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Species Composition, Seasonal Variation and Roles of *Anopheles* Mosquitoes in the Transmission of Malaria in Koka villages, Central Ethiopia

By
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ABSTRACT

Entomological studies on the species composition, seasonal variation and infection rates of *Anopheles* mosquitoes were conducted at four selected villages of Koka area, Central Ethiopia, between December 2006 and November 2007. Collection and identification of larvae of *Anopheles* from various breeding habitats during the study period indicated that there are three species, namely *Anopheles gambiae* s.l. (84.7%), *An. pharoensis* (14.4%) and *An. squamosus* (0.9%). Similarly, a total of 8, 279 adult anophelines mosquitoes representing three species were collected through indoor (7784) and outdoor (495) samplings. Overall, *An. gambiae* s.l. was the predominant species, making 90.1% of all collections followed by *An. pharoensis* (9.6%). Both species were abundant after the short rainy season and remained high through the wet season, and declined in the dry season. Peak indoor resting density was observed in May. The ratio of fed to half gravid and gravid combined for *An. gambiae* s.l. in indoor resting collection was 4.9:1, indicating high degree of exophily in the study area. This was also supported by high number of half-gravid and gravid *An. gambiae* s.l. in outdoor collection than indoor. Similarly, high exophilic behavior (3.5:1) for *An. pharoensis* was also observed.

Of 662 *An. gambiae* s.l. and 246 *An. pharoensis* dissected from all collections; the average parous rate was 31.4% and 28.0%, respectively. None of the 208 parous *An. gambiae* s.l and 69 parous *An. pharoensis* analyzed by ELISA to detect sporozoite antigen of *P. falciparum* and *P. vivax* were found infected. This seems to support the parasitological data during the study period. Although no sporozoite infection rates was detected *An. gambiae* s.l. (presumably *An. arabiensis*) seems the principal vector of malaria in the area.

1. INTRODUCTION

1.1 Current Global Malaria Situation

Malaria is major cause of morbidity and mortality in tropical and subtropical countries of the world despite the enormous investment towards its control. It remains to be a major public health problem and is endemic in more than 107 countries of the world. Today over 40% of the world's population, mostly those living in world's poorest countries are at risk of malaria due to complex combination of environmental, social, economic and climatic factors (Bennett, 1995).

There are four species of *Plasmodium* responsible for the cause of human malaria, namely *Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae*. *Plasmodium vivax* is the most extensively distributed and causes much debilitating disease. *Plasmodium falciparum,* which is also widely spread, results in the most severe infections and is responsible for nearly all malaria-related deaths. *Plasmodium ovale* which is mainly confined to Africa is less prevalent, while *P. malariae,* which causes the least severe but most persistent infections, also occurs widely (Bruce-Chwatt, 1993).

Worldwide the world the incidence of malaria is estimated to be 300-500 million clinical cases per year, of which 90% of the cases are in Africa. It is estimated 2.7 million people die worldwide each year, of which about 1million are African children under the age of 5year (WHO, 2000). This loss of valuable human resources is something that many African countries, already underdeveloped, simply cannot afford.

Malaria has recently become increased significantly in parts of the world beyond the tropics as seen even in the United States, where the number of infected Americans increased from 303 in 1980 to nearly 600 in 1989 (Lauerman, 1991) and in Europe where 9,000 cases were reported in 1992. This increase is due, in part, to increased overseas travel (Johnson, 1993). The significant rise is also due to an increased resistance of *Plasmodium* to anti-malaria drugs, and also increased resistance of mosquitoes to insecticides (Miller, 1992; Aldhouse, 1993). No longer can first world countries distance themselves from the problem of malaria as it begins to spread and invade even those countries once thought to be free from its harmful effects.

Malaria is not only a health problem but also a vital hindrance to socio-economic development in most developing countries. It imposes very significant economic costs on some of the poorest nations. The African region lost more than $ 2 billion because of malaria and malaria-related diseases in 1997 alone (Samba, 2000). The economic cost as determined from direct cost of care and control, and indirect costs due to losses in productivity and lost future earning from death of malaria in South African countries is conservatively estimated to be $ 20 million in 1997/98 (Tren, 2000 cited in Kibretu, 2005). The incidences of malaria in these areas have severe economic impacts and as in the past, continue to thwart economic development.

The global malaria situation is worsening, particularly in Africa, where some of the most severe malaria epidemics have taken place. Malaria epidemics usually result from climatic changes, human activities and from failed control policies. Epidemics take place

when the factors limiting transmission are altered as a result of either temporary climatic changes such as abnormal rains, long periods of increased humidity and high temperature, or more permanent changes of the microclimate such as the development of agricultural irrigation systems (Bouma and Vander Kaay, 1996). The future pattern of malaria might be affected by global warming. Estimating the potential impact of climate changes on malaria transmission has generated a great deal of interest because, in part, of the concern that this debiliting disease may emerge or re-emerge in many parts of the world (Bayoh *et al*., 2003).

Movement of population or migration for the purpose of settlement in new areas is a major part associated with malaria transmission. During the occupation of such areas by the migrants, major environmental transformations take place, thus fostering the proliferation of mosquito breeding sites, and resulting in major malaria outbreaks and make epidemic-prone situations more explosive (Meek, 1988). Migrants are most likely to lack immunity against the disease as well as appropriate knowledge of the transmission process. A similar epidemiological pattern accompanies development projects such as railway and road construction and large irrigation projects undertaken in highly malarious areas. Malaria epidemic continues to develop and threaten populations even with modern control measures and the wealth of resources available (Packard, 1986; Najera *et al*., 1996).

1.2. Malaria in Africa

Malaria, which accounts for 90% of disease in Africa, ranks third among major infectious disease threats in Africa after pneumococcal acute respiratory infections (3.5%) and tuberculosis (2.8%) (Nchinda, 1998). The vast majority of malaria deaths occur south of the Sahara, where it also presents major obstacles to social and economic development (RBM, 2001). It threatens the lives and livelihoods of more than 500million Africans and exerts such a huge health burden that it has been incremented in the continued under development of the continent as a whole (Breman *et al.,* 2001).

In tropical Africa, case fatality rates of 10 to 30 % have been reported among patients with severe malaria referred to hospitals. These rates are even higher in rual and remote areas where there is little access to adequate treatment (Collins and Paskewitz, 1995). In southern and eastern Africa the proportion of deaths caused by malaria has increased from 18% in the 1980 to 37% in the 1990s (Korenromp *et al*., 2003).

Malaria in Africa has the greatest toll among pregnant women and young children. Most of the people who live in regions where malaria is endemic gradually develop immunity, although parasites may still live in the host, the host no longer suffers overt disease symptoms (Tangley, 1987). But this natural immunity is disrupted during pregnancy, and it does not develop in children until they are about five years old.

Most of malaria infections in Africa are caused by *P. falciparum*, the most and life-threatening form of the parasite (RBM, 2001). In many parts of the region changes in the environment, population migration (for reasons of agriculture, commerce and trade) and

financial problems have led to serious increases in malaria (Tangley, 1987; Collins and Paskewitz, 1995). In addition, large scale water development schemes for cultivating crops can also sharply increase malaria transmission by creating ideal breeding sites for the major malaria vectors. Furthermore, economic and political stress often results in population migration, which increases movement of non-immunes into malarious areas or carriers into non-endemic areas (Collins and Paskewitz, 1995).

In Africa today, malaria is understood to be both a disease of poverty and a cause of poverty. Annual economic growth in countries with high malaria transmission has historically been lower than in countries without malaria. It is believed that malaria is responsible for a growth penalty of up to 1.3% per year in some African countries (RBM, 2001). Malaria also has a direct impact on Africa's human resources. Not only does malaria result in lost life and lost productivity due to illness and premature death, but it also hampers children's schooling and social development through both absenteeism and permanent neurological and other damage associated with severe episodes of the disease (RBM, 2001).

A more serious problem in Africa in the fight against malaria is resistance to antimalarials drugs by *Plasmodium* parasites (RBM, 2001). Spreading drug resistance is increasing the amount of suffering and death caused by malaria in Africa as well as incidence of the disease. The other factors influencing the control of malaria are the occurrence of insecticides resistant vectors and changes in the behaviour of the mosquitoes as the result of frequent indoor insecticides sprays (Toure, 1999).

1.3. Malaria Vectors

All over the world, there are 430 species of *Anopheles* mosquitoes. Many species of *Anopheles* mosquitoes are important vectors of malaria, filariasis and several arboviruses. About 70 species of *Anopheles* have been definitely incriminated as vectors of human malaria (Service, 2000). *Anopheles* mosquitoes are most frequent in tropical and subtropical regions of the world. They are also found in temperate climates and even in the Arctic during the summer. As a rule, *Anopheles* spp. are not found at altitudes above 2000-2500m (Bruce-Chwatt, 1993).

The abundance and distribution of *Anopheles* vectors depend primarily on by the accessibility of ideal breeding sites, and such sites sometimes created through human activities, thus enhancing a close contact between man and vectors (Muirhead-Thompson, 1951).

1.3.1. Feeding and Resting Behavior

Females of most species of *Anopheles* mosquitoes require a blood- meal to nourish the eggs, and this is taken either before or more usually after mating. They obtain their blood meal from a wide range of hosts. The frequency with which an anopheline species feeds on human hosts (anthropophilic feeding behavior) as opposed to other animals (zoophilic feeding behavior) can point out those mosquitoes most likely to be important vectors (Bennett, 1995). The choice of host varies with the mosquito species and the opportunity available to it. Although trophic choice is important even normally zoophilic mosquitoes may potentially become dangerous vector (Bennett, 1995). In the absence of the preferred

host, when the mosquitoes is found in great abundance, or in situation that drive normally zoophilic mosquitoes to exhibit anthropophilic behaviour, these species serve as efficient vectors (Fontenille *et al*., 1990). Along with host preferences, mosquitoes also exhibit preferences regarding feeding location (Janz and Ribeiro, 1990). Some mosquito species exhibit exophagic behavior (feeding outdoors) while others tend to be endophagic (feeding in human-made shelters such as homes, storehouses, or stables) (Janz and Ribeiro, 1990). Feeding location can play an important role in whether or not a given species is likely to be an important malaria vector, as those mosquitoes feeding indoors are in closer contact with humans, and thus are more likely to show anthropophilic behavior (Janz and Ribeiro, 1990). As the number of bites received increases, so do the chances of receiving an infective bite and thus it is important to identify where mosquitoes are most likely to bite and to minimize contact with mosquitoes in these locations (Janz and Ribeiro, 1990).

Closely related to feeding location preference is the resting habit of the mosquito. Some species of *Anopheles* have endophilic character, which means that mosquitoes may either take a meal within a shelter and stay there, or take a meal outside and move into a shelter to rest (Gillies and DeMillon, 1968; Gillies and Coetzee, 1987; Service, 2000). On the contrary exophilic mosquitoes rest outdoors in a variety of natural shelters, such as vegetation, cracks and crevices, and under bridges after taking a blood meal (Service, 2000). In addition to the above characters, some species of *Anopheles* mosquitoes exhibit both behaviors, spending part of their gonotrophic cycle within the confines of human-made shelters and the other part outdoors (Gillies and Coetzee, 1987). The frequency of

resting on different sites in indoor shelters can be influenced by several factors, such as type of tukul construction, building materials, presence of households objects, presence of fires, and the availability of preferred hosts. Determining a mosquito's resting behavior can be important when launching antimalarial campaigns. Knowing where the mosquitoes of each species rest can help determine which insecticides might work best (Bennett, 1995).

1.3.2. Parity and Sporozoite Infections

The relative proportions of the different ages of mosquitoes in a population are a measure of their survival probability; this is determined by detection of their age. One method of aging anophelines involves dissecting out the ovarioles from ovaries and detecting ovarial relics (Detinova, 1962). The other method depends on the tracheation of the ovaries. The tracheation method is the detection of tightly coiled tracheoles into 'skeins' on the ovaries of females that have not oviposited their first batch of eggs. Tracheoles remain stretched in females which have oviposited (Detinova, 1945 cited in Service, 1993a). Parous females are those that have taken a blood meal and oviposited at least once, while nulliparous mosquitoes are young ones that have not taken blood meal and have not laid eggs. Parous females are epidemiologically dangerous as they might have been infected by parasites (Service, 2000). High parous rates in mosquito population imply longer survival, more gonotrophic cycles, and therefore, capable of transmitting malaria parasites for long (Jensen *et al*., 1998).

The sporozoite rate is the proportion of *Plasmodium* infected individuals in a population of local vector species (WHO, 1975). In order to know the role of a certain species of

Anopheles in malaria transmission, there is a need to dissect females and investigate the presence of sporozoites (the infective stages) in the salivary glands (Holisten, 1954). The above being the classical method, immunological methods like enzyme-linked immuno sorbent assays (ELISA) and more recently molecular methods like polymerase chain reaction (PCR) are more effective methods (WHO, 2003). Determining which *Anopheles* mosquitoes have the parasite can facilitate which species to target for control and to decide where and when to direct anti-malarial measures. These measures could either involve the use of chemical insecticides or some other measures against the vector of malaria (Bennett, 1995).

1.3.3. Human Blood Index (HBI)

Determination of the human blood index is one of the important factors in determining the vectorial capacity of the mosquito species (Adugna and Petros, 1996). It is also vital to differentiate the host preference of a species as well as the proportion of anthropophagy and zoophagy in a wild population. It is therefore necessary to determine the blood meal of species. Numerous procedures have been used to determine blood meal sources of arthropod vectors. These are precipitin test, fluorescent antibody techniques, passive hemagglutination inhibition technique and latex agglutination test (Adugna and Petros, 1996). Among these, the most widely used procedure remains to be the precipitin test, and yet it lacks sensitivity and specificity, and is time consuming (Washino, 1952 cited in Adugna and Petros, 1996). After the introduction of the microplate enzyme-linked immunosorbent assay (ELISA) for determination of blood meal source and sporozoite antigen, the precipitin test became no more popular (Beier *et al*., 1988).

ELISA method has sensitivity and specificity, the potential to perform the test on fresh, dried or frozen specimens (Beier *et al*., 1988). The double immuno-diffusion (double gel diffusion), counter-current immuno electrophoresis and others molecular methods (PCR) which are said to be sensitive and specific have also been used (Balkew, 2001).

Before the advancement and use of these recent techniques, information on mosquito blood meals in Ethiopia was derived from precipitin tests. Krafsur (1971) and Krafsur and Armstrong (1978), upon precipitin testing found 100% positive for human blood index for *Anopheles gambiae* s.l., *An. funestus* group and *An. nili* that were all collected from indoors, in Gambella. White *et al.* (1980), also reported a similar result for indoor resting *An. gambiae* s.l. around Jimma, but in mixed dwellings and animal shelters HBI for this species were 46% and zero respectively. Adugna and Petros (1996) found 88% HBI for *An. arabiensis* using ELISA from collections in mixed dwellings; and for *An. pharoensis* it was 84%, made in Arabmich, Awassa, Metahara and Ziway. Using the double immuno-diffusion method, Balkew (2001) recorded an overall HBI of 65% for *An. arabiensis* collected from three dwelling types (human alone, mixed dwellings and cattle sheds) in Metahara area (Eastern Ethiopia). Host preference of the main vector in Ethiopia therefore, shows variation in HBI depending on the availability, location and density of the host population.

1. 4. Malaria in Ethiopia

The earliest important studies on the epidemiology of malaria in Ethiopia were conducted during the 1936-1941 Italian occupation (Nigatu *et al*., 1994). The British and other pioneers later made substantial contribution to the information available today (Melville *et al*., 1945; Covell, 1957; Balkew, 2001). The disease is mainly characterized seasonal and sometimes appears as epidemic. In most parts of the country, particularly in the highland fringes, unstable malaria transmission are common when climatic conditions are conducive for its transmission (Tulu, 1993a; Ghebreyesus *et al*., 2006). In the epidemic situation, communities that lack protective immunity are severely attacked.

Malaria causes major obstacles to social and economic development of the country. The presence of malaria in lowlands accounted to overcrowding of populations to the highlands of the country which in turn resulted in the destruction of the ecology, reduced productivity, and hence famine and poverty. In endemic areas peak transmission periods coincide with the planting and harvesting seasons that reduce productive capacity of agricultural work. It is also responsible for loss of earning, and high treatment cost (Tulu, 1993a).

In Ethiopia, malaria is caused by four species of *Plasmodium*: *P. falciparum*, *P. vivax, P. ovale* and *P. malariae*, of which the former two are epidemiologically the most dominant accounting for 60% and 40% of malaria cases, respectively. This proportion varies from place to place and from season to season (Tulu, 1993b; Abose *et al.*, 1998b; Ghebreyesus *et al*., 2006).

Due to diverse topography and associated rainfall patterns, malaria transmission in Ethiopia varies widely. About three - fourth of the landmass is potentially malarious and over 40 million people are at risk of infection (Ghebreyesus *et al*., 2006). There is no administrative region free of the disease. In fact some region for example, Afar and Somali are 100% malarious (MOVBDCU, 1999 cited in Kibretu, 2005). In areas lying below 2000m, malaria is common, but highly prevalent below 1500meters. Areas lying at altitudes of 1500-2000m are prone to irregular malaria epidemic (Ghebreyesus *et al*., 2006). It is absent in areas above 2500m as the mean annual temperature of 10-15^0 C is too low to support development and survival of the parasite in the mosquito vector (Ghebreyesus *et al*., 2006). Malaria surveillance in the country showed that the mean number of reported cases is rising from year to year, for instance during the year 1980-1984, the mean parasite rate increased from 8.9 to 33.1% per annum (Wondwossen, 1991 cited in Kibretu, 2005). Some of the reasons for the increase of transmission include large scale migration, instability in conflict areas and spread of chloroquine resistant *falciparum* malaria (Mengesha and Mekonnen, 1999).

The transmission of malaria in the country is greatly influenced by seasonal variation in rainfall. The main malaria transmission peaks are between September and December, immediately at end of main rains of June through September, and a second but less pronounced peak occurring during the second transmission season in April and May, following the short rains (Ghebreyesus *et al*., 2006).

It was considered that upper limit for malaria transmission as 2000m above see level, but different periodic epidemics were recorded above this level. Consequently, moderate to

sever malaria epidemics was known to occur in the county (Covell, 1957; Fontaine *et al*., 1961; Chand, 1965). It was a cause for the death of 7000 people on the Dembiya plain, between Lake Tana and Gondar, in 1953. In the same year, one fifth of the inhabitants of Kolladiba were reported to have died of malaria (Covell, 1957). The most significant and devastating epidemic was that of 1958, which resulted in an estimated 3 million cases about 150, 000 deaths (Fontaine *et al*., 1961; Ghebreyesus *et al*., 2006). In recent years, frequent malaria epidemics of cyclical patterns of variable magnitude were recorded. For example, the epidemics of the years, 1988, 1991, 1992, 1995 and 1998 in different part of the country. Among these epidemics, mortality in the 1998 was very high accounting 7,783 deaths occurred out of 222,992 cases in West and East Gojam zones (Ghebreyesus *et al*., 2006).

1.5. Malaria Vectors in Ethiopia

The basis for the information of anopheline identification and distribution in the country were the works of Verrone (1962a, 1962b), O'Connor (1967), and Gillies and DeMillon (1968). Today, 42 species and subspecies of anopheline mosquitoes have so far been recorded in Ethiopia, with distribution varying by altitudinal zone and microhabitats (White *et al*., 1980; Ghebreyesus *et al*., 2006). Forty of these are recorded as potential vectors among the 70, which are recognized to have potential roles in malaria transmission throughout the world (Service, 1993b). Of these, *An. gambiae* complex, *An. pharoensis*, *An. funestus* and *An. nili* are associated in the malaria transmission in Ethiopia, the former being the most important (Abose *et al.*, 1998b). There are also other species which are suspected in the transmission of malaria, for example *An. coustani*

(Tekie, 1989). The *An. gambiae* complex comprises seven sibling species of which only *An. arabiensis* and *An. quadriannuiatus* sp.B are known to exist in Ethiopia (Abose *et al.*, 1998b). The former is the principal vector of malaria in Ethiopia and also in some parts of Africa (Ghebreyesus *et al*., 2006), the later is regarded to be zoophilic and hence is not known to involve in the transmission of malaria (Abose *et al.*, 1998b). The other species (*An. pharoensis*, *An. funestus* and *An. nili*) are believed to play a secondary role in the transmission of malaria (Abose *et al.,* 1998b; Ghebreyesus *et al*., 2006). In some parts of the country reports revealed that, one or more of the secondary vectors may occur sympatrically with *An. arabiensis* (Nigatu *et al*., 1992; Abose *et al*., 1998b). This coexistence is much more frequent with *An. pharoensis* than with others.

An. quadrannulatus (later identified as *An. quadrannulatus* species B, Hunt *et al*., 1998) in Ethiopia was reported for the first time by Turner (1972) and its distribution was shown to be in the highlands of south western and northern region; co-existing with *An. arabiensis* (White, 1974; White *et al*., 1980).

White *et al*. (1980) observed that in an area where *An. arabiensis* and *An. quadriannulatus* sp. B coexisted, the latter out numbered the former in animal shelters, but it was less abundant in mixed dwellings (containing humans and domestic animals) and was totally absent from houses only occupied by humans. As opposite to the above authors, Pates *et al.* (2006) found that *An. quadriannulatus* sp. B was also found in the houses that are occupied only by humans, in the Jimma area. Comparatively little is known about the blood feeding behaviour of *An. quadriannulatus* sp. B.

White (1974) states that, although *An. quadriannulatus* sp. B from Ethiopia are principally zoophilic they also willingly bite man, indoors or outdoors, especially when located close to cattle. When Fettene *et al*. (2004) (cited in Pates *et al.,* 2006) checked the blood meals of some Ethiopian *An. quadriannulatus* sp. B, they found that, although almost all the specimens had fed on cattle, a few (<1.1%) had fed on humans.

Though the anopheline fauna of Ethiopia is highly diversified the most important vector of malaria is *An. arabiensis* (White *et al*., 1980; Abose *et al.,* 1998a; Abose *et al*., 1998b; Ghebreyesus *et al*., 2006). It is usually the vector of epidemic malaria. Mosquitoes of this species breeds in small, temporary, sunlit water collections created during and after the rains. It also breeds in a wide variety of other types of water bodies (Mekuria, 1983). Sometimes it also breeds in artificial breeding habitats that are created by human activity, for instance construction pits such as those used for plastering of houses, hoofprints of animals and human, tractors and other vehicles in agricultural development and irrigation canals.

Jolivet (1959) revealed that in Ethiopia, adults of *An. arabiensis* inhabit in and around human dwellings as well as in areas where human being are absent. The host preference of *An. arabiensis* depends on the availability of the host. Where the available hosts are only humans, the species derives its blood meal from humans. In areas where both humans and cattle are present, it feeds on both types of hosts with variable proportions (White, 1974; Balkew, 2001). This species is not strictly anthropophilic, endophagic and endophilic. It exhibits partial zoophily, feeds and rests both indoor and outdoors (White, 1974).

The *An. funestus* group is widely distributed and is known to be the second important vector of malaria in the country, sometimes even dominating in certain areas of endemic malaria, for example in Gambella where it was reported to be a major vector (Krafsur, 1971; Turner, 1972). It is regarded as the most important secondary vector of malaria after *An. gambiae* s.l. This species contributes substantially to the transmission of malaria, at least in the lowlands where it abounds (Mekuria, 1983).

Anopheles pharoensis has a wide distribution in Ethiopia. It is regarded as a weaker vector in tropical Africa, due to its short life (Mekuria, 1983). Based on its voracious feeding habit on human and on its abundance in certain localities of Ethiopia is believed as a secondary vector in some parts of country. Nigatu *et al*. (1994) in Gambella reported sporozoite rate of 0.47% from a total of 430 *An. pharoensis*. Abose *et al*. (1998b) found infection rate zero for this species in Ziway. Balkew (2001) found a sporozoite rate of 3.2% (2/63) for the same species in Metehara area.

The *An. coustani* group consists of four member species of the group. These are *An. coustani*, *An. ziemanni*, and *An. tenebrosus* and *An. paludis*. The group has wide distribution in the country (Mekuria, 1983; Tekie, 1989). Concerning about the sporozoite rate, one positive gland, was found from unknown number in *An. ziemanni*, which was thus considered as a suspected vector. In was observed to be antropophilic in Western Ethiopia (Krafsur, 1977).

In Ethiopia sporozoite infection rates in *Anopheles* sp. is very low (0- 3%) (Rishikesh, 1966; Mekuria, 1983; Ameneshewa, 1995; Abose *et al.,* 1998b; Balkew, 2001; Taye *et al*., 2006) and large numbers of mosquito specimens need to be tested by dissection or ELISA in order to be able to determine the actual infection rate (Abose *et al*., 1998a).

2. OBJECTIVES

2.1. General Objective

This study was aimed to understand the ecology, species composition, behavior, seasonal variation and vectorial role of the *Anopheles* mosquito in relation to malaria transmission in the Koka villages.

2.2. Specific Objectives

1. Study the species composition, relative abundance and the seasonal variation of *Anopheles* species in the Koka area.
2. Determine the age (parous) and sporozoite rates of the *Anopheles* spp.
3. To study the resting and feeding behavior of the *Anopheles* species.
4. To identify breeding sites of anopheline mosquitoes.

3. MATERIAL AND METHODS

3.1. Study Area

The study was carried out in Koka area as a part of bigger project on malaria control supported by WHO/ AFRO Region. Koka is situated at about 100 km southwest of Addis Ababa on the Addis-Awassa road. It is located at 8º 41' N and 39º 35' E at an altitude of 1700m a.s.l. The area is malarious where small scale irrigation and vegetable farming takes place mainly as a source of income. Fishing activity is also intensively carried out as a source of income. Overflooding of Awash River and the Koka dam during the rainy season and retreating of these waters during the dry season creates swampy and more or less permanent large pools of water during most of the year which appear as small lakes on either of the road. Recently, several floral farming enterprises are sprouting in the area and are operating in full swings. Each of these enterprises have about 200-300 migrant and non-migrant laborers. The irrigation agricultural activities and outlet canals from these floral farms, the edges of the lakes serve as potential breeding habitats for mosquitoes.

The inhabitants mainly belong to the Oromo ethnic group who live principally on farming and fishing. Most live in tukuls constructed of mud walls and thatched roofs with some living in iron-roofing. They also raise cattle, sheep and goats. The vegetation of the study area which is grossly affected by human activity, is typical savanna woodland dominated by scanty of *Acacia* spp. and bushes.

The present investigation was carried out in two farming communities located near Koka on the main road to Ziway-Shashemene, Southern Ethiopia. These were koka-Negewo

Farmers' Association and Malima Farmers' Association, situated before and after Awash bridge near Koka, respectively. There are 4-5 villages ('Gots') in each of the above farmers' association. For the entomological study, one 'Got' (village) from Koka-Negewo and three 'Gots' (villages) from Malima (Fig 1) were selected, based on proximity to potential breeding sites near Awash River and Koka lakes. The distance between each of these study localities ('Gots') ranges from 1-2 kms. The study was carried out between December 2006 and November 2007.

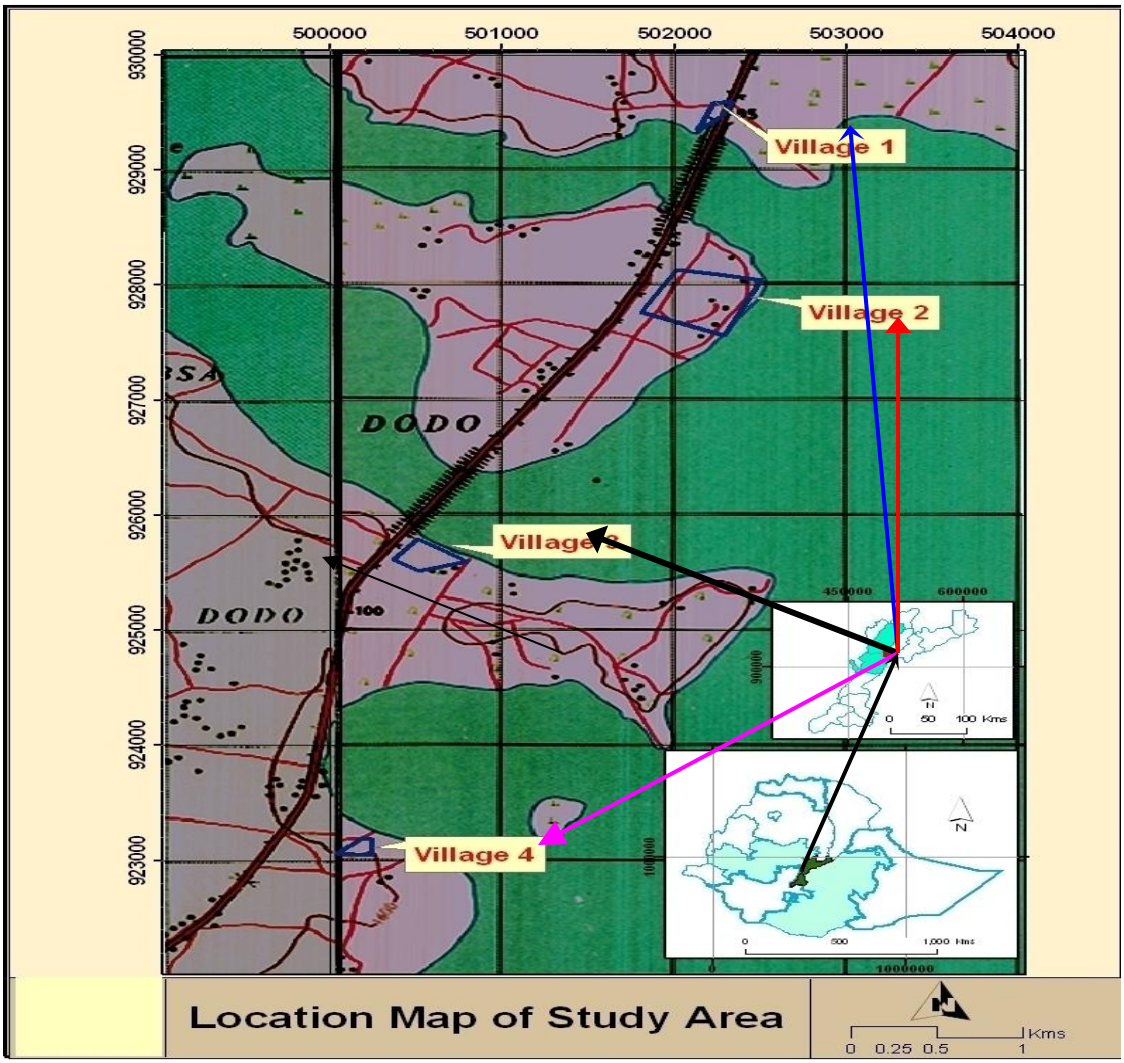

Figure 1. Map of the study area showing the four collection sites (villages)

3.2 Meteorological data

In order to see the relation of some weather conditions with the entomological observations, various weather variables (total rainfall of each month and mean monthly temperature) of the area for years (2003-2007) were kindly obtained from the National Meteorological Services Agency (Addis Ababa). In addition, as no relative humidity data could be obtained for Koka itself, data from the nearest weather station at Ziway was obtained still from the same agency.

The monthly mean temperature, relative humidity (RH) and rainfall data of the area for four years (2003-2007), kindly obtained from the National Metrological Services agency are depicted in Fig 2. The main feature is that there is one main rainy season during a year from June to September and a short rain between March and May. The average annual rainfall of the area is about 711.5mm. The mean monthly temperature fluctuates during the year being higher (22.5-24.4^{o}C) between February and May (dry season) and lower (20.5-21.4^{o}C) during the rainy season and early part of the dry season (October-December). RH was relatively stable throughout of the year (50-70%).

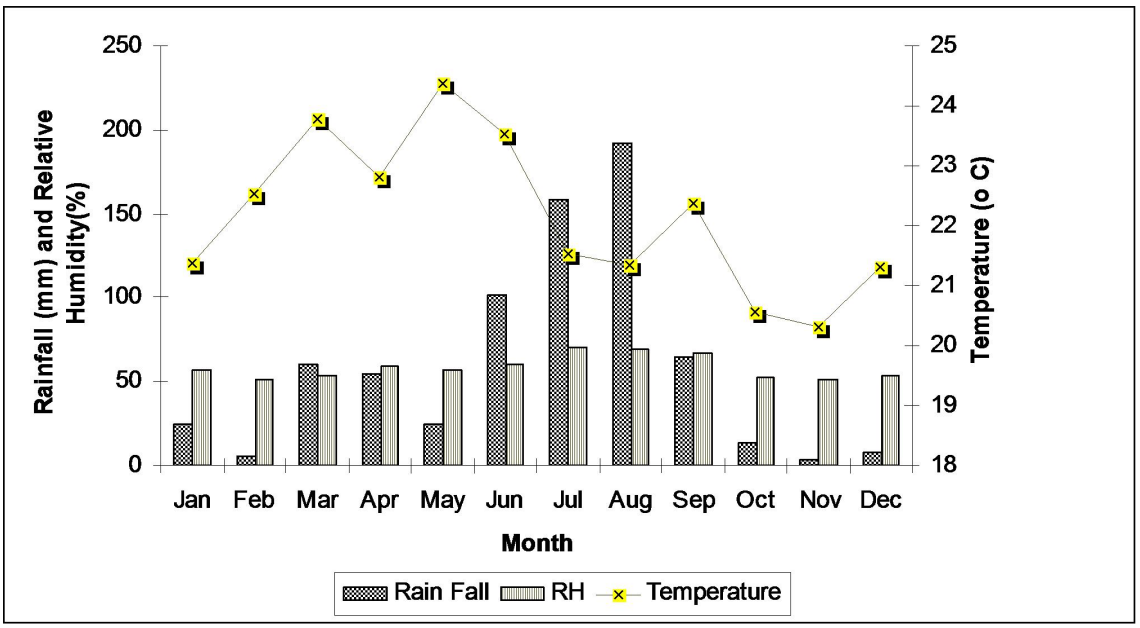

Figure 2. Monthly rainfall and mean temperature of Koka villages (2004-2007) and average relative humidity of Ziway (2003-2006)

Source: Compiled from data obtained from the National Meteorological Service Agency.

3.3. Entomological studies

3.3.1. Collection of immature stages and identification

To determine the species of *Anopheles* mosquitoes present and the preferred breeding habitats of the anopheline species, mosquito larvae and pupae were collected using appropriate ladles, pipettes and containers (Service, 1993a) from pools of temporary and permanent breeding habitats of the area such as hoof prints, brick pits (Plate 1), swampy areas, water canals (Plate 2), irrigation ditches and rain pools. *Anopheles* larvae collections were undertaken for about two hours in each 'Got' (village), usually between 8:00-10:00a.m. by two persons (myself and a field assistant). The larvae were brought to a field station and killed in warm water (about 60^0C) and preserved in small vials containing 70% ethyl alcohol (Service, 1993a) and transported to Addis Ababa. Each larva was mounted on a glass slide separately in a drop of gum- chloral mountant and covered with a cover slip (Lane, 1974). Identification of larvae was carried out based on keys by Verrone (1962b) and Gillies and Coetzee (1987) under a compound microscope. Pupal collections were allowed to emerge to adults in cages in the field, after which the adults were identified (Verrone, 1962a; Gillies and Coetzee, 1987).

Plate 1. Brick pit that serve as breeding habitat for *Anopheles* spp. in Koka villages

Plate 2. Water canal from the floral industry serving as a breeding site for *Anopheles* mosquitoes in Koka area

3. 3. 2. Collection of adults and identification.

3.3.2.1 Indoor collection

Anopheline mosquitoes were collected using indoors CDC light traps, aspirator and space spray methods (Service, 1993a).

3.3.2.1.1 CDC light trap collections

In each of the four selected 'Gots' in the study area, five tukuls were selected for the collection of endophilic and endophagic mosquito in which there were a few occupants in the tukul (2-3). In each tukul, the occupants were provided with untreated bed net to sleep under during the sampling night. For sampling night-biting mosquitoes in each tukul, a CDC light trap (Plate 3) was positioned at the foot end of the bed or floor and operated the whole night from dusk to dawn (6:00p.m -6:00a.m.)(Mboera *et al*., 1998). Mosquitoes collected on each trap were identified to the species using keys of Verrone (1962a) and Gilles and Coetzee (1987). Females collected were classified according to their blood digestion stages and dissected (WHO, 1975).

Plate 3. CDC light trap used to collect endophilic and/or endophagic night biting mosquitoes indoors

3.3.2.1.2 Aspirator collection

To asses the indoor resting mosquito, mosquitoes were collected using a mouth suction aspirator and a torch light from walls, eaves, hanging clothes and other household utensils (Service, 1993a). Such samplings were done in five tukuls in each village once a month during the study period. Collections were usually conducted in the morning hours (07.00-08.00) for about 10-20 minutes in each tukul. Collected female mosquitoes were identified, categorized according to their feeding status and processed for dissection (WHO, 1975).

3.3.2.1.3 Space spray method

In addition to aspirator collection, space spray method was employed to sample indoor resting anopheline mosquitoes (Service, 1993a). It was conducted early in the morning from 6:00-8:00 once a month in a tukul in each of the four 'Gots' of the study area during the study periods. For this purpose, all occupants, removable objects, exposed food and water were removed from the tukuls. Doors, windows and any opening were properly closed. The entire floor was covered with white plastic sheets (4×3m), after which an aerosol insecticides spray (Roach-Killer-M/s Kafr Elzayat, Maybanz Plc, Egypt) containing Fenitrothion, Cypermethrin, and Bioallethrin (2.3%) locally purchased was sprayed for about 5min inside the tukul. After spraying, the operator waited outside and the tukul remained closed for 15min to produce a knockdown effect. Then, the white plastic sheet was taken outside and all knocked down mosquitoes were collected using forceps and placed in test tubes.

All the female anopheline mosquitoes were identified to species level, categorized according to their feeding status and processed for dissection (WHO, 1975).

3.3.2.2. Outdoor collection

To sample outdoor resting mosquitoes, two methods were used: pit shelters (Service, 1993a) and Clay pots (Odiere *et al*., 2007). Attempts were also made to collect mosquitoes resting outdoors on vegetation using sweeping nets, but were unproductive on many occasions and were thus discontinued.

3.3.2.2.1 Collection in pit shelters

For collection in pit shelters, one rectangular pit shelter (1×1×1m) was dug in each selected 'Got' of the study area under shades of trees/bushes (Plate 4) near human dwellings and mosquito breeding sites (30-70metres). Inside the four sides of the pit, a horizontal hole of about10-15cm wide and 30cm length was prepared as hiding places for mosquitoes. Resting mosquitoes in these shelters were collected with an aspirator and torch once a month throughout the study period. Collection was done for about 10-20 minute in each pit. As above, the collected female mosquitoes were identified, categorized according to their abdominal status and dissected (WHO, 1975).

3.3.2.2.2 Collection in clay pots

The placement of clay pots (normally used for cooking or storing water), has been effectively used in collecting outdoor resting mosquitoes (Odiere *et al*., 2007). Two pots (one big with 10-15L capacity and a small one with 3L capacity) were placed in shaded places such as under tress and fences in each selected 'Got' (Plate 5), so that a total of eight clay pots were used in the study area. The pots were modified for mosquitoes sampling as follows. Three small holes per pot with 2 cm-diameters were made at the center of the base. These holes made the pot useless for cooking or to hold water, thereby limiting the likelihood of theft. Mosquitoes were sampled from the pots using an aspirator and torch or at times blown out through the holes made at the bottom into a mosquito cage. The collected female anophelines were identified, categorized according to the abdominal status and processed for dissection (WHO, 1975).

Plate 4. Pit shelters used to collect of outdoor resting mosquitoes

Plate 5. clay pots used to sample outdoor resting mosquitoes

3. 3. 3. Anopheline dissection for parity and sporozoite rates

After identification of the collected females *Anopheles* by the various methods described above, the abdominal status of each female *Anopheles* was categorized and recorded as freshly fed, half-gravid, gravid or unfed (WHO, 1975). The abdomen of each freshly fed mosquito was separately squashed on numbered portions of whatman No.1. filter paper using the wooden handle of a dissecting entomological needle under a cover slip. These were placed in an envelope and stored in -20^{o}C deep freezer in the laboratory of ALIPB for later blood meal analysis.

Unfed *Anopheles* females were dissected for parity rate (Plate 6). For this purpose, the individual mosquito which had previously been immobilized with chloroform vapor, was dissected in a drop of saline (0.85% sodium chloride) on a slide using two fine entomological needles under a dissecting microscope (Service, 1993a). The last 2-3 abdominal segments were pulled by the second needle and drawn away from the rest of the body to expose ovaries. The ovaries were left aside on the same slide to dry and observe the skeins of the tracheoles under microscope whether they were coiled or stretched for determination of parity (Detinova,1945 cited in Service, 1993a). Those mosquitoes which had the tracheoles coiled were recorded as nulliparous and those with stretched tracheoles as parous (Detinova, 1962). The parous females were preserved in labeled cryotubes containing silica gel for later sporozoite rate determination using ELISA; nulliparous females were recorded and discarded.

Plate 6. Identification and dissection of *Anopheles* spp.

3. 3. 4. Sporozoite rate determination using ELISA

Parous *Anopheles* mosquitoes were tested for sporozoite rate using enzyme-linked immunosorbent assay (ELISA) following the procedure of Wirtz *et al*. (1985; 1987) at the laboratories of EHNRI and ALIPB. Monoclonal *Plasmodium falciparum*, and *P. vivax*-210 and *P. vivax*- 247 capture- and peroxidase-labelled antibodies were used for the detection of sporozoite antigens in dried mosquitos. 50ml of the capture monoclonal antibodies (MAb) of *P. falciparum* (0.2ug/50u1 PBS) and *P. vivax* (0.025/ug/50ul PBS) were absorbed to wells of a microtitre plate. Following 30-minute incubation or overnight incubation at room temperature to bind the MAb to the plate, the well contents were aspirated and the remaining active binding sites on the plates blocked with 200u1 blocking buffer. The head-thorax of the dried mosquitos were ground in blocking buffer containing Igepal-630 diluted with more blocking buffer and aliquot and then added to the wells. Positive control and negative controls (these are triturate laboratory reared, known uninfected female mosquitoes from the insectary of ALIPB) were added to specific wells at this time. After two hour of incubation, the mosquito triturate was aspirated and the wells washed with PBS containing Tween-20. The respective peroxidase linked MAb was then added to the wells. After one hour, the plate was washed three times with PBS-Tw. and peroxidase substrate solution (ABTS and hydrogen peroxide) was added. A dark green product would indicate the presence of the CS protein after enzyme reaction for one hour (Wirtz *et al*., 1987; Abose *et al*., 1998b).

4. RESULTS

4.1. Species composition of *Anopheles*

4.1.1 Larval collections

During the study period, a total of 2,472 anophelines larvae were collected from different breeding habitats (Table 1). Three anopheline species were encountered. *An. gambiae* s.l. was the predominant species (84.7%); followed by *An. pharoensis* (14.4%) and *An. squamosus* (0.9%).

Both *An. gambiae* s.l. and *An. pharoensis* were found together in most of the breeding habitats searched for larvae, although there were differences in density. Among the different breeding habitats, brick making pits were the most favored sites by larvae of *An. gambiae* s.l., (χ^2 = 10.39, df=1, P= 0.001), while marshy areas were most favored by larvae of *An. pharoensis* (χ^2 = 65.5, df=1, P<0.001). The water canals/ditches from horticulture industry serve as artificial breeding habitats for both species. Monthly abundance and variation of the three species of *Anopheles* larvae is shown in Tables 2

and 3. It can be seen that larvae of *An. gambiae* s.l. were common in most habitats during the months of April and July (wet months) in most habitats except the pool of rain water which were created during the peak of the rainy season in August and served as the most favorable breeding habitat for *An. gambiae* s.l. larvae. During the months of September to March, although much of the area remains covered by large body of water, they were unproductive during most larval surveys in all accessible and likely habitats. Larvae of the second abundant species (*An. pharoensis*) were most abundant in marshy habitats in the months April and July as for *An. gambiae* s.l.

Table 1. *Anopheles* larvae/pupae collected from various breeding habitats in Koka villages (Dec. 2006 - Nov. 2007)

Breeding habitat	Species			Total
	An. gambiae s.l.	*An. pharoensis*	*An. squamosus*	
Hot spring	39	11	0	50
Water canals	193	15	0	208
Marsh	429	199	5	633
Pool/rain water	611	9	0	620
Bricks pit	729	67	0	796
Water pump pits/holes	16	37	0	53
Edge of lake	76	19	17	112
Total (%)	2093 (84.7)	357 (14.4)	22 (0.9)	2472

Table 2. Monthly variation of *An. gambiae* s.l. larvae/ pupae in different breeding habitats in Koka villages (Dec. 2006 – Nov. 2007)

Breeding habitat	Monthly abundance of *An. gambiae* s.l. larvae/ pupae												
	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Total
Hot spring	0	0	0	0	39	0	0	0	0	0	0	0	39
Water canals	0	0	0	0	0	50	0	0	0	76	67	0	193
Marsh	0	0	0	0	90	65	188	86	0	0	0	0	429
Pool/rain water	0	0	0	0	0	40	0	0	571	0	0	0	611
Bricks pit	0	0	0	0	0	326	367	36	0	0	0	0	729
Water pump pits/holes	0	0	0	0	14	0	0	2	0	0	0	0	16
Edge of lake	0	0	0	12	64	0	0	0	0	0	0	0	76
Total	0	0	0	12	207	481	555	124	571	76	67	0	2093

Table 3. Monthly variation of *An. pharoensis* larvae/ pupae in different breeding habitats in Koka villages (Dec. 2006- Nov. 2007)

Breeding habitat	Monthly abundance of *An. pharoensis* larvae /pupae												
	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Total
Hot spring	0	0	0	0	11	0	0	0	0	0	0	0	11
Water canals	0	0	0	0	0	14	0	0	0	1	0	0	15
Marsh	0	0	0	0	14	35	27	123	0	0	0	0	199
Pool/rain water	0	0	0	0	0	0	0	0	9	0	0	0	9
Bricks pit	0	0	0	0	0	23	7	37	0	0	0	0	67
Water pump pits/holes	0	0	0	0	1	0	0	36	0	0	0	0	37
Edge of lake	0	0	0	0	19	0	0	0	0	0	0	0	19
Total	0	0	0	0	45	72	34	196	9	1	0	0	357

4.1.2. Adult collections

During the study period, a total of 8, 279 adult anophelines were colleted comprising of three species based on all methods of collection (Table 4). These included *An. gambiae* s.l., *An. pharoensis* and *An. ziemanni.* As in larval collection, the most abundant adult species was *An. gambiae* s.l (90.1%), followed by *An. pharoensis* (9.6%) and *An. ziemanni* (0.3%). No adult *An. squamosus* was collected despite its larval stages being found. The majority of mosquitoes were collected from indoor collections, most being collected by CDC light traps.

Table 4. Adult *Anopheles* mosquitoes collected by different methods in Koka villages (Dec. 2006 -Nov. 2007).

	Indoors			Outdoors		
Anopheles spp.	Space spray	Aspirator	CDC light trap	Pit shelter	Clay pots	Total (%)
An. gambiae s.l.	638	351	5974	443	52	7458(90.1)
An. pharoensis	4	9	782	3	0	798 (9.6)
An. ziemanni	0	0	23	0	0	23 (0.3)
Total *An.* sp. (%)	642 (7.7)	360 (4.3)	6779 (82)	446 (5.4)	52 (0.6)	8279

4.1.2.1. Indoor collections and seasonal changes

Tables 5 and 6 summarize indoor collections using space spray, aspirator and CDC traps from selected houses from December 2006 to November 2007, for *An. gambiae* s.l and *An. pharoensis*, respectively. The former two methods of collections represent the actual indoor resting mosquitoes while the latter can be those that are host seeking and/or resting mosquitoes indoors. The results showed that *An. gambiae* s.l was the predominant species resting or host seeking in human dwellings (Table 5) than *An. pharoensis* (Table 6). The bulk of the collections of both species came from CDC light traps followed by space spray and aspirator collections. All of the small numbers of *An. ziemanni* were only collected using the CDC light trap. The density of *An. gambiae* s.l varied between the months of the sampling period; higher densities occurred from April to September with peak densities in May (70.4 mosquitoes/hut/day for space spray, 7.95 mosquitoes /hut/day for aspirator collections) and; May and June (72.4 to 80.7 mosquitoes /trap/hut/day) for CDC trap (Table 5). Space spray and aspirator collections of *An. pharoensis* were much lower than CDC light trap catches which revealed higher densities between March and September with its highest peak at 12.2 mosquitoes /trap/hut/day in July (Table. 6).

Table 5 Indoor collection of *An. gambiae* s.l. using three methods of collection (Dec. 2006 - Nov. 2007).

Month & Year	Space spray	Aspirator	CDC trap	
	Total coll. (# /hut)	Total coll. (#/hut)	Total coll. (#/trap/hut)	**Total**
Dec. 2006	-	1 (0.25)	13 (0.65)	14
Jan. 2007	-	17 (0.85)	15 (0.75)	32
Feb.	47(11.8)	0	67 (3.35)	114
Mar	40 (10.0)	19 (0.95)	40 (2.0)	99
Apr	33 (8.4)	56 (2.9)	244(12.2)	**333**
May	282 (70.4)	159 (7.95)	1447 (72.35)	**1888**
Jun.	117 (31.6)	40 (2.0)	1614 (80.7)	**1771**
Jul	34 (8.5)	23 (1.15)	1381 (69.05)	**1438**
Aug.	27 (6.75)	19 (0.95)	393 (19.65)	**439**
Sep	52 (13.25)	15 (0.75)	507 (25.35)	**574**
Oct	1 (0.25)	1 (0.05)	247 (12.35)	249
Nov.	5 (1.25)	1 (0.05)	6 (0.3)	12
Total	638 (13.29)	351 (1.56)	5974 (24.89)	6963

Note: Numbers in parenthesis are density of mosquitoes/hut/

Table 6 Indoor collection of *An. pharoensis* using three methods of collection

(Dec.2006-Nov. 2007)

	Space spray	Aspirator	CDC trap	Total
Month & Year	Total coll. (# /hut)	Total coll. (#/hut)	Total coll. (#/trap/hut)	
Dec. 2006	-	0	20 (1.0)	20
Jan. 2007	-	0	9 (0.45)	9
Feb.	0	0	33 (1.65)	33
Mar	2 (0.5)	1 (0.05)	42 (2.1)	45
Apr	1 (0.25)	2 (0.1)	65 (3.25)	68
May	0	3 (0.15)	37 (1.85)	40
Jun.	0	0	130 (6.5)	**130**
Jul	0	0	243 (12.15)	**243**
Aug.	1 (0.25)	1(0.05)	83 (4.15)	**85**
Sep	0	2 (0.1)	70 (3.5)	**72**
Oct	0	0	30 (1.5)	30
Nov.	0	0	20 (1.0)	20
Total	4 (0.08)	9 (0.04)	782 (3.26)	795

Note: Numbers in parenthesis are density of mosquitoes/shelter trap/

4.1.2.2 Outdoor resting collections and seasonal changes

The outdoor resting *An. gambiae* s.l. and *An. pharoensis* densities from pit shelters and clay pots are shown in Tables 7 and 8, respectively. As in indoor collections, *An. gambiae* and *An. pharoensis* were the only species collected, the former species being the predominant species (495 *An. gambiae* s.l. vs. 3 *An. pharoensis*), but with much lower densities than indoors. *Anopheles gambiae* s.l. peak densities occurred between May and August; highest at 21.3 mosquitoes/pit shelter/day) in June. Pit shelters were more productive than clay pots (χ^2 =308.8, df =1, P< 0.001) in which collections were limited to the months of May and July when overall mosquito density generally increased.

Table 7. Outdoor collection of *An. gambiae* s.l. from the pit shelters and clay pots (Dec. 2006 – Nov. 2007)

Month & Year	**Pit shelter**	**Clay pot**	**Total**
	Total coll. (# /pit shelter)	Total coll. (#/pot)	
Dec. 2006	0	0	0
Jan. 2007	49 (12.3)	0	49
Feb.	28 (7.0)	0	28
Mar	36 (9.0)	0	36
Apr	34 (8.5)	0	34
May	62 (**15.5**)	31(4.4)	93
Jun.	85 (**21.3**)	17 (2.4)	102
Jul	64 (**16.0**)	4 (0.6)	68
Aug.	64 (**16.4**)	0	64
Sep	19 (4.8)	0	19
Oct	1 (0.25)	0	1
Nov.	1 (0.25)	0	1
Total	443	52	495

Note: Numbers in parenthesis are density of mosquitoes/pit shelter or clay pot

Table 8. Outdoor collection of *An. pharoensis* from the pit shelters and clay pots (Dec. 2006 – Nov. 2007)

Month & Year	Pit shelter	Clay pot	Total
	Total coll. (# /pit shelter)	Total coll. (#/pot)	
Dec. 2006	0	0	0
Jan. 2007	0	0	0
Feb.	0	0	0
Mar	2 (0.5)	0	2
Apr	1(0.25)	0	1
May	0	0	0
Jun.	0	0	0
Jul	0	0	0
Aug.	0	0	0
Sep	0	0	0
Oct	0	0	0
Nov.	0	0	0
Total	3	0	3

Note: Numbers in parenthesis are density of mosquitoes/pit shelter

4.2 Meteorological pattern and seasonal changes of the mosquito densities

The weather variables (rainfall, temperature and RH) were correlated with the density of mosquitoes in the various methods of collection to see any influence of the weather variables on the seasonality of mosquitoes. For *An. gambiae* s.l., it was observed that only temperature has a positive and significant correlation with the density of mosquito in all the collection($r = 0.602$-0.607; $P<0.05$) except in CDC light trap catches($r =0.2$; $P= 0.45$). Rainfall has a negative association, though weak, in space spray ($r = -0.08$; $P= 0.8$) and in aspirator collection ($r = -0.068$; $P =0.89$) but a weak positive association in CDC catches ($r = 0.331$; $P =0.293$). In general, for all meteorological factors, temperature ($r = 0.319$; $P= 0.313$), rainfall ($r= 0.276$; $P=0.384$) and humidity ($r = 0.443$; $P =0.150$) have positive association with total *An. gambiae* s.l. collected by all methods, but these were insignificant. Similarly for *An. pharoensis*, temperature for aspirator collection ($r = 0.578$; $P=0.049$) and rainfall for CDC collection ($r =0.695$; $P= 0.012$) have strong positive association and significant on the density of mosquitoes. In other cases there were both negative and positive relationships but the association was not significant. In general, *An. pharoensis* has negative association with temperature ($r = -0.039$; $P= 0.905$) and strong positive association with rainfall ($r = 0.695$; $P= 0.012$).

4.3. Abdominal status of anophelines collected from indoors and outdoor collections.

4.3.1 Indoor collections

The abdominal status of female *An. gambiae* s. l., collected indoors based on space spray, aspirator and CDC light trap collection is presented in Table 9. Of 638 *An. gambiae* s.l obtained from space spray, the great majority were freshly fed (77.6%), followed by half-gravids (15.0%), unfeds (6.6%) and gravids (0.8%). Of 351 *An. gambiae* s.l collected based on the aspirator indoors, a similar higher percentage of freshly feds (75.8%) were observed, followed by half-gravids (13.1%), unfeds (8.5%) and fully gravids (2.6%). These observations indicate the preference of freshly fed females to stay indoors soon after feeding at least for some time. Furthermore, analysis of these values using the method of Krafsur (1977) gave the degree of exophily (DE) of the mosquito based on the following formula:

Degree of exophily (DE) = 1- (1/ F: HGG) x 100

Where F= freshly fed; HGG= half-gravids and gravids combined; F: HGG >1, and the F and HGG components are obtained from the space spray.

The results indicate that the *An. gambiae* s.l. population in the area was largely exophilic (79.6%). Using the same formula, analysis of the aspirator collection also revealed a higher degree of exophily for *An. gambiae* s.l (79.2%).

Only thirteen females of *An. pharoensis* were collected in the indoor resting collection (Table 10). Although the number was too small, the majority of them were also freshly fed in both collections. Calculation of degree of exophily revealed that *An. pharoensis* population in the area was also largely exophilic (71.3%).

The abdominal status of *Anopheles* spp. collected in CDC light traps indoors is shown in Tables 10 and 11. The majority of both species were unfed (64-68%) followed by freshly feds (30-35%). Gravids and half-gravids formed the lowest proportion (<1%).

Table 9. Abdominal status of *An. gambiae* s. l. collected by various methods of indoor collections in Koka villages (Dec. 2006-Nov. 2007).

Abdominal Status	Space spray	Aspirator	CDC light trap
Unfed (%)	42 (6.6)	30 (8.5)	3846 (64.4)
Fed (%)	495 (77.6)	266 (75.8)	2088 (34.9)
Half-gravid (%)	96 (15.0)	46(13.1)	34 (0.6)
Gravid (%)	5 (0.8)	9 (2.6)	6 (0.1)
Total	638	351	5974
F: HGG	4.9	4.8	

Fed, HG- Half gravid and G- Gravid

Table 10. Abdominal status of *An. pharoensis* collected by various methods of indoor collections in Koka villages (Dec. 2006-Nov. 2007).

Abdominal Status	Space spray	Aspirator	CDC light trap
Unfed (%)	1 (25)	0	534 (68.3)
Fed (%)	3 (75)	7 (77.8)	237(30.3)
Half-gravid (%)	0	2 (22.2)	8 (1.0)
Gravid (%)	0	0	3 (0.4)
Total	4	9	782
F: HGG	_	3.5	

4.3.2 Outdoor collections

The abdominal status of anophelines collected from outdoor sites (pits shelters and clay pots) is summarized in Table 11. As in indoor collections of both space spray and aspirator, the majority of *An. gambiae* s.l were still freshly feds (41.6%), although the proportion was much lower. These were followed by half gravids (30.9%), unfeds (19.6%), and gravids (8.1%), the proportion of which were all higher than indoor collections.

Table 11. Abdominal status of *An. gambiae* s.l. collection from pit shelters and clay pot in Koka village (Dec. 2006-Nov. 2007)

Abdominal status	Pit shelter	Clay pot	Total
No. unfed (%)	88 (19.9)	9 (17.3)	97(19.6)
No. fed (%)	178 (40.2)	27 (51.9)	205 (41.4)
No. half-gravid (%)	137 (30.9)	16 (30.8)	153 (30.9)
Gravid (%)	40 (9.03)	0	40(8.1)
Total	443 (89.5)	52 (10.5)	495

4.4. Parity rates of *An. gambiae* s.l. and *An. pharoensis*

During the study period, a total of 603 unfed *An. gambiae* s.l. and 246 unfed *An. pharoensis* from indoor collections of (space spray, aspirator and CDC traps) were dissected for parity determination (Tables 12 and 13). The parous rates were 31.2% for *An. gambiae* s.l. and 28.0% for *An. pharoensis*. Similarly, a total of 59 *An. gambiae* s.l. from the outdoor was dissected, giving parous rate of 33.9% (Table 14); only 3 *An. pharoensis* were collected outdoors which were all freshly fed. For *An. gambiae* s.l. the rates recorded in indoor and outdoors were not significantly different (χ^2 =0.061, df= 1, P =0.806), the overall was 31.4% (Table 15). Furthermore, parous rates exhibited some seasonal trend for both species, being lower (0-20%) during the dry season and higher (37-67%) during the wet season in *An. gambiae* s.l. For *An. pharoensis* higher parous rates (38-40%) were recorded between August and October indoor situation (Fig. 3). This was more evident in the CDC collections.

Assuming a gontrophic cycle of three days for both species (Ameneshewa, 1995; and Abose *et al*., 1998b), daily survival rates (p) of the two species are the cubic roots of the parous proportions, or 0.68 for *An. gambiae* s.l. and 0.65 for *An. pharoensis*.

Table 12. Monthly parous rates of indoor resting *An. gambiae* s.l. based on three methods of collection (Dec. 2006-Nov. 2007).

Method	Months												Total
	D	J	F	M	A	M	J	J	A	S	O	N	
CDC light trap													
No. dissected	0	3	17	23	65	153	59	108	37	55	15	0	535
No. par (%)	0	1(33.3)	3(17.6)	4(17.4)	8(27.7)	32(20.9)	21(35.6)	49(45.4)	26(70.2)	28(50.9)	7(46.7)	0	179(33.4)
Space spray													
No. dissected	0	0	3	7	6	9	13	1	2	0	0	0	41
No. par (%)	0	0	0	0	0	0	6(46.1)	0	0	0	0	0	6(14.6)
Aspirator													
No. dissected	0	2	0	7	10	8	0	0	0	0	0	0	27
No. par (%)	0	0	0	0	0	3(37.5)	0	0	0	0	0	0	3(11.1)
Total													
No. dissected	0	5	20	37	81	170	72	109	39	55	15	0	603
No. par (%)	0	1(20)	3(15.0)	4(10.8)	8(9.8)	35(20.5)	27(37.5)	49(44.9)	26(66.7)	28(50.9)	7(46.7)	0	188(31.2)

Table 13 Monthly parous rates of indoor resting *An. pharoensis* based on three methods of collection (Dec. 2006-Nov. 2007).

	Months												
Method	D	J	F	M	A	M	J	J	A	S	O	N	Total
CDC													
No. dissected	0	3	10	14	30	20	37	67	16	38	10	0	245
No. par (%)	0	1(33.3)	2(20.0)	2(14.3)	6(20.0)	4(20.0)	12(32.4)	17(25.3)	6(37.5)	15(39.5)	4(40.0)	0	69(28.2)
Space spray													
No. dissected	0	0	0	0	0	0	0	0	1	0	0	0	1
No. par (%)	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspirator													
No. dissected	0	0	0	0	0	0	0	0	0	0	0	0	0
No. par (%)	0	0	0	0	0	0	0	0	0	0	0	0	0
Total													
No. dissected	0	3	10	14	30	20	37	67	17	38	10	0	246
No. par (%)	0	1(33.3)	2(20.0)	2(14.3)	6(20.0)	4(20.0)	12(32.4)	17(25.3)	6(35.3)	15(39.5)	4(40.0)	0	69(28.0)

Table 14 Monthly parous rates of outdoor resting *An. gambiae* s.l. based on two methods of collection (Dec.2006-Nov. 2007).

Method	Month												Total
	D	J	F	M	A	M	J	J	A	S	O	N	
Pit shelters													
No. dissected	0	5	7	8	6	12	0	6	10	0	0	0	54
No. par (%)	0	2(40.0)	0	3(37.5)	0	3(25.0)	0	4(66.7)	5(50.0)	0	0	0	17(31.5)
Clay pot													
No. dissected	0	0	0	0	0	0	5	0	0	0	0	0	5
No. par (%)	0	0	0	0	0	0	3(60.0)	0	0	0	0	0	3(60.0)
Total													
No. dissected	0	5	7	8	6	12	5	6	10	0	0	0	59
No. par (%)	0	2(40.0)	0	3(37.5)	0	3(25.0)	3(60.0)	4(66.7)	5(50.0)	0	0	0	20(33.9)

Table 15 Results of female anophelines dissections in Koka village (Dec.2006-Nov. 2007)

Collection Sites	*Anopheles gambiae* s.l		*An. pharoensis*	
	No. dissected	No. parous (%)	No. dissected	No. parous (%)
Indoor	603	188(31.2)	246	69 (28.0)
Outdoor	59	20 (33.9)	0	0
Total	662	208 (31.4)	246	69 (28.0)

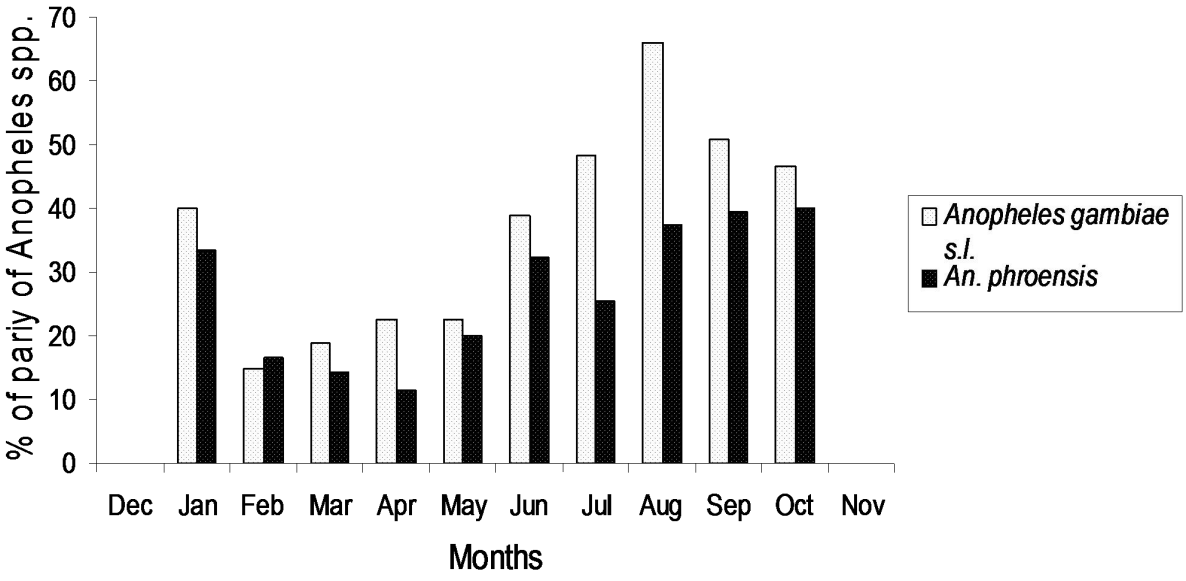

Figure 3 Monthly parity rates of *Anopheles* mosquitoes in Koka villages with different methods of collection

4.5 Sporozoite infection rates of *Anopheles* species

During the study period, a total of 208 parous *An. gambiae* s.l and 69 parous *An. pharoensis* were tested to detect sporozoite antigens of *Plasmodium falciparum* and *P. vivax* using ELISA method. The results showed that none of them was infected.

5. DISCUSSION

Anopheles larvae breed in various types of habitats, varying from large permanent to small temporary water collections (Service, 2000). The larval breeding habitats in the study area (Koka villages) included outlet and water collections from hot spring, water canals, marshy areas, rain water pool, man-made pits for brick construction, and holes for water pumps, and edge of Koka reservoir. It was found that both *An. gambiae* s.l. and *An. pharoensis* breed sympatrically, although the former was most abundant. Marshy areas and edge of reservoir served as breeding habitats for three species (*An. gambiae* s.l, *An. pharoensis* and *An. squamosus*).

In the current study *Anopheles* larvae were found to breed in artificial breeding habitats created as the result of human activities. In Koka area brick pits were dug to make bricks for building houses (tukuls) near the edge of the Lake would soon be filled with groundwater especially on the dry season when water from the lake regresses. These were seen to be important breeding sites for both of *An. gambiae* s.l. and *An. pharoensis*. The other breeding site was water canal that used to discharge industrial liquids and toxic substances (presumably chemical pesticides) from floral industries also served as breeding habitats. The distance between the canal and tukuls was approximately 10-15m, this short distance from breeding habitats to community, may increase exposure to mosquitos' bite and malaria infection. The abundance and distribution of *Anopheles* vectors depend primarily on the accessibility of ideal breeding sites, and such sites are occasionally created by human activities, enhancing close contact between man and vectors (Muirhead-Thompson, 1951; Mutuku *et al*., 2006).

Prior works in Ethiopia indicated that larvae of *An. gambiae* s.l. breed in small, temporary, often sun-lit pools of water, of the type formed after rains, or receding river beds, discarded tires and artificial containers, shores of lakes, and marshy areas. (Mekuria, 1983; Tulu, 1993b; Ghebreyesus *et al*., 2006). Larvae also breed in slow moving hot springs and swampy area (Ameneshewa and Service, 1996). *An. pharoensis* however breeds in permanent habitat such as lakes and irrigated areas covered with vegetations (O'Connor, 1967; Mekuria, 1983). In general, larval densities of anopheline species were closely associated with the accessibility of temporary suitable breeding places in the Koka villages. Thus, for *An. gambiae* s.l., brick pits, marshy and water canals could be the main target for any larval control during the dry season, while temporary collection of rain water could be the main target during the wet season. Marshy areas and brick pits could be the main target for the control of *An. pharoensis* in Koka area. *Anopheles squamosus* which were only encountered as larval have no known medical importance.

Adult anopheline mosquitoes were collected by using different techniques during the study period. A total of 8, 279 female anophelines were collected from their resting places (indoors or outdoors) and while they were seeking for host, only three species of *Anopheles* were found, namely *An. gambiae* s.l. (presumably *An. arabiensis*), *An. pharoensis* and *An. ziemanni*. *Anopheles gambiae* s.l. (*An. arabiensis*), the principal vector of malaria in Ethiopia (Mekuria, 1983; Ghebreyesus *et al*., 2006), was by far the most abundant anopheline found in the present study. *Anopheles pharoensis* was the second abundant mosquito in the study area and it is considered as secondary vector

together with *An. funestus* and *An. nili* in the country (Mekuria, 1983; Ghebreyesus *et al*., 2006). The largest numbers of *An. gambiae* s.l. was caught in May and June, then after a rapid decline in July, remained low the rest of the year, even in main rainy season.

In space spray and aspiration collections, *An. gambiae* s.l. was the most abundant species resting indoors followed by *An. pharoensis*. Although there was monthly variation in the densities of indoor resting *An. gambiae* s.l., the maximum density was observed in May (70.4 mosquitoes/hut/day for space spray, 7.95 mosquitoes/hut/day for aspirator collections); this might be due to the increases of breeding habitats following the short rains in March and April. During the main rainy season, the indoor densities of *Anopheles* were very low compared to short rainy season. This might be due to lower temperature during the wet season (21-22^{o}C), so unimodal peak was apparent in the present study in the indoor collection.

Large number of *An. gambiae* s.l. was collected in those tukuls with unplastered walls than mud walled tukuls. In addition, a higher number of indoor resting mosquitoes were found in the poorly constructed tukuls that have incomplete construction with thatched roofs and a lot of openings. These openings of the walls and thatched roof are less efficient barriers for the mosquitoes. Though this observation need further detail investigation, it agrees with the work of Konradsen *et al.* (2003). They found 30% higher risk for harboring the local main malaria vectors with poorly constructed tukuls than those that were complete and built with permanent materials in Sri Lanka. Furthermore, it was observed that the indoor resting mosquitoes were much higher in space spray method

than aspirator collection, showing that aspirator collection may underestimate the indoor resting density mosquitoes. Similar observation has been noted by Abose *et al*. (1998b) in Ziway.

Regarding the abdominal status of *An. gambiae* s.l. in the indoor resting collections more freshly fed females (77.6% in space spray catches and 75.8% in aspirator) were sampled in study area. In contrast to fresh feds, the numbers of gravid females were extremely low in the indoor collections. This indicates that high proportion of *An. gambiae* s.l. left human dwelling before they digest their blood meal completely. A high degree of exophily (79.6%) was observed in the *An. gambiae* s.l. population in the current study. In Metehara and surrounding areas, Balkew (2001) observed exophilic behavior of *An. arabiensis* ranging from 18% in insecticide untreated villages to 69.4% in insecticide treated villages. Ameneshewa and Service (1996) in Gergedi, Upper Awash documented 56% of *An. arabiensis* resting indoors comprising of fresh feds. They also observed partially exophilic behavior of this vector (37.5%). Bockarie *et al*. (1994) also noted a high degree of exophily (76.1%) for *An. gambiae* s.l. in Sierra Leone. In Sudan this vector has become increasingly exophilic as a result of the irritant effect caused by DDT (Zahar, 1985 cited in Shililu *et al*., 2004). In contrast to our observation, Abose *et al.* (1998b), in Ziway found low number of fresh fed *An. arabiensis* as compared to the combined half-gravid and gravid, indicated that this malaria vector is endophilic in this study area. In western Kenya this species also exhibit largely endophilic nature (Githeko *et al*., 1994).

The highest proportions of mosquitoes were collected by CDC light traps placed near sleeping persons protected by untreated nets than the other methods, including those which were found at only very low densities, *An. ziemanni*. The human baits method was considered as standard to measure human biting rate and human-vector contact (Service, 1993a). However, it has some drawbacks. It is so tedious, uncomfortable, exhausting for the catchers and it is also too expensive (Lines *et al*., 1991). Some people are more attractive to the vectors than others. The most serious problem of this method arises when entomological study is conducted in the area of drug resistant malaria occurs (Lines *et al*., 1991). Due to this and other ethical reasons there is a growing need to replace of human bait by using CDC traps hang near sleeping persons protected by untreated nets, which are ethically acceptable, not expensive and need less labor (Lines *et al*., 1991; Maxwell *et al*., 1998). It is also important in sampling different species of mosquitoes that are found even in small density (Bockarie *et al*., 1994). Ameneshewa (1995) at Gergedi found that light traps were more efficient than other methods and collected mosquitoes even in low densities. In the present study, the proportion of unfed mosquitoes in CDC light traps were much higher (64-68%) in both species (*An. gambiae* and *An. pharoensis*) than those obtained in space spray and aspirator collection. This indicates light trap placed indoors were recruiting most of the host seeking hungry mosquitoes.

The highest numbers of mosquitoes were caught in CDC light trap where cattle are kept inside the human dwellings. Most of these were freshly fed; this might expose the residents to frequent bites. Seyoum *et al.* (2002) in Ziway reported that *An. arabiensis* was attracted more to humans in mixed dwellings than cattle. The rate with which an

anopheline species feeds on human hosts as opposed to other animals can indicate those mosquitoes most likely to be imperative vectors (Bennett, 1995), and the number of malaria infections might be higher in those people who keep cattle. Contrary to the above authors, Minakawa *et al.* (2002) showed in the Mwea highland area of Kenya, in irrigated areas where the local anopheline population such as *An. arabiensis* in mixed dwellings shelters readily chooses to feed on cattle rather than on human hosts. Likewise, Hadis *et al*. (1997) from blood meal analysis revealed that the existence of cattle near humans protects people from mosquitos' bites in Southern, Eastern and Western Ethiopia. In the study area, the density of mosquitoes in light traps, space spray and aspirator collections were high soon after the short rainy season and remained high until the end of wet season. In the dry months, however the mosquito density became small and reached to zero.

Small numbers of *An. gambiae* s.l. were caught resting in clay pots in outdoor collection, showing little attraction to the habitat. In Western Kenya, clay pots were favored better than pit shelters, accounting for 37% of all the mosquitoes taken in the study period by using different techniques to collect both indoor and outdoor (Odiere *et al*., 2007).

In outdoor resting collections, *An. gambiae* s.l still outnumbered *An. pharoensis*. The low number of *An. pharoensis* in the outdoor collection may be due to the tendency of the mosquito to rest in cattle shed or in vegetations along the shores of the lake which were not accessible for collection. In general the outdoor resting sites of mosquitoes were so numerous, diverse and difficult to locate one on many trials using sweep nets. In Ziway,

72% of the outdoor collected *An. pharoensis* females were resting in the cattle shed (Abose *et al*., 1998b).

The abdominal status of outdoor collection also indicated more number of fresh fed females than the others. The numbers of half gravid and gravid female *An. gambiae* s.l. were much higher in the outdoor collection than the indoor collection. This agrees with the observation by Ameneshewa and Service (1996). The occurrence of relatively high number of half-gravid and gravid mosquitoes in outdoor collection further reveals the more exophilic nature of *An. gambiae* s.l. in the area.

The density of *Anopheles* mosquitoes are affected by meteorological factors. In the present study meteorological factors have positive association with the density of mosquitoes; however this relationship was not significant. This might be due to there are other factors that contribute for the rise and fall of mosquito density together with meteorological factors.

Based on abdominal conditions or blood digestion stages, unfed *Anopheles* mosquitoes were dissected to determine parous rates. For both *An. gambiae* s.l. and *An. pharoensis,* there was a clear seasonal trend in parous rates, being higher during the wet season. The highest (66.7%) was observed in August for *An. gambiae* s.l and the highest for *An. pharoensis* was observed in October (40.0%). This is in line with Abose *et al*. (1998b). They found the highest parity rate (57.1%) in August. The proportion of parous *An. arabiensis* exhibited seasonal tendencies, in general being higher in the wet season than

dry season (Ameneshewa, 1995). The overall parity rate of the two species showed slight difference being higher for *An. gambiae* s.l. (31.4%) than *An. pharoensis* (28.0%). This perhaps indicates that *An. pharoensis* is relatively short-lived in comparison with *An. gambiae* s.l., and has a lower vectorial capacity compared to *An. gambiae* s.l. Abose *et al*. (1998b) also found a parous rate of 43.2% for *An. arabiensis* and 36.5% for *An. pharoensis* in Ziway. From the same area, Rishikesh (1966) also reported 65-70% for *An. gambiae* s.l and 34-43% for *An. pharoensis*. Ameneshewa and Service (1996) also reported an overall parous rate of 58.3% for *An. arabiensis* at Gergedi in the Awash valley. Balkew (2001) also reported higher parous rate for *An. gambiae* s.l (45.1%) than *An. pharoensis* (30.2%). In general, the parous rates in the present study were lower than other parts of Ethiopia for both species (Rishikesh, 1966; Abose *et al.,* 1998b; Taye *et al*., 2006). This might be due to the majority of the present dissection were performed in those mosquitoes that were collected in light traps. Lines *et al*. (1991) revealed that the parous rate of *An. gambiae* s.l. caught by indoor light traps were significantly lower than in corresponding human biting catches, probably because of parous females may be light shy. In general, epidemiologically parous females are hazardous as they could be infected by parasites and carry them to human hosts (Service, 2000). High parous rates in mosquito populations imply longer survival, more gonotrophic cycles, and therefore, capable of transmitting malaria parasites for long (Jensen *et al*., 1998).

The daily survival rates of *An. gambiae* s.l. (0.68) and *An. pharoensis* (0.65) in the present study is close to that reported for *An. arabiensis* (0.76) and *An. pharoensis* (0.71) in Ziway by Abose *et al.* (1998b). Krafsur (1970) estimated daily survival rates of *An.*

arabiensis in Gambella area of southwestern Ethiopia to be 0.89 during the wet season and 0.79 during the dry season.

None of the 208 parous *An. gambiae* s.l. and 69 parous tested by ELISA revealed any sporozoite infection with either *P. falciparum* or *P. vivax*. This further explains the low prevalence of malaria in the human population in the study area during the study period and might be due to the current ITNs campaign in the country or might be due to other factor not immediately obvious. However, absence of sporozoite infection or low infection rates in anopheline vectors is not uncommon in Ethiopia or elsewhere. Rishikesh (1966) found a very low sporozoite rate (9/4573) in *An. gambiae* s.l. through dissection of the salivary glands and none among 2577 *An. pharoensis*. Nigatu *et al*. (1994) in Gambella reported sporozoite rate of 0.77% from a total of 261 *An. gambiae* s.l. and 0.47% from a total of 430 *An. pharoensis*. In Gergedi (Wonji area) Ameneshewa (1995) tested a total of 3626 *An. arabiensis* using ELISA and found infection rate of 1.52% (*P. falciparum*). On the other hand in Ziway, Abose *et al*. (1998b) tested a total of 334 *An. arabiensis* and 272 *An. pharoensis* by the ELISA method for both *P. falciparum* and *P. vivax* antigens and found no infection. The highest infection rate recorded for *An. gambiae* s.l in Ethiopia so far is 3% (3/100) in Kobo-Chercher in Wollo (O'Connor, 1967; Mekuria, 1983). Mekuria (1983) suggested for such low rates probably lie in the relatively low malaria endemicity existing in Ethiopia compared to other highly endemic regions of tropical Africa. In general, it can be concluded from these studies, sporozoite

infection rates in Ethiopia are very low and large numbers of specimens need to be tested in order to determine the actual infection rate.

6. CONCULSIONS AND RECMMENDATIONS

6.1 Conclusions

- ✓ Larval collections from marsh area, brick pits, hot spring, water canals, edge of lake, and holes made for water pumping engines areas revealed the presence of three species (*An. gambiae* s.l., *An. pharoensis* and *An. squamosus*). *An. gambiae* s.l. was the most abundant followed by *An. pharoensis*. These habitats might be targeted for larval control in addition to ITNs.
- ✓ A total of 8,279 adult anophelines representing three species (*An. gambiae* s.l., *An. pharoensis* and *An. ziemanni*) were collected using CDC light traps, space spray and aspirator methods. In all collection *An. gambiae* s.l. was the predominate species.
- ✓ *An. gambiae* s.l. in the present study showed higher degree of exophilic nature that accounted to 79.6% of all mosquitoes taken in the study period.
- ✓ In the study area, the sporozoite rate for *An. gambiae* s.l and *An. pharoensis* were zero, whereas the parity rate for the two species is 31.4% and 28.0%, respectively.
- ✓ In the study area, it can be concluded that *An. gambiae* s.l.(presumably *An. arabiensis*) is the most important malaria vector as in the rest of Ethiopia and that control measures should target this species.

6. 2. Recommendations

- Extensive entomological works should be done to determine the biting and outdoor resting behaviour of the main vectors and their role in malaria transmission.
- Encourage the use of non-persistent larvicidal chemicals at the breeding habitats of *Anopheles* mosquitoes to decrease the adult population, especially the brick pits and water canals.
- Social mobilization is important to reduce the breeding habitats of *Anopheles* such as brick pits that are formed by human activity.
- The floral farming industries that are booming in the area should be very careful not to create additional breeding sites for mosquitoes. They should use appropriate means of discharging industrial chemicals and other wastes, to reduce breeding habitats of *Anopheles*.

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APPENDICES

Appendix 1. Species of *Anopheles* larvae collected from various breeding sites in the four study sites

Species	Study and collection sites									
	Shulki				Got-1	Got-2		Got-3		
	Hot spring	Water canals	Marsh	Pool/rain water	Marsh	Brick pits	Holes made for water pumps	Brick pits	Edge of lake	**Total**
An. gambiae s.l.	39	193	157	611	272	328	16	401	76	2093
An. pharoensis	11	15	75	9	124	0	37	67	19	357
An. squamosus	0	0	0	0	5	0	0	0	17	22
Total	50	208	232	620	401	328	53	468	112	**2472**

Appendix 2. Monthly variation of *An. squamosus* larvae/ pupae in different breeding habitats in Koka villages (Dec. 2006- Nov. 2007)

Breeding habitats	Monthly abundance of *An. squamosus* larvae/ pupae												Total
	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	
Hot spring	0	0	0	0	0	0	0	0	0	0	0	0	0
Water canals	0	0	0	0	0	0	0	0	0	0	0	0	0
Marsh	0	0	0	0	0	4	1	0	0	0	0	0	5
Pool/rain water	0	0	0	0	0	0	0	0	0	0	0	0	0
Bricks pit	0	0	0	0	0	0	0	0	0	0	0	0	0
Holes for water pumps	0	0	0	0	0	0	0	0	0	0	0	0	0
Edge of lake	0	0	0	0	17	0	0	0	0	0	0	0	17
Total	0	0	0	0	17	4	1	0	0	0	0	0	22

Appendix 3: Summary of monthly indoor collected adult Anopheline using CDC light trap from the four study sites (Dec. 2006-Nov.2007)

CDC		Study sites				Total Tot (Avr.)
		Shulki	Got-1	Got-2	Got-3	
		Tot (Avr.)	Tot (Avr.)	Tot (Avr.)	Tot (Avr.)	
Dec	AG	7(1.4)	6(1.2)	0	0	13(0.65)
	AP	18(3.6)	0	2(0.4)	0	20(1.0)
	AZ	0	0	0	0	0
Jan	AG	6(1.2)	9(1.8)	0	0	15(0.75)
	AP	6(1.2)	3(0.6)	0	0	9 (0.45)
	AZ	0	0	0	0	0
Feb	AG	10(2)	51(10.2)	6(1.2)	0	67(3.35)
	AP	3(0.6)	28(5.6)	2(0.4)	0	33 (1.65)
	AZ	0	7(1.4)	0	0	7(0.35)
Mar	AG	6(1.2)	32(6.4)	0	2(0.4)	40(2.0)
	AP	14(2.8)	28(5.6)	0	0	42 (2.1)
	AZ	0	0	0	0	0
Apr	AG	43(8.6)	168(33.6)	12(2.4)	21(4.2)	244(12.2)
	AP	4(0.8)	42(8.4)	19(3.8)	0	65 (3.25)
	AZ	0	0	0	0	0
May	AG	635(127)	795(159)	5(1.0)	12(2.4)	1447(72.35)
	AP	17(3.4)	19(3.8)	1(0.2)	0	37 (1.85)
	AZ	0	2(0.4)	0	0	2(0.1)
Jun	AG	891(178.2)	613(122.6)	30(6.0)	80(16.0)	1614(80.7)
	AP	85(17)	38(7.6)	6(1.2)	1(0.2)	130 (6.5)
	AZ	0	0	0	0	0
Jul	AG	607(121.4)	598(119.6)	171(34.2)	5(1.0)	1381(69.05)
	AP	11(2.2)	179(35.8)	53(10.6)	0	243 (12.15)
	AZ	2(0.4)	3(0.6)	0	0	5(0.25)
Aug	AG	200(40)	182(36.4)	11(2.2)	0	393(19.65)
	AP	7(1.4)	72(14.4)	4(0.8)	0	83 (4.15)
	AZ	0	4(0.8)	0		4(0.2)
Sep	AG	351(70.2)	149(29.8)	7(1.4)	0	507(25.25)
	AP	38(7.6)	25(5.0)	7(1.4)	0	70 (3.5)
	AZ	3(0.6)	2(0.4)	0	0	5(0.25)
Oct	AG	242(48.4)	5(1.0)	0	0	247(12.35)
	AP	20(4.0)	10(2.0)	0	0	30(1.5)
	AZ	0	0	0	0	0
Nov	AG	6(1.2)	0	0	0	6(0.3)
	AP	20(4)	0	0	0	20(1.0)
	AZ	0	0	0	0	0

Note: AG- *Anopheles gambiae* s.l.
AP- *An. pharoensis*
AZ- *An. ziemanni*

Appendix 4 Summary of monthly indoor collected adult Anopheline using space spray from the four study sites (Dec. 2006-Nov.2007)

Space spray		Study sites				Total Tot (Avr.)
		Shulki	Got-1	Got-2	Got-3	
		Tot (Avr.)	Tot (Avr.)	Tot (Avr.)	Tot (Avr.)	
Dec	AG	0	0	0	0	0
	AP	0	0	0	0	0
Jan	AG	0	0	0	0	0
	AP	0	0	0	0	0
Feb	AG	1(1.0)	45(45.0)	1(1.0)	0	47(11.8)
	AP	0	0	0	0	0
Mar	AG	0	37(37.0)	0	3(3.0)	40 (10.0)
	AP	0	0	1(1.0)	1(1.0)	2(0.5)
Apr	AG	5(5.0)	17(17.0)	1(1.0)	10(10.0)	33 (8.4)
	AP	0	1(1.0)	0	0	1(0.25)
May	AG	40(40.4)	181(181.0)	5(5.0)	56(56.0)	282 (70.4)
	AP	0	0	0	0	0
Jun	AG	0	78(78.0)	3(3.0)	36(36.0)	117 (31.6)
	AP	0	0	0	0	0
Jul	AG	2(2.0)	21(21.0)	0	11(11.0)	34 (8.5)
	AP	0	0	0	0	0
Aug	AG	2(2.0)	21(21.0)	1(1.0)	3(3.0)	27 (6.75)
	AP	0	0	0	1(1.0)	1(0.25)
Sep	AG	13(13.0)	31(31.0)	3(3.0)	5(5.0)	52 (13.25)
	AP	0	0	0	0	0
Oct	AG	0	1(1.0)	0	0	1(0.25)
	AP	0	0	0	0	0
Nov	AG	0	1(1.0)	4(4.0)	0	5 (1.25)
	AP	0	0	0	0	0

Note: AG- *Anopheles gambiae* s.l.
AP- *An. pharoensis*

Appendix 5 Summary of monthly indoor collected adult Anopheline using aspirator from the four study sites (Dec. 2006-Nov.2007)

Aspirator		Study sites				Total Tot (Avr.)
		Shulki	Got-1	Got-2	Got-3	
		Tot (Avr.)	Tot (Avr.)	Tot (Avr.)	Tot (Avr.)	
Dec	AG	1(0.2)	0	0	0	1 (0.25)
	AP	0	0	0	0	0
Jan	AG	11(2.2)	6(1.2)	0	0	17 (0.85)
	AP	0	0	0	0	0
Feb	AG	0	0	0	0	0
	AP	0	0	0	0	0
Mar	AG	6(1.2)	13(2.6)	0	0	19 (0.95)
	AP	0	1(0.2)	0	0	1(0.05)
Apr	AG	0	45(9.0)	0	11(2.2)	56 (2.9)
	AP	0	2(0.4)	0	0	2(0.1)
May	AG	13(2.6)	111(22.2)	20(4.0)	15(3.0)	159(7.95)
	AP	0	3(0.6)	0	0	3 (0.15)
Jun	AG	9(1.8)	17(3.4)	4(0.8)	10(2.0)	40(2.0)
	AP	0	0	0	0	0
Jul	AG	7(1.4)	11(2.2)	0	5(1.0)	23 (1.15)
	AP	0	0	0	0	0
Aug	AG	4(0.8)	8(1.6)	5(1.0)	2(0.4)	19(0.95)
	AP	0	1(0.2)	0	0	1(0.05)
Sep	AG	7(1.4)	4(0.8)	2(0.4)	2(0.4)	15 (0.75)
	AP	0	2(0.4)	0	0	2(0.1)
Oct	AG	1(0.2)	0	0	0	1 (0.05)
	AP	0	0	0	0	0
Nov	AG	0	1(0.2)	0	0	1 (0.05)
	AP	0	0	0	0	0

Note: AG- *Anopheles gambiae* s.l.
AP- *An. pharoensis*

Appendix 6 Summary of monthly indoor and outdoor collected adult Anopheline from Koka village (Dec. 2006- Nov. 2007)

Species	Months												Total
	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct	Nov	
An. gambiae s.l.													
Indoor	14	32	114	99	333	1888	1771	1438	439	574	249	12	**6963**
Outdoor	0	49	28	36	34	93	102	68	64	19	1	1	**495**
Total	14	81	142	135	367	1981	1873	1506	503	593	250	13	**7458**
An. pharoensis													
Indoor	20	9	33	45	68	40	130	243	85	72	30	20	**795**
Outdoor	0	0	0	2	1	0	0	0	0	0	0	0	**3**
Total	20	9	33	47	69	40	130	243	85	72	30	20	**798**
An. ziemanni													
Indoor	0	0	7	0	0	2	0	5	4	4	0	1	**23**
Outdoor	0	0	0	0	0	0	0	0	0	0	0	0	**0**
Total	0	0	7	0	0	2	0	5	4	4	0	1	**23**

Appendix 7. Summary of different abdominal status of *An. gambiae* s.l. indoor collection by various methods.