ADDIS ABABA UNIVERSITY
FACULTY OF SCIENCE
DEPARTMENT OF BIOLOGY

Entomological Studies on the Species Composition and the Significance of Insecticide Treated Mosquito Nets (ITNs) Against Malaria Vector in Guragie Zone, Southern Ethiopia

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I, the undersigned, declare that this thesis is my original work and it has not been presented in other universities, college or institutions, seeking for similar degree or other purpose. All sources of material used for the thesis have been duly acknowledged.

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Abstract

Studies on species composition and significance of Insecticide Treated mosquito Nets (ITNs) for malaria vector control were conducted in four randomly selected localities in Jolie Peasant Association, Meskan Woreda, Guragie Zone, Southern Ethiopia. Larval and adult collections were carried out, from different sites throughout the study period to identify species composition, thus three *Anopheline* species: *Anopheles gambiae s.l., Anopheles pharoensis* and *Anopheles christyi* were identified. *An. gambiae s.l.* was the principal vector responsible for the transmission of malaria in the study area. The main identified breeding sites in the study area includes rain pools, river pockets, ponds, marshes and pools of water from spillage of canals which are the result of human activities rather than environmental causes.

To investigate the effect of Insecticide (Permethrin) Treated Mosquito Nets (ITNs), 20 houses with and without ITNs were paired. Malaria incidence, resting density and parity rate were assessed. The result of malaria incidence showed that the difference was not significant at ($\chi^2 = 0.6247$, $P > 0.05$). However, a clear reduction in the density of indoor resting population of *An. gambiae s.l.* were found in houses with ITNs ($t = 10.0278$, $df = 38$, $p < 0.05$). Similarly, the parous rates were significant at ($\chi^2 = 4.657$, $p < 0.05$).

Further entomological studies are required to determine the vectorial status of the *Anophelines* in this area. Besides, in providing an enabling environment for scaling-up actions, governmental sectors need to focus on creating awareness on utilization and demand for ITNs through health information channels and mass media.

At the end of 2004, 107 countries and 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*. Malaria causes more than 1 million deaths each year, out of which 90% of the malaria cases and deaths occur in Africa. It also contributes indirectly too many additional deaths, mainly in young children, through synergy with other infections and illnesses (World Malaria Report, 2005; TDR, 1997-98).

In Africa, Southeast Asia and Eastern Mediterranean region estimated number of deaths due to malaria in 2002 indicates that, 89% (1,136,000), 5.1% (65,000), and 4.6% (59,000), respectively. Malaria control in Africa is less successful because of the occurrence of drug resistant parasites and insecticide resistant vectors, changes in the resting behavior of mosquito (from endophily to exophily) as the result of frequent indoor insecticide sprays, lack of efficient infrastructure, shortage of trained man power, lack of equipment, financial constraints, lack of appropriate management and inability to integrate several method of control (World Malaria Report, 2005; Toure, 1999).

1.1. Epidemiology of Malaria in Ethiopia

Ethiopia is one of the countries in Sub-Sahara Africa where the prevalence of malaria is intense. Almost 65% (40 million) of the population live in malaria prone area and affects about 4-5 million people annually. Large scale migration due to resettlement, instability to conflict areas and speared of chloroquine resistant *P. falciparum* malaria were thought to be reasons for the increase of transmission in the low land

(MOH, 2004, 2000; Gebre-Mariam, 1984; Gebre-Mariam *et al.*, 1988; Tulu, 1993a, 1993b; Mengesha and Mekonnen, 1999).

A study carried out in Gambela showed that *Plasmodium falciparum* and *Plasmodium vivax* are the major pathogens of malaria in that region as well as for the country. The distribution of malaria in Ethiopia is related to variation in altitude, topography and climate. Malaria is common in areas lying below 2000 meters, but highly prevalent below 1500 meters. Areas lying at altitudes of 1500-2000 meters are prone to occasional malaria epidemics. It is absent in areas above 2500 meters where the climatologically factors inhibit the survival of vector species and the development of the parasite in the mosquito. The country experiences three locally known climatic zones cold "Dega", temperate "Woyna Dega" and warm "Kola" climatic zones (Nigatu *et al.*, 1992; MOH, 2004; Gebre-Mariam, 1984; Gebre-Mariam *et al.*, 1988; Tulu, 1993a ,1993b).

However, the upper limit for malaria transmission was considered as 2000 meters, periodic epidemics were recorded above this level. Consequently moderate to sever malaria epidemics was known to occur in the country. In 1958 there was an epidemic in several regions affecting about 3 million people out of which 150,000 died. Moreover, the epidemics in Ethiopia can be substantiated by its repeated events with similar feature, but of lesser intensity in 1965, 1973, 1981 and 1982. Recently frequent malaria epidemics of cyclical patterns of variable magnitude were recorded for example in the year 1988, 1991, 1995 and 1998 in different parts of the country (Gebre-Mariam *et al.*, 1988; MOH, 1999; Covell, 1957; Chand, 1965; Fountaine *et al.*, 1961; Gebre-Mariam *et al.*, 1988).

Malaria occurs immediately after the small rainy seasons of March and April as well as after the long rain of June through September. Peak transmission occurs during the months of September through November. Malaria transmission continues less intensely in the wet seasons, several epidemics in the high land fringe areas caused numerous deaths (MOH, 2000; Tulu, 1993a).

The disease has been reported as the first cause of morbidity and mortality accounting for 20.4% admission and 27% in patient deaths. In non-epidemic year; 5-6 million clinical malaria cases reported from health facilities, however, the number of malaria cases were reported from health facilities is only a portion of the actual magnitude. Children who seem to lack primary immunity and pregnant women are the main victims of the disease. In general, the problem of malaria in this country is aggravated by population movement to the lowland in agricultural and agro-developmental projects, urban developments as well as settlement operation (Abosse *et al.*, 1998b; MOH, 2004; Nega and Haile-Meskel, 1991).

1.2. Malaria Vectors in Ethiopia

The distribution and density of the *Anopheles* vectors are determined primarily by the availability of the suitable breeding sites, and such sites often arise through human activities enhancing a close association between man and the vectors (Muirhead-Thomson, 1951; Service, 2000).

Although, study on the distribution of the mosquito species in Ethiopia began at the beginning of the twentieth century by the British and Italian expatriates in a series of journey. Verrone (1962a and 1962b) prepared for the first time identification keys for adults and immatures of 34

Anopheline species. These keys are still in use for morphological identifications, in spite of the addition of some morphological characteristics that increased the number of species (Gillies and Coetzee, 1987). Later, O'Connor (1967) provided distributional maps of 34 *Anopheline* species in the country and also reviewed sporozoite rates in *An. gambiae s.l.,* following the changes made on the taxonomy of *Anophelines.*

Mekuria (1983) listed 44 species and subspecies of Anopheles mosquitoes in Ethiopia. Until now the Ethiopian species and subspecies of *Anophelines* are 42, but it is noted that the list slightly differs from the previous authors. Therefore, based on the assessment of the available literature on the subject (O'Connor, 1967; White *et al.*, 1980; Mekuria, 1983; Gebre-Mariam *et al.*, 1988; Hunt *et al.*, 1998).

The 42 species of *Anopheles t*hat occur in Ethiopia is part of the world's 430 species of the genus in which 70 are recognized to potential role in malaria transmission, 40 of theses are recorded as vectors (Service, 1993a). More than 83% of the primary vectors belong to species complexes (Besansky and Collins, 1992) in which sibling species exhibit biological, morphological and behavioral differences. Species within the complex show marked differences in resting, feeding and other behaviors.

The *An. gambiae* complex comprises five known by name and two yet to be named sibling species, and a number of incipient species (Gillies and Cotezee, 1987; Hunt *et al.*, 1998) which include *An. gambiae s.s, An. arabiensis, An. quadrannulatus* species *A, An. quardiannulatus species B, An. melas* Theobald, *An. merus* Donitz *and An. bwambae* White. Two or more members of the complex are sympatric in their geographical distributions. The coexistence is most notable between *An. gambiae s.s* and *An. arabiensis*, (Coetzee *et al.,* 2000). The two species are efficient and dominant vectors in the continent (White, 1974).

Hunt *et al.,* (1998) showed that *An. quadriannulatus* contained two distinct species and provisionally named them as *An. quadriannulatus* species A from South Africa and *An. quadriannulatus* species B from Ethiopia. The South African species was regarded as the same species to that of Ethiopia, like wise, they were considered as epidemiologically unimportant, but Coetzee *et al.*, (2000) has suggested to further studies on the behavior and vectorial capacity of *An. Quadriannulatus* species B. Work is also needed to clarify the occurrence or absence of the other species in Ethiopia. *An. quadriannulatus* in Ethiopia was reported for the first time by Turner (1972) and its distribution was shown to be in the highlands of southwestern and northern regions, coexisting with *An. arabiensis* (White, 1974; White *et al.*, 1980).

Reports indicate that of the seven species of the *An. gambiae* complex, *An. arabiensis* and *An. quadriannulatus* species B are the only two species found in Ethiopia (Mekuria, 1983; Gebre-Mariam, 1984; Gebre-Mariam *et al.*, 1988, Tulu, 1993a; Nigatu *et al.*, 1992; Ameneshewa, 1995; Abose *et al.*, 1998b). Outside of *An. gambiae* complex *An. pharoensis. An. funestus* and *An. nili* are regarded as secondary vectors (Krafsur, 1970; Krafsur, 1977; Mekuria, 1983; Ameneshewa, 1995; Abose *et al.*, 1998b). *An. funestus* is associated with endemic malaria in the western part of the country (Jolivet. 1959).

One or more of the secondary vectors may occur in sympatric with *An. arabiensis* as has been reported in Gambela (Krafsur, 1970; Krafsur, 1977; Nigatu *et al.*, 1992), Gergedi (Amenshewa, 1995) and Zeway (Abose *et al.*, 1998b). The coexistence is much more frequent with *An.*

pharoensis than the others. *An. pharoensis* might be responsible for the transmission of malaria in the absence or low number of *An. arabiensts*, particularly in the dry season (Nigatu *et al.*, 1992; Ameneshewa, 1995; Abose *et al.*, 1998b).

Environmental changes brought by various human activities in Gambela altered the vectorial importance of some species (Nigatu *et al.*, 1992). Krafsur (1970; 1977) reported *An. gambiae s.l. (An. arabiensis), An. funestus. a*nd *An. nili* in that order of importance as vectors of malaria in Gambela. However, a study carried out twenty years later in the same area incriminated *An. arabiensis* and *An. pharoensis* as the only two vectors for *P. falciparum* and *P. vivax,* respectively (Nigatu *et al.*, 1992).

In the previous study the contribution of *An. pharoensis* in malaria transmission was considered insignificant. Indoor insecticide sprays might have eliminated the indoor frequenting *An. funestus*, which is reported to be highly sensitive to such control measures. Jolivet (1959) in Gambela noted the complete disappearance of the species from insecticide sprayed dwellings, while few catches were made from unsprayed dwellings. The importance of this species as a vector in areas where insecticide sprays have been practiced for a long time needs further investigation.

Reports so far (Nigatu *et al.*, 1992; Ameneshewa, 1995; Abose *et al.*, 1998b) have confirmed that *An. arabiensis* followed by *An. pharoensis* are responsible for the transmission of malaria in Ethiopia. As to the distribution of the vectors, documents show that *An. arabiensis* and *An. funestus* occur in all administrative regions while, *An. nili* is reported from the regions of Amhar, Western Oromia, Gambela and Southern Nations Nationalities and Peoples Regional State. *An. pharoensis* is found in all regions except the Bale zone of Oromia. Gebre-Mariam *et al.,* (1988) presented distributional maps based on the administrative regions of the country as set up by the previous government.

The vectorial role of the rest of the *Anophelines* is not yet completely known due to several limitations. Most of the entomological information currently available are emanated from areas where there are accessible means and hence is incomplete. Shortage of communication in inaccessible areas has hindered the research on malaria and its vectors. Parasite susceptibility studies complemented by field observations can lead to the finding of other potential vectors (Gebre-Mariam *et al.*, 1988).

In an experiment in which more than 500 wild caught *An. tenobrosus* Donitz fed on *P. falciparum* gametocyte positive patients in Sille, south Ethiopia. Adugna *et al.*, (1998) reported infection rates of 15.8% by dissection and 7% by DNA hybridization, suggesting the need to observe the role of this species in the epidemiology of malaria. The same authors proposed similar studies to be carried on *An .christyi* (Newstead and Carter), *An. paludis* Theobald, *An. rhodesiensis* Theobald, *An. maculipalpis* Gilles and others, which exhibit anthropophilic tendencies. In a similar study, it was shown that 33-80% of *An. quadriannulatus* species A from South Africa exhibited susceptibility to *P. falciparum* infection (Takken *et al.*, 1999). It would thus be interesting to study the vectorial status of *An. quadriannulatus* species B in Ethiopia. The existence of *An. arabiensis* and *An. quadriannulatus* species B have long been known (White *et al.*, 1980; Mekuria, *et al.*, 1982; Abose *et al.*, 1998a; Hunt *et al.*, 1998).

Anopheles gambiae in Ethiopia seems to inhabit in and around human dwellings as well as in areas where human habitation is absent. Jolivet

(1959) collected the larvae of these species 30 kilo meters away from human dwellings. This species in its feeding and resting behavior is not strictly anthropophilic, endophagic and endophilic. It exhibits partial zoophily, feeds and rests both indoors and outdoors (White, 1974). It shows plasticity in its feeding behavior depending on the availability of host and the prevailing environmental situations. Where available hosts are only humans, the species derives its blood meal from humans. In area where both humans and cattle are present, it feeds on both with variable proportions (White, 1974; Braack *et al.*, 1994).

Mosquitoes of this species rest in human dwellings, animal shelters and also outdoors in ditches, vegetation, trees holes and others (White, 1974). While resting indoors, it shows preference for walls than ceilings. Smoke tends to drive it from ceilings. In houses where there is no smoke it is abundantly found on the walls and ceilings (Jolivet, 1959). Collections made in the 1958 malaria epidemics revealed the average hut resting density to vary from 100 to 150 (Fountaine *et al.*, 1961). This figure remains the highest in published reports in Ethiopia in Gambela, Krafsur (1971-1977) reported the average hut resting density less than 30. White *et al.*, (1980) near Jimma (Giligil Ghibe River valley) found the density to vary from less than 1 in January-March to greater than 100 in July-October.

Frequent insecticide sprays affect the feeding and resting behavior of *An. arabiensis* either by reducing the number or altering the behavior. The portion of the population frequenting indoors would be eliminated giving a rise in the outdoor population. The repellent effect of insecticides enforces the species to avoid insecticide sprayed human dwellings (Zahar, 1985 citied in Ameneshewa and Service, 1996). In this case it increasingly rests outdoors.

In other situations, it may be found in sprayed dwellings resting on the unsprayed part of the house, hanging on cloth and household utensils (Jolivet, 1959; Ameneshewa and Service, 1996). Based on studies conducted in the country between 1984-1988 by Tulu (1993a) indicated 72.4% exophgagy and 80.3% endophily. Earlier, Krafsur (1977) reported 20-40% of the species leaving indoor environments after it took its blood meal. Similarly, Ameneshewa and Service (1996) noted 37.5 % exophily in an insecticide sprayed village in the Upper Awash Valley. This shows that the species is increasingly exhibiting exophily, as the result, it is becoming more difficult to eliminate it by chemical insecticides applied indoors.

1.3. Sporozoite and Parity Rates of *Anophelines*

The sporozoite rate is the proportion of *Plasmodium* infected individuals in a population of local vector species (WHO, 1975). Traditionally, the sporozoite rate is measured by dissecting the salivary glands and detecting parasites under the microscope. This method is laborious and time consuming requires expertise, demands fresh specimens and does not distinguish between different species of malaria parasites. Such limitations were later solved by the development of the Enzyme Linked Immunosorbent Assay (ELISA). The advantage of these methods is largely related to the suitability and simplicity to process large specimens. The ELISA is easy to perform, utilizes stable reagents, requires less expensive equipment and is relatively sensitive and specific (Burkot *et al.*, 1984, Wirtz *et al.*, 1987; Boudin *et al.*, 1988).

However, as many diagnostic methods, the ELISA is not far from problems. This technique does not distinguish the mosquito with active parasites from the one with past infections. False positivity has been recorded in swine and bovine blood engorged mosquitoes (Somboon *et al.*, 1993).

Recently, it has been attempted to use the ELISA technique (Nigatu *et al.*, 1992; Ameneshewa, 1995; Abose *et al.*, 1998b). A study describing sporozoite rates of *An. arabiensis* and other *Anophelines* in Ethiopia is in *An. arabiensis* varied from 0 to 3% despite, the incrimination of *An. arabiensis* as the principal vector in Ethiopia, the sporozoite rates so far detected is very low. Sporozoite rates in the 3 other secondary vectors namely; *An. funestus, An. nili* and *An. pharoensis* were reported to be 1.23-1.4%, 1.29% and 0-0.45%, respectively (Krafsur, 1977; Nigatu *et al.*, 1992).

1.4. Insecticide Treated Mosquito Nets (ITNs)

The development of synthetic pyrethroid insecticides which are stable and remain effective for long periods enabled entomologists to test the idea of impregnating mosquito nets of various textures and fabrics as a vehicle for residual insecticide. Much has been written about this technique, which is now well understood and has proven effective (Curtis, 1996; Snow *et al.*, 1987b).

Bed nets treated with insecticide offers much greater protection against malaria, not only does the net act as barrier to prevent mosquitoes biting, but also the insecticide repels, inhibits, or kills any mosquitoes attracted to feed (MacCormack and Snow, 1986; Robert and Carnevale, 1991; Aikins and Pickering, 1994).

Malaria control is an increasingly important focus for the international body concerned with public health and disease control. In response, the

Roll Back Malaria (RBM) initiative has arisen with backing from the United Nations Development Program, World Bank, United Nations Children's Fund, and WHO (Nabarro, 1999). The initiative is examining various strategies to do this, one of which is the promotion of Insecticide Treated Mosquito Nets (ITNs).

In April 2000, African heads of state participating in the Abuja Summit agreed that at least 60% of those at risk for malaria, particularly children under 5 years of age and pregnant women, are to benefit from the most suitable combination of personal and community protective measures such as ITNs by 2005 (WHO, 2000; The African Malaria Report, 2003).

Trials in African settings of different transmission intensities have shown that ITNs can reduce the number of under-5 deaths by around one-fifth. The incidence of clinical episodes of *Plasmodium falciparum* infection is reduced by 50% on average. When used by pregnant women, ITNs are also efficacious in reducing maternal anaemia, placental infection, and low birth weight (Lengeler, 2001; Garner and Gulmezoglu, 2000).

In a Tanzanian project, household coverage approached 75%, and contributed to a reduction in slide positivity and improved weight gain in children younger than 5 years who slept under treated nets (Shiff *et al*, 1996). In the Gambia, the National Impregnated Bed net Programme achieved an 83% net treatment rate and reported 77% of under-5s and 78% of women of childbearing age sleeping under ITNs, overall under-5 mortality fell by 25%, and case–control studies suggested that there were 59% fewer episodes of uncomplicated malaria in ITN users (Cham, 1996; D'Alessandro, 1997).

There was also a major impact on the density of infected mosquitoes in the protected areas (Temu *et al*, 1999). The use of ITNs could be integrated into any national malaria control strategy. Depending on the behavior patterns of the local vector species, ITNs could replace indoor spraying in many instances (Misra *et al*, 1999). The main problem to be addressed is the process of implementing and sustaining operations.

Curtis (1999) explain that, study in Tanzania indicated that 65% reduction of incidence between children aged <6 years who slept using treated mosquito nets. Similarly Alonso (1999) Expressed that study conducted in Gambela region indicated that 60% reduction mortality among children aged 1- 4. Although the effectiveness of the ITNs is highest, crucial point or more attention should be given for effective implementation and appropriate utilization of the net.

Some studies of insecticide-treated bed nets have shown a 'mass-killing effect' with reductions in survivorship, sporozoite rate and density of the mosquito vector population at a village level (Magesa *et al.*, 1991). When individual bed nets were treated with permethrin or placebo, and used by less than 10% of the children in one village, those who slept under treated bed nets had fewer clinical episodes of malaria than control children, but other features of malarias infection, such as splenomegaly and parasitaemia, were not altered significantly (Snow et al., 1987a).

Efficacy of ITNs, reduced mosquito burden extends to households and communities without nets. Results, illustrates a protective effect of ITNs on compound lacking ITNs located within 300 meters of compound with ITNs. The protection afforded to non-users in the vicinity is difficult to quantify, but it appears to extend over several hundred meters. The ITNs trials achieved their impact with close to 100% of households possessing nets and 50–75% of under-5 years sleeping under them, a level of use similar to the Abuja target of 60%. Where lower coverage and use rates are achieved, the impact on mortality will be less. On the otherhand

Quinones *et al.*, (2003) showed that, the parous rate of *An. gambiae s.l.* collected in exit trap 4 months after treatment were significantly lower than in untreated villages.

Most African households in malaria risk areas do not possess any net, whether treated with insecticide or not. To achieve adequate coverage most countries will require many more nets; to cover all Africans at risk, an estimated total of 260 million nets would be needed (The Africa Malaria Report, 2003).

Long-Lasting Insecticidal Nets (LLINs) solve the need for re-treatment, unlike conventional ITNs, LLINs resist washing and reduces both human exposure at any given time, and the risk of environmental contamination. There is also scope to increase the use of ITNs by providing insecticide treatment for any untreated nets already in houses (Guillet, *et al.*, 2001).

To use of ITNs, the habit of the vector would be Anthropophagic, Endophilic and Endophagic, thus, the control measurer will be more effective. The time people spend in the net and the habit of mosquito biting time has to be taken in to consideration. In line with this appropriate type of the shape of nets, must be selected, unless the sleeper more likely to come in contact with the nets and being bitten (Misra *et al*, 1999; Eshuis and Manschot, 1993).

The type of vector species, their vector status, distribution, biting and resting habits are necessary to select appropriate type of control strategy then this offer opportunity of controlling the disease (Eshuis and Manschot, 1993).

2. OBJECTIVE

2.1. General Objective

To study species composition and significance of Insecticide Treated Mosquito Nets (ITNs) against malaria vectors.

2.2. Specific Objectives

1. To find out species composition and breeding sites of *Anopheles* mosquitoes in the study area.

2. To determine density, parity and sporozoite rate of *Anopheles* mosquitoes among households with and without of ITNs.

3. To compare morbidity (Incidence) of malaria among households with and without ITNs.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study site Jolie Peasant Association is found in Meskan Woreda, Guragie Zone, and Southern Nation Nationalities People Regional State (SNNPRS). The study was conducted from October 2006 to June 2007.

The study site is found at an average altitude of 1840 meters above sea level. Its distance from Addis Ababa is about 120 kilometers and 18 kilometers from Butajira town (Butajira located at Latitude: 8.117 and Longitude: 38.367). The population in the Jolie Peasant Association is estimated to be 7,454. There are 1,133 households. The dwellers are engaged in cultivating maize and cash vegetables such as onion, pepper, tomato, potato and cabbage.

The area has two major rainy seasons; the main rainy season starts in June and ends in mid September, while the short rains occur in March and April. Meteorological data obtained from the National Meteorological Services Agency for three years between 2003 and 2005 revealed that the area received an annual rainfall amounting to 1167.4, 898.7 and 1702 mm, respectively. The respective average temperature was 25.91, 26.27 and 26.37^{0}C (National Meteorological Services Agency, 2006).

The vegetation in the study area is composed of mainly the sparsely distributed Acacia woodlands, non- indigenous trees and shrubs Water from the Meke River has made it possible to irrigate and develop a small scale farms owned by private peasants (Plate 1).

Plate 1. Overview of the study area and irrigation farm.

All study localities have few corrugated iron sheet houses, but dominantly with tukuls. The tukuls in both villages are similar in structure. They are circular shaped types made from wood and mud; but some tukuls are not plastered with mud. The construction materials for the roofs are grasses. Each hut has a 2 meter high door, but no window instead, each hut has a small opening to allow the entrance of light and air. Incomplete partitions are formed inside each hut to delineate the bedroom from animals. In some huts, a small room for small goats is included. Mixed dwellings with either goats or calves are not uncommon.

Plate 2. Identified breeding site (River pocket).

In recent years, some of the inhabitants (peasants) are producing vegetables by practicing irrigations. Water is drawn from Meke River either by large or small surface pump to the small canals and the vegetable farms. Pools of stagnant water left in the small canals, river pockets and marshy areas formed from spillage of water from the big canals together with pools of rain water have become suitable breeding habitats for mosquitoes (Plate 2). Mosquitoes in the dry season seem to breed in all localities.

3.2. Study on Incidence of Malaria Parasites

The incidence of malaria parasites among individuals that live in houses with and without ITNs were determined (Plate 3) by taking thick and thin blood smear from individuals included in the study. Infection with *Plasmodium* species was determined from the thick smear slides stained with giemsa. While species identification was carried out using thin blood smear slides stained with giemsa.

Relevant data on the average monthly malaria case distribution and prevalence of malaria parasites in the study area for the study period has been gathered from relevant health facilities to show malarial infection and the species of *Plasmodium* responsible for the cause of malaria in the study areas. Jolie Health Post provides relatively efficient treatments for malaria cases on clinical grounds and sometimes by using Rapid Diagnostic Test (RDT) kit.

Plate 3. House, without Insecticide Treated Mosquito Nets (ITNs).

3.3. Collection and Identification of Immature Stage *Anopheles* Mosquitoes

Collection of immature stage of *Anopheles* mosquitoes collections were conducted to determine the species composition of *Anopheles* mosquito species in the study area to supplement the data of adult collection regarding to the study of species composition. Larval sample collection was carried out in a variety of aquatic habitats of the study area. *Anopheles* larvae collections undertaken for four days per month deploying two collectors.

Mosquito larvae were collected using dippers, pipettes and containers from pools of temporary and permanent breeding habitats from all sites. The breeding sites included ponds, pools of rain water, water from broken pipes, River pocket, stagnant water in irrigation canals and marsh (Plate 4).

Identifications were made with fourth instar larvae. The larvae were killed in hot water (about 50^0 C) and preserved in small vials containing 70% ethyl alcohol (Service, 1993b) and transported to Addis Ababa. Each larva was then mounted on a glass slide separately in a drop of gum chloral mountant and covered with a piece of cover slip (Lane, 1974). Each cover slip measuring 22x22 mm was cut into four parts and one piece was used for a single specimen. The gum chloral mountant was prepared in the laboratory as described in Gordon and Lavoipierre (1969). The constituents are 25 ml distilled water, 160 gm crystal chloral hydrate, 15 gm gum Arabic, 10 gm glucose syrup and 5 ml glacial acetic acid which were then mixed in the above order in a water bath at temperature at about 80^0C. At all times, the mountant was kept in a dark well-screwed bottle. Identification of larvae to the species level was based on Verrone (1962b) and Gillies and Coetzee (1987) using a compound microscope.

Plate 4. Identified breeding site (Pond) in the study area.

3.4 Collection and Identification of Adult Anopheles Mosquitoes

Adult *Anopheles* mosquitoes sampling were conducted in four localities (village) namely Gurambar, Akemuj, Megazen and Jolie in the study area. Adult Anopheles mosquitoes were collected from ten houses (5 houses that use ITNs and 5 houses that do not use ITNs) randomly selected in each of the four villages in the study area. Houses selected for adult mosquito collection comprised tukuls and houses with corrugated iron roofs.

Indoor and outdoor mosquito collections were conducted once a week using CDC light traps, mouth suction aspirators, and space spray methods, respectively (WHO, 1992; Service, 1993c) from October 2006 to June 2007.

Female mosquitoes collected using aspirators, CDC light traps and space spray methods were sorted, counted, categorized as unfed, fed, half gravid, and gravid, and finally placed in separate labeled paper cups for identification.

All collected female mosquitoes from households with ITNs and without ITNs from the four villages using different methods were identified based on keys provided by Verrone (1962a) and Gillies and Coetzee (1987).

Unfed mosquitoes were dissected to determine parity rate while the fed, half-gravid and gravid were discarded after identification. An enzyme-Linked Immunosorbent Assay (ELISA) were used to test heads and thoraces of dried unfed female mosquitoes from collections for the presence of *Plasmodium falciparum* and *P. vivax* circumsporozoit proteins as described by Wirtz et al. (1987).

3.4.1. Space Spray Collection and Identification

Space spray collections of indoor resting female mosquitoes were carried out once a week early in the morning beginning 6:00 am to 10:00 am. All human occupants, animals, domestic utensils, exposed food and water were voluntarily moved out of the dwellings prior to spraying. The entire floor was covered with white cloth sheets cut to 1.2m x 2.20m and all household items were underneath. After spraying, the dwellings were left closed for 20 minutes to produce a knockdown effect (Plate 5). All knocked down mosquitoes were collected from the white cloth sheets using torch, forceps and paper cups.

Plate 5. Waiting for knock-down collection.

3.4.2. Indoor and Outdoor Mouth Suction Aspirator Collection and Identification

Aspirator collections of female mosquitoes were carried out on a different day once a week early in the morning beginning 6:00 am to 10:00 am. Mouth suction aspirators were used to collect indoor resting mosquitoes from walls, under roofs, hanging clothes, household utensils and dark corners. Outdoor resting female mosquitoes were collected from tree holes, dark or concealed places in the four villages using aspirators.

3.4.3. Indoor and Outdoor CDC Light Trap Collection and Identification

CDC light traps were used to collect female mosquitoes on yet another day once a week from February to May 2007 (Plate 6). CDC light traps were positioned indoors and outdoors at selected houses from 07:30-12:00 pm at night.

Plate 6. CDC Light traps collection in the study area.

3.5. Entomological Study on Effects of ITNs on Malaria Vector

A cross-sectional study on 40 randomly selected rural households was carried out in Jolie Peasant Associations. As described in the previous sections, all houses were visited between October 2006 and May 2007 to collect *Anophelines.* The total numbers of An. gambiae s.l. collected by indoor space spray method in households with and without ITNs collected were compared (Plate 7). *Anopheles* mosquitoes collected from households with ITNs and without ITNs from each village using space spray catch were then compared to evaluate the difference or significance of using ITNs on mosquito densities, parity and sporozoite rates.

Plate 7. House with Insecticide Treated Mosquito Net (ITN).

3.6. Parity and Sporozite Determination

Unfed female *Anophelines* caught by the methods of space spray, CDC light traps and aspirator collection were subjected for dissection to determine parity and sporozoite infection rates (Service, 1993c). Parous mosquitoes are those that have at least fed and oviposited once. So, parous mosquitoes are blood seeking insects, which are epidemiologically the most dangerous group of a population since they are potentially infective (Service, 1993c). However, in other species, this is not always the case. Parity in *Anophelines* is determined by two methods: (i) Tracheation of the ovaries: based on the observation of Detinova (1945 citied in Service, 1993c), 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. The tracheoles remain stretched in females who have oviposited. The former are known as nulliparious, while the latter are parous females. By this method one is unable to determine the number of times a female has oviposited. (ii) Ovariolar dilatation this method depends on the counting of dilatation on the ovarioles as the result of development and oviposiion of eggs (Detinova, 1992). Each mosquito was placed in a drop of fresh physiological saline (0.85% Sodium chloride solution) on a slide and dissected under a dissection microscope. Using two entomological needles mounted on slim wooden handle the legs and wings were first removed. With one needle pressed gently against the thorax, the last 2-3 abdominal segments were pulled out by the second needle and drawn away from the body to expose the gut and ovaries. Of the two ovaries, one was left aside on the same slide to dry (to observe the skeins of the tracheoles), while the other was further dissected to see the presence or absence of ovarian dilatations and relics (Detinova, 1962). The ovaries for parity were examined under the 10x and 40x objectives of a compound microscope.

For detection of sporozoites, the salivary glands were separately dissected out either attached to the head or by pulling them from the anterior thorax sometimes with the front coax. An Enzyme – Linked Immunosorbent Assay (ELISA) were used to test heads and thoraces of dried unfed female mosquitoes from collections for the presence of *Plasmodium falciparum* and *P. vivax* circumsporozoite proteins as described by Wirtz *et al.*, (1987). The parity and sporozote rate of *Anophelines* were compared to those households with and with out ITNs from each village.

3.7. Data Analysis

All data were analyzed using statistical soft ware (SPSS In., Version 13, 2004). The association of vector density between households with and with out ITNs were determined by a paired t test using P-value, the parity rate and morbidity of malaria in households with and without ITNs were statistically tested for significance using chi-square test.

4. RESULT

4.1. Study on Incidence of Malaria Parasites

To determine incidences of malaria two surveys conducted in October 2006 and April 2007. Out of the total 182 blood smear examined in the two surveys, 38 were positive for malaria parasites. 57.9% of the total positive blood films were due to *P. falciparum* and 42.1% were due to *P. vivax* (Appendix 5). The incidence of malaria in households with and without ITNs by locality shown in Table 1.

Table 1. Incidence of malaria in households with and without ITNs by locality in the study area 2007.

Localities	Positive/by species				
	Pf		*Pv*		Total
	HWI	HWOI	HWI	HWOI	
Megazen	2	3	1	3	9 (23.68%)
Gurambar	2	3	2	1	8 (21.05%)
Akemuja	2	2	1	1	6 (15.79%)
Jolie	3	5	3	4	15 (39.47%)
Total	9	13	7	9	38 (100%)

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However, differences in malaria incidence were observed among different localities of Jolie study area a chi-square test (χ^2) showed that, there were no statistically significant differences observed among households with and without ITNs ($\chi^2 = 0.6247$, $p > 0.05$) (Table 2).

Table 2. Analysis of a chi-square (χ^2) test on incidence of malaria in households with and without ITNs.

Group	Positive	Negative	Number	χ^2	p
HWI	16 (18.16)*	71 (68.83)*	87		
HWOI	22 (19.83)*	73 (75.16)*	95		
Total	38	144	182	0.6247	0.445

*Figure in parenthesis denote expected values

4.2. Larval Mosquito Collection and Identification

A total of 2108 *Anophelines* larvae were collected from different types of breeding sites. The majority (43.3%) were collected from ponds which served as a major breeding site during the dry season, while the remaining sites served as breeding sites during the rainy season as well as the dry season (Table 3).

Table 3. Total larvae sampled in the study area from October 2006 to May 2007.

	Number of *Anopheles* mosquito larvae sampled monthly								
	2006			2007					
Breeding sites	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Total
Pond	210	53	10	8	15	139	287	192	914 (43.3)
Irrigation canals	14	12	6	4	12	10	79	22	159 (7.5)
River pocket	110	60	49	22	21	22	104	61	449 (21.3)
Rain pools	142	22	0	0	0	14	110	0	288 (13.7)
Swamps/Marsh	172	20	16	10	12	10	12	46	298 (14.2)
Total	648	167	81	44	60	195	592	321	2108 (100)

*Figures in parenthesis denote percentage

Of the total collected, 1665 larvae, three species were identified namely: *An. gambiae s.l.*, *An. pharoensis* and *An. christyi*. About 43 of the larvae could not be identified because of bad preparation and mechanical damage and the rest 400 *Culex* discarded. *Anopheles gambiae s.l.* is the most abundant which accounts 98.8%, the rest were scarce; *An. Pharoensis* and *An. Christyi* made up 1.02% and 0.18% of the larvae identified, respectively (Table 4).

Ponds were found to be a typical breeding site to *An. gambiae s.l. An. pharoensis* and *An. christyi* co-existed in small interrupted irrigation canals and River pockets. Except the canals all the sites were sunlit and devoid of vegetation. *Anopheles pharoensis* seem to breed mainly in irrigation canals but also in ponds and river pocket during the dry season. *Anopheles christyi* was found breeding in River pockets, pond and irrigation canals. This species was the least abundant relatively compared to *An. gambiae s.l.* and *An. pharoensis.* Based on monthly larval collection data, *An. gambiae s.l* was observed to breed through out the year in all identified breeding sites.

Table 4. Monthly distribution of *Anopheles* mosquito larvae by species in different identified breeding sites of the study area from October 2006 to May 2007.

Breeding sites/spp	2006 Oct	Nov	Dec	2007 Jan	Feb	Mar	Apr	May	Total
An. gambiae									
Pond	208	51	11	6	16	139	287	192	910
Irrigation canals	14	12	6	4	12	10	6	10	74
River pocket	92	59	42	21	19	21	98	48	400
Rain pools	11	0	0	0	0	0	26	16	53
Swamps/Marsh	101	16	14	10	9	10	19	29	208
Total/%	426	138	73	41	56	180	436	295	1645 (98.8%)
An. pharoensis									
Pond	0	0	0	0	1	1	0	0	2
River pocket	0	0	0	3	3	6	2	1	15
Total	0	0	0	3	4	7	2	1	17 (1.02%)
An. christyi									
Pond	0	0	0	1	0	0	0	0	1
River pocket	0	0	0	0	0	1	0	0	1
Irrigation canals	0	0	0	0	1	0	0	0	1
Total	0	0	0	1	1	1	0	0	3 (0.18%)

4.3. Adult *Anophelines* Collection and Identification

To identify the species, two methods were employed; adult female *Anopheles* mosquitoes collected from the study sites and adult that emerged from larvae collected at different breeding sites. A total of 1715 adult *Anophelines* were collected from all the study localities. Of which 1487 *Anophelines* were used for identification and the rest 228 *Culex* were discarded. The species, number and methods of adult *Anophelines* collected from the four sites are shown in Table, 5 and 6. At least three species, *An. gambiae s.l., An. pharoensis and An. christyi* were identified.

Anopheles gambiae s.l. was the predominant species in the whole study area, making up 96.5% of the whole *Anophelines* collected, followed by *An. pharoensis* (3.2%). *An. christyi* species were rarely found. Both *An. gambiae* and *An. pharoensis* occurred in all of the four localities. The majority of the former species was collected from Megazen and Jolie study localities, while *An. christyi* was collected only from Megazen and Jolie (Table 5).

Table 5. Species and number of adult *Anophelines* caught from the four study sites by various collection methods in the study area between October 2006 and May 2007.

	Sites				
Species	Akemuja	Gurambar	Megazen	Jolie	Total %
An. gambiae s.l,	283	349	377	426	1435 (96.5)
An. pharoensis	3	5	17	23	48 (3.2)
An. christyi	-	-	2	2	4 (0.3)
Total	286	354	396	451	1487 (100)

Table 6. Adult *Anopheles* mosquitoes collected by different methods in the study area from October 2006 to May 2007.

Anopheles Spp	Space Spray	CDC	Aspirator	Total
An. gambiae s.l	209	262	114	585 (91.83%)
An. pharoensis	3	42	3	48 (7.53%)
An. christyi	0	4	0	4 (0.64%)
Total *An.* Sp.	212	308	117	637 (100%)

4.3.1. Space Spray Collection and Identification

Only two species, *An. gambiae s.l.* and *An. pharoensis* were found resting indoors, the former was caught abundantly in all localities. The average hut density of *An. gambiae s.l.* varied from 0.2 to 1.62 mosquitoes/hut/day in eight months of Jolie study area. The monthly pattern of *An. gambiae s.l.* resting in huts is presented in Table 7. The highest density of the species was recorded in April and May in the four study localities, coinciding with high rainfall in the area. In the months between October 2006 and May 2007, the average densities in Akemuja, Gurambar, Megazen and Jolie study localities were 7, 7.1, 5 and 7, respectively. There were only three fed *An. pharoensis* in space spray collected.

The catches of space spray were categorized on abdominal status as unfed, fed, half-gravid and gravid. The results showed that there was scarcity of unfed females compared to freshly fed mosquitoes in all sites (Appendix 8).

Table 7. Monthly densities of A*nopheles gambiae s.l.*/hut/day collected using space spray catch in the study area between October 2006 to May 2007.

Months/ Years	No. Houses searched	*An. gambiae*	
		No. Collected	density/ hut/day
Oct. 2006	40	22	0.55
Nov. 2006	40	21	0.52
Dec. 2006	40	10	0.25
Jan. 2007	40	11	0.27
Feb. 2007	40	6	0.15
Mar. 2007	40	34	0.85
Apr. 2007	40	39	0.97
May. 2007	40	66	1.65
Total	320	209	5.21

4.3.2. Indoor and Outdoor Mouth Suction Aspirator Collection and Identification

In the Jolie study area, the average hut density of *An. gambiae s.l.* in the eight months ranged from 0.05 to 0.35 mosquitoes /hut/ day (Table 8). *An. gambiae s.l.* was found to rest more frequently on walls, the lower parts of roofs, hanging utensils and clothes. It avoided the upper part of the roof where this part was usually covered with soot. The species preferred mud as its resting site because the rough features are more favorable than the smooth surfaces of walls and roofs. Two fed *An. Pharoensis* collected from Megazen study locality.

Regarding the abdominal status of females, the number comprising the half-gravids and gravids was much lower than the fresh feds, similar to the collection made by the space spray method. The number of unfed females was similarly very low (Appendix 9).

In the four study localities Akemuja, Gurambar, Megazen and Jolie *Anophelines* aspirated from partially destroyed huts, which served as cattle sheds and other cattle shelters (Table 9). The largest groups of *An. gambiae* collected from these resting sites were half-gravids and gravids, showing the tendency of females in the two abdominal states to leave human dwellings, perhaps driven by smoke or by the inherent exophilic behavior of the species. A small number of *An. gambiae* from the Megazen locality was also aspirated from tree holes, worn out old house and ground burrows. Only one half-gravid *An. Pharoensis* were collected from outdoor resting sites.

Table 8. Monthly densities of *Anopheles An.gambiae* /hut/day collected using indoor mouth aspirator collection method in the study area between October 2006 to May 2007.

Months/ Years	No. Houses searched	*An. gambiae s.l.*	
		No. collected	density/ hut/day
Oct. 2006	40	14	0.35
Nov. 2006	32	6	0.19
Dec. 2006	40	3	0.07
Jan. 2007	36	3	0.07
Feb. 2007	38	2	0.05
Mar. 2007	40	4	0.1
Apr. 2007	40	12	0.3
May. 2007	40	9	0.22
Total	306	53	1.35

Table 9. Number and abdominal status of *Anopheles gambiae* collected by mouth aspiration from outdoor sites between October 2006 and May 2007.

Species and Abdominal Status		**Sites**			
	Akemuja (cattle shed)	Gurambar (cattle shed)	Megazen tree hole/borrow	Jolie (cattle shed)	**Tota l**
An. gambiae					
Total mosquitoes	12	13	11	25	
No unfed	-	3	2	4	**61**
No. fed	1	2	1	3	**9**
No. half-gravid	8	5	6	6	**7**
No. gravid	3	3	2	12	**25**
					20

4.3.3. Indoor and Outdoor CDC Light Trap Collections and Identification

Anophelines entering huts or resting in huts were caught in CDC light traps (Table 10). Two species of *Anophelines, An. gambiae* and *An. pharoensis* were caught by CDC light traps indoors, but the two species were not always caught together in some villages such as in Gurambar. Light traps caught more *An. gambiae* in four months between February and May 2007 when the indoor density was relatively high, due to the presence of small rain season. Two half gravid *An. Pharoensis* were caught in light traps in the Jolie locality. The majority of mosquitoes attracted to light traps were fed females comprising 52.29% of all collections. The second largest was half gravid (29.93%) (Appendix 10).

CDC light traps placed outdoor catch *An. gambiae s.l., An. Pharoensis and An. christyi*. Throughout the study period, *An. gambiae and An. pharoensis* were the two commonly encountered species collected both indoors and outdoors in all the study sites. However, the former species was the commonest biting species. Thus, considering *An. gambiae s.l.* a total of 23, 26, 45 and 59 were caught in Akemuja, Gurambar, Megazen and Jolie, respectively (Table 11). The number of *An. pharoensis and An .christyi* caught and abdominal status are shown in Appendix 11.

Table 10. Monthly indoor collections of *An. gambiae s.l.* using CDC light traps in four sites from February to May 2007.

Months/ Methods of collection	No. Houses searched	*An.gambiae* No. Collected	l density/ hut/day
Feb. 2007	40	16	0.4
Mar. 2007	40	19	0.47
Apr. 2007	40	48	1.2
May. 2007	40	26	0.65
Total (%)	160	109	2.72

Table 11. Monthly outdoor collections of *An. gambiae s.l.* using CDC light traps in four sites from February to May 2007.

Months	No. *An. gambiae*	No. *An. pharoensis*	No. *An. christyi*
Feb. 2007	18	21	2
Mar. 2007	38	12	2
Apr. 2007	58	7	0
May. 2007	39	0	0
Total (%)	153	40	4

4.4. Entomological Study on effect of ITNs on Malaria Vector

A total of 371 *An. gambiae s.l.* and 7 *An. pharoensis* were collected by indoor space spray, aspirator and CDC light trap collection methods. The percentage of *An. gambiae s.l.* in households with and with out ITNs collected were 14.55% and 85.44% respectively. The number of *An. gambiae s.l.* on monthly basis in the four sites shown in Table 12.

Anophelines collection in Households with and without ITNs intended to indoor resting mosquitoes by knock down space spray catches are shown in Appendix 6.

The total number of *An .gambiae s.l.* collected by space spray catch in Households with and without ITNs was tested by a paired t test. It showed that the difference was significant, ($t = 10.03$, $df = 38$, $p < 0.05$) thus, the difference between Households with and without ITNs in the total number of *An. gambiae s.l.* collected was significantly fewer *An. gambiae* s.l. were found resting in rooms with ITNs (Appendix 6). The result of analysis shown in Table 13.

Table 12. Number and percentage of *An. gambiae s.l.* collected by indoor space spray, Aspirator and CDC light trap collection from the four study localities in households with and without ITNs from October 2006 to May 2007.

Months	Sites and ITNs Status									
	Akemuja		Gurambar		Megazen		Jolie		Total	
	HWI	HWOI	HWI	HWOI	HWI	HWOI	HWI	HWOI	HWI	HWOI
Oct.	0	15	1	21	1	17	4	25	6	78
Nov.	2	6	1	7	0	6	3	10	6	29
Dec.	0	0	0	4	0	5	1	8	1	17
Jan.	1	2	0	3	0	3	1	7	2	15
Feb.	0	3	2	2	0	2	1	6	3	13
Mar.	2	6	0	6	2	5	5	11	9	28
Apr.	3	12	2	13	1	26	5	29	11	80
May.	6	13	3	14	3	15	4	15	16	57
Total (%)	14	57	9	70	7	79	24	111	54 (14.55)	317 (85.44)

Table 13. Analysis of a paired t test on density of *An. gambiae s.l.* in households with and without ITNs.

Group	Number	Mean ± Std. Err	t	p
HWOI	20	9.55 ± 0.85		
HWI	20	0.90 ± 0.14		
Difference		8.65 ± 0.86	10.39	0.000*

*P < 0.001

4.5. Parity and sporozoite Rates

Unfed females of *An. gambiae, An. pharoensis* and *An. christyi* from aspirator, CDC light trap and space spray collection were dissected to determine the parous rates in the population. The result of the dissection of *Anopheles* species caught in the four sites is shown in Table 14. A total of 262 *An. gambiae* were dissected, of which 164 (44.93) parous. A total of 21 and 4 *An. pharoensis* and *An. christyi* dissected, respectively, none were parous. Of the examined 82 parous *An. gambiae* for sporozoite, none were found harboring sporozoites in their salivary glands. The parous rates from the samples collected by space spray catches were variable from households to households in the four study localities (Appendix 7). The effects of ITNs were observed on parous rate, which were reduced in households with ITNs. The result of chi-square (χ^2) test showed that statistically significant ($\chi^2 = 4.657$, $p < 0.05$), the result of analysis shown in Table 15.

Table 14. Parity rate of *Anopheles* species in the four study localities from October 2006 to May 2007.

Species	Akemuja	Gurambar	Megazen	Jolie	Total
An. gambiae					
No.dissected	60	66	53	73	252
No.parous	31	30	46	57	164
parous%	(51.66)	(45.45)	(86.79)	(78.08)	(6508)
An .pharoensis					
No.dissected	0	0	9	12	21
No. parous	0	0	0	0	0
An. christyi					
No.dissected	0	0	2	2	4
No. parous	0	0	0	0	0
Total					
No.dissected	60	66	53	73	277
No. parous	31	30	46	57	164
	(51.66)	(45.45)	(86.79)	(78.08)	(6508)

Table 15. Analysis of a chi-square test on parity of *An. gambiae s.l.* in households with and without ITNs.

Group	Parous	Nulliparous	Number	χ^2	p
HWI	1 (33.3%)	2 (66.67)	3		
HWOI	28 (84.85)	5 (15.15)	33		
Total	29	7	36	4.65	0.032

5. DISCUSSION

Malaria in the study area prevailed both in the wet and dry months showing the perennial transmission of the disease. The intensity of transmission during the dry months was relatively lower; however, malaria during this period remained to be a significant cause of morbidity in the population. It is known that, in Ethiopia malaria occurs immediately after the small rainy seasons of March and April as well as after the long rain of June through September. Peak transmission occurs during the months of September through November. Malaria transmission continues less intensely in the wet seasons, several epidemics in the high land fringe areas caused numerous deaths (MOH, 2000; Tulu, 1993a).

Monthly morbidity report of malaria cases treated based on clinical and laboratory diagnosis in Meskan Woreda from 2001 to 2006 is shown in Appendix 1. More over infants (9.3%) and children aged between one and four (18.05%) were more affected compared to other age groups. Similarly studies indicated that, children who seem to lack primary immunity and pregnant women are the main victims of the disease. Malaria also contributes indirectly too many additional deaths, mainly in young children, through synergy with other infections and illnesses (World Malaria Report, 2005; TDR, 1997-98; Abose *et al.*, 1998b; MOH, 2004; Nega and Haile-Meskel, 1991).

Similar to other parts of Ethiopia, malaria in the study area was predominantly caused by *P. falciparum* followed by *P. vivax*. The two malarial parasites are known to be the main cause of mortality and morbidity, respectively. According to Butajira Health center laboratory diagnosis report between 2001 and 2006, *P. falciparum* and *P. vivax* constituted 2883 (60.4%) and 1890 (39.6%) of all malaria patients, respectively. This figure is almost the same to the average prevalence rate of *P. falciparum* (60%) common in most parts of the country. Likewise, the prevalence rate of *P. vivax* 39.6% was also almost to the known average rate of 40%. In line with this, other studies showed that *P. falciparum* and *P. vivax* are the major pathogens of malaria in country (Nigatu *et al.*, 1992; MOH, 2004; Gebre-Mariam, 1984; Gebre-Mariam *et al.*, 1988; Tulu, 1993a). The appearance of *P. vivax* malaria in the dry months might be related to either relapses or new infections from the bite of infective mosquitoes. The presence of *P. falciparum* infections in the dry season is an indication of the continuation of active transmission during this period.

In this household-level study involved 40 houses, incidence of malaria in households with and without ITNs was determined from the surveys in the study area. Out of the total blood film examined in two survey, 38 (20.87%) were infected. 22 (57.9%) and 16 (42.1%) were due to *P. falciparum* and *P. vivax,* respectively. The result of chi-square test showed that there were not significant differences observed in malaria parasites among households with and without ITNs, since the value of $P > 0.05$.

Similarly, study conducted on insecticide-treated bed nets when individual bed nets were treated with permethrin or placebo, and used by less than 10% of the children in one village, those who slept under treated bed nets had fewer clinical episodes of malaria than control children but other features of malarias infection, such as splenomegaly and parasitaemia, were not altered significantly (Snow et al., 1987). Misra et al., (1999) suggested that, to use ITNs the habit of the vector would be Anthropophagic, Endophilic and Endophagic, thus, the control measurer will be more effective. The time people spend in the net and the habit of mosquito biting time has to be taken in to consideration (Misra *et al*, 1999). In line with this appropriate type of the shape of nets, must be selected, unless the sleeper more likely to come in contact with the nets and being bitten. Selecting appropriate and feasible vector control method should be relying on the type of vector species, their vector status, distribution, biting and resting habits and breeding places, are necessary to select appropriate type of control strategy then this offer opportunity of controlling the disease effectively (Eshuis and Manschot, 1993). Thus these factors should be considered in the study area.

Unique ecological and hydrological factors in the area contributed to the persistence of populations of mosquito vectors in the dry months to maintain infection and transmission as well. The persistence of malaria transmission throughout the year in the study area was associated with irrigation practices; the existence of permanent breeding habitats created by poor management of water supported the survival of vector species throughout the year. Rain pools in the wet months formed additional breeding sites. Muirhead-Thomson (1951) indicated that, the distribution and density of the *Anopheles* vectors are determined primarily by the availability of the suitable breeding sites, and such sites often arise through human activities enhancing a close association between man and the vectors (Service, 2000).

Of the three identified species, *An. gambiae s.l.* was found in appreciable density. The immature forms of the species invaded various breeding habitats in all months and it mainly occurred in the Megazen and Jolie study localities. Looking at the evidences from the present data, no other malaria vector is important than *An. gambiae s.l.* in the study area. It is relevant that this species is the principal vector of malaria in Ethiopia

(Tulu, 1993a; Ameneshewa, 1995; Abose *et al.*, 1998b) and elsewhere in east, West and south Africa (e.g. Gillies and Coetzee, 1987; Fontenille and Lochouran, 1999; Coetzee *et al.*, 2000).

The presence of this species in the study area during the dry months was an evidence for the uninterrupted malaria transmission throughout the year. *An. pharoensis* was also found but the density of this species was very low in the study period. Therefore this species can be considered as a secondary vector of malaria in the study area as in other parts of Ethiopia (Nigatu *et al.*, 1992; Ameneshewa, 1995; Abose *et al.*, 1998b).

The rest *An. christyi* in the area might have little or no role in the transmission of malaria. In line with this, an experiment in which more than 500 wild caught *An. tenobrosus* Donitz fed on *P. falciparum* gametocyte positive patients in Sille, south Ethiopia, Adugna *et al.*, (1998) reported infection rates of 15.8% by dissection and 7% by DNA hybridization, suggesting the need to observe the role of this species in the epidemiology of malaria. The same authors proposed similar studies to be carried on *An. christyi* (Newstead and Carter), *An. paludis* Theobald, *An. rhodesiensis* Theobald, *An. maculipalpis* Gilles and others, which exhibit anthropophilic tendencies.

The monthly variation, in the abundance of *An. gambiae* both as larval and adult forms may be associated with climatologically factors such as amount of rain, temperature and relative humidity. The larval breeding sites in the study area included rain pools, river pockets, ponds, pools of water from spillage of canals and marshes. The breeding habitats of *An. gambiae* in the study localities particularly in the dry season are due to human activities rather than environmental causes. Water accumulated from overflow of large canals, interrupted canals, marshy areas and ponds remain the most important breeding sites created by human negligence. Similarly, larval habitats of *An. gambiae* in the other parts of Ethiopia constituted sunlit rain pools, receding river beds, discarded tyres and containers, edges of lakes, side pools of rivers, marshes, swamps and irrigation ditches (White *et al.*, 1974; Jolivet, 1959; Tulu, 1993a). It also appears also to proliferate in waters polluted by urine (Jolivet, 1959). Jolivet (1959) further reported that the species breeds on the shores and side pools of the fresh waters of Lakes Zeway, Abaya and Awassa. The brackish water of Lake Awassa was known to support the co-breeding of the species with *An. pharoensis* (Jolivet, 1959). The margins of other brackish water lakes including Lake Langano and Abidjata were suggested to favors the breeding of this species when the margins are flooded with rain (Jolivet, 1959). Swamps created from the hot springs at Sodere and Gergedi are also permanent breeding sites (Jolivet, 1959; Ameneshewa, 1995).

Adult *Anophelines* were collected from resting sites. The largest catch was made on CDC light trap; the contribution of the other methods was comparatively low. The human bait method was not used, even it, it is considered as a standard method to measure human biting rate and human-vector contact (WHO, 1975; Service, 1993c). However, the appearance of drug resistant parasites in malaria endemic countries, made this method ethically unacceptable to apply it in routine entomological studies. Moreover, the method is tedious, labour intensive, expensive and unacceptable as the intervention of collectors disturbs the privacy of residents. This method is not far from bias arising from variations in human attractiveness to mosquitoes (Service, 1993c).

Some people are more attractive to vectors than others. Because of such factors, there is a growing need to replace the human bait method with other collection methods such as CDC traps, which are ethically unproblematic, cheap and demand less labor (Lines *et al.*, 1987). At the four study localities, CDC traps were more efficient than the other methods and collected mosquitoes even in low densities (Ameneshewa, 1995).The pyrethrum spray method is used to sample indoor resting mosquitoes (Service, 1993c). Some workers (Krafsur, 1977) used collections made by this method to estimate human biting rates.

An .gambiae was caught from indoor resting sites by space sprays, CDC light trap, and aspirators, but there was variation in its density between the four study sites. In general the resting density of *An. gambiae* indoors in the study area was lower, compared to other localities in Ethiopia (Fountaine *et al.*, 1961; White *et al.*, 1980; Ameneshewa and Service, 1996). Eventhough, the species was caught indoors, during the study period the most productive months were April and May, and even in these months the average density was less than 9.6 mosquitoes/house/ day in the study area. In the dry months, the density fell to zero inspite of the existence of the species as evidenced from larval and adult collections. Similarly, in Zeway, the highest density from hand capture was estimated to be 7.23 mosquitoes per hut per day. The highest density 100-150 females per house per day, in Ethiopia were documented in the malaria epidemic year of 1958 (Fountaine *et al.*, 1961). White *et al.* (1980) reported that the indoor resting *An. arabiensis* reached to 100 per hut.

Although, in the study area, the density of *An. gambiae* varied month to month. *An. gambiae* still remained dominant in abundance. The second abundant *Anopheline* was *An .pharoensis*, but it has never outnumbered *An. gambiae*, even in the dry months as it has been documented in the Zeway area (Abose *et al.*, 1998b).

An. arabiensis was not found to be deterred to enter, feed and rest in insecticide treated huts. In a similar observation at Gergedi, Ameneshewa and Service (1993c) noted this species to rest in DDT – sprayed huts avoiding surfaces of the wall sprayed with the insecticide. Insecticide sprays are said to affect the indoor resting habit of mosquitoes by increasing the rate of exophily (Pant *et al.*, 1981). Under normal circumstances, however *An. arabiensis* exhibits partial exophiliy (White, 1974). In Gambela, the proportion resting outdoors reached between 20 to 40% (Krafsur, 1977). In an area where insecticide sprays is practiced 37.5% of the population showed exophily (Ameneshwa and Service, 1996).

In line with this, in the study area fresh fed females of *An. gambiae* comprised the highest number in space spray, CDC light trap and aspirator collections in human dwellings showing the tendency of gravids to leave these resting locations and rest in outdoor shelters. Krafsur (1977) documented 45-75% of the population of *An. gambiae s.l* being comprised of fresh feds resting indoors. Similarly, Ameneshewa and Service (1996) reported the composition of these gonotrophic stages of *An. arabiensis* to be 56% in unsprayed huts and 54.4% in sprayed huts.

In the study sites, few outdoor resting sites were located. Animal shelters, worn out old houses, tree and excavated burrows represent the main outdoor resting sites in the four study localities. The vegetation could serve as one but in spite of the application of different collection methods, the yield was nil. In other areas in Ethiopia, *An. arabiensis* was shown to prefer cow sheds and ditches (Abose *et al.*, 1998b, Ameneshewa and Service 1996). *An. gambiae* demonstrated behavioral variation in its indoor and outdoor resting behaviour in the study sites.

Insecticide sprays have an excito-repellency effect on the indoor resting mosquitoes (Pant *et al.*, 1981). Mosquitoes tend to develop behavioral avoidance of human dwellings with ITNs. As the result, the densities of the *An. gambiae* in households without ITN exceed that of the households with ITNs. This could be attributed to the efficient coverage (76.8%) of ITNs at this site. Eventhough, data is not available on the density of this species before and after the distribution of ITNs in the four localities to compare with the results produced in this study, it can be speculated that the three-year interruption of ITNs has effect in the density of indoor resting population. It is obvious that in households without ITNs *An. gambiae* population out number those of *An. gambiae* in households with ITNs ($p < 0.05$) (Table 13). Similarly, Temu *et al*, (1999) indicated that, there was a major impact on the density of infected mosquitoes in the protected areas. Besides, Misra *et al*, (1999) suggested that, the use of ITNs could be integrated into any national malaria control strategy depending on the behavior patterns of the local vector species, ITNs could replace indoor spraying in many instances. In line with this, a major reduction in the vectorial capacity of the vector population is the most desirable effect of using an insecticide for malaria control. Insecticide-treated bed nets may be able to provide this as well as giving personal protection. Some studies of insecticide-treated bed nets have shown reductions in survivorship, sporozoite rate and density of the mosquito vector population at a village level (Magesa *et al.*, 1991; Snow *et al.*, 1987; MacCormack and Snow, 1986; Robert and Carnevale, 1991; Aikins and Pickering, 1994).

The physiological age of mosquitoes was determined by ovarian dissection as there is no a method of choice other than the method described in Detinova (1962). Ovarian dilation counts are made based on the number of ovipositions completed, and age of the individual mosquito is determined by the number of dilatations completed, and age of the individual mosquitoes determined by the number of dilatations as 1 – parous, 2-parous, etc. the number of ovipositons so far recorded for *An. gambiae s.l* is six (Detinova and Gillies, 11964). Faced by the difficulty in ovarian dissection, the oldest female detected in this study was 2-parous.

Parous females are epidemiologically dangerous because these are the most important to carry malaria parasites to humans. They acquire malaria infections from repeated blood meals. Parous females of *An .gambiae* in households with and without ITNs was significantly lower in household with ITNs ($p < 0.05$) (Table 15). Similarly Quinones *et al.*, showed that, the parous rate of *An. gambiae s.l.* collected in exit trap 4 months after treatment were significantly lower than in untreated villages. In line with this, Snow *et al.*, (1987) showed, significantly fewer unfed females resting in bed nets, fewer unfed females resting in treated rooms, and a higher percentage of females exiting from treated rooms measured as the ratio ETC/ETC+PSC.

In all the study sites there was not found parous *An. pharoensis,* which was similar to other observations (Mekuria, 1983; Ameneshewa, 1995; Abose *et al.*, 1998b). The lower parous ratio in this species is an indicative of the short life. Such mosquitoes are less dangerous than with longer survival rates. The sporozoite rates detected in *An. gambiae* and *An. pharoensis* in the four sites were nil; in agreement with other studies in Ethiopia (O'connor, 1967; Ameneshewa, 1995; Abose *et al.*, 1998b).

6. CONCLUSSIONS AND RECOMMENDATIONS

6.1. Conclusions

In Jolie study area, *An. gambiae s.l.* still remained dominant in abundance. The second abundant *Anopheline* was *An. pharoensis*, but it has never outnumbered *An. gambiae s.l.*, even in the dry months. The third, *An. christyi* in the area might have little or no role in the transmission of malaria. The larval breeding sites in the Jolie area included rain pools, river pockets, ponds and pools of water from spillage of canals. The breeding habitats of *An. gambiae s.l.* in the study localities particularly in the dry season are due to human activities rather than environmental causes.

Entomological studies intended to the collection of indoor resting mosquitoes collected by knock-down space spray catches [SSC]. The results of the study showed significantly fewer females resting in households with ITNS than without ITNs. The composition of parous females of *An. gambiae s.l.* in the four sites showed site variation, and the difference in households with ITNs and households without ITNs was significant. The sporozoite rates in *An. gambiae s.l.* in the four sites were nil. Although, the results of incidence were not significant, differences in malaria parasites were observed among households with and without ITNs.

1.2. Recommendations

1. Detailed further entomological studies are required on species composition, host preferences and sporozoite rate to determine the vectorial status of the *Anophelines* in this area.

2. Active involvement of the community in the control programme, social mobilization will be necessary to reduce the larval breeding sites in the Jolie area such as rain pools, river pockets, ponds and pools of water from spillage of canals which are the result of human activities rather than environmental causes.

3. In providing an enabling environment for scaling-up actions, governmental sectors need to focus on creating awareness on utilization and demand for ITNs through health information channels and mass media.

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Appendices

Appendix 1. Age distribution of malaria cases treated based on clinical and laboratory diagnosis in Meskan Woreda between 2001 and 2006.

	Age group				
Year	>1	1to4	5to14	15+	Total
2001	121	230	280	621	1252
2002	139	196	263	465	1063
2003	226	516	532	1421	2695
2004	271	980	1190	2836	5277
2005	538	845	810	1638	3831
2006	606	912	1149	3592	6259
Total	1901	3679	4224	10573	20377

Source: Meskan Woreda Health Office monthly morbidity report.

Appendix 2. Age distribution of *Plasmodium falciparum* based on laboratory diagnosis in Butajira Health Center between 2001 and 2006.

	Age group				
Year	<1	1- 4	5-14	15+	Total
2001	4	17	26	93	140
2002	23	27	46	64	160
2003	22	63	109	258	452
2004	21	112	169	482	784
2005	0	16	24	77	117
2006	17	100	116	373	606
Total	87 (3.85)	335 (14.83)	490 (21.69)	1347 (59.63)	2259 (100)

Source: Butajira Health Center monthly outpatient morbidity report.

Appendix 3. Age distributions of *Plasmodium vivax* based on laboratory diagnosis in Butajira Health Center between 2001 and 2006.

	Age group				
Year	>1	1-4	5-14	15+	Total
2001	3	42	49	81	175
2002	4	17	29	50	100
2003	5	27	51	86	169
2004	9	72	74	207	362
2005	5	30	32	70	137
2006	51	189	230	477	947
Total	77 (4.07)	377 (19.95)	465 (24.60)	971 (51.37)	1890 (100)

Source: Butajira Health Center monthly outpatient morbidity report.

Appendix 4. Age distribution of clinically treated malaria cases in Jolie health post between 2004 and 2006.

Age group

Year	<1	1-4	5-14	15+	Total
2004	4	16	47	87	154
2005	9	26	64	119	218
2006	18	57	153	192	420
Total	31 (3.91)	99 (12.5)	264 (33.33)	398 (50.25)	792 (100)

Source: Jolie Health Post annual morbidity report.

Appendix 5. Incidence of malaria by locality in Jolie study area 2007.

Localities	Number	*Plasmodium* spp	
		Pf	*Pv*
Gurambar	8	5	3
Megazen	9	5	4
Akemuja	6	4	2
Jolie	15	8	7
Total %	38 (100)	22 (57.9)	16 (42.1)

Pf= *Plasmodium falciparum* *Pv*= *Plasmodium vivax*

Appendix 6. Number of *An. gambiae s.l.* per room resting indoors collected using space spray catches in the four study localities in households with and with out ITNs from October 2006 to May 2007.

Locality	House hold with ITNs (Intervention)				Households without ITNs (control)			
	No. houses visited	No. *An. gamb*	*An. phar*	Density	No. houses visited	No. *An. gamb*	*An. phar*	Density
Akemaja	10	7	0	0.7	10	49	0	4.9
Gurambar	10	3	0	0.3	10	54	0	5.4
Megazen	10	2	0	0.2	10	38	2	4.0
Jolie	10	6	0	0.6	10	50	5	5.5
Total	40	18	0	1.8	40	191	7	19.8

Appendix 7. Parous rate *of An. gambiae s.l.* collected by space spray catches in the four study localities from October 2006 to May 2007.

Locality	No Houses	Houses			
		With ITNs		Without ITNs	
		Examined	Parous	Examined	Parous
Akemuja	10	1	0	7	6
Gurambar	10	0	0	8	6
Megazen	10	1	0	6	5
Jolie	10	1	1	12	11
Total	40	3	1	33	28

Appendix 8 The abdominal status of indoor resting *An. gambiae s.l.* based on space spray, collections in the four sites from October 2006 to May 2007.

Abdominal Status	Sites				
	Akemuja	Gurambar	Magazen	Jolie	Total
Total *An .gambiae s.l.*	56	57	40	56	209 (100)
No. unfed (%)	3	12	8	6	29 (13.9)
No. fed (%)	29	23	22	27	101 (48.3)
No. half-gravid (%)	17	17	6	16	56 (26.8)
No. gravid (%)	7	5	4	7	23 (11)

No = Refers to number

Appendix 9 The abdominal status of indoor resting *An. gambiae s.l.* based on mouth aspirators collection in four sites from October 2006 to May 2007.

Abdominal Status	Sites				
	Akemuja	Gurambar	Magazen	Jolie	Total
No. gravid (%)	2	2	2	4	10 (18.87)
No unfed (%)	1	1	2	3	7 (13.21)
No. fed (%)	4	6	7	9	26 (49.06)
No. half-gr (%)	2	2	3	3	10 (18.87)
Total flies	9	11	14	19	53 (100)

Appendix 10. The abdominal status of indoor resting *An. gambiae* based on CDC light trap collection method in four sites from February to May 2007.

Abdominal Status	Sites				
	Akemuja	Gurambar	Magazen	Jolie	Total
No unfed (%)	1	3	3	8	15 (13.76)
No. fed (%)	6	9	17	25	57 (53.39)
No. half-gr (%)	4	3	7	11	25 (22.93)
No. gravid (%)	1	2	5	4	12 (11)
Total flies	12	17	32	48	109 (100)

Appendix 11. The abdominal status of outdoor resting *An. gambiae, An. pharoensis and An. christyi* based on CDC light trap collection in four sites from February to May 2007.

Abdominal status	Sites								
	Akemuja	Guramabar	Megazen			Jolie			Total
	An.gambia	*An.gambia*	*An.gambia*	*An.phar*	*An.christyi*	*An.gambia*	*An.phar*	*An.christyi*	
Unfed	2	2	6	9	2	6	12	2	41(20.81)
Fed	12	14	20	0	0	32	0	0	78(39.6)
Half gravid	6	7	12	2	0	11	2	0	40 (20.3)
Gravid	3	3	7	6	0	10	9	0	38(19.29)
Total	23	26	45	17	2	59	23	2	197 (100)