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STUDIES ON ANOPHELES MOSQUITOES HOST PREFERENCE AND MALARIA TRANSMISSION INTENSITY USING IMMUNOLOGICAL DIAGNOSTIC METHODS IN GILGEL-GIBE DAM AREA, SOUTHWESTERN ETHIOPIA

BY

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TABLE OF CONTENTS

Contents **Pages**

LIST OF TABLES

Tables **Pages**

LIST OF FIGURES

Figures **Pages**

LIST OF PLATES

Plates **Pages**

LIST OF APPENDICES

Appendices **Pages**

LIST OF ABRIVIATIONS

ANOVA	Analysis of variance
HBI	Human blood index
EIR	Entomological inoculation rate
ORC	Outdoor resting collection
HPI	Host preference index
LTC	Light trap catches
HCC	Hand capture collection
IRC	Indoor resting collection
CDC	Center of diseases prevention and control

ABSTRACT

The Host feeding pattern and sporozoite rate of Anopheline mosquitoes were determined for mosquitoes collected from September to November 2010 in Gilgel-Gibe hydroelectric dam area, southwestern Ethiopia. Mosquitoes were collected from 16 villages surrounding the dam by hand-capture and light trap catches. Mosquitoes resting outdoors were also collected from potential natural resting sites and artificial pit shelters. Overall, 520 anopheline mosquitoes were collected. Anopheles arabiensis was the most predominant (88.65%) species followed by An. coustani s.l (7.12%) and An. demelloni (4.23%). All the 520 mosquito specimens were tested for circumsporozoite protein (CSP) and host feeding pattern using ELISAs. Of those tested for CSP, 10 specimens were found positive for Plasmodium vivax infection. None were found positive for P. falciparum. The CSP rate was 1.92 and entomological inoculation rate (EIR) was 9 infectios bites/person/month. Moreover, a single specimen of An. coustani s.l was screened positive for Plasmodium CSP. Results of blood meal ELISA showed that An. arabiensis have a wider host range and more opportunistic feeder than the other two anopheline species. The human blood index (HBI) of An. arabiensis was 4.12%, but no human blood was found in the other two species. The host preference index and forage ratio were higher for Equine for all tested species, indicating that equine was the most preferred host by the three mosquito species. An. arabiensis fed more on equine, bovine and ovine than human though the number of humans was significantly higher in the study area. Thus, zoo prophylaxis can be employed as possible vector control strategy. Multiple blood feeding was observed in all the 3 mosquito species and this multiple blood feeding behavior especially, in An. arabiensis population might have resulted in elevated EIR in contrast to the low HBI.

Keywords: Anopheles, Blood meal, ELISA, *An. arabiensis* , sporozoite

1. Introduction

1.1. Background

Anopheles mosquitoes belong to Phylum Arthropoda, class Insecta, order Diptera, family culicidae and genus Anopheles. There are different species of mosquitoes throughout the world (Richards and Davies, 1977; Harbach, 2007). Several insects are known to be vectors of human diseases. Mosquitoes in particular take the highest share of having been the first insects to be associated with the transmission of a disease. It now seems almost incredible; it was not until near the end of the last century that blood-sucking insects were conclusively shown to be vectors of diseases, although for many hundreds of years previously they had been seen with suspicion. Malaria for instance was believed in both Africa and India to be mosquito borne at a time when in Europe it was confidently attributed to be Miasma, bad air "Mal'air" arising from marshes (Askew, 1971). In this world, diseases that can spread by mosquitoes are: Malaria, Murray Valley encephalitis, Dengue fever, Japanese encephalitis and several viral diseases like West Nile Virus, Ross River Virus, Barmah Forest Virus with malaria being the most life treating disease transmitted by mosquitoes (Askew, 1971; Tolle, 2009). According to Stacey *et al*. (2009), mosquitoes are not only vector of human diseases, but also vector a variety of pathogens of medical and veterinary importance.

Worldwide, there are many species of mosquitoes. However, only females of one genus, *Anopheles*, transmit human malaria. Out of the total of 430 *Anopheles species* documented, 30 to 40 species are known to transmit malaria (IWMI, 2010). Climate change effects on African malaria vectors shift their distributional potential from west to east and south, which has implications for overall numbers of people exposed to these vector species. *Anopheles gambiae s.s* and *An. arabiensis* are the two most important malaria vectors in Africa, having a broad distribution.

Current human populations coincide spatially with the inferred geographic distributions of these vectors (Peterson, 2009). The distribution of the *Anopheles gambiae* complex of malaria vectors in Africa is uncertain due to under-sampling of vast regions. Distribution of seven members of the complex (*An. gambiae s.s*, *An. arabiensis*, and *An. quadriannulatus species A, An. bwambe, An. melas, An. merus and An. quadriannulatus* species B) were predicted. Predictions correspond well to previous estimates, but provide detail regarding spatial discontinuities in the distribution of *An. gambiae* s.s. that are consistent with population genetic studies. These predictions also identify large areas of Africa where the presence of *An. arabiensis* is predicted, but few specimens have been obtained, suggesting under-sampling of the species (Levine *et al.*, 2004). The collection of information from 44 countries in Africa containing 4,234 geo-referenced, independent sites of predicted geographic reference for Africa, indicated *An. arabiensis*, *An. funestus*, *An. gambiae*, *An. melas*, *An. merus*, *An. moucheti* and *An. nili*, are the predicted malaria vectors distributed in Africa (Sinka *et al.*, 2010).

In sub- Saharan Africa, *An. gambiaes.s and An. arabiensis are* considered the most efficient vector of malaria due to their socio economical importance. Vector control interventions, combining insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS) have suppressed *An. gambiae* populations in some malaria-endemic areas in sub-Saharan Africa. However, in other areas, such as the western part of Kenya, while the impact of ITNs and IRS on *An. gambiae* has been dramatic, these intervention methods have had very little impact on the populations of *An. arabiensis.* There is growing evidence that the void created by the low population s of *An. gambiae* is being filled by *An. arabiensis* since current malaria infections in these areas are caused by the latter rather than the former species (Ignell, 2010). Historically, members of the *An. gambiae* complex from Ethiopia have been identified chromosomally as either *An. arabiensis* or *An. quadriannulatus* species B (Hunt *et al.*, 1998).

1.2. Malaria disease

Malaria (mal' air, bad air), a life- threading disease is probably one of the oldest diseases known to mankind. The parasite responsible for malaria are *Plasmodium* species, represented by 150 species, transmitted by the bites of mosquito vectors to human, simians, rodents, birds and reptiles (Krettli *et. al.*, 2004)

Globally, at the end of 2004, 107 countries' territories had areas at risk of malaria transmission. Some 3.2 billion people lived in areas at risk of malaria transmission. An estimated 350–500 million clinical malaria episodes occur annually; most of these are caused by infection with *P. falciparum* and *P. vivax*. Falciparum malaria causes more than 1 million deaths each year (WHO, 2005). Malaria is one of the world most serious and complex public health problems (Ashenafi Woime, 2008). It contributes indirectly to many additional deaths, mainly in young children, through synergy with other infections and illnesses. Malaria is also a major cause of anaemia in children and pregnant women, low birth weight, premature birth and infant mortality (WHO, 2005).

About 60% of the cases of malaria worldwide, about 75% of global falciparum malaria cases and more than 80% of malaria deaths occur in Africa south of the Sahara. *P. falciparum* causes the vast majority of infections in this region and about 18% of deaths in children under 5 years of age. In endemic African countries, malaria imposes a great burden on already fragile health-care systems (WHO, 2005). It is still one of the leading causes of morbidity and mortality in developing countries (Tilahun Nigatu *et al.*, 2009). World Health Organization (WHO) estimates that malaria kills an African child every 30 seconds (WHO, 2003). It causes human misery and is a serious burden to the very often fragile economies in the tropical regions (Leopold and Milek, 1999). It is the most well known of the climate sensitive diseases.

Epidemics of malaria are often triggered by climate anomalies and climate informed early warning systems have been advocated in recognition of this (Connor *et al.*, 2007).

Africa bears the greatest burden of malaria worldwide and it is estimated that almost 125 million people live in areas prone to malaria epidemics (Connor *et al.*, 2007). As well as bringing numerous benefits, increased dam-building in Africa will also bring costs, which are often borne by poor people living close to the dams and the reservoirs created. One of the most lethal consequences of dams is increased malaria prevalence (IWMI, 2010). In sub-Saharan Africa, malaria epidemics arise suddenly in mostly remote, disadvantaged settings without effective alert systems (Checch *et al.*, 2006)

1.3. Anopheline mosquitoes and malaria transmission

Mosquitoes go through four stages in their life cycle: egg, larva, pupa and adult. The first three stages live in water and last for 5 to 14 days in tropical settings, depending on the species and environmental factors (IWMI, 2010). Scientists have known for decades that mosquitoes are drawn to carbon dioxide, exhaled in abundance by the animals that hungry mosquitoes favor. Carbon dioxide doesn't tell the whole story. Mosquitoes, after all, tend to bite people on the arms and legs due to lactic acid produced by the skin (Corinnawu, 2000).

During the adult (flying insect) stage (which lives up to a month in favorable conditions) the female mosquitoes bite people in order to ingest the blood they need to produce eggs. Stimuli from the ingested blood meal are required for the proportion of ovarian development. The two types of stimulus, physical and chemical have been proposed to explain the triggering of hormone release by the blood meal and there is experimental evidence that both abdominal digestion and exposure to digestion products are involved.

Both male and female feed on plant juice and it is the only energy source for males (Clemets, 1992). Anophelines generally feed just once per gonotrophic (oviposition) cycle. Newly emerged insects, however, may feed two or more times during their first oviposition cycle thus increasing the likelihood of becoming infected (Charlwood *et al.*, 2003). This provides the link between the human host and female *Anopheles* mosquitoes in the malaria parasite life cycle (IWMI, 2010). Hence, malaria transmission involves interactions between the malaria parasite (i.e. *Plasmodium*), *Anopheles* mosquitoes and people.

When a female *Anopheles* mosquito takes blood from a malaria-infected person, together with the blood she also swallows the *Plasmodium* parasite (IWMI, 2010). Over a period of 2 to 4 weeks, large numbers of sporozoites (i.e., the infective stage of *Plasmodium*) are formed and migrate to the salivary gland of the mosquito. Whenever this mosquito takes blood from a person, she injects her saliva containing the malaria parasites into the person's bloodstream (IWMI, 2010).

Within the infected person, the *Plasmodium* parasites multiply in red blood cells causing symptoms (IWMI, 2010). Fever, headaches, chills and shivering were the most frequently mentioned symptoms of malaria (Wakgari Dheresa *et al.*, 2000). If this person is bitten by another mosquito, then the cycle is repeated (IWMI, 2010). People are familiar with the symptoms of malaria and to a lesser degree, are aware of an association between mosquito and malaria (Wakgari Dheresa *et al.*, 2000).

Control measures against infective mosquito bites have a major beneficial impact on malaria morbidity and mortality. Implemented vector control strategies should provide a community-wide health impact that will vary effectively between localities with consideration of high proportions of the disease vector abundance and transmission level.

Malaria vector feeding and resting behavior should be taken into consideration for initiation of malaria control strategies (Kweka *et al.*, 2008). Understanding the factors that determine the feeding behavior of vectors is of paramount importance for reducing the impact of disease caused by vector borne pathogens (Kilpatrick *et al.*, 2007).

2. Literature Review

2.1. Malaria in Ethiopia

Ethiopia is the second most populous country in Africa. Due to widespread poverty combined with highland and desert-fringe terrain, it is the country with the highest proportion of the population (more than two thirds) (Figure 1) living at risk of malaria epidemics (Connor *et al.*, 2007). Almost 68% of the populations in Ethiopia live in malarious areas covering almost 75% of the land (FDROEMOH, 2006; PMI, 2010). The diverse eco-climatic conditions in the country make the malaria transmission pattern seasonal and unstable usually characterized by frequent focal and cyclic widespread epidemics (FDROEMOH,

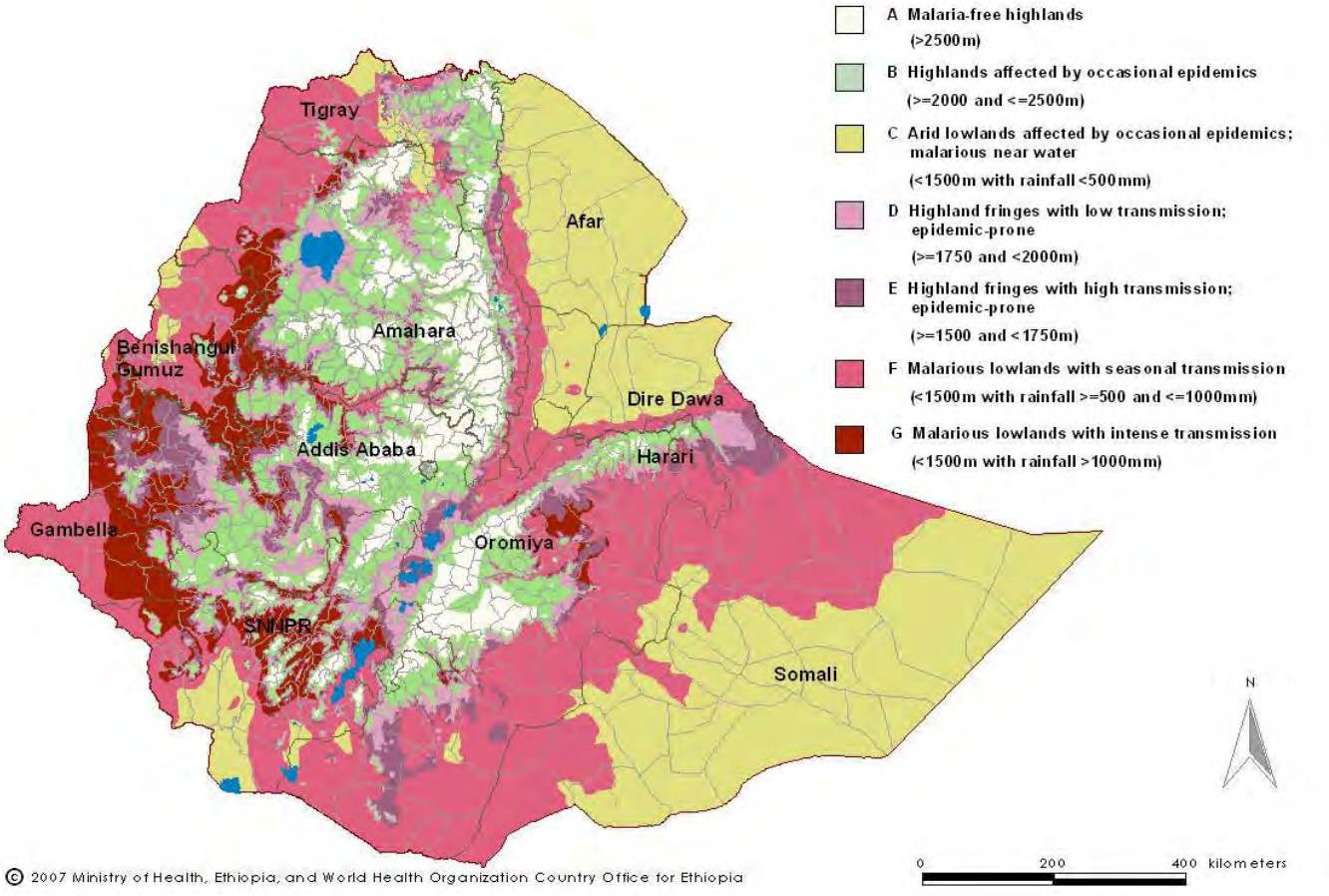


Figure 1: Distribution of endemic malaria in Ethiopia (*source*: Ethiopian Ministry of Health and World Health Organization, 2007).

Malaria is a major concern in the country since it is one of the leading causes of morbidity and mortality (Tilahun Nigatu *et al.,* 2009; PMI, 2010). Annual malaria mortality is about 70,000 and in a non epidemic year, 5-6 million clinical malaria cases and over 600,000 confirmed cases are reported from health facilities. However, the actual number of malaria cases in the country is expected to be more than ten-fold of those captured through the routine public health surveillance system (Tilahun Nigatu *et al.,* 2009). Over the last five years (2001 – 2005) the proportion of malaria in out patient department, admission and in-patient deaths has been increasing with the highest being recorded in 2003 and 2004 while a slight reduction was observed in 2005. An estimated 36% of the population is out of the reach of the health service coverage. Based on these scenarios, the actual number of malaria cases that might occur annually throughout the country is estimated to be higher. The highest burden of malaria (30-34% increase) was observed in 2003 and 2004. The high case fatality rates were particularly observed during the 2003 epidemics, particularly in the Oromiya, Amhara, Tigray, and SNNP regions (FDROEMOH, 2006).

Despite the current efforts to control malaria in Ethiopia, the situation has not improved mainly due to the increasing problems of insecticide resistance (Meshesha Balkew *et al.,* 2010; Delenasaw Yewhalaw *et al.,* 2010a and 2011) and drug resistance (Checchi, *et al.*, 2006; Tilahun Nigatu *et al.,* 2009). According to Tilahun Nigatu *et al.* (2009), vector resistance to insecticides, low coverage of malaria preventive services, poor access to health care, rudimentary health service infrastructure, large population movements and limited financial and human resources also impose other burdens in controlling malaria.

Transmission is seasonal and predominantly unstable. The major transmission of malaria follows the June – September rains and occurs in the period from September – December, while the minor transmission season occurs in April – May following the February – March rains. The bimodal malaria transmission pattern is limited to areas that receive the small "*Belg"* rains and are mainly located in the eastern part of Ethiopia, while the major malaria transmission occurs in all areas at risk of malaria (FDROEMOH, 2006).

2.2. Anopheline species and their distribution in Ethiopia

In southern Ethiopia, seven *Anopheles* species were identified from all night landing collections, conducted from 18:00 to 06:00 h between October 2001 and August 2002. The predominant species was *An. arabiensis* (55.8%), followed by *An. coustani* (31.5%), *An. pharoensis* (9.5%), *An. funestus* (2.2%), *An. nili* (0.5%), *An. marshallii* (0.4%) and *An. demeilloni* (0.2%) *(*Aseged *et al.*, 2006). Again in Sille, south western part of Ethiopia, *An. gambiae s.l.* was the principal species collected, followed by very low densities of *An. funestus*, *An. nili* and *An. pharoensis* (Ribeiro *et al.*, 1996). According to Birhan Teklu *et al.* (2010), four *Anopheles* species were identified in Ziway area of central Ethiopia: *An. pharoensis*, *An. gambiae* s.l. *An. coustani* and *An. squamosus*.

At least eight anopheline species which differed in their habitats were found to be prevalent in Gambella region. These are: *An. gambiae, An. pharoensis*, *An. funestus*, *An. nili, An. coustaini, An. pauraludis*, *An. ziemanni* and *An. squamasus* (Wandatir Nigatu, 1994).

The major malaria vector known in Ethiopia is *An. arabiensis.* In some areas *An. pharoensis*, *An. funestus* and *An. nili* also transmit malaria (FDROEMOH, 2006). In addition to these, *An. coustaini, An. pauraludis*, *An. ziemanni* and *An. d`thalia* have been recorded to possess vector capacity (Ashenafi Woime, 2008).

2.2.1. ***Anopheles arabiensis***

An. arabiensis is considered as a major malaria vector and is widely distributed in Ethiopia (Nyanjom *et al.*, 2003; Ashenafi Woime, 2008). In the study conducted in Ziway reservoir area of central Ethiopia, the mean larval density of *An. gambiae* s.l. Was higher in slightly turbid and shallow aquatic habitats than in turbid and relatively deep aquatic habitats .The occurrence of *An. gambiae* s.l. was also greater than that of the non-reservoir village in which the effect of the dam was not observable. *An. gambiae* s.l. occurred in shallow slightly turbid habitat with emergent vegetation and open sunlit conditions (Birhan Teklu, *et al.*, 2010). It breeds in small, temporary, sunlight pools and become abundant at the beginning and end of the big rainfalls. It is found in both lowland areas as well as in the highland areas up to 2000 m above sea level (Ashenafi Woime, 2008).

2.2.2. Anopheles pharoensis

It is the second most frequent and widely distributed vector of malaria in Ethiopia. It breeds in large, permanent and shaded water bodies with emergent vegetation. Lake shores and irrigation canals are also favorable breeding places. The 1984-88 study shows that 18.9 % of the collected specimens were *An. pharoensis* and of which 65.1 % feed outdoors and 88.5 % rest indoors. It is found along river Baro and Awash, in Lake Tana and in the rift valley lake regions (Ashenafi Woime, 2008).

In Ziway, central Ethiopia, the occurrence of *An. pharoensis* was greater in the villages neare to reservoir than in the villages farther away from the resservoir mainly because of the availability of shoreline puddles suitable for breeding. *An. pharoensis* was greater in turbid aquatic habitat with floating vegetation (Birhan Teklu *et al.*, 2010).

2.2.3. Anopheles funestus

It is the third most common vector of malaria comprising 3.2 % of all the specimens collected during the period 1984-88. It dominates in areas where malaria is endemic especially along rivers, around lakes and shaded swamps in the lowlands where altitude is between 1000 m and 1500 m. About 76.2 % of the specimens collected feed outdoor and 63.5 % of them rest outdoors. This vector is found along river Baro, Lake Tana and the lake regions of Shoa and Sidamo (Ashenafi Woime, 2008).

2.2.4. Anopheles nili

It is the least common and more localized species and is not adequately studied except in Gambella. It is found in the south western, western and north western parts of Ethiopia along river Bilate, the Segan river valley and the Baro River (Ashenafi Woime, 2008).

2.3. Plasmodium species in Ethiopia

Virulent Plasmodium species include *Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Plasmodium falciparum*. Most cases of malaria are caused by *P. falciparum* and *P. vivax*, with *P. falciparum* inducing the most severe symptoms (Tran and Saier, 2004).

Three human malaria species are confirmed to be present in the country and transmitted between individuals by *Anopheles species*. *P. falciparum* and *P. vivax* are the most dominant malaria parasites in Ethiopia. They are predominant malaria parasites in the country and they account for 60% and 40% of the malaria cases, respectively (FDROEMOH, 2006) but their relative prevalence rate of the two species varies according to localities and seasons (Delenasaw Yewhalaw *et al.,* 2009). *P. malariae* accounts for less than 1% and *P. ovale* has never been reported from health facilities with the only report being that of Armstrong in 1969 (FDROEMOH, 2006).

2.4. Plasmodium life cycle

Malaria infection occurs when an infected Anopheline mosquito injects sporozoites into the skin of a mammalian host while probing for a blood meal. The initial phase of malaria infection is the pre-erythrocytic phase, which begins when parasites are injected by the mosquito into the dermis and ends when parasites are released from hepatocytes into the blood (Amino *et al.,* 2007). These sporozoites enter the blood stream and are transported to the liver, where they invade hepatocytes and transform into exoerythrocytic forms (EEFs). After multiple rounds of replication, each EEF contains thousands of merozoites which are released into the blood stream and rapidly invade erythrocytes, thus initiating the erythrocytic stage of the life cycle which is responsible for the symptoms of the disease (Sinnis and Coppi, 2007).

Within minutes after a mosquito ingests an infected blood meal, gametocytes emerge from red blood cells and differentiate into male and female gametes. After fertilization, zygotes differentiate into ookinetes that move within the blood bolus. After crossing the peritrophic matrix and the midgut epithelium, the ookinetes lodge beneath the basal lamina, facing the hemocoel, and differentiate into oocysts. Each oocyst undergoes about 12 rounds of nuclear divisions to produce thousands of sporozoites that, upon oocyst maturation, are released into the hemocoel **(**Eappen *et al.,* 2004: Figure 2)

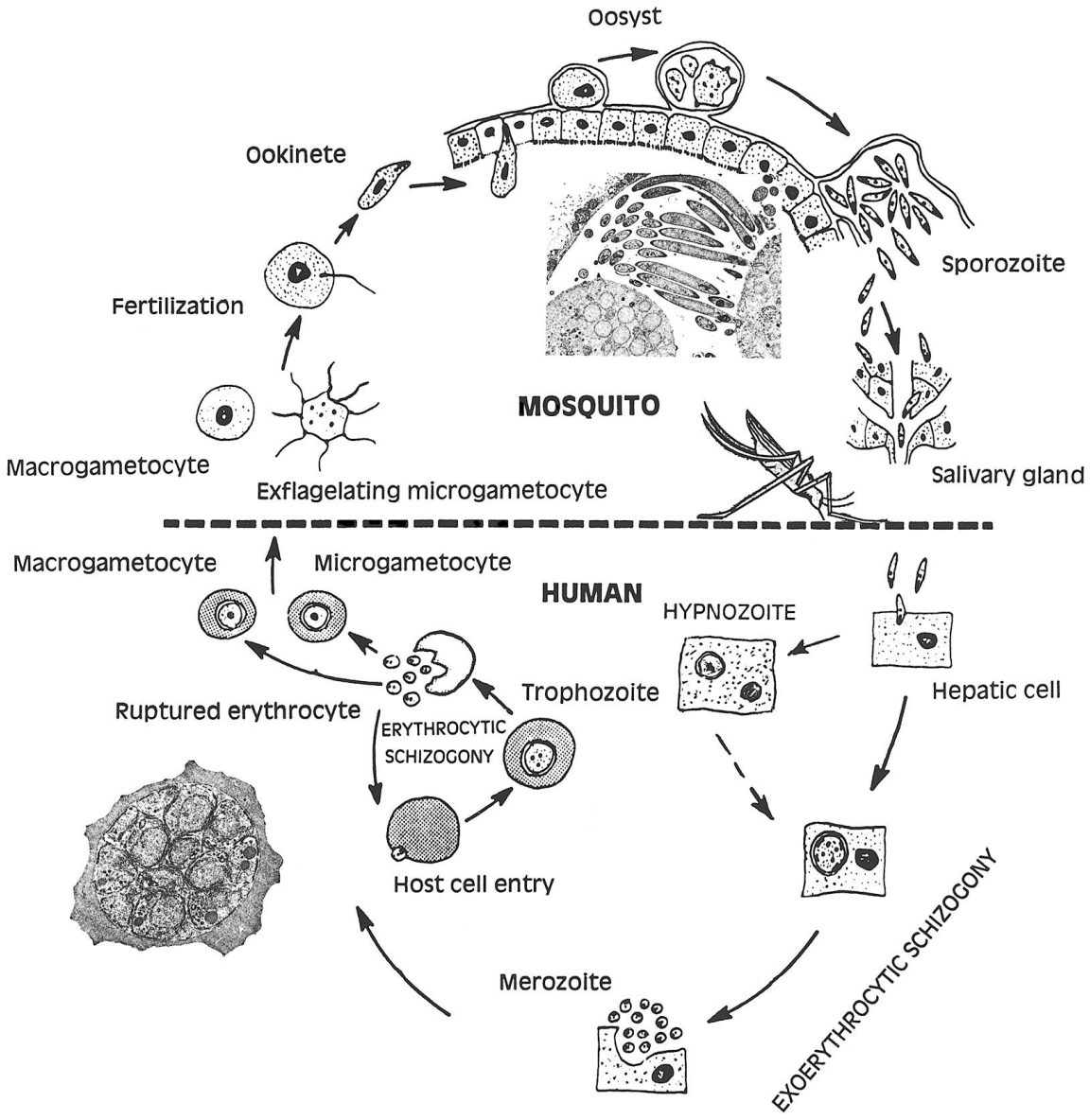


Figure 2: Malaria disease parasite structure and life cycle
Source: Fujiokaa and Aikawab (2002).

In summary, malaria sporozoites bind tightly to the sinusoidal cell layer, cross Kupffer cells, and leave behind a trail of dead hepatocytes when migrating to their final destination in the liver (Frevert *et al.*, 2005).

Development of a vaccine against malaria presents formidable obstacles in terms of parasite Biology and host immune responses. Different Antigens (Ags) that may be targeted by protective immune responses are expressed during the different stages of the parasite's complex life cycle, and only a limited number of epitopes are contained within a relatively small number of Ags (Hoffman *et al.,* 2000).

2.4.1. Plasmodium diagnostics

Enzyme-linked immunosorbent assays (ELISAs) were developed to detect *Plasmodium* circumsporozoite (CS) proteins in malaria-infected mosquitoes. The sensitivity and specificity of the ELISAs are based on the monoclonal antibodies (Mabs) used. The ELISAs detect CS proteins, which can be present in the developing oocysts, dissolved in haemolymph, and on sporozoites present in the haemocoel or in the salivary glands. ELISAs can be carried out on fresh, frozen, or dried mosquitoes. If specimens are to be dried, they must be processed quickly and kept dry (stored with desiccant) to prevent microbial growth that can affect results. After the substrate has been added, 30 and or 60 minutes later, results are read visually or at 405-414 nm using an ELISA plate reader (Wirtz *et al.,* 2007).

Results of ELISA were almost similar with PCR-RFLP (27% versus 34%) (Maleki-Ravasan *et al.*, 2009). Without an adequate DNA extraction protocol, the identification of *Plasmodium* species in whole mosquitoes by PCR is difficult because of the presence of reaction inhibitors from the insects (Lardeux *et al.*, 2008). Anopheline mosquitoes contain substances that can inhibit PCR amplification aimed at detecting *Plasmodium* parasites.

However, a loss in the sensitivity of parasite detection (<1000 parasites) was observed only when the insect material from which the DNA template was purified included the head and the thorax.

The inhibitors are present mainly in the hard exoskeleton of the head and the thorax. The inhibition associated with the use of non dissected specimens could be abrogated by some but not all DNA template preparation methods. Sensitive detection of malaria parasites in their insect vector can be achieved using whole mosquitoes, provided that the method used to obtain the DNA template is tested for its efficacy at removing inhibitors (Arez *et al.*, 2000).

The present protocol is based on a chelex extraction which overcomes the PCR inhibition phenomenon. The semi-nested multiplex PCR technique detects and distinguishes among the four human *Plasmodium species* in single mosquitoes and in pools of up to 100 mosquitoes (Lardeux *et al.*, 2007a).

The other option in diagnosing plasmodium in mosquitoes is dissection. Dissection of the mid-gut to observe oocysts should be performed 5-7 days after infection occurs. The precise number of days that is best will vary with parasite species (Benedict, 2007).

In most mosquitoes varying numbers of encapsulated and unencapsulated (normal) oocysts are found. Encapsulated oocysts appear as disk-shaped structures in the mid gut (small melanized particles) (Figure 3, right panel). Normal oocysts appear as large spherical objects, usually slightly lighter in coloration and attached to the apical surface (Figure 3, left panel) (Benedict, 2007).

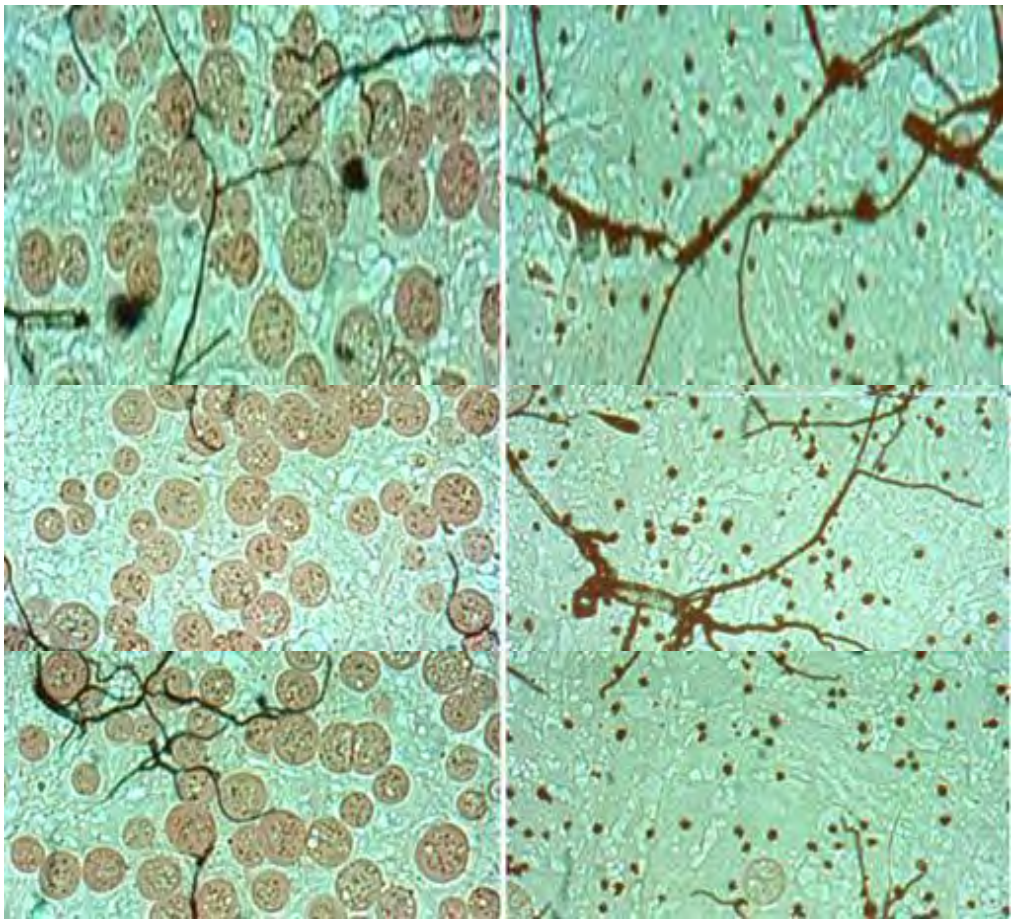


Figure 3: Microscopic structure of sporozoite in mosquito gut
Source: (Collins *et al.* 1986: cited in Benedict (2007))

According to Wakgari Dheresa (2010), diagnostic tools of malaria for suspected human patient includes: Microscopy, rapid diagnostic test (RDT) and clinical diagnosis. The most reliable method for diagnosing malaria is microscopic examination of patient's stained blood film by a trained microscopist (WHO, 2004).

3. Objectives

3.1. General objective

- To assess host-feeding pattern of anopheles mosquito species and malaria transmission intensity in Gilgel-Gibe dam area, southwestern Ethiopia in order to recommend malaria vector control intervention strategies in the study area.

3.2. Specific objectives

- To determine host preference and human blood index of mosquitoes using blood meal ELISA in the study area.
- To determine infectivity rate of anopheline mosquitoes using sporozoites ELISA.
- Malaria transmission intensity in the study area.

4. Materials and methods

4.1. Study area and period.

The study area is located 260 km south-west of Addis Ababa in Oromiya Regional State, southwestern Ethiopia, near Gilgel-Ghibe hydroelectric dam, which started operating in 2004. The study area lies between latitudes 7°42’50"N and 07°53’50"N and longitudes 37°11’22"E and 37°20’36"E, at an altitude of 1,672-1,864 m above sea level. The study villages are located in Sekoru, Tiro-Afeta, Omo-Nada and Kersa districts (woredas). Houses are traditional type constructed of mud and wood, the majority with thatched roofs and a few with corrugated iron sheets (Plate 2A).

The area has a sub-humid, warm to hot climate, receives between 1,300 and 1,800 mm of rain fall annually and has a mean annual temperature of 19°C. The rainfall pattern of the area is similar to other parts of Ethiopia with the long rainy season starting in June and extending up to September, while the short rainy season begins in March and extends to April/May. Vegetation type of the area is mainly of broadleaved type consisting of trees like *Cordia africana* and eucalyptus trees planted by farmers (Plate 1). Where there is very low vegetation cover little grass land type predominates. Soil type is arid red clay soil with available under ground water within about nine meters of depth which is characterized by high water retention capacity. Local farmers usually prepare traditional bricks from this soil for ornamental house decoration purpose.

The main economic activities of the local communities are mixed farming involving the cultivation of crops and animal rearing. Staple crops like maize (main crop), teff and sorghum are cultivated in combination with cattle, sheep, goat and small traditional poultry rearing.

The main cash crops of the area are coffee and pepper (*Capsicum annuum*). Other fruit crops like papaya, mango, avocado and banana are also supporting the farmer's economy in the area.

Plate 1: Maize and Sorghum plot indicating Agro-ecological picture of the study area (photograph taken from study site)

4.2. Mosquito sampling and identification

Monthly entomological surveys were conducted from September to November 2010 in sixteen villages around Gilgel-Gibe hydroelectric dam area, Southwestern Ethiopia. Mosquitoes resting indoor were collected using indoor resting catches (IRCs) aspirator and by light trap catches (LTCs) using CDC light traps from sentinel houses selected randomly in each of the sixteen villages. For hand capture collection, 10 sentinel houses were selected randomly in each of the sixteen villages and mosquito collection was done by two collectors who spent 15-20 minutes inside each house using mouth aspirators early in the morning (Plate 2A). Mosquitoes were also collected from two additional sentinel houses selected randomly in each of the sixteen villages by LTCs using CDC miniature light traps (John W. Hock Company, Model 512).

Traps operated by batteries were hung 1.5 meters from the bed/sleeping ground of the sleeper in each house and run from 6:00pm to 6:00am (plate 2C). Outdoor resting catches (ORCs) was carried out from all potential natural resting sites (animal burrow, tree hole, tree buttress, among vegetation) and from standard artificial pit shelters (WHO, 1975) dug in 4 villages by aspirator (Plate 2B).

A

B

C

Plates 2: The three mosquito collection methods (A=IRCs, B=ORCs,C= LTCs) **:**photographs taken from collection sites

All mosquitoes collected from all villages and by all collection methods were then sorted by sex and kept in paper cups plugged with moist cotton. Mosquitoes were morphologically identified at Asendabo vector biology laboratory using standard keys (Gillies and Coetzee, 1987). The identified samples were then labeled and kept individually in eppendorf tubes over silica gel for further immunologic and molecular assays.

Potential hosts and humans slept inside each sentinel house of the sixteen study villages were recorded. Attempts were also made to record available hosts found outside each sentinel houses. Potential hosts recorded in the study villages included: dogs, chickens, equines (horses, and donkeys) bovines (cow ox and calf) and ovines (sheep and goats)

4.3. ELISA diagnostic tests

4.3.1. Sporozoite ELISA

All Adult female *Anopheles* species collected during the 3 months entomological survey were tested by Enzyme Linked Immunosorbent Assay (ELISA) for Plasmodium circumsporozoite protein following the method described in Wrtiz *et al.* (1987). The head- thorax of each mosquito specimen was separated and then ground in blocking buffer containing IGEPAL CA-630 to make mosquito homogenate using a pestle and 1.5 ml grinding tube. 50 µl of species- specific monoclonal antibody (Mab) was added to each wells of micro ELISA plate. After the wells of the plate were coated with capture Mab, the well contents were aspirated and the remaining binding sites were blocked with blocking buffer. After incubation at room temperature for 1 hr, the mosquito homogenate was transferred to the wells followed by aspiration and washing. Peroxidase-linked Mab was then added to the wells. After 2 hours, the well contents were aspirated and washed again.

Finally, Peroxidase substrate solution, ABTS (Kirkegaard and Perry Laboratories Inc.) was added.

Positive and negative controls were also added to specific plate wells. The plates were incubated for 1 hr and positive wells were read visually.

4.3.2. Blood meal ELISA

Each mosquito abdomen separated from head-thorax was homogenized in Phosphate buffer saline (PBS) using a pestle and 1.5 ml grinding tube. The homogenized samples in PBS were diluted to 1:50. Then, 50 µl of the diluted sample was added into wells of micro ELISA plate, After incubation at room temperature for 3 hrs, the homogenate was discarded and the plate was washed with PBS–Tween20 (two times for each sample). 50 µl peroxidase conjugate antibodies of human, dog, horse, chicken, goat and 50 µl phosphatase conjugate antibodies of bovine (SIGMA-ALDRICH) were added to respective wells of micro ELISA plate. After adding peroxidase conjugated antibodies, plates were covered and incubated for 1 hr at room temperature and washed three times with PBS–tween 20. Then, 100 µl ABTS peroxidase substrate solution was added and the results were read visually after 30 minutes. Content of wells turned green color were considered as positive for the test. In this assay, double testing system was employed for humans and bovine as described in *Beier et al.* (1988). Thus, after reading the results for human blood, the wells of micro ELISA plate were washed again three times with Tween-20 and then 100 µl pNPP phosphatase substrate solution (SIGMA_ALDRICH) was added to wells of micro ELISA plate in which primarily human peroxidase conjugate and phosphatase conjugates were added. Finally, results were read again visually after incubating for an hour and development of yellow color in the content of wells were considered as positive for bovine blood meal.

Blood samples collected from local butcher houses on whatman paper from live hosts were used as positive control. Humans' blood smear was also taken by finger-brick and put on pieces of Whatman (Plate 3).

Then, a piece of Whatman paper with blood smears was macerated in a tube using 50 µl PBS and the homogenate was diluted to 1:50.

Wells of the micro ELISA plate were coated by adding 50 µl of the diluted homogenate. PBS was used as negative control in the assay (Birley and Charlwood, 1986; Lardeux, 2007b).

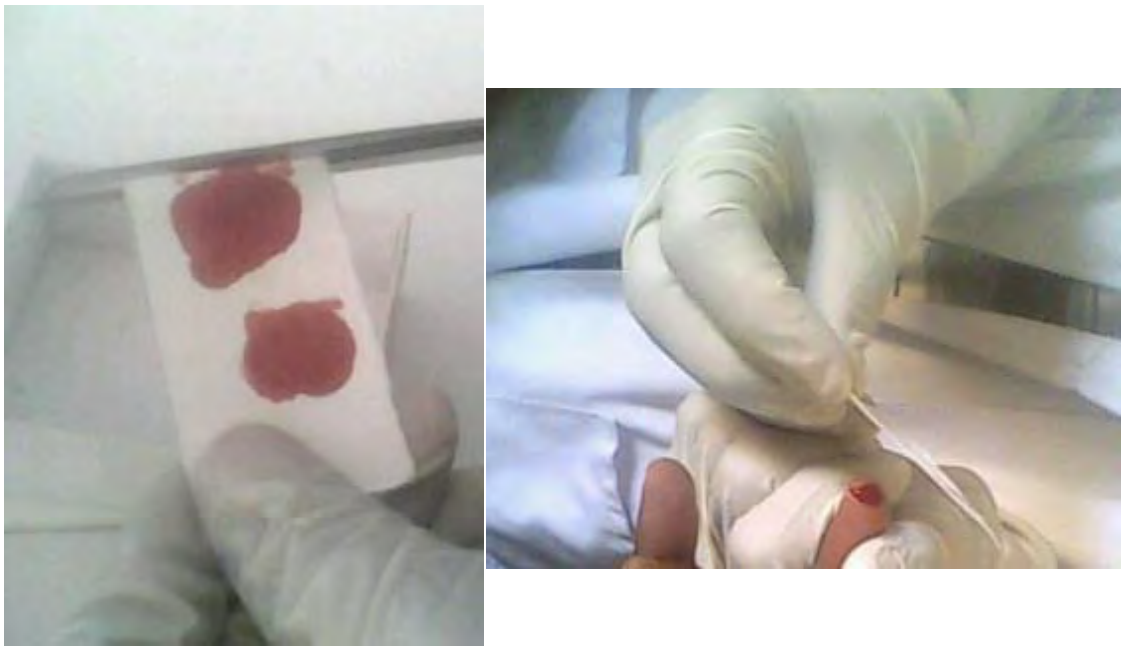


Plate 3: Human blood smears preparation for positive control in blood meal ELISA (photographs taken during laboratory work)

4.4.1. Data analysis

Data were entered into a computer and cleaned and checked for completeness and consistency. Then, it was analyzed using Excel and SPSS version 16.0 software packages and one-way ANOVA was used for multiple mean comparisons. Infectivity rate, host blood antigen detected and host proportion were expressed as percentage. $\alpha < 0.05$ was considered significant during the analysis .

4.4.2. Entomological data analysis

Data were presented graphically for vector and potential host distribution and blood feeding pattern. Host preference index (HPI) was calculated following a standard formula (WHO, 1975) and forage ratio (FR) was also calculated as described in Hess *et al*. (1968).

Entomological inoculation rate (EIR) was calculated using the formula described in Drakeley *et al*. (2003) and modified by Kulkarni *et al.* (2006), for infectious bits per person per year (i/p/yr):
EIR (i/p/yr)=1.6 (number of sporozoite positive ELISA test from CDC)/ number of CDC light trap catches x 365. Similarly, EIR was calculated per month using the same formula.

Sporozoite rate (SR) = number of sporozoite positive ELISA tests from all collection techniques/ total number of tests x 100.

4.5. Ethical Clearance

Ethical clearance was obtained from WHO/TDR review ethics committee and Jimma University research and ethics committee.

5. Results

5.1. Species composition, abundance and distribution of anopheles mosquitoes

A total of 520 mosquitoes were collected from September to November 2010 from all sixteen study villages using three collection techniques. Of these, 461 (88.65%) *An. arabiensis* Patton , 37 (7.12%) *An. Coustan*i s.l Laveran and 22 (4.23%) *An. demeilloni* Evans were morphologically identified. The majority (98.85%, 514/520) of the mosquitoes were collected from inside houses. Of these indoor collected mosquitoes, 48.07% (250/520) were from Indoor resting catches and 50.77% (264/520) from light trap catches. Outdoor collected mosquitoes accounted only 1.15% (6/520) of the total mosquito collection (Table 1).

Anopheline mosquitoes showed a reduction in number from September – November, 2010 and the highest number of *An. arabiensis* was recorded in September which was 403 and reduced to 3 in November 2010. Almost similar pattern was seen in vector reduction for *An. coustani* s.l which was 12 in September and reduced to 2 in November but highest number for this species was recorded in October (Appendices, 1, 2 and 3).

Table 1: Mosquito species composition and abundance in16 villages in Gilgel-Gibe hydroelectric dam area, southwestern Ethiopia (September-November, 2010)

Locality	*An. arabiensis*			*An. coustani* s.l			*An. demeilloni*		
	IRC	LTC	ORC	IRC	LTC	ORC	IRC	LTC	
Ossobili	37	25	3	3	0	0	0	0	
Budo*	15	6	0	1	1	0	20	0	
Gelan*	2	5	0	2	1	0	0	0	
Dogosso*	1	2	0	1	0	0	0	1	
Koticha*	5	5	0	0	0	0	0	0	
Togo*	13	14	0	0	0	0	0	0	
Gommo*	1	6	0	0	0	0	0	1	
Dalu*	24	5	1	0	0	0	0	0	
Bisyola	35	37	0	0	1	0	0	0	
Yebo	20	7	0	1	3	0	0	0	
Yasso	17	26	0	0	6	0	0	0	
Abbayota*	13	36	2	2	1	0	0	0	
Warsu	14	43	0	0	0	0	0	0	
Kobi*	0	1	0	0	0	0	0	0	
Dora*	2	2	0	0	1	0	0	0	
Kara	21	15	0	0	13	0	0	0	
Grand total	220	235	6	10	27	0	20	2	
Mean+SE	13.75±2.94	14.69±3.53	0.37±0.22	0.62±0.24	1.69±0.85	0	1.25±1.25	0.13±0.09	

IRCs=in-door resting catches, LTCs= light trap catches, ORCs= Out-door resting catches.

*= Deltamethrin was sprayed in September 2010 during the survey period.

The number of mosquitoes collected using different methods from September to November 2010 indicated the nature of the three anopheline mosquitoes resting behavior. Though the number of *An. coustani* s.l and *An. demeilloni* was small in the 3 months collection period, the two species showed endophilic behavior unlike *An. arabiensis* which showed wide ranging (both endophilic and exophilic) behavior (Figure 4).

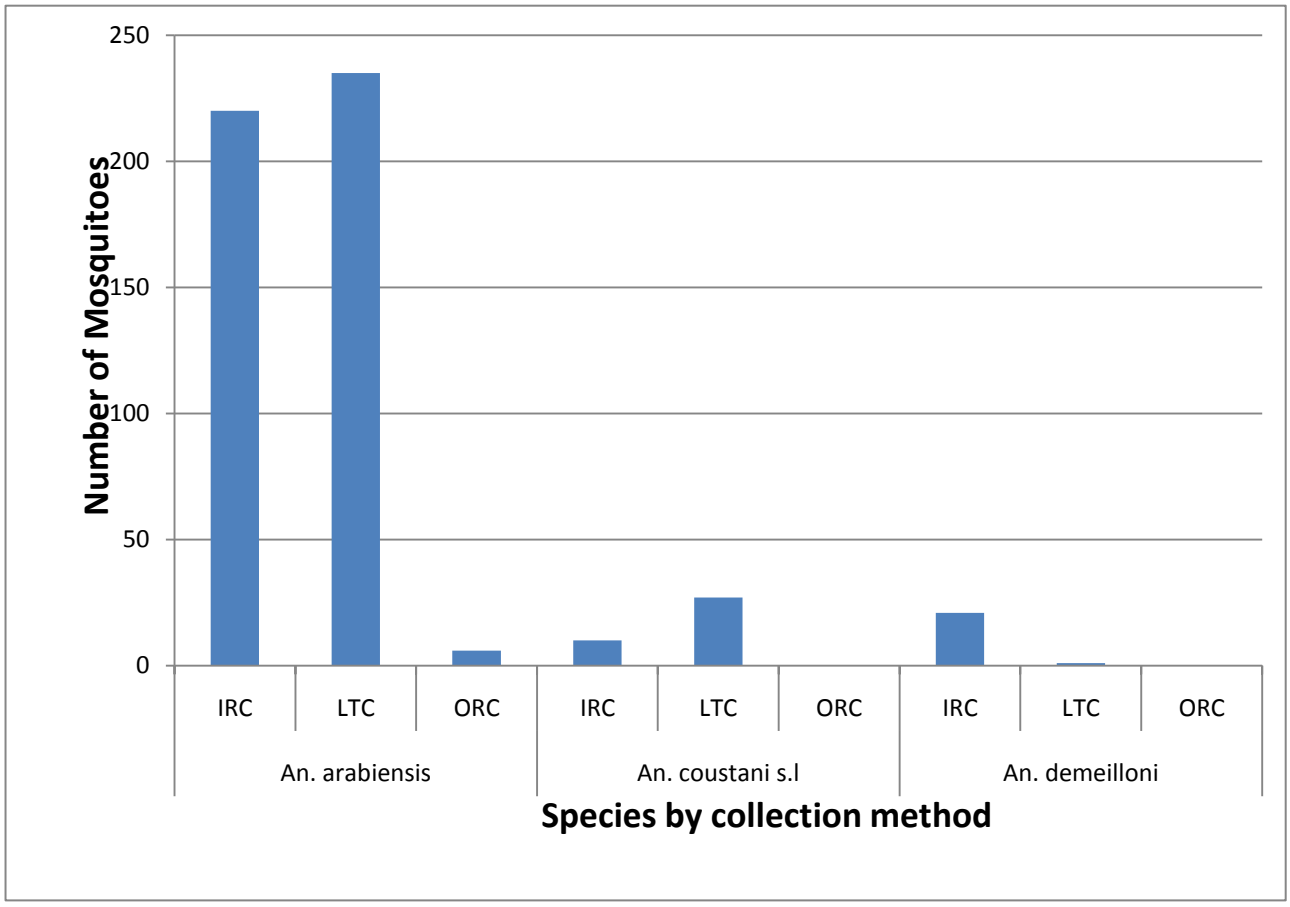
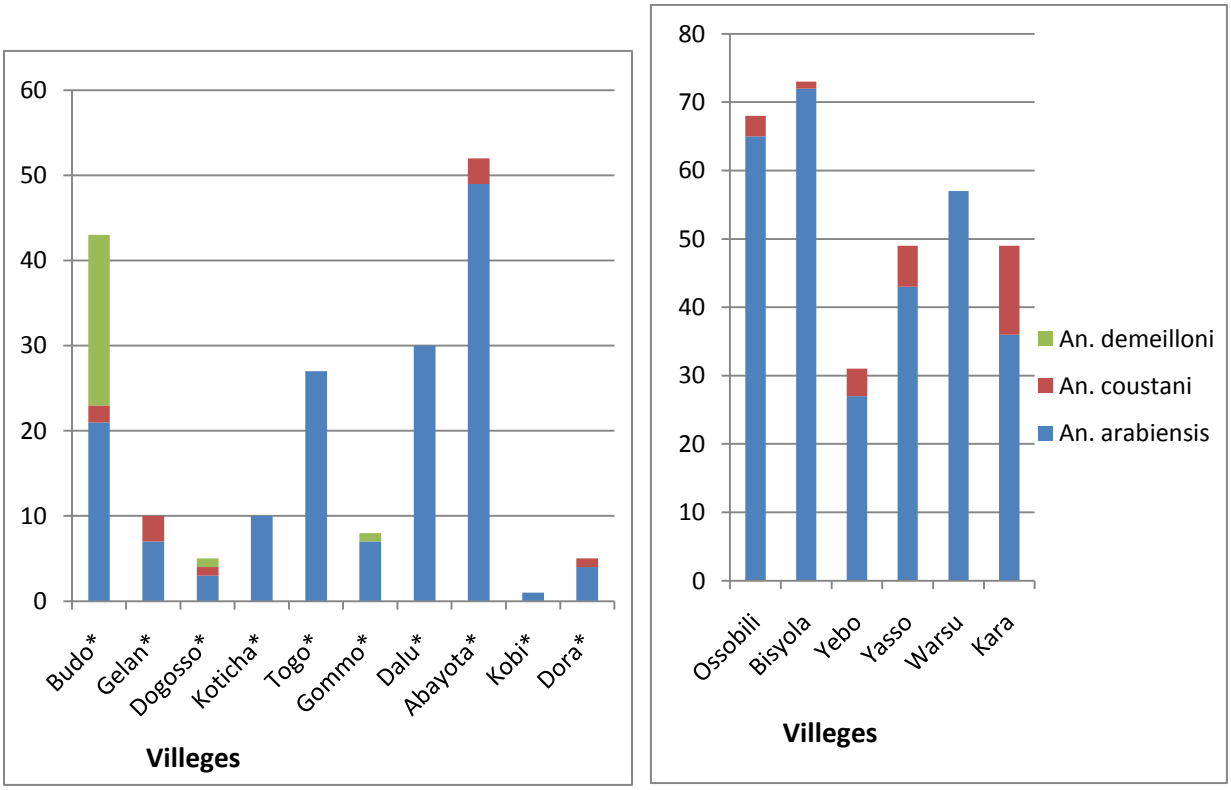


Figure 4: Mosquito distribution by collection methods in Gilgel-Gibe hydroelectric dam area, southwestern Ethiopia (September –November 2010)
IRCs=in-door resting catches, LTCs= light trap catches, ORCs= Out-door resting catches

Mosquito density of the 3 anopheline species is presented in Figure 5. *An. arabiensis* was almost appeared in all the villages with some numerical differences among villages. *An. coustani* s.l also showed similar pattern in their density with some inclination to the distant villages from the dam. Unlike the two species, *An. demeiloni* completely showed different pattern in being confined to villages close to the dam. After insecticide spray (October to November 2010), the highest number of mosquitoes was observed in Budo followed by Ossobili which is also very much closer to the dam. On the other hand some villages at a relatively far distance from the dam also retained somewhat large number of mosquitoes. In the unsprayed Villages large number of mosquito density was observed (Figure 5).

Figure 5: Mosquito species distributions by village, in Gilgel-Gibe hydroelectric dam area, southwestern Ethiopia (September-November, 2010)



A **B**

*=Deltamethrin sprayed in September 2010 survey period (A left) B= unsprayed

5.2. Infectivity rates of anopheles species

Of the 520 mosquitoes tested for CSP antigen by sandwich ELISA, *Plasmodium vivax* infection was detected in 4 (0.87%) *An. arabiensis* and 1 (2.7%) *An. coustani* s.l. ten specimens (4 *An. arabiansis* and 1 *An. coustani* s.l) were found positive, (4 for *P. vivax* (Pv. 210) and 1 for *P. vivax* VK247). No mosquito was found positive for *P. falciparum* circumsporozoite-protein antigen. All the positive mosquitoes were from September 2010 collections. Moreover, all positive mosquitoes for CSP antigen were from indoor resting collections (3 from LTCs and 2 were from IRCs). Sporozoite rates during the collection period were 0.87% for *An. arabiensis* and 2.7% for *An. coustani* s.l. The overall sprozoite rate of anopheline species was 0.96%. Three of the CSP positive mosquitoes for *P. vivax* 210 were from the same house. Entomological inoculation rate (EIR) of the study area was 4.5 infectious bites/person/month for *An. arabiensis* during the study period (Table 2).

Table 2: Circumsporozoite protein rate of anophelines mosquitoes, in Gilgel-Gibe hydroelectric dam area, southwestern Ethiopia (September-November, 2010)

Species	No. tested	Reactive circumsporozoite protein for				Infection rate
		P. falciparum	*P. vivax* 210	*P. vivax* VK247	Mixed	
An. arabiensis	461	0	3	1	0	0.87
An. coustani s.l	37	0	1	0	0	2.70
An. demelloni	22	0	0	0	0	0
Total	520	0	4	1	0	0.96

5.3. Feeding behavior and Blood meal source of anopheles species

In total 520 mosquito specimens were assayed for mosquito blood source, of these, 43 (11.35%) *An. arabiensis*, 3 (10.71 %) *An. coustani* s.l and 1 (4.76%) *An. demeiloni* specimens were found positive for bovine blood. Only specimens from *An. arabiensis* were positive for human 19 (4.12%) and chicken 5 (1.08%) throughout the tests in both mixed blood meal and single meal results (table 3). Among the 3 anopheline species tested, *An. arabiensis* was the only species fed on all the six hosts. This species was much more opportunistic in their feeding habit than any other species as they fed on all the hosts tested. Nevertheless, they show more preference to mammalian blood than chicken blood meal in the presence of 734 (23.68%) chicken potential hosts in the study area. *An. coustani* s.l and *An. demeiloni* showed narrow range of hosts. These groups of species were limed to bovine, equine and ovine hosts. Bovine hosts were the largest host blood antigen detected for *An. arabiensis* 77(20.32%) and for *An. coustani* s.l 5(13.51%) (Table 3).

Total of 45 (9.76%) *An. arabiensis* specimens from indoor resting collections by both IRCs and LTCs were positive for all antigens of the six hosts tested (human, bovine, ovine, equine, canis and chicken) except IRC which was negative for canis. Of the six out door resting collected specimens, only a single specimen of *An. arabiensis* was positive for dogs (canis). No specimen from outdoor resting collection was positive for others. Mixed blood meal was found in 8.24% (38) LTCs and 2.82% (13) IRCs specimens, no mixed blood meal was detected from ORCs (Table 3).

Table 3: Host blood feeding pattern of mosquito species, in Gilgel-Gibe hydroelectric dam area, southwestern Ethiopia (2010)

Species	Human		Bovine		Equine		Ovine		Canis		Chicl
	N	%	N	%	N	%	N	%	N	%	N
An.arabiensis	11/461	2.39	43/379	11.35	11/461	2.39	43/461	9.33	5/461	1.09	2/461
An. arabiensis mixed fed	8/461	1.74	34/379	8.97	11/461	2.39	37/461	8.03	16/461	3.47	3/461
Total	19/461	4.12	77/379	20.32	22/461	4.77	80/461	17.35	21/461	4.56	5/461
An. coustani s.l	0/37	0	3/28	10.71	0/37	0	1/37	2.7	0/37	0	0/37
An. coustani s.l mixed fed		0	2/28	7.14	2/37	5.41	1/37	2.70	1/37	2.70	0/37
Total	0/37	0	5/28	13.51	2/37	5.41	2/37	5.41	1/37	2.70	0/37
An. demeiloni	0/22	0	1/21	4.76	0/22	0	2/22	9.1	0	0	0/22
An. demeiloni mixed fed	0/22	0	2/21	9.52	1/22	4.55	1/22	4.55	0/22	0	0/22
Total	0/22	0	3/21	14.29	1/22	4.55	3/22	13.64	0/22	0	0/22

Table 4: Blood source of anophelines mosquitoes by collection method, Gilgel-Gibe dam area, southwestern Ethiopia (2010)

Species	Collectin method	Human		Bovine		Ovine		Equine		Canis		Chic
		N	(%)	N	%	N	%	N	%	N	%	N
An. arabiensis	IRC	6/461	1.3	15/379	3.96	15/461	3.25	8/461	1.75	0/461	0	1/461
	CDC	5/461	1.09	28/379	7.39	28/461	6.07	3/461	0.65	4/461	0.87	1/461
	ORC	0	0	0	0	0	0	0	0	1/461	0.22	0
An. coustani s.l	IRC	0	0	0	0	0	0	0	0	0	0	0
	CDC	0	0	3/28	10.71	1/37	2.70	0	0	0	0	0
	ORC	0	0	0	0	0	0	0	0	0	0	0
An. demeiloni	IRC	0	0	1/21	4.76	1/22	4.55	0	0	0	0	0
	CDC	0	0	0	0	1/22	4.55	0	0	0	0	0
	ORC	0	0	0	0	0	0	0	0	0	0	0

Out of six host's blood tested throughout the experiment, the maximum number of mixed host blood antigen detected in a single mosquito specimen was four mixed hosts' blood antigen in an *An. arabiensis* specimen and only two mixed hosts' blood antigen were detected in a single *An. coustani* s.l and *An. demeiloni* specimens.

Of all the positive 183 specimens, 126 (68.9%) were single and 57(31.1%) were mixed. Both in a single and mixed host detected cases, *An. arabiensis* species showed wider host range followed by *An. coustani* s.l. In contrast, in both single and mixed host detected, *An. demeiloni* species were found to have narrowest host range with large blood antigen being that of ovine in *An. demeilloni* specimens. Large proportion of mixed host blood antigen detected was that of *An. coustani* s.l 5 (13.51%), with narrow host range (Figure 6).

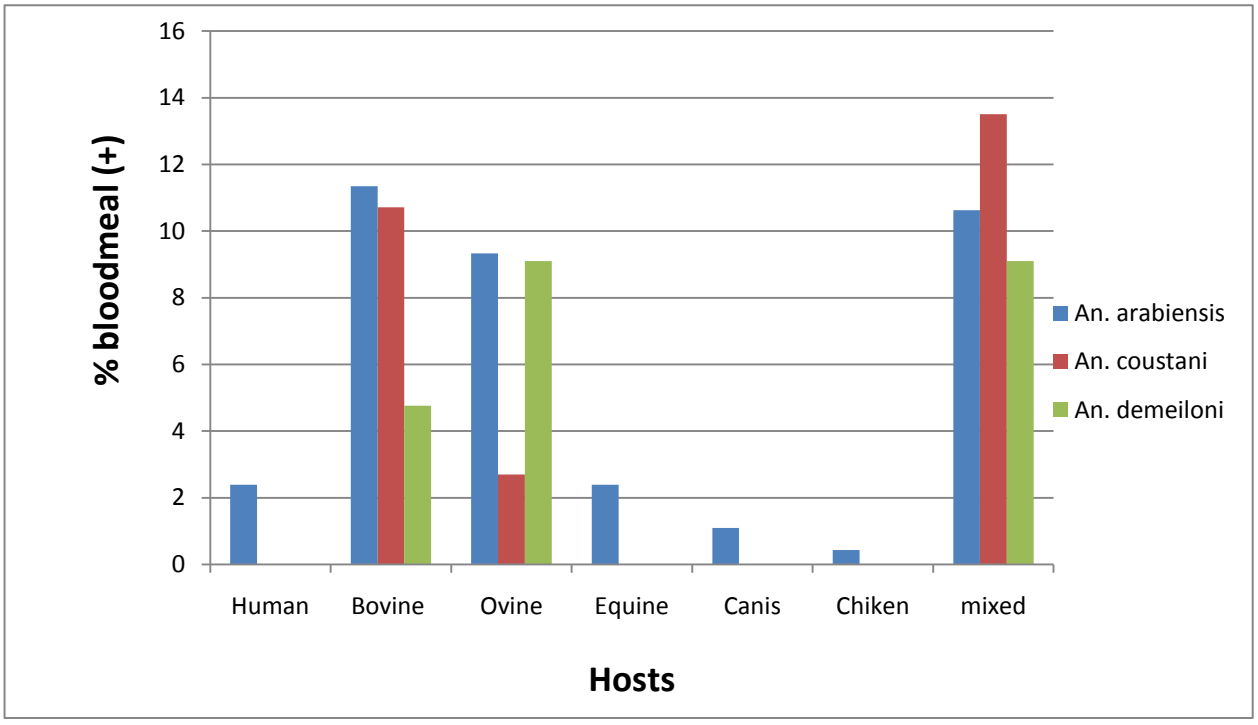


Figure 6: Host feeding pattern of anophelines mosquitoes, in Gilgel-Gibe hydroelectric dam area, southwestern Ethiopia (September-November, 2010).

On average 3,100 potential hosts were surveyed from September to November 2010 along with mosquito collection. About 1057 (34.1%) human, 734(23.68%) chicken, 731 (23.58%) bovine, 455 (14.68%) ovine, 44(1.42 %) equine and 79 (2.55%) canis hosts resting inside all the sentinel houses of the villages were recorded. All the potential hosts of the villages were almost evenly distributed through out the study area except for Ossobili, Budo, Dalu, Warsu and Kobi villages where no equines resting in the houses were recorded. With exception of Kobi which significantly differ from other villages, significance difference was not observed among most of the villages in potential host abundance. In relation to other hosts, the numbers of equine and canis recorded in the houses were significantly small (Appendix 5). Large numbers of hosts were from that of human, bovine ,chicken and ovine with significantly higher number of humans sleeping in the houses (Tables 5 and 6).

Table 5: Average number of humans and animals in the farmers house with in the villages, in Gilgel-Gibe hydroelectric dam area, southwestern Ethiopia, (2010) (N= 192)

Hosts	Mean ± SE*	95% CI
Human	5.5 ± 0.16a	5.18, 5.82
Bovine	4.8 ± 0.22b	4.36, 5.26
Ovine	2.5 ± 0.21c	2.10, 2.93
Equine	0.2 ± 0.04d	0.15, 0.31
Dogs	0.2 ± 0.04d	0.10, 0.26
Chicken	3.7 ± 0.30e	3.17, 4.36
Total	2.8 ± 0.10f	2.64, 3.03

N = number of cases (houses)

95%CI = confidence interval

* Means followed by the letter(s) are not significantly different from each other.

Table 6: Distribution of potential hosts among study villages during mosquito collection, in Gilgel-Gibe hydroelectric dam area, southwestern Ethiopia (2010)

Village name	no. of humans	no. of bovines	no. of ovines	no. of chickens	no. of equines	no. of canis
Ossobila	61	14	15	16	0	6
Budo	66	20	35	47	0	6
Gelan	76	87	29	50	3	6
Dogosu	76	22	27	66	1	3
Koticha	60	26	13	30	1	5
Togo	62	8	43	41	6	6
Gomo	57	81	66	43	2	6
Dalu	66	86	25	50	0	8
Bissola	59	55	28	40	4	5
Yebo	70	60	26	52	9	7
Yasso	66	88	32	79	6	7
Abbayota	82	12	28	90	5	2
Warsu	68	58	24	43	0	4
Kobe	57	18	7	26	0	2
Dora	65	43	28	42	1	3
Kara	66	53	29	19	6	3
Total	1057	731	455	734	44	79
Average	66.06	45.69	28.44	45.88	2.75	4.94
% Proportion	34.1	23.58	14.68	23.68	1.42	2.55

The ratio of number of human to other hosts ranged from ≈2:1 to ≈23:1 showing higher number of humans sleeping in the house than any other hosts (Table 7). But this ratio did not account for other outdoor resting hosts which might have had impact on the ratio of humans to other hosts. In contrary to this high ratio, in only single and including mixed (total) host blood detected conditions, human blood index was the third and the fourth ranked respectively with percentages 2.39% for single and 4.12 % for total result.

Monthly estimate of available human and other hosts in selected households during the study period were done and the ratios were used to determine host preference index (HPI) and to calculate the forage ratio among blood fed mosquitoes positive for each of the hosts (human, bovine, ovine equine, canis and chicken) (Table 7).

In contrast to the lowest ratio of equine hosts in the study area, there was high host preference index for equine for all the three species of mosquitoes. HPI for equine of the three species were; *An. arabiensis* (1.83), *An. coustani* s.l (3.81) and *An. demeilloni* (3.2). These HPI results for the entire three species were greater than one indicating the most preferred host was equines. HPI for chicken was 0.05 for *An. arabiensis* and zero for the other two mosquito species, which means avoidance of this host by the three mosquitoes' species in the presence of other hosts. HPI for canis by *An. coustani* s.l and HPI for bovine and ovine by *An. arabiensis* were nearly one, indicating neither avoidance nor preference of these hosts. Where as, the HPI for human and chicken by *An. arabiensis* HPI for human, bovine, ovine and chicken by *An. coustani* s.l and *An. demeilloni* was less than one showing host avoidance (Table 8).

The forage ratio (FR) was also determined according to Hess *et al*. (1968). This ratio was calculated for each host category as the percentage of positive blood meals divided by the percentage of available hosts, with ratio >1 indicating preference ,<1 indicating avoidance and ratio approaching 1 little preference or avoidance . Like HPI the highest FR was observed for equine among engorged mosquitoes showing high preference. The FR for human, bovine, ovine, equine, canis and chicken ratio was: 0.3, 1.97, 3.16, 9.62, 4.71, and 0.12, respectively. The higher FR and HPI were suspected due to significantly low numbers of equine hosts in the houses of the study area (Table 7 and 8).

Table 7: Ratio of potential hosts available in 16 study villages in Gilgel-Gibe hydroelectric dam area, southwestern Ethiopia (2010)

Ratio	no. of Human	no. of Bovine	no. of ovine	no. of Chickens	no. of Equine	no. of Canis
Human		1.45:1	2.32:1	1.44:1	22.55:1	13.37:1
Bovine			1.61:1	1:1	16.61:1	9.25:1
Ovine				0.62:1	10.34:1	5.76:1
Chicken					16.68:1	0.11:1
Equine						0.56:1
Canis						

Table 8: Host preference index (HPI) and forage ratio (FR) of anopheline mosquitoes, in Gilgel-Gibe hydroelectric dam area, southwestern Ethiopia (2010)

Species	Human		Bovine		Ovine		Equine		Canis		Chicken	
	HPI	FR	HPI	FR	HPI	FR	HPI	FR	HPI	FR	HPI	FR
An. arabiensis	0.12	0.25	0.86	1.46	1.18	2.64	3.36	6.92	1.79	3.68	0.05	0.09
An. coustani s.l	0	0	0.57	2.12	0.37	1.36	3.81	14.08	1.06	3.92	0	0
An. demeilloni	0	0	0.61	1.82	0.31	2.92	9.61	10.06	0	0	0	0
Total FR	-	0.3	-	1.97	-	3.16	-	9.62	-	4.71	-	0.12

HPI = host preference index
FR = forage ratio

6. Discussions

According to this study, mosquito density was the highest in September which is in agreement with peak malaria transmission season in the study area (FDROEMOH, 2006). This is at the end of main rainy season of the study area, which is consistent with other similar studies by Ribeiro *et al.* (1996). Pyrethrum spray collection was not employed for mosquito collection as the local mosquito population showed low susceptibility to major insecticides used in public health (Delenasaw Yewhalaw *et al.*, 2010a and 2011; Meshasha Balkew *et al.*, 2010). On the other hand indoor residual spraying during our data collection especially in September 2010 might have underestimated the distribution of mosquitoes. As the result, some mosquito density irregularities have been observed in villages near by the dam.

The over all sporozoite rate of this area was 0.96% for the 3 months of survey. A higher sporazoite rate (2.26%) was observed in southern Ethiopia for *An. arabiensis (*Aseged *et al.,* 2006). Again in tropical rain forest of Nigeria 2.3% rate was observed for *An. arabiensis* (Oyewole *et al.*, 2005).

The sporozoite rate showed variability for the past three years from 1.67% in 2008 down to 0.37% in 2009 (Delenasaw Yewhalaw, unpublished data). Again, increased to 0.96% in 2010.

The study villages were close to Gilgel_Gibe dam which was became operational since 2004. According to Delenasaw Yewhalaw *et al.* (2009), it is clear that large numbers of people are currently at greater risk of malaria transmission due Gil-gale Gibe dam construction.

Studies results in the vicinity of the Koka reservoir in Ethiopia by Solomon Kibret *et al.* (2009), also highlighted the role of the reservoir in increasing malaria transmission.

Out of 37 adult *An. coustani* s.l specimens tested for CSP one specimen was found positive for *P. vivax* (Pv.210). *An. coustani* species was considered as a suspected vector which had not been proved to have a role in malaria transmission in Ethiopia (Ashenafi Woime, 2008; WHO, 2009). The role of this species in local malaria transmission was not well investigated in Ethiopia.

None of the *An. coustani* s.l and *An. demeiloni* was positive for human blood. Nevertheless, both *An. coustani* s.l and *An. demeilloni* tend to be endophilic from our indoor resting collection as LTC from farmers' bed room was higher for these species. *An. coustani* s.l somehow showed wider host range than *An. demelloni* in both mixed and single host blood antigen detection. This could have implication that they might have fed on humans when other hosts were not readily available, thereby increased the probability of picking up malaria parasite and became positive for CSP. In support to this idea, the feeding behavior of vectors influences the likelihood of pathogen invasion and the exposure of humans to vector-borne zoonotic pathogens, genetically influenced feeding behavior may have significant impacts on the probability of invasion of a pathogen (Kilpatrick *et al.*, 2007).

In the study area, the number of *An. coustani* s.l was relatively fewer. Beyond their feeding habit their number could also be one of the factors that had limited their being a major malaria vector in the area and resulted in zero HBI. This has supporting evidence from the work of Wadatir Nigatu *et al.* (1994), *An. coustani* s.l fed on human blood was 16.7%. In a similar way to our work result, the feeding frequency for *An. coustani* s.l was suspected because of low numbers. This evidence tells us they could feed on humans but their number could limit the expected result. Transmission of the parasite is influenced by different factors other than mosquitoes feeding habit.

The net impact depends on other aspects of transmission such as vector abundance and survival and host and vector competence (Kilpatrick *et al.*, 2007).

In comparison with *An. coustani* s.l and *An. demeilloni*, *An. arabiensis* appeared in high abundance in the study area which can easily increase the risk of bites to humans by this species. This species was the only species fed on humans in the study area. Their feeding habit on humans and high abundance than *An. coustani* s.l and *An. demeilloni* species of mosquitoes', endow them the chance to pick up malaria parasite and lead to multiplication of the parasite in them that results in increase of malaria epidemic in the area. Obviously, for a parasite where humans are an important amplification host i.e. malaria, in that feeding on humans increases both the probability of an epidemic and subsequent exposure of humans (Kilpatrick *et al.*, 2007).

Indoor human to other hosts ratio of the study area was higher for all the hosts compared, this would have led to higher human blood index (HBI), but low level of HBI was observed in the tested mosquitoes. Our samples had higher number of *An. arabiensis* that showed unique behavior, from the results of Tirados *et al.* (2006), it was surprising that the proportion of human blood meals in the samples collected indoor was so low, given that livestock were rarely kept indoor; in our case large numbers of livestock were kept indoor with significantly high number of humans but HBI was still low. A similar low HBI (7.3%) was reported in populations of *An. arabiensis* from southwestern Ethiopia (Messay Fettene *et al.*, 2004). *An. arabiensis* from Eretria also showed similarity with our findings where humans were not the predominant blood source for mosquitoes caught either outdoors or indoors (Waka *et al.*, 2005). Similarly, HBI was lowest in Jarso, Burkina Faso where human cattle ratio was 17:1 (Tirados *et al.*, 2006; Muriu *et al.*, 2008).

Thus, the feeding tendencies of *An. arabiensis* are conducive for zoo prophylaxis vector control strategy (Mahande *et al.,* 2007).

The maximum number of host blood antigen detected in a single mosquito was up to four host blood antigen in one *An. arabiensis* specimen. Our results demonstrated that *An. arabiensis* move fast from one host to another during blood feeding which means they feed not only on hosts of different species but also on different individuals of the same host species (Tirados *et al.*, 2006).

High percentages of *An. arabiensis* and *An. coustani* s.l fed on bovine, but *An. demeiloni* which was a rather none malaria vector showed a different host feeding pattern. This species showed a narrow host range, (fed only on bovine, ovine and equine). This indicates the species preferred large mammals like bovine and equine though the number of equine was significantly small in the study area. The FR and HPI result indicate that the most preferred host was equine for the entire mosquito species tested. This might be due to the large amount of carbon dioxide produced by these big mammals that could attract them to wards such a large host (Corinnawu, 2000). Over all result showed that, *An. arabiensis* fed on wide range of hosts followed by *An. coustani* s.l and the list being *An. demeiloni*. This host feeding pattern shows that *An. arabiensis* could feed on any available host at a time. As described in Clemets (1992), stimuli from the ingested blood meal are required for the proportion of ovarian development leading to oviposition. The digestion product of blood is again used for energy source. Blood is not the only source of energy for adult mosquitoes. Thus, in the absence of the preferred host, in most cases *An. demeilloni* and *An. coustani* s.l must have reverted to other food for energy source since they have narrower host range than *An. arabiensis* in blood feeding.

7. Conclusions and recommendations

7.1. Conclusions

- Sporozoite and entomological inoculation rates of the study area were 0.96 % and 4.5 /infectious bites/ person /month, respectively.
- Our result is the first to report positive Plasmodium infection in *An. coustani* s.l in Ethiopia. There is little or no information on its biology and its role in malaria transmission.
- Large proportion of *An. arabiensis* fed on bovine and ovine though significantly higher number of humans was recorded in the houses than others. But, no significant difference was observed in abundance among these vertebrate hosts in the study area villages except for Kobi village which deviate significantly from the other.
- Multiple blood feeding was observed in the entire (*An. arabiensis, An. coustani* and *An. demeilloni*) mosquito species with the highest mixed feeding (up to four host's blood) in a single *An. arabiensis* specimen. In the other two up to two mixed host antigen were detected. *An. arabiensis* fed on all the tested hosts showing wide range of hosts.
- HBI for *An. arabiensis* was 4.12%, but Zero for the other two *species* tested. FR and HPI result showed higher preference for equines for all the three species of mosquitoes in the study area.
- Narrow host range was observed for *An. coustani* s.l and *An. demeiloni* species.

7.2. Recommendations

- In the study area sporozoite rate was high for the study period of 2010. Therefore, malaria prevention strategies of the government and NGOs should give due attention to this area as this area is becoming one of the top malarous area of Ethiopia.

- As their feeding and resting habit is variable depending on host availability and accessibility, vector control strategies that involve *An. arabiensis* has to consider all mosquito host resting places so as to reduce their feeding on alternative hosts that leads to the perpetuation of their species thereby increasing malaria disease.

- The current study confirmed *An. coustani* s.l being positive for malaria parasite; hence, mosquito vector control program has to include this species in the study area for malaria vector control strategy that specifically suit the control of this species and further contribution of this species to local malaria transmission also needs to be investigated**.**

- As the feeding tendencies of *An. arabiensis* are conducive for zoo prophylaxis, zoo prophylaxis can be employed as possible vector control strategy in the area in addition to the current vector control interventions as well as it is recommendable that in areas with a predominant *An. arabiensis* population in Ethiopia, cattle should be placed close to dwelling houses in order to maximize the effects of zoo prophylaxis. Hence, protective effects of human from malaria can further be enhanced by keeping cattle in surroundings of residences.

- Other dam constriction areas where mosquitoes can reproduce should be assessed for malaria epidemic occurrence due to damming effect.

References

Amino, R., Thiberge, S., Blazquez, S., Baldacci, P., Renaud, O., Shorte, S. and Me´nard, R. (2007). Imaging malaria sporozoites in the dermis of the mammalian host. *Nature protocols* **2**: 1705-1712.

Arez, A. P., Lopes, D., Pinto, J., Franco, A. S., Snounou, G. and do Rosa´rio, V. E. (2000). *Plasmodium* sp.: Optimal Protocols for PCR detection of low parasite numbers from Mosquito (*Anopheles* sp.) samples. *Experimental Parasitology* **94:** 269–272.

Aseged Taye, Mamuye Hadis, Nesibu Adugna, Dejene Tilahun and Wirtz, A. R. (2006). Biting behavior and *Plasmodium* infection rates of *Anopheles arabiensis* from Sille, Ethiopia. *Acta Tropica* **97**: 50-54.

Ashenafi Woime (2008). *Changes in the Spread of Malaria in Ethiopia:Case Study from Awassa and Hossana Area 2006-2007.* M.Sc. thesis, Telemark University College, Telemark, Norway.

Askew, R. R. (1971). *Parasitic Insects*: Heinemann Educational Books LTD, London.

Beier, J.C., Perkings, P. V., Wirth, R. A., Koros, J., Diggs, D., Garganii, T. P. and Koech, D. K. (1988). Blood meal identification by direct enzyme linked immonosorbent assay (ELISA), tested on Anopheles (Diptera Culicidae) in Kenya. *Journal Medical Entomology* **25**: 9-16.

Benedict, M. (2007). *Methods in Anopheles Research.* CDC, Atlanta .

Birhan Teklu, Habte Tekie, McCartney, M. and Solomon Kibretl (2010). The effect of water physical quality and water level changes on the occurrence and density of larvae of *Anopheles* mosquitoes around the shoreline of the Koka reservoir, Central Ethiopia. *Hydro Earth System Science Discussion* **7:** 6025–6055.

Birley, M. H. and Charlwood, J. D. (1986). Vectorial capacity**.** *Parasitology Today* **2:** 248.

Charlwood, J., Pinto, J. D., Sousa, C.A., Ferreira, C., Petrarca V. and Rosario, V. E. (2003). A mate or a meal' – Pre-gravid behaviour of female *Anopheles gambiae* from the islands of São Tomé and Príncipe, West Africa. *Malaria Journal* **2:** 1475-2875.

Checchi, F., Cox, J., Balkan, S., Abiy Tamrat, Priotto, G., Alberti, P. K., Zurovac, D., and Guthmann J. P. (2006). Malaria epidemics and interventions, Kenya, Burundi, Southern Sudan, and Ethiopia, 1999–2004. *Emerging Infectious Disease* **12**: 1477-1485.

Clemets, A. N. (1992). *The Biology of Mosquitoes: Development Nutrition and Reproduction*. Chapman and Hall, London.

Connor, S. J., Tufa Dinku, Tsegay Wolde-Georgis, Endalkachew Bekele and Daddi Jima (2007). *A Collaborative Epidemic Early Warning and Response Initiative in Ethiopia.* National Meteorological Agency, Addis Abab.

Corinnawu (2000). Mosquito Magnets your skin chemicals lure blood-sucking insects to their next meal (culicidae). *Science News* **157**: 268-672.

Delenasaw Yewhalaw, Worku Legesse, Bortel, W. V., Solomon Gebre-Selassie, Kloos, H., Duchateau, L. and peybroeck, N. (2009). Malaria and water resource development: the case of Gilgel-Gibe hydroelectric dam in Ethiopia. *Malaria Journal* **8**:21.

Delenasaw Yewhalaw, Bortel, V. W., Denis, L., Coosemans, M., Duchateau, L. and Speybroeck, N. (2010a). First evidence of high knockdown resistance frequency in Anopheles arabiensis (Diptera: Culicidae) from Ethiopia. *American Journal of Tropical Medicine and Hygiene* **83:** (1)122-125.

Delenasaw Yewhalaw, Wondwossen Kassahun, Kifle Woldemichael, Tushune, K., Sudaker, M., Daniel Kaba, Duchateau, L., Bortel, B. W. and Speybroeck, N. (2010b). The influence of the Gilgel-Gibe hydroelectric dam in Ethiopia on caregivers' knowledge, perceptions and health-seeking behavior towards childhood Malaria. *Malaria Journal* **9**:47.

Delenasaw Yewhalaw, Fantahun Wassie, Steurbaut, W., Spanoghe, P., Bortel, V. W., Denis, L. W., Dejene Tessema, Yehenew Getachew, Coosemans, M., Duchateau, L. and Speybroeck, N. (2011). Multiple Insecticide Resistance: An Impediment to Insecticide-Based Malaria Vector Control Program. *PLoS One* **6**: (1) e16066. doi:10.1371/journal.pone.0016066.

Drakeley, C., Schellenberg, D., Kihonda, J., Sousa, C. A., Arez, A. P., Lopes, D., Lines, J., Mshinda, H., Lengeler, C. and Schellenberg J. A. (2003). An estimation of entomological inoculation rate for Ifakara: a semi-urban area in the region of intense malaria transmission in Tanzania. *Tropical medical institute of health* **8**:767-774.

Eappen, G. A., Islam, S., Srinivasan, P. Ghosh, A. K., Valenzuela, J.G., Ribeiro, J. M. C., Kafatos, F.C., Dimopoulo, G. and Jacobs-Lorena, M. (2004). Analysis of the *Plasmodium* and *Anopheles* transcriptional repertoire during Ookinete development and midgut invasion**.** *Journal of Biological Chemistry* **279:** 5573–5580.

FDROEMOH (2006). *Federal Democratic Republic of Ethiopia Ministry of Health: National Five-year Strategic Plan for Malaria Prevention and Control in Ethiopia 2006 – 2010* .Addis Ababa.

Frevert, U., Engelmann, S., Zougbe´de´, S., Stange, J. Ng. B., Matuschewski, K., Liebes, L. and Yee, H. (2005). Intravital observation of *Plasmodium berghei* sporozoite infection of the liver. *PLoS Biology* **3**: 1034-1046.

Perlmann P and Troye-Blomberg, M. (2002). Malaria immunology 2nd edn. **In**: *Malaria Parasite and Disease; Structure and Life Cycle*, PP.1–26, (Fujiokaa, F. and Aikawab, M. eds) Karger, Stockholm.

Gillies, M. T. and Coetzee, M. (1987). *A Supplement to the Anophelinae of Africa South of the Sahara (Afrotropical region).* Publications of the South African institute for medical research No. 55. Johannesburg.

Harbach, R. E. (2007). The Culicidae (Diptera): a review of taxonomy, classification and phylogeny. *Zootaxa* **1668**: 591–638.

Hess, A. D., Hayes, R. A. and Tempelis, C. H. (1968). The use of forage ratio techniquies in mosquito host preference studies. *Mosquito News* **28**: (3) 386-389.

Hoffman, L., Robert, A. S., Gramzinski, Mason, C., Koech, D., Yuichiro , S. I., Higashimoto, Maewal, A., Sidney, J., Appella, E., Gomez, E., Richards, A., Doolan, L. D., Southwood, S. and Chesnut, R. (2000). Class II alleles antigens restricted by multiple HLA *falciparum* pre-erythrocytic-stage epitopes from *Plasmodium. Journal of Immunology* **165:**1123-1137.

Hunt, R. H., Coetzee, M. and Messay Fettene (1998) .The *Anopheles gambiae* complex: a new species from Ethiopia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **92** :(2):231-235.

Ignell, R. (2010). *Chemo Ecological Management of Malaria Mosquitoes*. Swedish University of Agricultural Sciences (SLU), Stockholm.

IWMI. (2010). *Dams and malaria in Sub-Saharan Africa*. Colombo, Sri Lanka: International Water Management Institute (IWMI), Water Policy Brief 34.

Kilpatrick, A. M., Kramer, L. D., Jones, M. J., Marra, P. P., Daszak, P. and Fonseca, D. M. (2007). Genetic influences on Mosquito feeding behavior and the emergence of Zoonotic pathogens. *American Journal of Tropical Medicine Hygiene* **77** :(4) 667–671.

Krettli, U., Andrade-Neto, V., Brandao, M. and Ferrari, W. (2004). The search for new antimalarial drugs from plants used to treat fever and malaria or plants randomly selected: *Memórias do Instituto Oswaldo cruz* . **96**: 1033 -1042.

Kulkarni, M. A., Kweka, E. J., Nyale, E. M., Lyatuu, E. E., Mosha F. W., Chandramohana, D., Eraun M. and Drakeley, C. (2006). Entomologican inoculation of malaria vectors at different altitudes in Hai District Northeastern Tanzania. *Journal of medical entomology* **43**: 580-588.

Kweka, E. J., Nkya, W. M., Mahande, A. M., Assenga, C., Mosha, F. W., Lyatuu, E. E., Massenga, C. P., Nyale, E. M., Mwakalinga, S. B. and Lowassa, A. (2008). Mosquito abundance, bed net coverage and other factors associated with variations in sporozoite infectivity rates in four villages of rural Tanzania**.** *Malaria Journal* **7**:1475-2875.

Lardeux, F., Loayza, P., Bouchité, B. and Chavez, T. (2007b). Host choice and human blood index of *Anopheles pseudopunctipennis* in a village of the Andean valleys of Bolivia. *Malaria Journal* **6**: 6-8.

Lardeux, F., Tejerina, R. and Aliaga, C. (2007a). *Methods in Anopheles*: *Molecular Identification of Plasmodium spp. in Anophelines* CDC, Atlanta.

Lardeux, F., Tejerina, R., Aliaga, C., Ursic-Bedoya, R., Lowenberger, C. and Chavez, T. (2008). Optimization of a semi-nested multiplex PCR to identify *Plasmodium* parasites in wild-caught *Anopheles* in Bolivia, and its application to field epidemiological studies. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **102**: (5) 485-492.

Leopold, R. and Milek, B. (1999). *Major Transmission-Blocking Vaccine Candidate of the Human Malaria Parasite Plasmodium Falciparum***.** Leiden.

Levine, S. R., Peterson, T. A. and Benedict, M. Q. (2004). Geographic and ecologic distributions of the *anopheles gambiae* complex predicted using a genetic algorithm. *American Journal of Tropical Medicine Hygiene* **70**: (2) 105-109.

Mahande, A., Mosha, F., Mahande, J. and Kweka, E. (2007). Feeding and resting behavior of malaria vector, *Anopheles arabiensis* with reference to zooprophylaxis. *Malaria Journal* **6**:100 doi:10.1186/1475-2875-6-100.

Maleki-Ravasan, N., Oshaghi, M., Javadian, E., Rassi, Y., Sadraei, J. and Mohtarami, F. (2009). Blood meal identification in field-captured Sand Flies: comparison of PCR-RFLP and ELISA assays. *Iranian Journal of Arthropod-Borne Disease* ***3*:** (1) 8-18.

Meshesha Balkew, Ibrahim, M., Koekemoer, L. L., Brooke, B. D., Engers, H., Abraham Aseffa, Teshome Gebre-Michael and Elhassen, I. (2010). Insecticide resistance in *Anopheles arabiensis* (Diptera: Culicidae) from villages in central, northern and south west Ethiopia and detection of *kdr* mutation. *Parasites and Vectors* **3**:40.

Messay Fettene, Hunt R. H, Coetzee M. and Fikedu Tessema (2004). Behaviour of *Anopheles arabiensis* and *An. quaadriannulatus* sp. B mosquitoes and malaria transmission in southwestern Ethiopia. *African Entomology* **12**: (1) 83-87.

Muriu, M. S., Muturi, E. J., Shililu, J. I., Mbogo, C. M., Mwangangi, J. M., Jacob, B. G., Irungu, L. W., Mukabana, R. W., Githure, J. I. and Novak, R. J. (2008). Host choice and multiple blood feeding behavior of the malaria vectore anmd other amophelines in Mwea rice scheme, Kenya. *Malaria Journal* **7**:43-49.

Nyanjom, S. R. G., Chen, H., Gebre-Michael, T., Bekele, E., Shililu, J., Githure, J. Beier, J. C. and Yan, G. (2003). Population Genetic Structure of *Anopheles arabiensis* Mosquitoes in Ethiopia and Eritrea. *Journal of Heredity* **94:** (6) 457–463.

Oyewole, I. O., Ibidapo, C. A., Oduola, A. O., Obansa, J. B. and Awolola, T. (2005).Anthropophilic mosquitoes and malaria transmission in a tropical rain forest area of Nigeria**.** *Sacta Satech* **2:** (1): 6-10.

Peterson, A. T. (2009).Shifting suitability for malaria vectors across Africa with warming climates. *BMC Infectious Diseases* **9**:59 doi: 10.1186/1471-2334-9-59.

PMI (2010). President's Malaria Initiative (PMI) in Ethiopia, April 2010.

Ribeiro, J. M. C., Seulu, F., Abose, T., Gebramariam Kidane, and Addis Teklehaimanot (1996). Bulletin of the World Health Organization (WHO). **74** : 299-305.

Richards and Davies (1977). *IMMS, General Text Book of Entomology: Classification and Biology 10th edn*. Chapman and Hall. London, UK.

Sinka, M. E., Bang, M. J., Manguin, S., Coetzee, M., Mbogo, C. M., Hemingway J., Patil, A. P., Temperley, W. H., Gething, P. W., Kabaria, C. W., Okara, R. M., Boeckel, T. V., Godfray, H. C. J., Harbach, R. E. and Hay, S. I. (2010). The dominant Anopheles vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. *Parasites and Vectors* **3**:117.

Sinnis, P. and Coppi, A. (2007). *A Long and Winding Road: The Plasmodium Sporozoite's Journey in the Mammalian Host*. Elsevier Ltd., Ireland.

Solomon Kibret, McCartney, M., Lautze, J., and Jayasinghe, G. (2009). *Malaria Transmission in the Vicinity of Impounded Water: Evidence from the Koka Reservoir, Ethiopia*. Water Management Institute, Colombo. IWMI Research Report 132.

Stacey, L., Watts, Daniel, M., Fitzpatrick, James and Maruniak, E. (2009). Blood meal identification from Florida mosquitoes (Diptera: culicidae). *Florida Entomologist* **92:** 619-622.

Tilahun Nigatu, Berhane Haileselassie, Samuel Hailu and Dawit Seyum (2009). *Involving Community in the Fight Against Malaria in Ethiopia: AMRF Case Study, MOH, Health and Health-related Indicators Planning and Programming Department, Federal Democratic Republic of Ethiopia Ministry of Health, 2005/2006*, Addis Ababa.

Tirados, I., Costantini, C., Gibson, G. and Torr, S. J. (2006). Blood feeding behavior of the malaria mosquitoes *Annopheles arabiensis* : implication for vector control. *Medical and veterinary Entomology* **20**: 425- 437.

Tolle, M. A. (2009). Mosquito-borne diseases. *Current Problem in Pediatrics of Adolescence Health Care* **39**:97-140.

Tran, V. C. and Saier, M .H. (2004). *Microbiology Comment: the Principal Chloroquine Resistance Protein of Plasmodium falciparum is a Member of the Drug/ Metabolite Transporter Superfamily*. SGM, London.

Waka, M., Hopings, R. J., Akinpelu, O. and Curtis, C. (2005). Transmission of malaria in the Tesseney area of Eritrea: Parasite prevalence in children, and vector density, host preferences and sporozoite rate. *Journal of vector Ecology* **30**: (1) 27-32.

Wakgari Dheresa (2010). *Overview of Malaria Epidemiology in Ethiopia: Symposium on International Neuro Infectious Disease Conference*. Addis Ababa.

Wakgari Deressa, Ahmed Ali and Fikre Enquoselassie (2000). Knowledge, attituSde and practice about malaria, the mosquito and antimalarial drugs in a rural community. *Ethiopian Journal of Health Development* **17**:99-104.

Wandatir Nigatu, Beyene Petros, Mesfin Lulu, Nasibu Adugna and Wirtz, R. (1994). Species composition, feeding and resting behavior of the common anthropophilic anopheline mosquitoes in relation to malaria transmission in Gabella , southwestern Ethiopia. *Insect Science Applications* **15:** 371-377.

WHO (1975). Manual on Practical Entomology in Malaria. Part II Methods and Techniques. WHO offset publication 13, Geneva.

WHO (2003). World health organization (WHO) UN country team Ethiopia sep2003.

WHO (2004). *Basic Malaria Microscopy Part I. Learners Guide 2nd edn*. World Health Organization (WHO), Geneva.

WHO (2005). *World Malaria Report 2005*. World Health Organization(WHO) and UNICEF.

WHO (2009). *World Health Organization (WHO), world malaria report 2009.*

Wirtz, R., Avery, M. and Benedict, M. (2007). *Methods in Anopheles Reaserch: Plasmodium Sporozoite* ELISA. CDC, Atlanta.

Appendices

Appendix 1: September 2010 mosquito collection in Gilgel-Gibe hydroelectric dam area, southwestern Ethiopia (September, 2010)

Locality	*An. arabiensis*			*An. coustani*			*An. demeilloni*			Total
	IRC	CDC	ORC	IRC	CDC	ORC	IRC	CDC	ORC	
Ossobila	33	15	3	3	0	0	0	0	0	54
Budo	1	0	0	1	0	0	20	0	0	21
Gelan	2	5	0	2	1	0	0	0	0	10
Dogosu	1	1	0	1	0	0	0	0	0	3
Koticha	4	5	0	0	0	0	0	0	0	9
Togo	8	13	0	0	0	0	0	0	0	21
Gomo	0	6	0	0	0	0	0	1	0	7
Dalu	24	5	1	0	0	0	0	0	0	31
Bissola	35	28	0	0	0	0	0	0	0	63
Yebo	20	7	0	1	1	0	0	0	0	28
Yasso	17	22	0	0	0	0	0	0	0	39
Abbayota	13	36	2	2	0	0	0	0	0	53
Warsu	14	41	0	0	0	0	0	0	0	56
Kobe	0	0	0	0	0	0	0	0	0	0
Dora	2	0	0	0	0	0	0	0	0	2
Kara	21	15	0	0	0	0	0	0	0	24
Grand total	195	199	6	10	2	0	20	1	0	434

Appendix 2: October 2010 mosquito collection in Gilgel-Gibe hydroelectric dam area, southwestern Ethiopia (October, 2010)

Village	*An. arabiensis*			*An. coustani*			*An. demeilloni*			Total
	IRC	CDC	ORC	IRC	CDC	ORC	IRC	CDC	ORC	
Ossobila	4	10	0	0	0	0	0	0	0	14
Budo	14	5	0	0	0	0	0	0	0	19
Gelan	0	0	0	0	0	0	0	0	0	0
Dogosu	0	0	0	0	0	0	0	1	0	1
Koticha	1	0	0	0	0	0	0	0	0	1
Togo	5	1	0	0	0	0	0	0	0	5
Gomo	1	0	0	0	0	0	0	0	0	1
Dalu	0	0	0	0	0	0	0	0	0	0
Bissola	0	9	0	0	0	0	0	0	0	9
Yebo	0	0	0	0	2	0	0	0	0	2
Yasso	0	4	0	0	6	0	0	0	0	10
Abbayota	0	0	0	0	1	0	0	0	0	1
Warsu	0	2	0	0	0	0	0	0	0	2
Kobe	0	0	0	0	0	0	0	0	0	0
Dora	0	2	0	0	1	0	0	0	0	3
Kara	0	0	0	0	13	0	0	0	0	13
Grand total	25	33	0	0	23	0	0	1	0	82

Appendix 3: November 2010 mosquito collection in Gilgel-Gibe hydroelectric dam area, southwestern Ethiopia (November, 2010)

Village	*An. arabiensis*			*An. coustani*			*An. demeilloni*			Total
	IRC	CDC	ORC	IRC	CDC	ORC	IRC	CDC	ORC	
Ossobila	0	0	0	0	0	0	0	0	0	0
Budo	0	1	0	0	1	0	0	0	0	2
Gelan	0	0	0	0	0	0	0	0	0	0
Dogosu	0	1	0	0	0	0	0	0	0	1
Koticha	0	0	0	0	0	0	0	0	0	0
Togo	0	0	0	0	0	0	0	0	0	0
Gomo	0	0	0	0	0	0	0	0	0	0
Dalu	0	0	0	0	0	0	0	0	0	0
Bissola	0	0	0	0	1	0	0	0	0	1
Yebo	0	0	0	0	0	0	0	0	0	0
Yasso	0	0	0	0	0	0	0	0	0	0
Abbayota	0	0	0	0	0	0	0	0	0	0
Warsu	0	0	0	0	0	0	0	0	0	0
Kobe	0	1	0	0	0	0	0	0	0	1
Dora	0	0	0	0	0	0	0	0	0	0
Kara	0	0	0	0	0	0	0	0	0	0
Grand total	0	3	0	0	2	0	0	0	0	5

Appendix 4: Potential hosts available in the study area during mosquito collection in Gilgel-Gibe hydroelectric dam area, southwestern Ethiopia (September-November, 2010)

Village name	no. of Human	no. of Cattle	no. of Goats	no. of Sheep	no. of Chickens	no. of Cats	no. of Horses	no. of Dogs	Others
Ossobila	61	14e 60	5e 11	10	16	5	0	1e	0
Budo	66	20e 47	9	26	47	2	0	4e	0
Gelan	76	87	3	26	50	1e 9	3	5e 3	0
Dogosu	76	22e 46	4e 12	23	66	2e 7	1e 2	1e	0
Koticha	60	26e 62	6e 22	7e 9	30	3e 4	1	2	0
Togo	62	8e 60	21	22	41	5	6	1	0
Gomo	57	81	48	18	43	5	2	1e 6	0
Dalu	66	86	24	1	50	1e 3		7e 1	0
Bissola	59	55	12	16	40	4e 1	4	1e	0
Yebo	70	60	13	13	52	2e	9	5	0
Yasso	66	88	11	21	79	5	6	2	0
Abbayota	82	12e 52	2	26	90	1e 3	5	1e 8	0
Warsu	68	58	12	12	43	2e 2	0	2e	0
Kobe	57	18	3	4	26	0	0	2	0
Dora	65	43	12	16	42	0	1	3e 2	0
Kara	66	53	12	17	19	2e 3	6	1e 3	0

Note; e= animals resting outside the house during mosquito collection

Appendix 5: LSD Multiple Comparisons test result of average number of potential hosts per sentinel houses of villages in Gile-gale Gibe dam area, southwestern Ethiopia (2010)

(I) Type of host	(J) Type of host	Mean Difference (I-J)		95% CI	
			Std. Error	Upper Bound	Lower Bound
Human	Bovine	.69271(*)	.26634	0.1701	1.2153
	Ovine	2.98958(*)	.26634	2.4670	3.5122
	Equine	5.27083(*)	.26634	4.7483	5.7934
	Dogs	5.32292(*)	.26634	4.8003	5.8455
	Chicken	1.73437(*)	.26634	1.2118	2.2569
Bovine	Human	-.69271(*)	.26634	-1.2153	-.1701
	Ovine	2.29688(*)	.26634	1.7743	2.8194
	Equine	4.57813(*)	.26634	4.0556	5.1007
	Dogs	4.63021(*)	.26634	4.1076	5.1528
	Chicken	1.04167(*)	.26634	0.5191	1.5642
Ovine	Human	-2.98958(*)	.26634	-3.5122	-2.4670
	Bovine	-2.29688(*)	.26634	-2.8194	-1.7743
	Equine	2.28125(*)	.26634	1.7587	2.8038
	Dogs	2.33333(*)	.26634	1.8108	2.8559
	Chicken	-1.25521(*)	.26634	-1.7778	-.7326
Equine	Human	-5.27083(*)	.26634	-5.7934	-4.7483
	Bovine	-4.57813(*)	.26634	-5.1007	-4.0556
	Ovine	-2.28125(*)	.26634	-2.8038	-1.7587
	Dogs	.05208	.26634	-.4705	.5747
	Chicken	-3.53646(*)	.26634	-4.0590	-3.0139
Dogs	Human	-5.32292(*)	.26634	-5.8455	-4.8003
	Bovine	-4.63021(*)	.26634	-5.1528	-4.1076
	Ovine	-2.33333(*)	.26634	-2.8559	-1.8108
	Equine	-.05208	.26634	-.5747	.4705
	Chicken	-3.58854(*)	.26634	-4.1111	-3.0660
Chicken	Human	-1.73437(*)	.26634	-2.2569	-1.2118
	Bovine	-1.04167(*)	.26634	-1.5642	-.5191
	Ovine	1.25521(*)	.26634	.7326	1.7778
	Equine	3.53646(*)	.26634	3.0139	4.0590
	Dogs	3.58854(*)	.26634	3.0660	4.1111

* The mean difference is significant at the .05 level

Declaration

I, the undersigned, declare that this is my original work, has not been presented for a degree in any university that all sources of the materials used for this thesis have been duly acknowledged.

Name Musin Kelel Abas

Signature _____

Date _____