respectively. Animals were grouped into three age categories, calves < 1 year, young 1 to 3 years, Adult >3 years with the prevalence of 4.2, 8.5 and 20.5% respectively. Based on the body condition score, the prevalence of 8, 13 and 25.2% was recorded in good, medium and poor conditioned animals, and it was higher in females 15.7% than males 12.4%. The statistical analysis showed a significant association in the variation of age categories, body condition and among peasant associations ($p<0.05$). The result also showed a significant difference in packed cell volume (PCV) values between infected and non-infected cattle. In conclusion, the study showed that disease was higher in the area and had significant effect on the body condition and development of anemia. Therefore, the responsible organizations and the community should work on the control and prevention activities of the disease in environmental friendly manner.

Key words: Cattle, Dara, prevalence, *trypanosoma,* trypanosomiasis

INTRODUCATION

Trypanosomosis is the wide spread protozoan parasitic disease affecting cattle and other wide range of host, including humans in Sub-Saharan Africa. The course of the disease may run from an acute and rapidly fatal to a

*Corresponding author. E-mail: migkafe@gmail.com. Tel: +251910083862/+251919125288.

chronic long lasting one depending on the parasite-host interaction and the disease is characterized by intermittent fever, progressive anemia, loss of body condition of susceptible hosts, and this lead to heavy mortality if animals are untreated (FAO, 2005). Trypanosomosis in Africa livestock producers and consumers causes an estimated US $ 1 billion loss each year. It is a severe problem to agricultural production in widespread areas of the tsetse infected regions that accounts for over 10 million km^2 of the tropical Africa (Maudlin et al., 2004).

The disease occurs in some 240,000 km^2 area of Ethiopia and bout 10 to 14 million heads of cattle and a significant number of small ruminants and equines are under serious risk of contracting the disease of which 20,000 heads die every year (Solomon, 2006). Currently the country is infested with five species of tsetse flies namely *Glossina pallidipes, Glossina moristans*, *Glossina fuscipes*, *Glossina tachinoides* and *Glossina logispenis* (NTTICC, 2004). In Ethiopia, the most important trypanosome species that affects cattle, sheep and goats are *Trypanosoma congolense, Trypanosoma Vivax and Trypanosoma brucei* but *Trypanosoma evansi* and *Trypanosoma equiperdium* are for camel and equines respectively (Abebe, 2005).

Trypanosomiasis can be transmitted through cyclical or mechanical transmissions. In cyclical transmission there is always development and replication of parasite in intermediate hosts (tsetse flies) species like *Glossina m. submorsitans*, *G. pallidipes, Glossina fuscipes fuscipes and Glossina tachinoides*. These species of tsetse flies are distributed along the lowlands of western, southern and southwestern part of Ethiopia (wondewosen et al., 2012). The disease is also transmitted mechanically by biting flies of the genus *Tabanus*, *Haematopota*, *Chrysops* and *Stomoxys*. This type of transmission has caused the spread of *T. evansi* and *T. vivax,* which is found outside the tsetse belt areas (Oluwafemi et al., 2007). However, in very acute infections with highly susceptible exotic, animals infected with *T. vivax* can also pass through the placenta into the fetus in pregnant animals. As a result some cows abort and some calves are born before birth time (Abebe and Jobre, 1996).

In Ethiopia, trypanosomiasis is one of the most important disease limiting livestock productivity and agricultural development due to its high prevalence in the most arable and fertile land of south west and Northern part of the country following the greater river basins of Abay, Omo, Ghibe and Baro (Abebe and Jobre, 1996). The most estimates of the economic loss attributable to trypanosomiasis infection are based on the cost of mortality, reduced weight gain in animal grown for meat, reduced milk yield, draught output and impact on fertility (FAO, 2005). In addition to these, the disease is also responsible for an annual loss of millions of dollars in livestock production as a result of the cost related to treatment, prevention and vector control efforts (Samuel et al., 2001).

In Southern Nations Nationalities and people's regional state, animal trypanosomiasis results to socio-economic impact through debilitation and deaths of untreated animals and reduces production and productivity of affected animals (Waldeyes and Aboset, 1997); however the disease had not yet been assessed and there is no documented baseline data in Dara district. Therefore, this study was conducted to estimate the prevalence of trypanosomiasis and to identify the prevalent trypanosome species and the possible risk factors of the disease.

MATERIALS AND METHODS

Study area

The study was conducted in five purposively selected peasant associations (PAs), namely Safa, Adame, Odola, Machisho and Kumato found in Southern Nations, Nationalities, and Peoples' Region (SNNP) region, Sidama zone, Dara Woreda which is situated at 365 km from Addis Abeba and 76 km from Hawassa. The woreda is found in 6.47°N and 38.33°E and covers 27,000 hectare total area, which is sub-divided into 37 PAs. The altitude of the woreda ranges from 1400 to 2800 m.a.s.l and mean rain fall is 1200 to 1700 mm. The temperature of the woreda ranges from 20°C to 27°C. In the area mixed farming system is practiced and the grazing land is covered by different vegetation types mainly savanna grassland forest, and bush lands (MARDO, 2011**)**. Livestock population of the woreda are 21456 bovine, 5985 caprine, 8279 ovine, 2861 equine and 6468 poultry (CSA, 2012).

Study animal

The study was carried out on 384 indigenous Zebu cattle of both sexes; age groups and body condition of the animal were also considered. The animals in area mainly depend upon communal grazing fields and crop residues as feed source and watering paints are the Gidawo and Buna rivers which are infested with tse-tse flies.

Sampling methods and sample size determination

A cross-sectional study using simple random sampling technique was employed to determine the prevalence of bovine trypanosomiasis in the study area. The 5 PAs were selected purposively based on the availability of transportation and logistics as well as their agro ecological representativeness of 37 Pas of the district. From each selected PA, the farmers as well as the study animals were selected randomly in each household. During sampling, PAs, age, sex and body condition score (BCS) of the animal were recorded. The body condition score was grouped in to good, medium and poor conditioned animals based on the appearance of ribs and dorsal spines applied for zebu cattle (Nicholson and Butterworth, 1986). Age of the animal was estimated by dentition (De-lahunta and Habel, 1986) and owner's information. The sample size (n) was calculated according to the formula given by Thrusfield (2005), considering 50% expected prevalence (p), 95% confidence level and 5% desired absolute precision (d).

$$N = \frac{(1.96^2 \times P)\,(1-P)}{d^2}$$

Table 1. Prevalence of trypanososis and identified trypanosome species in the study area.

Peasant associations	No of examined	T. *congolense* (%)	T. *vivax* (%)	T. *bruci* (%)	T. *congolense* and *T. vivax* (%)	Total positives	Prevalence (95%CI)	X^2 (Pvalue)
Safa	74	10 (47.6)	7 (33.3)	2 (9.5)	2 (9.5)	21	28.4 (18.5-40)	20.6 (0.004)
Adame	81	8 (50.0)	5 (31.25)	2 (12.5)	1 (6.25)	16	19.8 (11.7-30.1)	
Odola	78	3 (33.3)	3 (33.3)	2 (22.2)	1 (11.1)	9	11.5 (5.4-20.8)	
Machisho	69	3 (42.8)	2 (28.6)	1 (14.3)	1 (14.3)	7	10.1 (4.2-19.8)	
Kumato	82	2 (50.0)	1 (25.0)	1 (25.0)	-	4	4.9 (1.3-12)	
Total	384	26 (45.6)	18 (31.6)	8 (14.0)	5 (8.8)	57	14.8 (11.4-18.8)	

Study design

The study was conducted by using simple random sampling techniques in order to determine the prevalence of trypanosomiasis in bovine species at study area. It was performed by parasitological survey and hematological procedures. Blood samples were obtained by puncturing the marginal ear vein with lancet.

Thin blood smear

A small drop of blood from a micro-haematocrit capillary tube was applied to a clean slide and spread by using another clean slide at an angle of 45 degree. The smear was dried by air and then fixed for 2 min in methyl alcohol. The thin smear was flooded with Giemsa stain 1:10 solution for 30 min. Then it was allowed to dry standing up right on the rack and examined under microscope oil immersion (100) objective lens (OIE, 2008).

Packed cell volume (PCV)

Blood was directly collected into heparinized capillary tubes, and the tubes were then sealed at one end with crystal seal. The capillary tubes were placed in micro-hematocrit centrifuge and allowed to centrifuge at 1500 revolution per minute (rpm) for 5 min. The centrifuged capillary tubers were placed on hematocrit reader, and measured for PCV. Animals with PCV less than 24% were considered to be anemic (OIE, 2008).

Buffy coat techniques

The capillary tubes were cut at 1 mm below the buff coat to include the upper layer of red blood cell and expressed onto a slide and then covered with cover slip, the slide was examined under 40× objective lens. Trypanosome species were identified according to their morphological descriptions of Giemsa stained blood film as well as movement in wet film preparations provided by Radostits et al. (2007).

Data analysis

The collected raw data and the results of parasitological and hematological examination were entered into a Microsoft excel spread sheet. Then the raw data was summarized using statistical package for the social sciences (SPSS) version 20. The presence of association between the prevalence of the diseases and the risk factors such as PAs, age, sex and body condition score were assessed by using chi-square test ($\times^2$). Mean PCV values of parasitaemic and non- parasitaemic animals were compared by independent t test. P-values less than 0.05 were considered as significant.

RESULT AND DISCUSSION

Out of the total of 384 cattle examined, 57 were positive for Trypanosomosis hence the overall prevalence of the study area was 14.8%. This result is closely related with the result of 14.2% reported in Arbaminch (Abraham and Tesfaheywet, 2012). The maximum prevalence was observed at Safa peasant association (28.4) followed by Adame (19.8%). This might be due to their location around Gidawo and Buna river belts respectively, where there is high tsetse flies distribution whereas Odola, Machisho and kumato showing 11.54, 10.14 and 4.88% respectively showed statistically significant difference ($p<0.05$). From the total trypanosome species identified, *T. congolense* 26/57(45.6%) was the most prevalent followed by *T. vivax* 18/57(31.6%) and *T. brucei* 8/57(14.0%) and 5/57(8.8%) mixed (*T. congolense and T. vivax*) infection. According to this result *T. congolense* was the dominant species in the study area (Table 1).

This finding is in agreement with the reports of Takile et al. (2014) with 53.33, 30 and 16.66% of the infections were due to *T. Congolese, T. Vivax* and *T. brucie* respectively, and also the reports of Habtamu et al. (2014) showed *T. congolense* (63.64%) followed by *T. vivax* (27.27%) and *T. brucei* (9%). The predominance of *T. congolense* infection in cattle suggests that the major cyclical vectors or *Glossina* species are more efficient

Table 2. Prevalence of Trypanosomiasis based on animal`s age, sex and body condition.

Variables	No. of examination	No. of positives (%)	(95% CI)	χ^2 (P-value)
Age				
<1year	48	2 (4.2)	0.5-14.5	
1-3 years	117	10 (8.5)	4.2-15.2	13.6 (0.01)
>3years	219	45 (20.5)	15.4-26.5	
Sex				
Female	287	45 (15.7)	11.7-20.4	0.63 (0.43)
Male	97	12 (12.4)	6.6-20.6	
BCS				
Good	112	9(8)	3.7-17	
Medium	196	22 (13)	8.3-19.0	13.36 (0.001)
Poor	103	26 (25.2)	17.2-34.8	
Total	384	57 (14.8)	11.7-18.8	-

Table 3. Comparison of mean PCV between parasitaemic and aparasitaemic cattle.

Condition	No. examined	PCV <24%	PCV >24%	Mean PCV (95% CI)	t-test (p-value)
Parasitaemic	57	39 (68.4)	18 (31.6)	22.7 (22.2-23.2)	7.5 (0.001)
Aparasitaemic	327	63 (19.3)	264 (80.7)	25.9 (25.5-26.2)	
Total	384	102 (26.6)	282 (73.4)	25.4 (25.1-25.7)	-

transmitters of *T. congolense* than *T. vivax* in East Africa and also due to the high number of serodems of *T. congolense* as compared to *T. vivax* and the development of better immune response to *T. vivax* by infected animals (Leak et al., 1999).

In the present study, higher prevalence was observed in females (15.7%) than males (12.4%) which is in agreement with the reports of Feyissa et al. (2011) with 15 and 13.7% in female and males respectively but there was no significant difference ($P > 0.05$) (Table 2). The possible explanation for relative increment of prevalence in female animals might be due to physiological differences (Torr et al., 2006).

Based on age category, 4.2, 8.5 and 20.5% prevalence was observed in animals less than one year, between one and three years and above three years of age respectively which revealed significant variation between calves less than one year and >3 years of age ($p<0.05$) (Table 2). This could be associated to the fact that adult animals travel long distance for grazing and draught as well as harvesting crops in areas of high tsetse challenge than calves (Ayele et al., 2012) which is supported by the results of Rowlands et al. (1995) in Ghibe valley which indicated that suckling calves don't go out with their dams but graze at homesteads until they are weaned off. Also, Fimmen et al. *(*1999) suggested that young animals are slightly protected by maternal antibodies.

In this study, out of the total animals examined, 26.6% were anemic having PCV <24 and 73.4%. On the other hand, out of the total 57 parasitaemic animals, 68.4% were anemic (PCV<24) and only 31.6% were not; whereas from 327 aparasitaemic animals only 19.3% were anemic (PCV<24) but 80.7% were not anemic. There was significant difference between the mean PCV values of parasitaemic and aparasitaemic animals ($t=7.5$, $p<0.05$) (Table 3). This lower PCV was reported in previous studies in different parts of the country like Nigatu (2004) and Abraham and Tesfaheywet (2012). This might be due to trypanosome infection which produces erythrophagocytosis anemia (destruction of red blood cells) carried out by enzymatic and immunological mechanism during infection in parasitaemic animals (Budovsky et al., 2006).

Conclusion

Trypanosomiasis is one of the major constraints of cattle production as well as agricultural productivity in the Dara woreda due the reduction of milk yield, loss of body condition, stunted growth in young animals, and low output of draught power. The result revealed that *T. congolense* was the most prevalent species in the study area and the infections significantly affect the PCV values

and body condition. Therefore, economical and environment friendly community based tsetse fly and trypanosomiasis control program should be designed and implemented in the area.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Entomological study on species composition, behavior, longevity and probability of surviving sporogony of Anopheles mosquitoes in Lare District, Ethiopia

Behailu Taye[1*], Mohammed Seid[2] and Adugna Gindaba[2]

[1]Department of Biology, Mettu University, Mettu, Ethiopia.
[2]Department of Wildlife and Ecotourism Management, Gambella University, Gambella, Ethiopia.

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In order to develop efficient malaria vector control strategies, this study was conducted to assess species composition, relative abundance and longevity of Anopheles mosquito malaria vectors in Lare district, south western Ethiopia. For this, Anopheles mosquitoes were collected using CDC light traps catch and pyrethrum spray collection for five months from May to September, 2016. Four kebeles (the smallest administrative unit in Ethiopia, three houses per each kebele) for CDC light trap catches collection and 20 households from each sampled kebeles for pyrethrum spray collection were selected randomly. A total of 2735 Anopheles mosquitoes belonging to four species were collected during the study period. *Anopheles gambiae* s.l. 1914 (69.9%) was the predominant malaria vector followed by *Anopheles pharoensis* 602(22%), *Anopheles nilli* 137(5%) and *Anopheles coustani* s.l. 82 (3.10%). Significant ($p<0.05$) variations existed in mean density of mosquitoes per month, but not in mean mosquito density per site (between sampled kebele). The highest mean longevity of *A. gambiae* s.l. was 9.4 days in July, while probability of surviving sporogony was 0.52 and 0.56 for *P. falciparum* and *P. vivax*, respectively. Thus, this study could contribute to the basic understanding of age, distribution and behaviour of anopheles mosquitoes in Lare district for evidence based malaria vector control program.

Key word: Anopheline species, longevity, sporogony, plasmodium, Ethiopia.

INTRODUCTION

Mosquitoes of the genus, *Anopheles* are important vectors of plasmodium parasites, causal agents of malaria. There are over 400 species of anopheles mosquitoes but only 30-40 of these acts as vectors of malaria. In the sub-Saharan Africa, approximately 20 out of 140 *Anopheles* species are known to transmit malaria to humans. Among these vectors, *Anopheles gambiae* Giles, *Anopheles cluzzii* Coetizee et al., *Anopheles. arabiensis* Patton and *Anopheles funestus* Giles are the most widely distributed and the most effective malaria

*Corresponding author Email. behailu27taye@gmail.com.

vector species in tropical Africa (Coetzee et al., 2013; Gillies and Coetzee, 1987).

The non-random distribution of both anopheles larvae and adults includes fresh or salt-water, marshes, mangrove swamps, rice fields, grassy ditches, stream edges, rivers and small temporary rain pools. Many species prefer habitats with vegetations, others prefer habitats that have none. Some breed in open, sunlit pools while others are found only in shaded breeding sites in forests. A few species breed in tree holes or the leaf axils of some plants (Nikookar et al., 2010).

The identification of mosquito species and associated distribution records are vital to build effective malaria control program and best indication for resistance of insecticide between species (Ramirez et al., 2009; Yehwalaw et al., 2010). In order to determine malaria transmission in a given area understanding vector behavior and diversity is crucial. To accomplish this goal, entomological study with Anopheles collection is important. The choice of method depends on the objectives of the study, even though human landing catch is golden standard; however, it has an ethical issue and less probability to catch different species of anopheles. On the other hand, CDC light traps has better access to catch more number with diversity of Anopheles mosquitoes (Ndiath et al., 2011). Coetzee (2004) reported that the distribution maps of mosquito vectors might not reflect the real species distribution in nature as it could be influenced by the collection efforts of entomologist. There is no doubt that entomological studies in Ethiopia are scarce and there is a need to focus on vector species biology and ecology. This is particularly important in the south western part of Ethiopia including Gambella region where malaria is endemic (Alemu et al., 2011; Krafsur, 1977).

The data from the district showed that malaria is one and the leading top ten diseases in the area with high incidence and morbidity (Lare District Report, 2016). This study aimed to assess the species composition, behavior, longevity and probability of surviving sporogony of Anopheles mosquito malaria vectors of Lare district, south western Ethiopia.

MATERIALS AND METHODS

Study area and period

The study was conducted in Lare district, Nuer zone, south west Ethiopia. Lare is bordered on the south and east by the Anuak Zone, on the west by the Baro river which separates it from Jikawo, and on the north by the Jikawo river which separates it from South Sudan. The study was conducted from April to September, 2016 G.C. (Figure 1).

Mosquito sampling and identification

Lare district has 28 (twenty eight) kebeles (the smallest administrative unit in Ethiopia) among these, 4 (four) kebeles were randomly selected. A total of 12 households (three household per each kebele) were selected for mosquito sampling. Adult mosquitoes were collected once per month from the selected households (the households chosen one at the center and the others at the periphery of the kebele) (WHO, 1975) from May to September, 2016 using CDC light traps and pyrethrum spray collections. The collected mosquitoes were identified to species and complex level morphologically using standard keys by Gillies and Coetzee (1987); they were counted and then stored in Eppendorf tubes with desiccant (silica gel) for further laboratory processing at Sokoru Tropical Infectious Disease Research Center, Jimma University (TIDRC JU).

The CDC light trap collection

CDC light traps were set indoors (inside bed room) and outdoors (within 15-20 m radius outside the house), human dwelling (WHO, 1975; Mboera, 2005) and run from 18:00-06:00 h.

Pyrethrum spray collection

Pyrethrum spray collection (PSC) was used to collect indoor resting *Anopheles* mosquitoes from 6:00-7:30 h in 20 houses from each kebeles. The monitored houses were different from those used for light trap catches. Sampling was done once per month from May to September, 2016. Anopheles mosquito was sampled from each house once per month, from May to September, 2016. Prior to PSC, the inhabitant was asked to empty the house, any openings that could allow mosquito escaping were closed and the entire floor was covered with a white cloth from wall to wall in a single room. Then, a protected sprayer sprayed the room with Mobil flit (Bioygon SC. Jonhanson and Sun. Inc. USA) for about 5 min; the sprayed room was left closed for 15 min. Subsequently, the sheet was brought outside the room and knock-down mosquitoes were counted and identified.

Method of parity status determination

Half of the unfed female *Anopheles* mosquito collected from light trap catches were dissected for parity status. The abdominal and ovary dissection was conducted following the standards of WHO (1975). Ovaries with coiled tracheal skeins was considered as nulliparous while those with stretched out tracheoles was taken as parous as described by WHO (1975) and Fils et al. (2010).

Data analysis

The data was analyzed using SPSS statistical software version 16.0 (SPSS Inc, Chicago, IL, USA). Before analysis it was normalized by transforming into Log n +1 in SPSS. The significance test p-value less than 0.05 was considered significant during the analysis.

Daily survival rate (S) = $\sqrt[gc]{PR}$ Where gc = Estimated gonotrophic cycle of *A. gambiae* s.l. of the population. The gonotrophic cycle in area was estimated to be 3 days based on Krafsur (1977) study in west Ethiopia. Life expectancy (LE) = 1/ -LnS (Davidson, 1954). Degree of exophily (De) was calculated following: DE = 1 − (1/F-HGG) (Ameneshewa and Service, 1996).

Ethical considerations

The study was legalized by research review and ethical committee of Gambella University. Permission from the community was sought

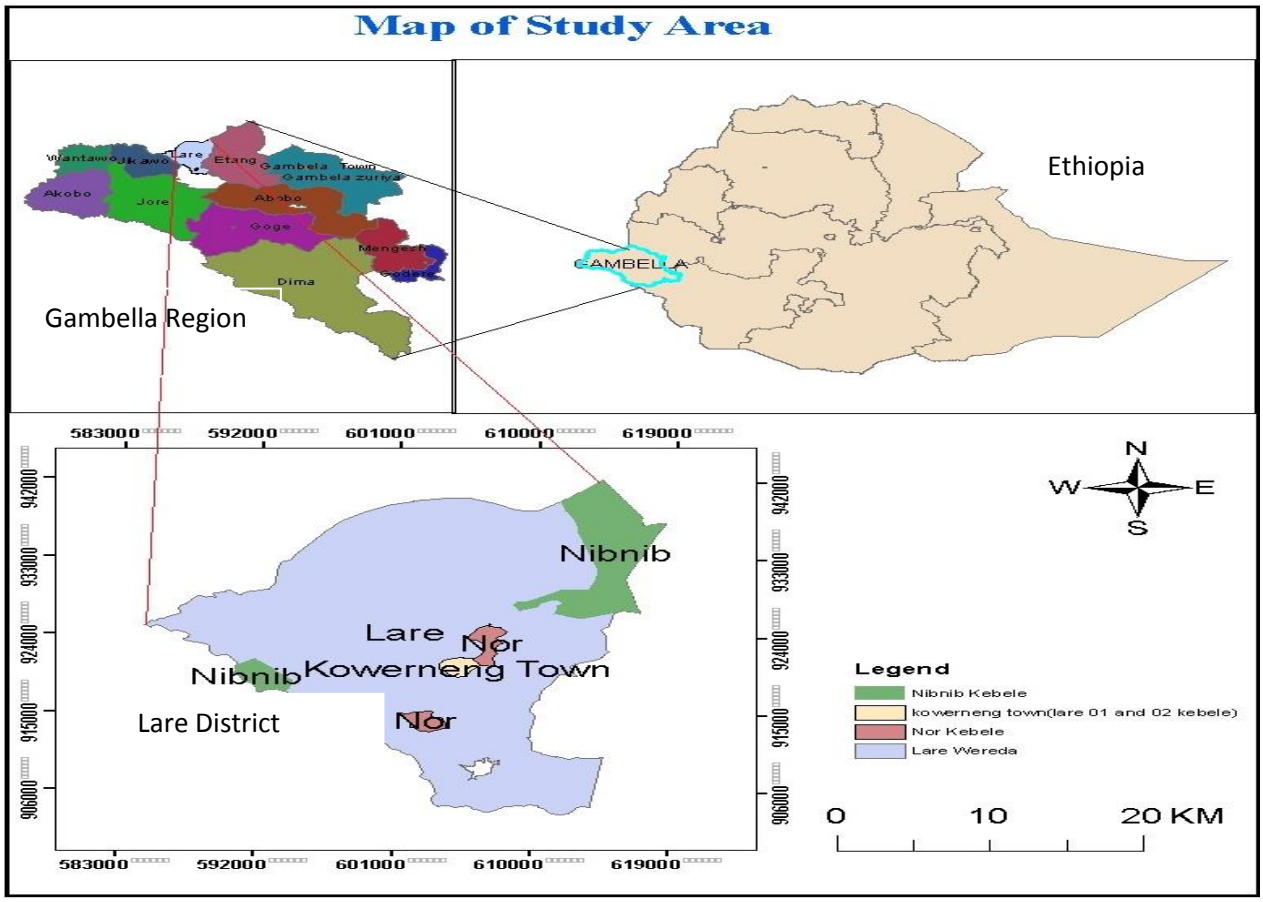

Figure 1. Map of south Western Ethiopia showing Lare district of the study area.

Table 1. Species composition and abundance of anopheles mosquitoes collected by CDC light trap and Pyrethrum spray catches in Lare district, southwest Ethiopia (May- September, 2016).

***Anopheles* spp.**	**Total collected**	**Percentage**
A. gambiae s.l	1914	69.90%
A. pharoensis	602	22%
A. nilli	137	5%
A. coustani s.l.	82	3.10%
Total	2735	100%

before initiating the study by communicating the responsible zonal and district administrative offices through official letters from Gambella University. Similarly, household agreement and local oral consent were sought.

RESULTS

Anopheles mosquitoes species composition and relative abundance

A total of 2735 anopheles mosquitoes belonging to four species: *A. gambiae s.l*., *A. pharoensis*, *A. nilli,* and *A. coustani* s.l. were collected from May to September, 2016 in the Lare district. Among the collected mosquitoes, the highest number of anopheles belonged to *A. gambiae* s.l (n= 1914; 69.9%) followed by *A. pharoensis* (n= 602; 22%) while the lowest density belonged to *A. coustani* s.l. (n= *82;* 3.1%) (Table 1).

Indoor and outdoor density of Anopheles mosquitoes

CDC light traps placed outdoors revealed higher mean number of mosquitoes per traps per night than CDC light

Table 2. Mean indoor and outdoor number of Anopheles mosquitoes/trap- night houses in Lare district, southwest Ethiopia (May- September, 2016).

		Mean	±Std. error mean	95% CI difference				
				Lower	Upper	T	Df	p-value
A. gambiae s.l	Indoor	0.8678	±.03467	-0.32876	-0.19494	-7.831	59	0.00
	Outdoor	1.1296	±.04485					
A. pharoensis	Indoor	0.3498	±.04884	-0.31238	-0.17434	-7.056	59	0.00
	Outdoor	0.5932	±.06775					
A. nilli	Indoor	0.5932	±.06775	0.22468	0.41487	6.729	59	0.00
	Outdoor	0.2734	±.04499					
A. coustani s.l	Indoor	0.041	±.01510	-0.16188	-0.03178	-2.979	59	0.04
	Outdoor	0.1378	±.03321					

*p<0.05

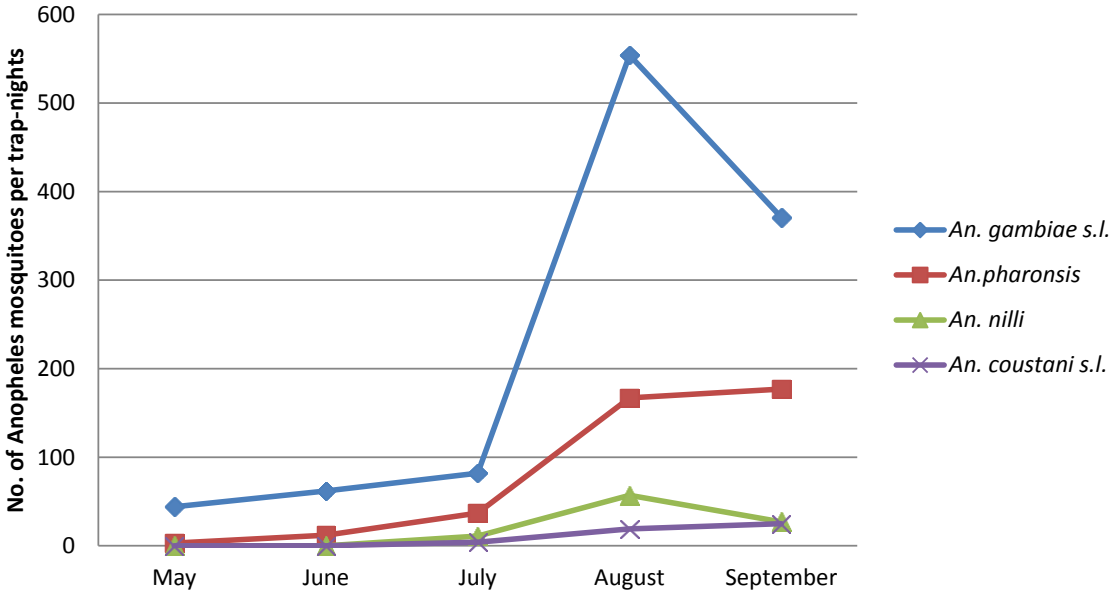

Figure 2. Monthly density of Anopheles *spp*. caught per CDC light trap per night in Lare district, south west Ethiopia (May- September, 2016).

traps placed indoor for each the four anopheles. Likewise, there were significant (p< 0.05) differences between indoor and outdoor mean mosquito number per trap per night for *A. gambiae s.l.* (t= -7.831, p < 0.00), *A. pharoensis* (t= -7.056, p = 0.00), *A. nilli* (t= 6.729, *p= 0.00*) and *A. coustani s.l*. (t= -7.056, p= 0.00), respectively (Table 2).

Monthly dynamics of Anopheles species

Over all, the highest, 841 (39.4%) and lowest, 115 (5.4%) anopheline mosquitoes densities were observed in August and May, respectively (Figure 2). The abundance of *A. gambiae* s.l and *A. nilli* peaked during August, while *A. pharoensis* and *A. coustani* s.l. peaked during September. One way ANOVA revealed that there were significant (p < 0.05) mean density differences between the monitored months for *A. gambiae* s.l (F= 27.247, p= 0.000), *A. pharoensis (*F= 65.648, p = 0.000), *A. nilli* (F=5.296, p= 0.000) and *A. coustani s.l.* (F= 13.874, p = 0.000), respectively.

Regarding mosquito abundance per sampled kebeles, total mean densities of *A. gambiae* s.l. (F= 0.184, p = 0.907), *A. pharoensis* (F= 0.038, p = 0.990), *A. nilli* (F= 2.038, p = 0.119) and *A. coustani* s.l. (F= 0.038, p = 0.485) were shown not to be significantly different between kebeles (Appendix Tables 1 and 2).

Table 3. Monthly parity rates, daily survival rates and life expectancy of *A. gambiae s.l.* in Lare district south west Ethiopia (May-September, 2016).

	Total dissected	No. of parous	Parity rate in (%)	Daily survival rate	Life expectancy
May	15	8	53%	0.81	4.7
June	24	16	67%	0.88	7.8
July	46	34	74%	0.9	9.4
August	107	70	65%	0.87	7.2
September	57	33	58%	0.83	5.4
Mean	49.8	32.2	63%	0.86	6.9

Table 4. Monthly parity rates, daily survival rates and life expectancy of *A. pharoensis* in Lare district south west Ethiopia (May-September, 2016).

	No. of dissected	No. of parous(P)	Parity rate (PR)	Daily survival rate(S)	Life expectancy in days (LE)
May	5	2	40%	0.74	3.3
June	11	5	45%	0.77	3.8
July	13	7	54%	0.81	4.7
August	14	9	64%	0.86	6.6
September	27	19	70%	0.89	8.6
Mean	14	8.4	55%	0.81	5.4

Parity rate and longevity of Anopheles mosquitoes

The highest parity rate (0.74, 0.70), probability of daily survival rate (0.9, 0.89) and life expectancy (9.4, 8.6) of *A. gambiae s.l.* and *A. pharoensis* were observed in July and September, respectively (Tables 3 and 4).

The highest mean probability of surviving sporogony (PSS) of *A. gambiae* s.l. and *A. pharoensis* for *Plasmodium falciparum* (0.52, 0.49) and *Plasmodium vivax* (0.56, 0.53) were observed in July and September, respectively, with corresponding highest expected infective life in days for *P. falciparum* (3.3, 2.4) and *Plasmodium vivax* (4.1 and 3.2) of the two *Anopheles* spp., respectively (Tables 5 and 6).

Indoor resting behavior and degree of exophily

Out of 602 collected anopheles mosquitos by pyrethrum spray catches, the highest (459) were *A. gambiae* s.l followed by *A. pharoensis* (93) (Table 7*).* Furthermore, fed to gravid ratios was 2.04:1, 3.04:1, 2.8:1 and 8:1 for *A. gambiae* s.l., *A. pharoensis*, *A. nilli* and *A. coustani* s.l., respectively (Table 3). The degree of exophily (DE) for predominant malaria vector was 0.51 (Table 7).

DISCUSSION

This study assessed the density of anopheles mosquitoes in selected site in Lare district and their potential role as malaria vectors. The distribution of anopheles mosquitoes in the four kebele revealed that *A. gambiae s.l., A. pharoensis, A. nilli* and *A. coustani* s.l. were found in sympathry. No significant differences in mosquito densities between kebeles were found. This could be because the study areas have similar environmental conditions, homogeneous people in socio cultural activities and the presence of several similar mosquito breeding sites. *A. gambiae* s.l. was the most abundant malaria vectors in the study area which is consistent with other parts of Ethiopia. Hence, *A. gambiae s.l*. is the principal vector of malaria in sub-Saharan Africa in general, East Africa and Ethiopia in particular (Taye et al., 2016; Coetzee, 2004; Tesfaye et al., 2011) it may be responsible for the presence of heavy burden of malaria incidence and morbidity in the area.

Significant variation existed in the density of *Anopheles* spp. during the season, with the highest densities being in August-September. The study demonstrated that all the four Anopheles mosquitoes collected during the study period exhibited significant outdoor biting activity. It is similar to Woyessa et al. (2004) studies in which one of *A. gambiae* s.l, *A. arabiensis* showed exophagic feeding behavior. The high outdoor biting density was observed by *A. gambiae s.l*, this contrasted with prior report by Fornadel et al. (2010a), in which outdoor biting was not observed. This implies a behavioral shift favoring outdoor host-seeking, possibly as the result of intense selection pressure imposed by the indoor application of insecticides. This observation was consistent with other studies both in Africa and elsewhere reported by

Table 5. Probability of surviving sporogony of *Plasmodium* species in *A. gambiae s.l.* by month in Lare district south west Ethiopia (May- September, 2016).

	Atom. Temperature (°C)	EIP pf in days	EIP pv in days	PSS of pf	PSS of pv	Expected of infective life days for pf	Expected of infective life in days for pv
May	35.25	5.8	5.1	0.3	0.34	-1	-0.3
June	34.33	6.1	5.3	0.46	0.51	1.8	2.5
July	34	6.2	5.4	0.52	0.56	3.3	4.1
August	33.67	6.3	5.5	0.41	0.46	0.9	1.7
September	34.11	6.1	5.3	0.32	0.37	-0.8	0.01
Mean	34.3	6.1	5.32	0.4	0.5	0.84	1.6

*EIP= Extrincic incubation period, pf = *Plasmodium falciparum, pv= Plasmodium vivax,* PSS = probability of surviving sporogony.

Table 6. Probability of surviving sporogony of *Plasmodium* species in *A. pharoensis* by month in Lare district south west Ethiopia (May- September, 2016).

Month	Temperature (°C)	EIP *Plasmodium falciparum* in days	EIP *Plasmodium vivax* in days	PSS *Plasmodium falciparum*	PSS *plasmodium vivax*	Expected of infective life days for *Plasmodium falciparum*	Expected of infective life days for *plasmodium vivax*
May	35.25	5.8	5.1	0.18	0.21	-2.4	-1.7
June	34.33	6.1	5.3	0.2	0.25	-2.2	-1.4
July	34	6.2	5.4	0.27	0.32	-1.4	-0.6
August	33.67	6.3	5.5	0.38	0.44	0.34	1.1
September	34.11	6.1	5.3	0.49	0.53	2.4	3.2
Mean	34.3	6.1	5.32	0.3	0.35	-3.26	-0.6

Table 7. Total collected anopheles mosquitoes by pyrethrum spray catches with their abdominal status in Lare district south west Ethiopia (May- September, 2016).

Abdominal status	*A. gambiae s.l*	*A. pharoensis*	*A. nilli*	*A. coustani s.l*	Total
Fed (F)	308	70	17	24	418
Have Gravid (HG)	117	16	4	2	140
Gravid (G)	34	7	2	1	44
Total	459	93	23	27	602
F: HG and G	2.04 : 1	3.04 :1	2.8 : 1	8 : 1	
Degree of Exophily	0.51	0.67	0.64	0.87	

Fornadel et al. (2010b) on an increased proportion of outdoor host-seeking in response to indoor residual spray or long lasting insecticide nets. It is now widely accepted that outdoor transmission will need to be addressed in order to achieve the goal of eliminating malaria from many endemic areas (Meyers et al., 2016). The finding showed relatively high degree of exophily observed for *A. gambiae s.l.,* this high degree of exophily indicates

A. gambiae s.l. revealed tendency to outdoor resting. However, the observed degree of exophily by *A. coustani s.l., A. nilli* and *A. pharoensis* was anticipated since the three Anopheles species are known exophilic species as reported elsewhere (Korgaonkar et al., 2012; Fils et al., 2010; Kibret et al., 2009; Walker, 2002).

The mean monthly parous rates were high, 50% and suggested that a large proportion of the *A. gambiae* s.l. and *A. pharoensis* of the study site had already practiced haematophagy. These findings are in agreement with those of Taye et al. (2016) and Olayemi and Ande (2008). In contrast to this, relatively low parity rate was reported by Ndoen et al. (2012). Mosquito lifespan is one component of the lifetime transmission potential of an individual mosquito. Hence, the longevity of an adult Anopheles may affect its power of transmitting malaria. The probability of daily survival of the mosquitoes remained very high throughout the five months, suggesting that *A. gambiae s.l. and A. pharoensis* are well adapted to the environmental conditions of the study area. *A. gambiae* s.l. and *A. pharoensis* are the principal and secondary malaria vectors in sub-Saharan Africa and Ethiopia, respectively. Therefore, they are well adapted to the prevailing tropical conditions in the region. Relatively higher mean life expectancy was observed in July for *A. gambiae s.l.* as compared to Taye et al. (2016). A long-lived adult female mosquito allows for increased opportunities to encounter an infected human host, the malaria parasites to multiply and reach the salivary glands after an infective blood meal (Susanna and Eryando, 2012).

Relatively high probability of surviving sporogony was envisaged in this study as compared to study conducted in Ethiopia (Taye et al., 2016) and Indonesia (Ndoen et al., 2012). This may be due to high mean annual temperature of the study period because temperature has an impact on the extrinsic incubation period of the *Plasmodium* species, as ambient temperature of the area increase, the extrinsic incubation period of *Plasmodium* species decrease (Susanna and Eryando, 2012; Olayemi and Ande, 2008).

In conclusion, this study showed that *A. gambiae* s.l. was the predominant malaria vector in the Lare district. This species is a major vector of malaria in East Africa, in particular, Ethiopia. The highest abundance parity rate of this major malaria vector was observed in August and July. Furthermore, the highest longevity and probability of surviving sporogony were in July. Consequently, national vector control programs for indoor residual spray intervention in the Lare district should be conducted in mid to late July.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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Appendix 1. Mean density/ trap nights of Anopheline mosquitoes by months (May- September / 2016) Lare District south west Ethiopia.

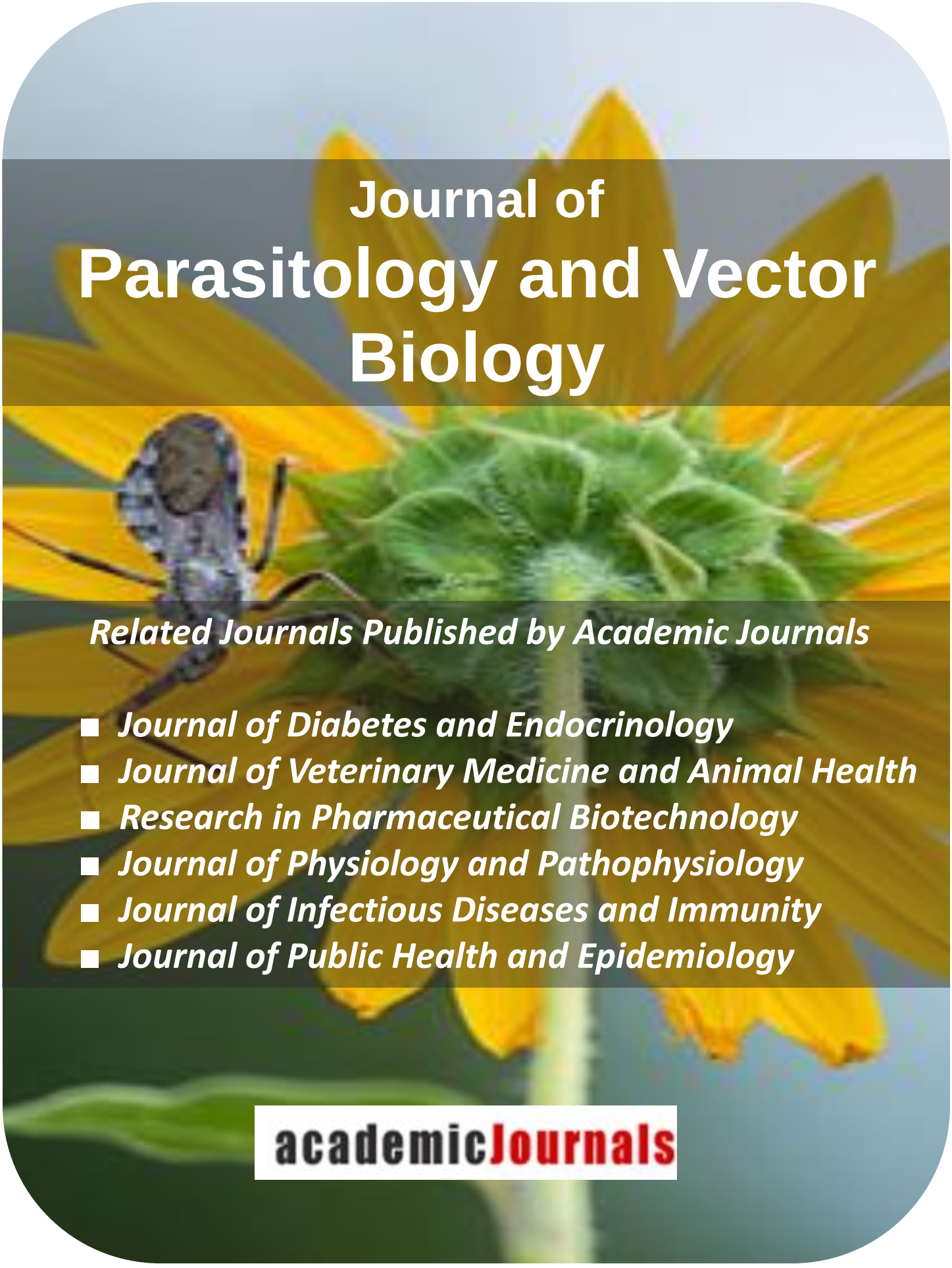
ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Total Mean density An.gambiae s.l.	Between Groups	2301.775	4	575.444	27.427	.000
	Within Groups	1153.958	55	20.981		
	Total	3455.733	59			
Total Mean density An.pharoensi	Between Groups	1076.725	4	269.181	65.648	.000
	Within Groups	225.521	55	4.100		
	Total	1302.246	59			
Total Mean density An.coustani s.l.	Between Groups	15.333	4	3.833	5.296	.001
	Within Groups	39.812	55	.724		
	Total	55.146	59			
Total Mean density An.nilli	Between Groups	66.975	4	16.744	13.874	.000
	Within Groups	66.375	55	1.207		
	Total	133.350	59			

Appendix 2. Mean density/trap-nights of Anopheline mosquitoes vs kebeles of the study site.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Total Mean density An.gambiae s.l.	Between Groups	33.787	3	11.262	.184	.907
	Within Groups	3421.946	56	61.106		
	Total	3455.733	59			
Total Mean density An.pharoensi	Between Groups	2.613	3	.871	.038	.990
	Within Groups	1299.633	56	23.208		
	Total	1302.246	59			
Total Mean density An.coustani s.l.	Between Groups	5.428	3	1.809	2.038	.119
	Within Groups	49.718	56	.888		
	Total	55.146	59			
Total Mean density An.nilli	Between Groups	5.651	3	1.884	.826	.485
	Within Groups	127.699	56	2.280		
	Total	133.350	59			



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