STUDIES ON THE ANOPHELINE MOSQUITOES OF METEHARA AND SURROUNDING AREAS IN RELATION TO MALARIA TRANSMISSION

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

Table Tage

LIST OF FIGURES

ABSTRACT

The ecology and behaviour of *Anopheles* mosquitoes was studied in four selected sites in the Metehara area, Upper Awash Valley, Eastern Ethiopia. The sites represented two insecticide unsprayed villages (Metehara and Gelcha) and two sprayed villages (the Sugar Estate and Buse). Information on the prevalence of malaria cases was also gathered from the Metehara Sugar Estate Hospital and the East Shoa Malaria Control Sector (Nazareth). The results showed that a total of 24,799 microscopically diagnosed malaria cases out of 68,000 blood samples were registered between July 1999 and September 2000 in the two health service rendering organizations. *Plasmodium falciparum* and *P. vivax* were found to be responsible for the disease and occur nearly in equal proportions (52.7% *P. falciparum* and 47.3% *P. vivax).*

Larval collections from the different breeding habitats revealed the presence of four species of which *Anopheles arabiensis* was the predominant followed by. *An. pharoensis.* Similarly, a total of 3639 adult anophelines representing at least eight species were caught using different methods from July 1999 to September 2000. *An. arabiensis* was the predominant species, forming 95% of all collections followed by *An. pharoensis* (3.8%).

The density of *An. arabiensis* resting indoors in the sprayed sites was much lower than the density in the unsprayed sites. Consequently, 18%, 21.3%, 66.1% and 69.4% of *An. arabiensis* exhibited exophily in Gelcha, Metehara, Buse and the Sugar Estate, respectively.

The indoor to outdoor biting ratio of *An. arabiensis* varied between villages: 0.53 in the Sugar Estate, 1.97 in Buse, 0.75 in Gelcha and 2.12 in Metehara town, showing that the species was more endophagic in Buse and Metehara and more exophagic in Gelcha and the Sugar Estate.

The highest man biting rate of 39.5, was recorded in Metehara town and the lowest 8.4 in Buse. *An. pharoensis* was mostly an outdoor feeder.

Of 864 *An. arabiensis* and 63 *An. pharoensis* dissected from human bait collections, the average parous rate was 45.1% and 30.2%, respectively, showing *An. arabiensis* to be longer-lived than *An. pharoensis.* The sporozoite rate of *An. arabiensis* was 0.77% in parous and 0.21% in nulliparous population, the overall being 0.46%. Similarly, *An. pharoensis* had sporozoite rate of 5.3% in parous and 2.3% in nulliparous population, the overall being 3.2%. The biting rhythm of *An. arabienisis* exhibited two to three peaks of activity before and after midnight. The highest biting density occurred after midnight indoors while variation was observed outdoors.

The average daily entomological inoculation rate (EIR) of *An. arabiensis* was 0.05 while that of *An. pharoensis* was 0.01.

The Human blood index (HBI) of *An. arabiensis* revealed variation between sites/villages being 1 in Metehara, 0.47 in Gelcha, 0.85 in Buse and 0.93 in the Sugar Estate, the overall being 0.65. The HBI varied also between dwelling conditions of mosquito sampling, being highest in human dwellings (0.78) and lowest in animal shelters (0.13) showing the opportunistic feeding behaviour *of An. arabienisis.*

Clearly, *An. arabiensis* is the most important vector and *An. pharoensis* a secondaiy vector of malaria in the Metehara area.

Insecticide susceptibility studies in Metehara showed that 30% arid 25% of *An. arabiensis* was resistant to DDT and pennethrin, respectively. The level of DDT and permethrin resistance in *An. arabiensis* does not seem to be epidemiologically dangerous, but requires frequent monitoring. *An. arabiensis* was found to be highly susceptible to propoxur (carbamate insecticide).

1. INTRODUCTION

Several countries in the tropics continue to be endangered by numerous vector-borne diseases that are considered to be the major public health problems, one of the factors for poverty and population displacement, as well as an impediment to social and economic development. The mosquito-transmitted malaria is one of the leading protozoan diseases to cause immense morbidity and mortality in humans. Many operational and envisaged social, agricultural, mining, water and other economic development efforts are hindered by malaria. The burden of the disease is much felt in the productive group of society. Children, who seem to lack primary immunity, are the main victims of the disease (Trigg and Kondrachine, 1998).

The countries in Europe and America for a long time enjoy a malaria free atmosphere due to its eradication by combined measures taken against the parasites and the anopheline vectors. The advent of Dichloro-diphenyl trichloro ethane (DDT) facilitated the vector control effort (Coluzzi, 1992; De Zueleta, 1999; Toure, 1999). However, this achievement could not be repeated in tropical countries. Consequently, the eradication program dramatically failed (De Zueleta, 1999) and in the latter years, the eradication approach was substituted by control. To the dismay of many malariologists and WHO, the control program faced a similar fate to that of eradication. Now, this disease has become a public health challenge because of its increased prevalence and spread on the globe. Evolution of drug resistant parasites, insecticide resistant vectors, international travel, global wanning and several other factors contributed to the rampant spread of the disease. Like several other infectious diseases, malaria has recently resurged, and its attack is targeted to both endemic and non-endemic nations of the world (Gratz, 1999).

Current reports show that malaria is prevalent in many countries and territories of the world. About 40% of the inhabitable land mass is infested by this disease and its anopheline vectors. The annual number of cases is estimated to be 300-500 million, out of which 1-2 million die of the disease. 90% of the malaria cases and deaths occur in Africa (TDR, 1997-98). The problem of the disease in Africa is exacerbated by climatic and massive ecological changes (deforestation, dams, irrigation and different constructions), population movements to developmental schemes, poverty, poor housing and lack of efficient control strategies. These important factors have also contributed to the spread of malaria to regions where it has never been known. Favourable breeding sites for the vectors are being created as the result of climatic and ecological changes (Trigg and Kondrachine, 1998).

In this continent all ages are at risk, but the most affected group are children and pregnant women.

The control of malaria and its anopheline vectors in Africa is less successful because of the occurrence of ding resistant parasites and insecticide resistant vectors, change in the resting behaviour of mosquitoes (from endophily to exophily) as the result of frequent indoor insecticide sprays, lack of efficient infrastructure and several other factors (Toure, 1999). Africa seems to depend predominantly on chemicals and imported technology including dings, impregnated nets, insecticides and application equipment. Traditional methods if any are either neglected or are greatly influenced by the western approach of disease control.

Situated in the sub-Saharan region where the prevalence of malaria is intense, two-third of Ethiopia is known to be malarious (Gebre-Mariam, 1984; Gebre-Mariam *et al.*, 1988; Tulu, 1993a). Of the Countiy's current 61, 672,000 populations, about 65 % (40

million) are at risk of malaria infection. It is estimated that 4 to 5 million are annually affected by the scourge (Malaria and other Vector-borne Diseases Control Unit [MOVBDCU], 1999/2000 unpublished report). There is no administrative region free of the disease. In fact some regions, for example, Afar and Somali are 100% malarious (MOVBDCU, 1999/2000 unpublished report). In recent years, malaria is expanding to many regions in the lowlands as well as to the highlands where transmission used to be absent or rare. Highland malaria commonly occurs in association with climatic changes, population movement, social calamities and ecological modifications (Tulu, 1993b). In general, the problem of malaria in this country is aggravated by population movements to the lowlands associated with agricultural and agrodevelopmental projects, urban developments as well as settlement operations (Nega and Haile-Meskal, 1991).

Large state and private owned agroindustrial schemes in the Awash Valley in the eastern part of the country particularly the major sugar producing factories attract labourers from all corners of the country. Using irrigation methods, cotton, horticultural crops, maize, fiber crops and sugar cane have been produced in the last three decades for export and local consumption. Intradomiciliary sprays have been practised to control indoor resting vectors of malaria. Malaria is a number one disease in the Awash valley (Meskal *et al.*, 1979 unpublished report cited in Mekuria *et al.*, 1982). Agricultural pests are controlled with the application of insecticides using organochlorines and organophosphates. Information is lacking on the consequence of long time application of these agricultural pesticides on vectors of diseases including anophelines. It is suggested that agricultural pesticides sometimes have contributed to the development of resistance in medically important vectors (FAO, 1987 cited in Service, 1993a).

In the Awash Valley, the presence of *An. arabiensis* Patton was reported by Mekuria

et al. (1982), Lulu *et al.* (1991) and Abose *et al.* (1998a). All identifications were based on cytogenetic studies from selected localities. Apart from these limited works, there has not been detailed published entomological information despite the long time residual indoor insecticide application in accessible localities. In view of this fact, it was proposed to conduct entomological studies in selected sites in the Metehara area. This area is site to the Metehara Sugar State Factory (the biggest agroindustrial complex in the country), Lake Beseka, Awash National Park and different government and private owned horticultural farms.

1.1 Objectives of the study

I) General

To study the ecology, behaviour, vectorial capacity and other aspects of *Anopheles* species which have importance in the transmission of malaria in Metehara and surrounding areas.

II) Specific

i) To study the species composition, abundance, biting and resting behaviour of anopheline mosquitoes.

ii) To determine the man biting rates, seasonality and human blood index (HBI) of vector species

iii) To determine the sporozoite rates and entomological inoculation rates(EIR).

iv) To determine the insecticide susceptibility/resistance level of the principal vector(s).

v) To investigate the breeding habitats of anopheline mosquitoes.

2. LITRATURE REVIEW

2.1 Malaria in Ethiopia

Published information by the Italian and British scientists starting in the mid 1930's and continued to the late 1950's showed the endemicity of malaria in Ethiopia (Lega *et al.*, 1937; Corradetti, 1938; Brambilla, 1940; Melville *et al.*,1945; Covell, 1957). The disease is mainly seasonal and occasionally manifests itself as epidemic. Unstable malaria occurs in most parts of the country particularly in the highland fringes when climatic conditions are conducive for its transmission. In the epidemic situation, communities that lack protective immunity are severely attacked. Some localities also experience perennial malaria, because the environmental and climatological situations permit the continual breeding of vectors in permanent breeding sites (Gebre-Mariam *et al.,* 1988; Tulu, 1993a; MOVBDCU, 1999).

The disease in man is caused by four *Plasmodium* species namely, *Plasmodium falciparum, P. vivax, P. malariae* and *P. ovale.* About 60% of infections are borne by *P. falciparum* while 40% are of *P. vivax.* The other parasites have little or focal significance. *Plasmodium falciparum* is the cause of severe malaria and accounts for high mortality rates. In contrast, high morbidity is recorded for *P. vivax* infections (Gebre-Mariam *et al.*, 1988; Tulu, 1993a).

The distribution of malaria in Ethiopia is related to variation in altitude, topography and climate (Gebre-Mariam, 1984). It is common in areas lying below 2000 meters, but highly prevalent below 1500 meters. Areas lying at altitudes of 1500-2000 are prone to occasional malaria epidemic. Unusual climatic phenomena such as abnormal rain coinciding with warm weather are thought to contribute to the severity of the disease in highland fringes. Malaria is absent in areas above 2500 meters where the climatological

factors inhibit the survival of vector species and the development of the parasite in the mosquito. It is reasoned that the concentration of the population to the highland regions in Ethiopia is associated with the avoidance of malaria and other tropical diseases. The lowland regions have been sparsely populated until recently when population pressure, repeated drought, famine and agroindustrial developments forced people to construct settlements, engage in agriculture and look for job opportunities in these areas (Gebre-Mariam, 1984; Nega and Haile-Meskal, 1991; Tulu, 1993a). In some parts of the country the disease occurs in two peaks, at the end of the wet seasons between September and November as well as the short rains in March and April. Malaria transmission continues less intensively in the wet seasons (Nega and Haile-Meskal, 1991).

In the historic events associated with malaria, the country has been affected by periodic outbreaks of the disease. In 1953, this disease was a cause for the death of 7,000 people on the Dembiya plain, between Lake Tana and Gondar. In the same year, one fifth of the inhabitants of Kolladiba were reported to have died of malaria (Covell, 1957). Five years later in 1958, there was an epidemic in several regions affecting about 3 million people, out of which 150,000 died (Fontaine *et al.*, 1961). There were also a number of epidemic episodes between 1960 and 1998 in the different parts of the country (Mengesha *et. al.*, 1998). In the last two decades, the number of malaria cases is rising from time to time despite drug treatment and vector control activities (Nega and Haile-Maskal, 1991; Tulu 1993a). It is known that malaria epidemics in this country occurred at intervals of 5-8 years, but in recent years, the frequency has significantly increased (MOVBDCU, 1999). Regional and global events, notably drought and famine as well as climatic phenomenon such as the El Niño southern oscillations attribute to the recent malaria epidemics (Trigg and Kondrachine, 1998; Mengesha *et al.*, 1998).

In addition to these environmental and climatological phenomena, the evolution of chloroquine resistant *P. falciparum*, population movement in the agrodeveloped areas, and development activities are factors responsible for the epidemics of malaria in the country (MOVBDCU, 1999).

Population pressure in the southern regions and repeated drought and famine in the north caused in the 1980's to the resettlement of highlanders in the fertile lowland regions in the west and north. Unequipped with natural immunity, several people were highly affected by malaria (Nega and Haile-Meskal, 1991). In Gambela, the settlement and agricultural activities altered the pattern of malaria transmission in the area (Nigatu *et al.*, 1992). A study carried in the latter years of 1960 in Gambela revealed that malaria was caused mainly by *P. falciparum* and *P. malariae*. The role of *P. ovale* and *P. vivax* was small (Armstrong, 1972). But a similar study 22 years later showed *P. falciparum* and *P. vivax* to be the major causes of malaria in that region (Nigatu *et al.*, 1992). Those same factors also brought changes on the roles of the vectors.

2.2 The anopheline species in Ethiopia

Observations on the distribution of the mosquitoes of Ethiopia began at the beginning of the twentieth century by the British and Italian expatriates in a series of expeditions initially intended for other purposes. The first record was that of Theobald (1904, cited in Bevan, 1937) who described specimens collected along the banks of the Baro and Pibor rivers in the Gambela. In the first and second decade of the century, other collectors also tried to provide descriptions, but according to Giaquinto-Mira (1950), mosquitoes were mistakenly identified and the information was fragmentary. Scott (1927) collected 13 species of mosquitoes, of which 5 were members of *Anopheles* including *An.*

gambiae Giles. Similarly, Bevan (1937) reported 11 species of *Anopheles,* including *An. gambiae.* Giaquinto-Mira (1950) noted that the Mission to the Oriental Africa of the Institute of Malariology of Rome carried the first systematic study on the distribution of mosquitoes. The result of the study was published by Lega *et al.* (1937) in which a list of 13 species was given detailing their biology and locality. Other Italian workers including Corradetti and Giaquinto-Mira carried several studies between 1936 and 1941. The results of the studies were documented by Giaquinto-Mira (1950) in which a critical description, geographical distribution and vectorial capacity of 31 species and varieties of *Anopheles* belonging to Ethiopia and Eritrea were given. Covell in 1952 (cited in 1957) collected 7 *Anopheles* species from the Lake Tana region in the north. Three of these were *An. gambiae* s.l, *An. funestus* and *An. pharoensis.* Jolivet (1959) undertook entomological studies in areas in the west and north of the country (Kobbo-Chercher plain, Dembia plain, Gambela area, and etc.) and presented a description of the bionomics and distribution of anophelines. The author made observations on 31 species based on his own findings and that of other workers before him, and he also for the first time presented sporozoite rates in *An. gambiae* s.l.. From 1955-1965, USAID workers were involved in malaria studies including its vectors in selected regions in the north. These workers recorded salivary gland infections, and their findings were compiled in unpublished reports, reviewed in O'Connor (1967). 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, inspite of the addition of some morphological characteristics that increased the number of species (Gillies and Coetze, 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 *Anopheles*

mosquitoes, Mekuria (1983) listed 44 species and subspecies in Ethiopia. The cessation of Eritrea brought changes in the number of *Anopheles* mosquitoes in Ethiopia due to the reduction of two species namely *An. culicifacies adenensis* Giles and *An. erythraeus* Corradetti, which were reported only from that country. With this in recognition, hitherto 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 assesment of the available litrature 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 anophelines presently found in Ethiopia are summarized in Appendix I.

2.2.1 Malaria vectors in Ethiopia

The 42 species of *Anopheles* that are found in Ethiopia are among the world's 422 species in the genus of which 70 are recognised to have potential roles in malaria transmission, and 40 of these are recorded as putative 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 behavioural differences. Species within complexes show marked differences in resting, feeding and other behaviours. Such species are difficult to identify with the classical morphological characteristics (Service, 1993a). The *An. gambiae* complex in Africa, the *An. maculipennis* complex in Europe, the *An. farauti* complex in the Australian and neighbouring islands, for example, contain a number of sibiling species incriminated as vectors. Conversely, some of the members of these complexes have so far no role in malaria transmission (Service, 1993a).

In Africa, members of the An. *gambiae* complex and *An. funestus* are widely

distributed and are responsible for the transmission of malaria in the region (White, 1974; Gillies and Coetze, 1987; Fontenille and Lochouarn, 1999). Others such as *An. nili* Theobald, *An. moucheti* Evans and *An. pharoensis* Theobald have focal importance (Gillies and Coetze, 1987; Fontenille and Lochouarn, 1999). Several other *Anopheles* species were found with sporozoite infections, however there are no conclusive evidences to consider them as vectors.

The *An. gambiae* complex comprises seven recognized sibling species of which two are yet to be named, and a number of incipient species (Gillies and Coetze, 1987; Hunt *et al.*, 1998) which include *An. gambiae* s.s, *An. arabiensis, An. quadriannulatus* species A, *An. quadriannulatus* species B, *An. melas* Theobald, *An. merus* Dönitz and *An. bwambae* White. The first 4 are freshwater species while *An. melas* and *An. merus* are salt water breeding species. *An. bwambae* breeds in geothermal mineral waters. 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). *An. melas* in the west and *An. merus* in the east serve as vectors in some localities in the coast regions (Davidson, 1967; White, 1974). The distribution of *An. bwambae* is limited to the forest locality in Uganda (White, 1973).

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. Before the separation of *An. quadriannulatus* to the two species, its distribution was noted in countries of eastern and southern Africa namely Ethiopia, Zambia, Zimbabwe and South Africa (White, 1974). The South African species was regarded as the same species to that of Ethiopia, and both species were

considered as epidemiologically unimportant, but Coetze *et al.* (2000) have suggested further studies on the behaviour and vectorial capacity of A*n. quadriannulatus* species B. Work is also needed to elucidate 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). It exhibits endophilic behaviour resting in animal shades and mixed dwellings. This species was also reported to feed on man either indoors or outdoors in the presence of cattle (White, 1974).

Recent reports indicate that *An. arabiensis* and *An. quadriannulatus species B* are the only two species of the *An. gambiae* complex that are found in Ethiopia. *An. arabiensis* is incriminated as the principal vector in all administrative regions of Ethiopia (Jolivet, 1959; O'Connor, 1967; Krafsur, 1977; Mekuria, 1983; Gebre-Mariam, 1984; Gebre-Mariam *et al.*, 1988; Tulu, 1993a; Nigatu *et al.*, 1992; Ameneshewa, 1995; Abose *et al.*, 1998b). Apart from the *An. gambiae* complex, *An. pharoensis, An. funestus* and *An. nili* are regarded as secondary vectors of malaria in Ethiopia (Krafsur, 1970; Krafsur, 1977; Mekuria, 1983; Gebre-Mariam, 1984; Gebre-Mariam *et al.*, 1988; Tulu, 1993a; Nigatu *et al.*, 1992; 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 sympatrically with *An. arabiensis* as has been reported in Gambela (Krafsur, 1970; Krafsur, 1977; Nigatu *et al.,* 1992), Gergedi (Ameneshewa, 1995) and Zeway (Abose *et al.*, 1998b). This coexistence is much more frequent with *An. pharoensis* than with the others. *An. pharoensis* might be responsible for the transmission of malaria in the absence or low number of *An. arabiensis*, particularly in the dry seasons (Nigatu *et al.*, 1992; Ameneshewa, 1995; Abose *et al.*, 1998b).

Environmental changes brought by various human activities in Gambela are believed to have altered the vectorial importance of some species (Nigatu *et al.*, 1992). Krafsur (1970; 1977) reported *An. gambiae* s.l (= *An. arabiensis*), *An. funestus,* and *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 to be insignificant. Indoor insecticide sprays might have eliminated the indoor frequenting *An. funestus,* which is reported to be highly sensitive to such control measures (Gillies and DeMeillion, 1968). 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 practised for a long time needs further investigation. Current 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.

Studies on the distribution of the vectors show that *An. arabiensis* and *An. funestus* occur in all administrative regions while *An. nili* is reported from the regions of Amahara, Western Oromia, Gambela and Southern Nations, Nationalities and Peoples Adiministrative Region. *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 emanates from areas where there are accessible means, and hence is incomplete (Gebre-Mariam *et al.*,

1988). Shortage of communication in inaccessible areas has hindered the research on malaria and its vectors.

Studies on susceptibility of mosquitoes to malarial parasites complemented by field observations can lead to the finding of other potential vectors. In an experiment in which more than 500 wild caught *An. tenebrosus* Dönitz 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 hybridisation, suggesting the need to observe the role of this species in the epidemiology of malaria. The same authors proposed similar studies to be carried out on *An. christyi* (Newstead & Carter), *An. paludis* Theobald, *An. rhodesiensis* Theobald, *An. maculipalpis* Giles 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.

Though the existence of *An. arabiensis* and *An. quadriannulatus* species B have long been known (White *et al.*, 1980; Mekuria, *et al.*, 1982; Lulu *et al.*, 1991; Abose *et al.*, 1998a; Lulu *et al.*, 1998; Hunt *et al.*, 1998), the presence or absence of *An. gambiae* s.s. in Ethiopia cannot be ruled out in view of the limited studies in different geographical regions of the country. Moreover, the methods of identification of sibling species were so far limited to the cytogenetics, which are performed by very few experts. Currently, there are various methods at hand to circumvent the problems of identification of isomorphic species within the *An. gambiae* complex.

2.3 Identification of sibling species in the *An. gambiae* complex

Distinguishing sympatric sibling species of a complex has paramount importance in

control operations with regard to making distinctions between the vector and non-vector members. It enables the applied entomologist to save time, money, labour and material. It would be a loss to expend on those mosquitoes, which are not involved in malaria transmission (Coluzzi, 1984; Coluzzi, 1992; Service, 1993a; Coetzee *et al.*, 2000). However, there have been difficulties in identifying sibling species of *An. gambiae* complex on morphological grounds alone, even though the saline species are distinguished from their fresh water siblings by some external morphological characteristics (White, 1974). In this respect, several genetic methods have been developed.

Differences among members of the complex were initially recognized by crossing experiments (Pant *et al.*, 1981). In the early times, the distribution of four members of the complex (*An. melas*, species A, B and C) was determined from identifications made by test cross (Davidson, 1967). Crossing experiments are laborious and difficult as a diagnostic technique (Service, 1993a), however, entomological laboratories still find this techniques valuable. Recently, *An. quadriannulatus* was separated to species A and B by breeding crosses in which the two species were found to be unable to produce fertile male progenies (Hunt *et al.* 1998). In addition to morphological characteristics, salinity tolerance test was used to distinguish the salt water species from the fresh water ones (Muirhead-Thompson, 1951). Of all the available genetic methods, cytogenetic techniques based on polytene chromosomal patterns prove to be valuable, and are used widely to identify sibling species of the African vectors (Coluzzi 1968 detailed in Coluzzi *et al.*, 1979). The technique requires the preservation of fourth instar larvae and half-gravid adults in Carnoy's fluid, because polytene chromosomes are found in the salivary glands and ovarian nurse cell tissues, respectively (Coluzzi, *et al.*, 1979). Identification is possible even from slide-mounted chromosomes after a long time (White *et al.*, 1980). With the required expertise

skill and experience, this technique adequately distinguishes all members except the two siblings of *An. quadriannulatus* (Hunt *et al*, 1998). Moreover, it is used in large-scale epidemiological studies (Coluzzi, 1992). In Ethiopia, the presence of *An. arabiensis* and *An. quadriannulatus* was repeatedly confirmed by cytogenetic studies (White *et al.*, 1980; Mekuria *et al.*, 1982; Lulu *et al.*, 1991; Abose *et al.*, 1998a; Lulu *et al.*, 1998). Ameneshewa (1995) applied both cytogenetic and DNA probes to distinguish the *An. gambiae* complex at Gergedi and found only *An. arabiensis* in the area.

Biochemical methods based on enzyme electrophoresis (zymotaxonomy) are also used to make identifications of isomorphic species (Miles, 1978; 1979). The tests require specimens that are either fresh or freshly frozen material and are applicable to large-scale identifications (Coluzzi, 1992). Marchand and Mnzava (1985) have prepared a biochemical key for members of the complex.

Gas chromatographic profiles of cuticular hydrocarbons of both adult and larval stages are reported to be able differentiate sibling species (Carlson and Service, 1980; Hamilton and Service, 1983), but the techniques are complex and difficult to apply in routine activities (Collins *et al.*, 1987).

DNA and RNA probes are developed to replace or complement the other methods (e.g. Collins *et al.*, 1987; Gale and Crampton, 1987). It is suitable to undertake tests on any part of the insect body in all forms.

The most recent technique that is more frequently applied in vector studies at present is the polymerase chain reaction (PCR) (Scott *et al.*, 1993). The advantage of PCR is in that, the tests are carried on a single part of the insect body, leg or wing (Scott *et al.*, 1993) leaving the other parts of the body for other studies such as blood meal analysis, detection of insecticide resistance and identification of sporozoite infection. Though PCR

differentiates *An. quadrriannulatus* from the other members of the group (Scott *et al.*, 1993), the technique could not separate the two species of *An. quadriannulatus* (Hunt *et al.*, 1998).

2.4 Behavioural studies

2.4.1 Resting and feeding site preference of *An. arabiensis*

Adults of *Anopheles arabiensis* in Ethiopia seem to inhabit in and around human dwellings as well as in areas where human habitation is absent. Jolivet (1959) collected the larvae of this species 30 kms away from human dwellings. This species 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 behaviour depending on the availability of 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). In South Africa this species is reported from a park area about 9 kms far away from human habitation, obtaining its blood meal probably from buffaloes (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 (Fontaine *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. In a place near Jimma (Gilgil Ghibe river valley), White *et al.* (1980) found the density to vary from less than 1 in January-March to greater than 100 in July-October.

Insecticide sprays affect the feeding and resting behaviour of *An. arabiensis* either by reducing the number or altering the behaviour. 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 cited 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 Tulu (1993a) indicated 72.4% exophagy 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.

2.4.2 Night biting rhythm of *An. arabiensis*

Published information on the biting rhythm of *An. arabiensis* in Ethiopia is that of Jolivet (1959), Krafsur (1977) and Abose *et al.* (1998b). In Sodore 86.4% of the fly population was observed to be active before midnight (Jolivet, 1959). Krafsur (1977) did not find differences in the time of biting activity both indoors and outdoors. In that locality the night activity of the species begins at dawn increasing in each hour of the night. The indoor peak was between 06:00 and 07:00 hours after sunset while the outdoor peak was

between 05:00 and 06:00 hours. In Zeway, the peak outdoor biting hour was recorded between 22:00 and 24:00 hours. Indoors, the species was most active between 18:00-20:00.

2.5 Sporozoite rate in *An. arabiensis* and other 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 determined by dissecting the salivary glands and detecting parasites under the microscope. This method is noted to have several drawbacks. It 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) [Burkot *et al.*, 1984; Wirtz *et al.*, 1987], DNA hybridisation (Delves *et al.*, 1989 cited in Lulu *et al.*, 1997; Lulu *et al.*, 1997), PCR techniques (Tassanakajon *et al.*, 1993; Stoffels *et al.*, 1995; Wilson *et al.*, 1998) and PCR-ELISA (Marinete *et al.*, 2000). The advantage of these methods is largely related to the suitability and simplicity to process large specimens. The ELISA is easy to perform, utilises stable reagents, requires less expensive equipment and is relatively sensitive and specific (Bouidn *et al.*, 1988). Moreover, because of its simplicity to handle large samples, it enables to obtain monthly sporozoite index as well as entomological inoculation raty (EIR) (Shililu *et al.*, 1998). The technique sufficiently detects circumsprozoite proteins (CS) produced from as few as 100 sporozoites. Since the ELISA is designed to analyse circumsporozoite proteins (CS) synthesised predominantly by sporozoites in the salivary glands, the head and/or thorax samples are used in the tests (Burkot *et al.*, 1984). However, this technique does not distinguish mosquitoes with active parasites from those with past

infections. As a result of this, sporozoite rates determined by ELISA are considered higher than the actual infection rate in the vector population (Lombardi *et al.*, 1987). In addition, false positive results have been recorded in swine and bovine blood engorged mosquitoes (Somboon *et al.*, 1993). Such limitations are circumvented by the combined techniques of ELISA and PCR. The same material can be used by both techniques, and the results obtained by ELISA can be adequately confirmed by PCR (Marinete *et al.*, 2000).

In Ethiopia, the work to determine sporozoite rates in vectors was largely dependent on the dissection method. However, attempts have also been made to use the ELISA technique in recent studies (Nigatu *et al.*, 1992; Ameneshewa, 1995; Abose *et al.*, 1998b).In addition, Lulu *et al.* (1997) investigated the potential application of DNA hybridisation to detect sporozoite infections in *An. arabiensis* in Ethiopia.

Studies describing sporozoite rates of *An. arabiensis* and other anophelines in Ethiopia is summarised in Appendix II. Most of the reports were made in the last three decades and rates 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 other secondary vectors namely, *An. funestus, An. nili* and *An. pharoensis* were reported to be 1.23-1.4%, 1.29% and 0-0.46%, respectively.

2.6 Entomological inoculation rate (EIR) of *An. arabiensis* and other vectors of malaria

Entomological inoculation rate, expressed as the number of infective bites a person receives per unit time is 'a comprehensive indicator of malaria transmission level' (Onori and Grab, 1980). In the EIR estimates the most important components are the man biting rate (MBR) and the sporozoite rate (Hay *et al.*, 2000). However, there is no general

agreement as to how EIR estimates can be made (Githeko *et al.*, 1996a). The sporozoite index is obtained from entomological observations carried at least for the transmission season or for a year (Hay *et al.*, 2000). The choice of collection method to derive the MBR seems to be left for the mosquito entomologist, eventhough, the human bait collections are indicated to directly measure the man biting rate (WHO, 1975). For example, Krafsur (1977), Babiker *et al.* (1997), and Shililu *et al.* (1998) used the space spray catches to measure the man biting rate. In some occasions light-trap catches were found valuable (Lines *et al.*, 1991; Babiker *et al.*, 1997). In the case of space spray catches, EIR estimates were performed by dividing the number of engorged females by the number of occupants and multiplying the resultant by the HBI and sporozoite rates (Krafsur, 1977; Shililu *et al.* 1998). Conversely, Babiker *et al.* (1997) omitted HBI values for the estimates. Krafsur (1970) used index of bites per man per night to calculate the EIR of *An. nili,* with respect to the exophilic behaviour of the species.

Recently Hay *et al.* (2000) presented a review on the annual *P. falciparum* EIR rates that were published for members of the *An. gambiae* complex in different countries in Africa from calculations based on different methods of mosquito collection and sporozoite detection. The review showed substantial cross-continent variation in EIR and revealed the highly heterogeneous malaria exposure risk throughtout the continent.

In Ethiopia, Krafsur (1970; 1977) was perhaps the first to estimate EIR of the three malaria vectors in Gambela town and the nearby villages. The estimates for *An. gambiae* s.l (presumably *An. arabiensis*) and *An. funestus* were made on monthly basis between July 1967 and December 1968. Because of the low number of *An. nili* dissected in that period, only average values for each year were given. A man living in Gambela town was calculated to receive 0-3.6 infective bites from *An. arabiensis,* 0-1.4 from *An. funestus* and

an average of 1.3-2.0 from *An. nili*. In the villages close to the Gambela River, the EIR of the three species was much higher, being 0-3.9, 0-29 and 20.6-32, respectively.

2.7 Host preference studies and human blood index (HBI) of *An. arabiensis*

In understanding the epidemiology of malaria, knowledge on the host preference of *Anopheles* mosquitoes is practically essential to identify the potential vector and promote the right control measure at the right time. It is necessary to distinguish the host preference of the species as well as the proportion of antropophagic and zoophagic in the wild population. It is thus important to determine the blood meal of these species. The human blood index (HBI) is used to show the proportion of mosquitoes, which feed on humans (Garrett-Johns, 1964). The HBI is determined by the collection and identification of blood meals using various methods. These techniques have undergone significant progress over time in simplicity, cost, sensitivity and specificity. Almost all the methods are, however, based on immunological (serological) techniques (Pant *et al.*, 1987). The oldest were precipitin tests with various modifications (reviewed by Pant *et al.*, 1987) and were largely in use before the early 1980's. One of the techniques, the precipitin ring test was found to be less sensitive and less specific even with the application of monoclonal antibodies (Pant *et al.*, 1987). As a result, this method is no more popular. There are other methods such as haemagglutination assays, latex agglutination test, complement fixation test, immunoflorescence techniques, counter current immunoelectrophoresis (CCIE), double immuno-diffusion (double-gel diffusion) techniques and enzyme linked immunosorbent assays. The advantages and disadvantages of each method are outlined by Pant *et al.* (1987). Of all these methods, the enzyme linked immunosorbent assay (ELISA) is now widely used to identify blood meals in tsetse, mosquitoes and other haematophagus species. This method is highly sensitive and specific and also requires less

expensive equipment and reagents. The CCIE is also in use to detect small and mixed blood meals in sandflies (e.g. Morsy *et al.*, 1993; Mamo, 1998). Though expensive electrophoretic equipment is needed, the technique requires less amount of material and is also easy to run the tests under laboratory conditions ((Pant *et al.*, 1987; Morsy *et al.*, 1993). The double immuno-diffusion method is much more easier to run sample tests as it requires few chemicals and equipment. Moreover, it is less expensive and allows testing large numbers of blood meals (Pant *et al.*, 1987).

Before the development and use of these recent techniques, information on mosquito blood meals in Ethiopia was derived from precipitin tests. The earliest observation on the *An. gambiae* s.l was perhaps that of Corradetti (1938) who recorded HBI of about 0.57. In mixed dwellings of unspecified localities, the proportion of *An. gambiae* s.l fed on humans was higher than those fed on cattle (Jolivet, 1959). Krafsur (1970) found the HBI of this species to be 100% in Gambela where there were no cattle. White *et al.* (1980) reported similar HBI in *An. gambiae* s.l caught from human dwellings near Jimma, but the average HBI in mixed dwellings was 46%. In another observation from mixed dwellings at Bako, Turner (1972) reported that, of the 144 tested blood meals of *An. gambiae* s.l, none was found fed on humans alone. Adugna and Petros (1996) observed an average 88% HBI in blood meal tests by ELISA from collections in mixed dwellings made in Arbaminch, Awassa, Metahara and Ziway. However, using the same method, Hadis *et al.* (1997) noted that in *An. arabiensis* trapped from Ledi, Alibeti, Sille, Erbore, Itang and Jawe, the HBI was 20.2% in mixed dwellings. Host preference of this species, therefore, seems to depend on the availability and density of the host population, whether it is human, cattle or both.

Genetic polymorphism of mosquitoes with variable host choice has been under investigation. Two chromosomal inversion polymorphisms of *An. arabiensis* have been found

to be associated with variations in host choice (Lulu *et al.,* 1998). In this investigation, it was found that mosquitoes with 3Ra inversion were prone to feed on cattle as 2Rb on humans.

2.8 Insecticide susceptibility/resistance of *An. arabiensis*

The intradomicillary application of residual insecticides for a long time had induced the development of insecticide resistance. Furthermore, agricultural pesticides are known to contribute to the rise of a number of resistant vectors (FAO, 1987 cited in Service, 1993a). The WHO (1970) test kits are usually utilised to constantly monitor or detect resistance in mosquitoes. The results produced from these tests provide crude information on the level of resistance in a certain species of mosquito to insecticides. Alternatively, biochemical, immunological and DNA probe methods for the detection of resistance have been developed (Brogdon, 1989).

Molecular tools have been recently developed to detect genes that confer resistance in the *Anopheles gambiae* s.s (Martinez-Tores *et al.*, 1998) and a gene is already characterised which is responsible for pyrethroid knockdown resistance (*kdr*). Work is in progress to investigate the existence of *kdr* in *An. arabiensis* (Dr. Carlos Costantini, Istituto di Parasitologia, Italy, personal communication).

In Ethiopia, DDT has been in use for more than 40 years to control indoor resting anophelines (Abose *et al.*, 1998b). Its long-lasting residual property and relatively low cost made this insecticide preferable to use for such a long time in this country. Although DDT and other insecticides have been in use for a long time, only few studies have so far been made to determine the insecticde susceptibility/resistance levels of *An. arabiensis* in Ethiopia. Abose *et al.* (1998b) compiled 16 susceptibility tests carried out in 13 localities between 1986 and 1995 by MOVBDU and others (Nigatu *et al.*, 1994; Ameneshewa, 1995).

Higher levels of DDT resistance of epidemiological significance were revealed in ArbaMinch (60%) and Gambela (76%). Moderate levels of DDT resistance were recorded in Nazareth (33%), Ziway (30%), and Enda-Selassie (23%). In other localities resistance to DDT was very low. In recent years, malathion is being used in localities where DDT resistance have been reported (Abose *et al.*, 1998b). At Gergedi, Ameneshewa (1995) noted 100% mortality in *An. arabiensis* tested against malathion.

To date, there are no reports on susceptibility/resistance to carbametes (e.g. propoxur) and pyretrhroids including permethrin and deltamethrin.

There are only two reports that indicate the status of susceptibility/resistance of secondary vectors to insecticides. *An. funestus* tested against DDT and Dieldrin in Gambela in 1959 was found to be highly susceptible to both insecticides (Jolivet, 1959). In Ziway, *An. pharoensis* was reported to be highly resistant (53%) to DDT but 100% susceptible to malathion (Wezam and Seulu, 1994).

3. MATERIALS AND METHODS

3.1 Description of the study area

The study area, Metehara, is situated in the Upper Awash Valley at latitudes 8^0.45'-8^0 .55' and longitudes of 39.50-40.00^0 E in the eastern part of Ethiopia. It is found at an average altitude of 1000 m. Its distance from Addis Ababa is about 200 kilometres. Metehara is one of the many fertile lowland areas in the country. According to the 1994 population and housing census the population in the district was estimated to be 60,048 (Central Statistical Authority, 1994) and is comprised of Oromos as well as various ethnic groups. The villages are inhabited by the Oromos (Kereyu) who mainly depend on cattle and camel keeping. The Itus (Oromos) and Somals who migrated from the lowlands of Harar also live in the villages with the Kereyus. Beside cattle and camel raising, Itus are engaged in cultivating maize and cash vegetables such as onion, tomato, capsicum, potato and cabbage. The population residing in the two urban localities, Metehara and Addis Ketema (Debre Selam) are members of different ethnic groups involved in civil service and other economic activities. Labourers from the different corners of the country inhabit the Metehara Sugar Estate. Metehara town is located on the main road and railway that extend to the ports of Assab and Djibouti

The vegetation in Metehara is mainly composed of sparsely distributed *Acacia* woodlands. Along the bank of the Awash River, *Acacia* and big trees such as *Ficus* form patchy forests. Large irrigated orchard and sugarcane farms as well as non-indigenous trees and shrubs have modified the ecology of the Sugar Estate.

Water from the Awash River has made it possible to irrigate and develop a number of large and small-scale farms owned by governmental and private enterprises. One of the

largest agro-industry developments in the area is the Metehara Sugar Factory known to be Ethiopia's largest sugar estate.

The Metehara area is home to Lake Beseka (known also as Lake Metehara). Beseka is a salt-water lake, but there are conflicting reports about the level of its salinity. Kebede *et al.* (1994) noted that the saline content has decreased 10 times in the last 30 years reaching to 5.3 g l^{-1} in 1991. According to Hammer's definition of salinity classes (1986 cited in Kebede and Willen, 1998) the lake is, therefore, in the category of hyposaline (3-20 g l^{-1}). Recent measurement (October, 1999) taken by myself using a salinity metre on the northern tip (on the side of the Metehara town) revealed a salinity level of 3 g l^{-1}. These two values show that the lake is being constantly diluted. In another unpublished study (Abejehu, 1995; Abejehu and Dilsebo, 1998) the lake was found to contain high amounts of sodium and bicarbonate ions demonstrating the high salinity of the Lake in the southern and eastern sides.

The present entomological studies covered the area that extends from Metehara town in the north, to the camps of the Sugar Estate in the south. The west limit is Lake Beseka, while in the east is the village Gelcha (Fig.1). For the purpose of sampling mosquitoes, initially two sites were selected based on the presence or absence of insecticide sprays. The two sites were the villages Gelcha and Buse, where the former has been withdrawn from insecticide spray operation program for four years between 1996 and 1999. The persistent refusal of the inhabitants, claiming that DDT exacerbates the problem of domestic pests has been a cause for the withdrawal of insecticide sprays. Buse has been included in the annual operation despite limited coverage (Dereje Olana, personal communication, East Shoa Malaria Control Sector). Two other sites, Metehara town and the Sugar Estate were included making the total sites four. The two sites were added because

sampling of mosquitoes was deemed necessary from urban and agro-industrial environments, respectively. The houses in Metehara town are excluded from the national spray operation program. Almost all the houses in the Sugar Estate are under spray two times a year at intervals of 6 months, formerly with DDT and in the last two years with malathion. DDT was last utilized in July-August 2000. DDT was replaced by malathion because of refusal of inhabitants who claim that the insecticide has lost its effectiveness against mosquitoes (Alemu Berihun, personal communication, Metehara Sugar Factory Hospital). Different agricultural pesticides are also applied in the farms of the Sugar Estate and the privately owned farms in Gelcha and Buse.

Gelcha is a large village with several huts grouped in small patches. It adjoins in the east partially and south with the sugarcane farms and the North Camp (labourers' residential camp), Addis Ketema in the west and the railroad in the north.

Buse is located in the premises of the Sugar Estate. The sugar farms in the east and north, Awash River in the west and Aliyu and Godo villages in the south are the boundaries of Buse. Grouped in six patches of huts, it retained its name from the oromo word 'busaa', which literally means 'malaria'.

The tukuls in both villages are similar in construction. There are circular and dome-shaped types made from wood and mud; but some tukuls are not plastered with mud. The construction material for the roofs are, either grass (Cyperaceae: *Cyprus* sp.) or sugar cane leaves or mud. Each hut has a 1-meter high door but no window. Instead, each hut has a small opening usually in the bedroom to allow the entrance of light in the morning.

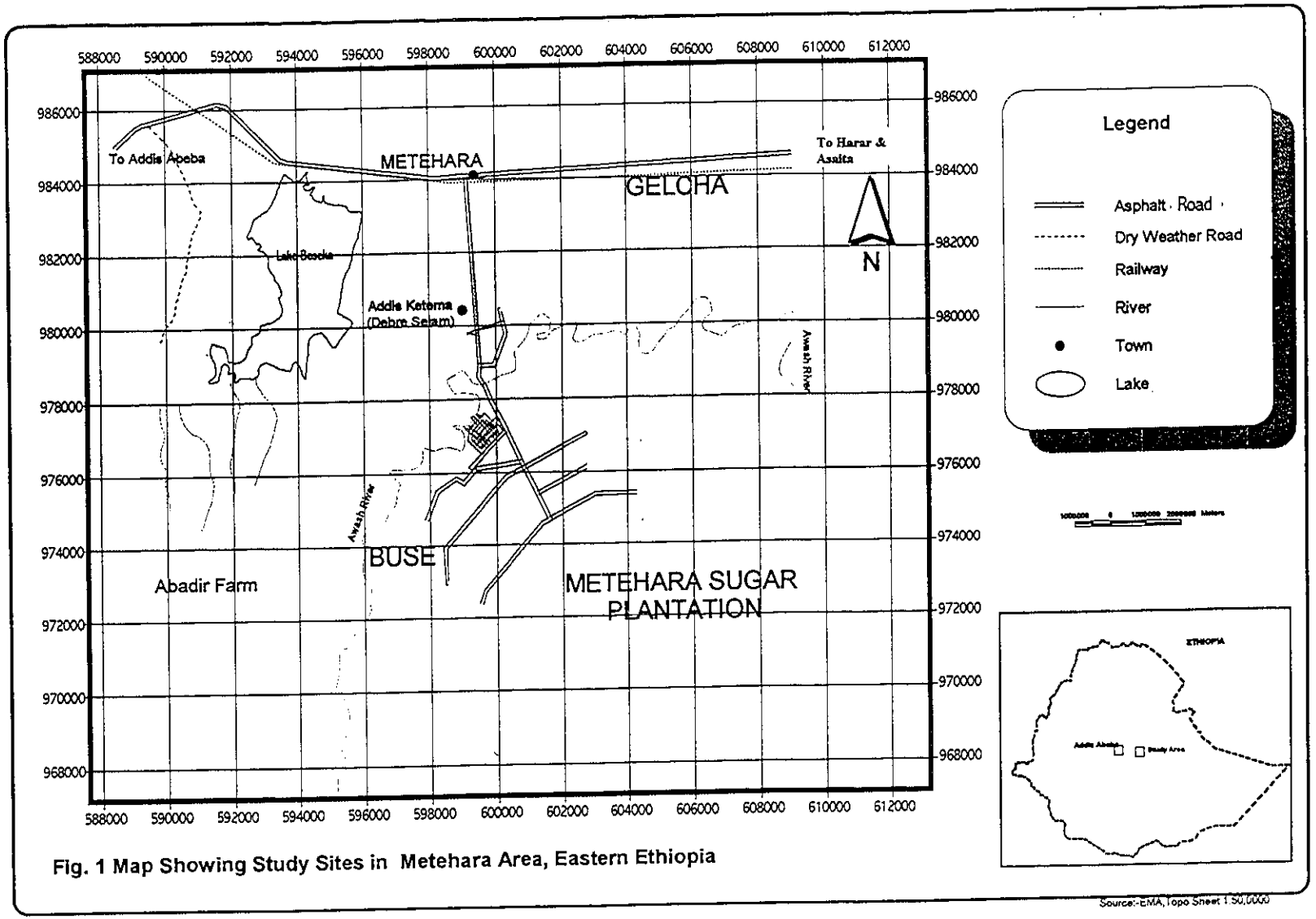

Fig. 1 Map Showing Study Sites in Metehara Area, Eastern Ethiopia

The openings are closed in the evenings with a piece(s) of cloth. In some huts, a small room for small goats is included. Mixed dwellings with either goats or calves are not uncommon.

Cattle are sheltered in open circular shades made of thorny branches of *Acacia* very near to the huts of the owners. There are also rectangular shelters in which the walls and roofs are constructed from wood and grass. Small shelters are either attached to the main hut or separately built for small goats and chicken in cases when the huts could not accommodate humans and animals. Partially destroyed huts also serve as shelters for goats and calves.

The Sugar Estate owns a total of 10 residential camps situated not far apart from each other. These are the Main Camp (Awash Camp), North Camp, Birtukan Sefer, Genet Sefer, Chore, Kikan (Third Camp), First, Second, Third and Fourth Abadir Camps. Wood and mud, and brick form the construction material of houses in the camps. Cattle are kept in animal shelters constructed far away from human habitations. During the rainy seasons small vehicles as well as tractors leave impressions where pools of water are formed in which mosquitoes breed. Pools of water are also created by accumulation of floods in the low-lying sites. Broken pipes also contribute for the breeding of mosquitoes. The sewage from Genet Sefer partially goes through an open ditch to drain into the Awash, but where there is a blockage, the sewage accumulates in the ditch, and it also spills to the surrounding land. The sewage diluted by water from another irrigation canal serves as breeding site for the indomitable anophelines. Water spillages from big canals on ground as well as stagnant water left in canals are other favourable breeding sites.

3.2 Meteorological data

As in most parts of Ethiopia, the area has two major seasons; the rainy season starts in June and lasts in mid September. The dry season is between October and March, which then followed by the short rains in mid-March and April. Meteorological data obtained from the National Meteorological Services Agency for three years between 1997 and 1999 revealed that the area received an annual rainfall amounting to 473.5, 495.1 and 494.5 mm, respectively. The respective average temperature was 25.9, 25.8 and 25.3°C. Similarly, the relative humidity was recorded as 57.8, 59 and 53%, respectively.

The monthly rainfall, average temperature and relative humidity during the study period are shown in Appendix III. The main rainy season was between June and September. Small rains also occurred in April and May. The area received a total of 788.8 mm rain in the study period. The average monthly temperature ranged between 21.3^{0}C in December to 28.7^{0}C in June and the relative humidity was between 43.2% (March 2000) and 65.5% (July 2000).

3.3 Gathering information on malaria case distribution and prevalence of malaria parasites

Relevant information on the average monthly malaria case distribution and prevalence of malaria parasites in the study area for the study period has been gathered from the Metehara Sugar Factory Hospital and the East Shoa Malaria Control Sector (Nazareth) to show malarial infection and the species of *Plasmodium* responsible for the cause of malaria in Metehara. These centres provide relatively efficient parasitological diagnostic service through the conventional microscopy method. The Metehara Health center also

treats malaria cases on clinical grounds. Thus, the data from this center and a number of private clinics in Metehara town and Addis Ketema was not included.

3.4 Mosquito surveys

Mosquito surveys of both immature and adult forms were made in the selected sites/localities using various methods as described below.

3.4.1 Collection of immatures

Mosquito larvae and pupae were collected using appropriate scoops, pipettes and containers from pools of temporary and permanent breeding habitats from all sites. The breeding sites included pools of rain water, water from broken pipes, washing water accumulated in ditches, stagnant water in irrigation canals, stagnant water in Lake Beseka, marshy areas, shore of Lake Beseka as well as small pools created nearby the lake.

To identify the species, two methods were employed. One of the methods was to rear adults in a cage both in the field and in the laboratory of the Institute of Pathobiology from immature stages (larvae and pupae) collected in the field. Identifications of the adults was made under a stereoscopic microscope with a magnification power of 20x and 40x using keys of Verrone (1962a) and Gillies and Coetzee (1987). Identifications were also made with fourth instar larvae. The larvae were killed in hot water (about 60^0C) and preserved in small vials containing 70% ethyl alcohol (Service, 1993b) and transported to Addis Ababa. The preserved materials were soaked for 12 hours in watch glasses containing distilled water. 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 Institute 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 are 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 and well-screwed bottle. Identification of larvae to the species was based on Verrone (1962b) and Gillies and Coetzee (1987) under a compound microscope.

3.4.2 Collection of adults

Adult mosquito collections were accomplished using a number of conventional methods including indoor and outdoor man biting/landing collection, CDC light traps, mouth aspirators and space sprays from tukuls, brick houses, animal shelters and outdoor sites (WHO, 1992; Service, 1993c) from July 1999 to March 2000 and July 2000 to September 2000. Attempts were also made to make collections of mosquitoes resting on vegetation with sweep net and drop net.

3.4.2.1 Night biting collections

Night biting mosquito collections were carried out indoors and outdoors in all sites to identify the anthropophilic species and their hourly biting cycles as well as to determine the rate of endophagy and exophagy (Service, 1993c) from insecticide sprayed and unsprayed human dwellings. Captures of mosquitoes by human biting method were done in Buse and Gelcha between July 1999 and March 2000, and in Metehara town and the Sugar Estate at two periods between October 1999 and March 2000 and July 2000 and September 2000.

In each month between July 1999 and October 1999 mosquitoes in Buse and Gelcha were caught for 2 nights at each site. But, in the following months, collections were made for a night only. A pair of volunteer human baits who were positioned indoor and outdoor for the first half of the night and replaced by a similar number for the rest of the night served to catch man biting mosquitoes. In the town and Sugar Estate, the indoor and outdoor collections were performed by single human bait at each site who was replaced by another one for the second half of the night. Naked parts of the leg and arm were exposed to attract mosquitoes. The collectors changed places between indoor and outdoor at the end of each collection hour to minimise differences in their attractiveness to mosquitoes (Service, 1993c). The collection period was from 18:30 to 06:00. The human baits were trained, to minimise variations in individual efficiency. The collectors were put on chloroquine prophylaxsis starting a week before the commencement of collection and thereafter.

Anophelines attempting to feed on human baits were captured using torches and test tubes. Hourly catches were kept separately in labelled paper cups and provided with 20% sugar solution. In the laboratory, mosquitoes were identified to the species and grouped according to abdominal content as unfed, fresh fed half gravid and gravid. The unfed females were dissected (see 3.5 below) while the rest were discarded.

The monthly man biting rate (MBR) which is the number of mosquito bites per person per night, was estimated as the number of mosquitoes captured on human bait divided by the number of person-nights during the month. MBR was determined for both indoor and outdoor collections.

3.4.2.2 CDC light trap collections

CDC traps were positioned indoors, outdoors and in animal shelters of all the sites.

However the number of traps and nights varied in view of the transmission season, site condition and abundance of anophelines. As the result, in the first five months (July-November) 4 traps (2 indoors and 2 outdoors) on each night for two nights in each month were placed in Buse and Gelcha. But for the last four months the number of collection nights was one and the number of traps were reduced to 2 indoors and 1 outdoors. In Metehara and the Sugar Estate, 2 CDC traps were set indoors and 1 outdoors for a night in each month between October and March while the number of traps was raised to 2 at each site during the wet months in 2000.

Females in light trap collections were also grouped as unfed, fed, half-gravid and gravid and their number was recorded. The unfed females were dissected and the freshly fed females were sorted out for blood meal analysis (see 3.7 below). The rest of the females with different physiological status were discarded.

The average number of mosquitoes per CDC light trap was estimated from geometric mean transformations ($\log_{10}$ (x+1)).

3.4.2.3 Aspirator collections

Mouth-operated aspirator was used to make catches of indoor resting mosquitoes in a randomly selected five tukuls each month from walls, roofs, clothes hanging material and household utensils as well as outdoor resting anophelines in tree holes and ditches (Service, 1993c) from Buse, Gelcha and the Sugar Estate. No attempt was made to use this method in the town. As in all collections, females from aspirator collections were categorized according to their abdominal status. Fresh fed females were sorted out for later blood meal analysis (see 3.7 below), the unfeds were dissected (see 3.5 below) while the half-gravids and gravids were discarded.

3.4.2.4 Space spray collections

The space spray method as described by Service (1993c) was employed to sample indoor resting anophelines. Sampling was performed in Buse and Gelcha for a total of 8 months (August 1999–March 2000), in the Sugar Estate for a similar 9 months (October 1999-March 2000 and July -September 2000) and in the town for 3 months only (July-September 2000).

Each month anophelines were collected from a randomly selected five dwellings early in the morning from 06:00 to 08:00 with the application of Mobil® - an aerosol containing 0.2% tetramethrin, 0.12% phenothrin and 0.25% allethrin. In Buse, Gelcha and Metehara and partly in the Sugar Estate the whole of the tukuls/houses were investigated. Because of the presence of large rooms in brick houses, in the Sugar Estate, mosquitoes were collected from the bedrooms only. After permission was granted from the inhabitants, all occupants, animals if any, removable objects, exposed food and water were removed from the dwellings. Doors and windows were closed; openings and eaves were properly covered. The entire floor was covered with white cloth sheets cut to sizes of 1m x 2 m, and all household items were underneath. The insecticide was sprayed for about 3-5 minutes on walls and roofs. After the spray, the operator (self) waited outside and the tukul remained closed for about 15 minutes to produce a knockdown effect. After 15 minutes, all knocked down mosquitoes were collected from the white cloth sheets still in place using torch, forceps and test tubes. In the laboratory, the mosquitoes were first separated into males and females and then identified to the species. The females were categorised according to the abdominal status and the freshly fed were again sorted out for later blood meal analysis (see 3.7 below). The half-gravids and gravids were also discarded.

The mean number of anophelines per hut per day was estimated by geometric mean after transforming the counts by ($\log_{10}$ (x+1)). Compared to the other averages, geometric mean is more reliable to estimate densities of mosquitoes, which have uneven distribution.

The degree of exophily of the principal vector was estimated as follows according to the method suggested by Krafsur (1971):

Degree of exophily = [1-(1/ratio of fresh fed to gravid females)] x 100.

3.5 Anopheline dissections

Unfed female anophelines caught by the methods of man biting and light traps were dissected to determine parity and sporozoite infection rates (Service, 1993c). Parous flies are those that have at least fed and oviposited once. So, parous mosquitoes in blood sucking insects are epidemiologically the most important 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 cited 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 nulliparous 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 oviposition of eggs (Detinova, 1962). The dilatation technique helps to identify the number of batches of eggs laid, and hence the female can be referred to as

1-parous, 2-parous etc.

The female anophelines were first identified to respective species before the act of dissection commences according to Service (1993c). The individual mosquito in a test tube was immobilised with either ethyl acetate or chloroform depending on availability of the chemicals. 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 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 and the gut for oocyst infection 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 coxa. Covered with a piece of cover slip, the salivary glands were observed for the presence or absence of sporozoites under 10x and 40x of object of the compound microscope. Infected salivary glands were fixed with methanol, stained with Giemsa and safely placed in a slide box.

3.6 Blood meal collection and identification

To determine the Human Blood Index (HBI) of the major vector in the study area, blood meals were collected from the various collection methods described above. Antisera

against human and bovine sera were raised in rabbits and the blood meals were tested by the double immuno-diffusion described by Hornbeck (1991) but slightly modified (Prof. Morten Harboe, AHRI, personal communication).

3.6.1 Blood meal collections

Freshly fed mosquitoes were sorted out from mosquitoes captured by aspirator, CDC light traps and space sprays from the inside of human and mixed dwellings and animal shelters. After identification of the mosquitoes, each blood meal was separately squashed with the handle of dissecting needle and a piece of cover slip on a divided and numbered Whatman No. 1 filter paper. After drying in the air, the filter papers were separately sealed in polythene bags and kept at 4^0C for a few days at the Metehara Hospital and later transferred to -20^0C in the laboratory of the IPB.

3.6.2 Production of antisera

Antisera against bovine and human sera were produced in 3 and 2 rabbits, respectively in the Institute of Pathobiology as described in Harlow and Lane (1988). Bovine serum was obtained from Addis Ababa Abattoir while human serum was drawn from volunteers. Initially, each rabbit was immunized with 1 ml of a mixture of equal amount of serum and Freund's complete adjuvant (*Sigma*). Thereafter, at intervals of two weeks a maximum of 5 booster doses were administered, each dose containing the above amount but this time the complete adjuvant was replaced by incomplete adjuvant. Before immunization and each booster, 0.5-1 ml antiserum was collected from blood drawn from the blood vessels in the ears or directly from the heart with 5 ml syringe and 20 gauge x 1.1/2" needle. The collected antisera were placed in 1.5 ml Eppendorff tubes and then kept

in -20^{0}C.

3.6.3 Testing the blood meals

To standardize and also determine the titer qualitatively, four tests, two each for human serum and two for cattle serum in replicates were carried out (Appendix IV). At one test, the human serum was tested against anti-human and anti-cattle by diluting the antiserum with saline (Appendix IV 2Ai). Correspondingly, the human serum was tested against the anti-human by absorbing the cross reacting antibodies and similarly by testing against diluted human serum as well as diluted cattle serum (Appendix IV 2A ii). In another test, cattle serum was tested against anti-cattle in two ways as described above for testing the human serum with its homologous and heterologous antiserum (Appendix IV 2B).

The test procedure was as follows. Where the human or cattle serum was tested against its corresponding homologous and hetrologous antiserum by the dilution method, the antiserum was diluted with saline (1:1 dilution). For the test, each slide was plated by 2% agar (BDH) and allowed to solidify. The slides were stored in a refrigerator at 4^0 C up to 4 days or were used on the same day of preparation. Wells on the slide agar were made using a puncher (with 3 mm diameter) and each slide was kept in the open for 30 minutes to dry out the fluid from the wells. In the dilution method, 5 µl of a 1:50 diluted serum was pipetted in two vertical punched wells at a distance of 4 mm between the upper and lower well, and a similar amount of physiological saline was added in opposite (horizontal) wells (Apx IV 2Ai and 2Bi). The distance between the wells containing the serum and the saline was 2 mm. After the saline was dried, 5 µl each of the homologous and heterologous antiserum were added to the first and second wells, respectively. Here, precipitin lines are expected to be formed on both reactions due to cross reacting antibodies. On another slide,

the same diluted serum at the same amount was pipetted in three wells and the same serum at the same dilution and amount was added in the first opposite well, a similar amount and dilution of a different serum in the second well, while the third well was filled with the same amount of saline (control). After the contents were dried, 5 µl of the homologous antiserum was added. The expected results are formation of precipitin lines only in the last two reactions. The middle is formed because of the absorption of non-specific antibodies. Both slides were then kept for 20-24 hours in a chamber made of petri-dishes where humidity was maintained with wet filter papers placed at the bottom of the petri-dishes. The slides were raised from the surface of the petri-dishes with small sticks. Slides were observed for the formation of white arcs on the agar gel against a light background. Where there were no such arcs, the test result was negative. A test sample either positive or negative by both methods was sufficient to accept the result as satisfactory.

Before tests were carried on the blood meals of mosquitoes collected from the field, the method was further standardized with known mosquito blood meals fed on human blood, rabbit and pig.

Each blood meal was eluted in 125 µl saline in Eppendorf tube for a night at 4 °C and kept at -20 ° C. Each meal was tested four times against the anti-sera of human and cattle by both dilution and absorption methods (Apx IV 2C). Each of 5 µl of the elute was added in four wells, two on one slide and two on another slide to test it against anti-human and anti-cattle sera and the slides were labelled accordingly. Where the dilution method was employed, 5 µl of each saline was pipetted in two opposite wells and allowed to dry for a few minutes on the open. Then in the well where the test is to be done against human antiserum, a similar amount of antiserum was added. Similarly, the same amount of cattle antiserum was added into the well on a separate slide left to test the blood meal against

cattle antiserum. In the absorption method, the opposite of the rest two wells were filled with the same amount of 1:50 diluted serum, which was heterologous to the test antiserum. In other words, if the blood meal was tested against anti-human, the antiserum was absorbed by diluted cattle serum and the vice versa. Finally, slides in petri-dishes were kept as described above and test results were observed. A blood sample positive by the dilution method and negative in the absorption was repeated by increasing the amount of the blood meal and anti-serum by two fold. This was done to increase the sensitivity of the method. Each day about 50 blood samples were tested, each slide accommodating 5 samples on a total 20 wells.

3.7 Insecticide susceptibility tests

To evaluate the level of resistance to selected insecticides in populations of the principal vector, susceptibility tests were undertaken in the field. The field situation was presumed favourable to generate reliable data. The tests were conducted according to the methods cited in WHO (1970; 1998).

3.7.1 Mosquito source

Insecticide susceptibility tests were performed twice in the field during December 1999 and January 2000 on populations of the major vector originated from the Metehara Sugar Estate. Emphasis was given to this site because of continued indoor sprays with DDT and malathion as well as utilization of agricultural pesticides for several years (Alemu Berihun, Metehara Sugar Factory Hospital, personal communication).

For the tests, adults were reared from fourth instar larvae and pupae scooped from

pools of water created from canal spillage and broken pipe. The pupae were separated, pipetted into a cup and then placed in a mosquito cage to allow the emergence of adults while the larvae were placed in a breeding tray to transform to pupae. Tests were conducted using one to two days old, blood unfed females (WHO, 1998).

3.7.2 The tests: techniques and procedures

Three insecticides namely DDT, propoxur and permethrin at discriminating doses of 4, 0.1 and 0.75%, respectively were used (WHO, 1998). These three compounds were chosen because DDT represents the Chlorohydrocarbons (CHC); propoxur is a carbamete which shares similar mode of action with that of organophosphates and permethrin is one of the synthetic pyrethroids. The impregnated papers were WHO formulations and were kindly obtained from Prof. Chris Curtis (London School of Hygiene and Tropical Medicine, U.K). Malathion impregnated papers could not be obtained for the test.

A well-aerated and illuminated room was chosen to undertake the tests. For each test and the control, 20 blood unfed females were used (WHO, 1998). The controls constituted mosquitoes that were not exposed to insecticide impregnated papers, but remain in tubes to the end of the test, lined with insecticide free papers. Before the commencement of the actual experiment, mosquitoes were aspirated from the breeding cage and placed in green labelled holding tubes in which the interior parts were lined with clean, insecticide free pieces of paper similar to the size of the insecticide impregnated papers, and left for one hour to check if there is external morphological damage and death. Leaving the controls in the same tube, the well looking test mosquitoes were transferred to red labelled tubes lined with insecticide impregnated papers for the period of one more hour of exposure. The tubes were held in vertical position as proposed in WHO (1998). Mosquitoes in the control

tubes were occasionally checked between and at the end of the experiment. Then the test mosquitoes were transferred to holding tubes (with green labels) for a recovery period of 24 hours, and were supplied with 20% sugar solution. The controls were also fed on the sugar solution. The tubes were then kept in separate semi-dark carton boxes. Humid micro-environment was created from wet cotton pads placed on the top of tubes as well as wet towels placed in each box and were constantly checked. The temperature of the room was at the same time checked and records were taken. At the termination of each test (i.e at the end of 24 hrs), the numbers of dead and surviving mosquitoes were counted. Tests for permethrin and DDT were done in 4 replicates, while the test for propoxur was limited to 2 replicates due to insecticide losses from the impregnated paper by evaporation. Attempts were made to breed surviving mosquitoes in the laboratory in Addis Ababa to repeat the test on the progeny to establish the existence of true resistance (Prof. C. Curtis, personal communication).

Samples of mosquitoes preserved in 70% ethanol and Carnoy's fluid were sent to Istituto di Parasitologia, Univerisita La Sapienza' in Rome for the detection of a gene responsible for knock down resistance (*kdr*) by molecular techniques (Martinez-Torres *et al.*, 1998).

4. RESULTS

4.1 Malaria cases and plasmodia infecting humans

During the study period, a total of 68,000 febrile cases from the four sites visited the Metehara Sugar Estate Hospital and the Metehara Malaria Sector (Appendix. V). Of these, 24,799 were confirmed acquiring malaria infections. The highest number of patients was recorded between July and December. The figure, however progressively decreased between January and May. The disease manifested in the population of Metehara town and the Sugar Estate in nearly all months of the year showing the existence of active transmission in the dry months in these two particular sites. In the same period, few cases were reported from Gelcha and Buse.

The laboratory examinations discerned that *P. falciparum* and *P. vivax* were the only two human *Plasmodia* to be prevalent in this area. Comparing cases from the study sites, *P. falciparum* infection was highest (49.4%) in Gelcha to be followed by Metehara (31.5%), Buse (24.5%) and the Sugar Estate (18.2%). *P. vivax* infection was highest in Metehara (20.5%) to be followed by the Sugar Estate (17.1%), Buse (12.6%) and Gelcha (10.3%). Falciparum infections took place in almost all months in the Sugar Estate and Metehara town, but the number of infected cases was relatively higher in the former than the latter site. Falciparum infection was completely absent from Gelcha in the months between April and July 2000. In the dry months in Buse, there was not a single malaria case infected by *P. falciparum,* the exception was January when 3 cases were found. In general *P. falciparum* is accounted for 19.2% malaria infections while 17.2% cases were infected by *P. vivax*.

4.2 Species composition and abundance of anophelines

4.2.1 Anophelines based on larval collections

The number and composition of anopheline species collected from various breeding sites and identified as larvae and adults bred from immatures are summarized in Table 1. At least four species of anophelines were identified, the predominant species being *An. arabiensis*. The second but less abundant species was *An. pharoensis*. The *An. coustani* group occurred in smaller numbers than the former two species. This group contains several species, but it is normally difficult to distinguish the members at larval stages.

An. arabiensis was observed to breed through- out the year including the dry season. The margins of Lake Beseka mainly in the wet season, and sewerage during the dry months in the Sugar Estate were favourite breeding sites for *An. arabiensis*. The sewerage was also favoured by *An. pharoensis* in the Sugar Estate. In the same site, the two species along with the *An. coustani* group co-existed in small interrupted irrigation canals. Except the canals all the sites were sunlit and devoid of vegetation. There were some *Anopheles* larvae collected from Lake Beseka, which could not be identified with the available conventional keys (Verrone, 1962b; Gillies and Demillion, 1968; Gillies and Coetzee, 1987). The species were found to breed in the lake in all the study period.

Table 1. Anopheline breeding sites identified from collections and breeding of immatures (July 1999 – March 2000, July – September 2000).

Species	Study and Collection sites										Total
	Buse	Gelcha		Sugar Estate					Metehara		
	Pool/rain water	Pool/rain water	Marsh	Pool/broken pipe	Pool/rain water	Sewerage	Canal	Canal spillage	Edge of Beseka	Pool/rain water	
An. arabiensis	70	217	100	308	815	1200	40	670	556	229	4205
An. pharoensis	0	0	6	0	0	406	15	0	0	0	427
An. coustani group	0	0	2	0	0	0	16	0	0	0	18
An. species	0	0	0	0	0	0	0	0	120	0	120
Total	70	217	108	308	815	1606	71	670	676	229	4770

4.2.2 Adult anophelines

During the investigation reported here, a total of 3,639 adult anophelines were collected from all the study sites in Metehara. The species and number of adult anophelines collected from the four sites are shown in Table 2. At least seven species, *An. arabiensis, An. pharoensis, An. tenobrosus, An. coustani* Laveran, *An. ziemanni* Grünberg, *An. ardensis* (Theobald), and *An. natalensis* (Hill & Haydon) were identified. Another *Anopheles* species collected from Metehara could not be reliably identified but all specimens appeared to be morphologically similar. The identity of *An. arabiensis* was based on reports of Mekuria *et al.* (1982), Ameneshewa (1995), Abose *et al.* (1998a) and Lulu *et al.* (1998) in which its presence in the Upper and Middle Awash was confirmed by chromosomal studies from collections made in different localities around Metehara. No other member of the complex so far was encountered in the area.

Anopheles arabiensis was the predominant species in the whole study area, making up 95% of the whole anophelines collected, followed by *An. pharoensis* (3.8%). The other species were rarely found. Both *An. arabiensis* and *An. pharoensis* occurred in all of the four localities. The majority of the former species was collected from Gelcha and Metehara town while *An. pharoensis* was collected from the Sugar Estate.

Table 2. Species, number and relative abundance of adult anophelines caught from the four study sites by various collection methods in the Metehara area between July 1999 and March 2000, July 2000 and September 2000.

Species	Sites				
	Buse	Gelcha	Sugar Estate	Metehara	Total (%)
An. arabiensis	764	1336	316	1042	3,458 (95)
An. pharoensis	20	42	62	14	138 (3.8)
An. tenebrosus	0	1	11	0	12 (0.33)
An. coustani	4	2	0	0	6 (0.16)
An. ziemanni	4	1	0	0	5 (0.14)
An. ardensis	1	1	0	0	2 (0.05)
An. natalensis	1	0	0	0	1 (0.03)
An. species	0	0	0	0	17 (0.47)
Total	794	1383	389	1073	3,639 (100)

4. 3 Indoor and outdoor resting densities of anophelines

4.3.1 Space spray collections

Only two species, *An. arabiensis* and *An. pharoensis* were found resting indoors, the former was caught abundantly in all the sites. Results of space spray collections of *An. arabiensis* in the four sites are depicted in Table 3.

The average hut density of *An. arabiensis* varied from 2.3 mosquitoes/ hut/day in nine months in Buse to 17.8 mosquitoes/hut/day in three months in Metehara town (Table 3). The overall density of *An. arabiensis* in the sprayed village of Buse and the Sugar Estate were much lower than the densities in the unsprayed village of Gelcha and Metehara town.

The seasonal pattern of *An. arabiensis* resting in huts can be seen in Table 3. Highest density of the species was recorded in August in Buse, Gelcha and the Sugar Estate, coinciding with high rainfall in the previous months in the area (Appendix. III). In Metehara town the highest density recorded was in September. In the months between August and October, the average densities in Buse, Gelcha and the Sugar Estate were 4.8, 20.8 and 5.2 females/hut/day, respectively.

There were only two *An. pharoensis* in space spray collections.

Table 3. Monthly indoor totals and hut density (in parentheses) of *An.arabiensis* based on space spray collections in the four sites (July 1999-Mar. 2000, July-Sept. 2000).

Month	Sites			
	Buse	Gelcha	Sugar Estate	Metehara Town
Aug. 1999	27 (7.3)	100 (39.7)	-	-
Sept.	13 (2.6)	57 (12.6)	-	-
Oct.	13 (2.6)	37 (10.1)	16 (4.7)	-
Nov.	26 (3.7)	39 (8.4)	7 (2.9)	-
Dec.	5 (1.5)	7 (2.4)	0	-
Jan. 2000	3 (0.8)	28 (7.3)	4 (3.9)	-
Feb.	0	2 (1.4)	0	-
Mar.	0	2 (1.4)	0	-
July	-	-	11 (2.3)	48 (7.9)
Aug.	-	-	18 (7.0)	9 (3.3)
Sept.	-	-	8 (3.4)	244 (42.3)
Overall	87 (2.3)	272 (10.4)	64 (2.6)	301 (17.8)

The gonotrophic status results of *An. arabiensis* showed that there was scarcity of unfed females with a preponderance of freshly fed flies in all sites (Tables 4). In general, the proportion of fresh feds was higher than the gravids, but there were variations between the study sites. The ratio varied from 1.22 in Gelcha (untreated village) to 3.27 in the Sugar Estate (treated village). The ratio of fed to gravids did not show any distinct seasonal variation in most study sites. Fresh fed females comprised 74.7, 54.4, 76.7 and 53.8% of all collections in Buse, Gelcha, the Sugar Estate and Metehara, respectively. There was no marked difference in the percentage of fresh feds between insecticide untreated villages and treated ones.

One of *the An. pharoensis* was found to be fresh fed while the other was gravid.

The results indicated that *An. arabiensis* was 18%, 21.3%, 66.1% and 69.4%

exophilic in Gelcha, Metehara town, Buse and the Sugar Estate, respectively.

Table 4. The abdominal stages of indoor resting *An. arabiensis* based on space spray, aspirator and CDC trap collections in the four sites (July 1999- Mar. 2000, July-Sept. 2000).

Abdominal status	Sites										
	Buse			Gelcha			Sugar Estate			Metehaea town	
	SS	AP	CD	SS	AP	CD	SS	AP	CD	SS	CD
Total mosquitoes	87	35	163	272	59	132	64	6	16	301	30
No.unfed (%)	0	3 (8.6)	58 (35.6)	3 (1.1)	11 (18.6)	48 (36.3)	0	2 (33.3)	12 (75)	11 (3.7)	14 (46.7)
No. fed (%)	65 (74.7)	19 (54.3)	95 (58.3)	148 (54.4)	23 (39.0)	10 (7.6)	49 (76.5)	3 (50.0)	3 (18.3)	162 (53.8)	4 (13.8)
No. half-gr. (%)	19 (21.8)	4 (11.4)	7 (4.3)	99 (36.4)	17 (28.8)	24 (18.2)	12 (18.8)	1 (16.7	1 (6.2)	118 (39.2)	0
No. gravid (%)	3 (3.5)	9 (25.7)	3 (1.8)	22 (8.1	8 (13.6)	50 (37.9)	3 (4.7)	0	0	10 (1.27)	12 (40.0)
Fed/gravid	2.95	1.46	-	1.22	0.92	-	3.27	3.0	-	1.27	-

SS= Space spray AP= Aspirator CD= CDC light trap

4.3.2 Aspirator collections

Both *An. arabiensis* and *An. pharoensis* were caught resting in human dwellings and outdoors in cattle sheds, tree holes and man made burrows. Mouth aspiration was not as efficient as the space spray method in the collections of anophelines from resting in huts/houses. From the results obtained, the indoor and outdoor resting rates of anophelines are separately treated below.

4.3.2.1 Indoor aspirator collections

In the two villages, Gelcha and Buse, the average hut density of *An. arabiensis* in the five months between July and November respectively was 2.8 flies/hut/day and 1.9 mosquitoes/hut/day (Table 5).

Table 5. Monthly total and indoor density (in parentheses) of *An. arabiensis* based on aspirator collections in Buse and Gelcha (July- November 1999).

Month	Sites	
	Buse	Gelcha*
July 1999	13 (2.7)	11 (1.9)
Aug.	4 (1.6)	15 (3.6)
Sept.	4 (1.6)	6 (1.9)
Oct.	5 (1.7)	14 (3.3)
Nov.	9 (2.1)	13 (3.1)
Overall	35 (1.9)	59 (2.8)

*= Only a single *An. pharoensis* was aspirated in August

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 (Table 4). The number of unfed females was similarly very low.

4.3.2.2 Outdoor aspirator collections

In Buse and Gelcha outdoor resting *An. arabiensis* and *An. pharoensis* were aspirated from partially destroyed huts, which served as cattle sheds and other cattle shelters (Table 6).

The largest group of *An. arabiensis* 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 behaviour of the species. As shown in section 4.3.1 and 4.3.2.1 above, there was deficiency of half-gravids and gravids in indoor collections by both space spray and mouth aspiration.

A small number of *An. arabiensis* from the Sugar Estate was also aspirated from tree holes and ground burrows. Very few *An. pharoensis* (2 females in Buse and 2 in Gelcha) were also caught from outdoor resting sites. Both species could not be caught from other outdoor resting sites despite intensive effort.

Table 6. Abdominal status of *An. arabiensis* collected by mouth aspiration from outdoor sites (July 1999- March 2000, July-September 2000).

Species and abdominal status	Sites		
	Buse (cattle shed)	Gelcha (cattle shed)	Sugar Estate (tree hole and burrow)
An. arabiensis			
No. unfed (%)	0	6 (4.5)	1 (20)
No. fed (%)	4 (9.5)	6 (4.5)	3 (60)
No. half-gravid (%)	34 (81.0)	88 (66.7)	0
No. gravid	4 (9.5)	32 (24.3)	1 (20)
Total flies	42	132	5

4.3.3 CDC light trap collections

4.3.3.1 Indoors

Anophelines entering huts or resting in huts were caught in CDC light traps. Two species of anophelines (*An. arabiensis* and *An. pharoensis*) were caught by light traps indoors but the two species were not always caught together in some villages such as in Buse (Table 7).

The highest density (4.8 mosquitoes/trap/day) was recorded for Buse while the lowest (1.2 mosquitoes/trap/day) was for the Sugar Estate (Table 7). The density difference between the insecticide untreated and treated villages was small.

Light traps caught more *An. arabiensis* in the wet months between July and September when the indoor density was relatively high.

With regards to the gononotrophic stage, the majority of mosquitoes attracted to light traps in Buse were fresh fed females comprising 58.3% of all collections (Table 4). The second largest were unfed females (35.6%). In the Sugar Estate 75% of the collections comprised females, which have not taken blood meals. In the other two sites, Gelcha and Metehara town, the proportions of unfed and gravid females was almost similar, both constituting the highest rates (Table 4).

Few *An. pharoensis* were caught in light traps in the Sugar Estate, Gelcha and Metehara town (Table 4).

Table 7. Monthly indoor collections of *An. arabiensis* and *An. pharoensis* based on CDC light trap catches in the four sites (July 1999- March 2000, July- September 2000); ( ) = density.

Month	Sites							
	Buse		Gelcha		Sugar Estate		Metehara town	
	An. ara	An. pha.	An. ara	An. pha.	An. ara	An. pha.	An. ara	An. pha.
July 1999	40 (6.5)	0	71 (6.8)	1 (1.0)	-	-	-	-
Aug.	34 (10.5)	0	40 (4.6)	0	-	-	-	-
Sept.	46 (11.1)	0	1 (1.2)	0	-	-	-	-
Oct.	28 (7.6)	0	6 (2.0)	0	1 (1.4)	2 (1.1)	0	0
Nov.	12 (3.4)	0	7 (2.4)	0	0	0	0	0
Dec.	1 (1.4)	0	0	2 (1.1)	0	0	0	0
Jan. 2000	1 (1.4)	0	4 (2.8)	0	0	0	0	0
Feb.	1 (1.4)	0	3 (2.0)	0	0	0	0	0
Mar.	0	0	0	0.	0	0	0	0
July	-	0	-	-	2 (1.7)	1 (1.0)	21 (8.7)	1 (1.0)
Aug.	-	0	-	-	10 (5.2)	0	1 (1.2)	0
Sept.	-	0	-	-	3 (2.4)	0	8 (4.6)	0
Overall	163 (4.8)	0	132 (2.4)	3 (1.0)	16 (1.2)	3 (1.0)	8 (4.6)	1 (1.0)

An. ara = *An. arabiensis* An. pha.= *An. pharoensis*

4.3.3.2 Outdoors

CDC traps placed outdoors failed to catch a single anopheline in Buse, however the traps in Gelcha caught 2 *An. arabiensis*, 3 *An. pharoensis* and one *An. ardensis*. Similarly, in the Sugar Estate the traps caught 2 *An. arabiensis*. In Metehara, 14 specimens of the

unidentified *Anopheles* species was attracted to light traps placed outdoors very near to breeding sites.

4.4 Night biting catches of *Anopheles*

Throughout the study period, *An. arabiensis* and *An. pharoensis* were the two commonly encountered species on human bait catches both indoors and outdoors in all the study sites. However, the former species was the commonest biting species. Thus, considering *An. arabiensis* a total of 437 in Buse, 711 in Metehara, 223 in the Sugar Estate and 740 in Gelcha were caught (Tables 8, 9, 10 & 11). Similarly, the number of *An. pharoensis* caught from the respective sites was 17, 13, 54 and 36. The other anophelines *An. ardensis*, *An. natalensis, An. ziemanni*, *An. coustani*, An. *tenebrosus* and the unidentified *Anopheles* species were rarely collected.

4.4.1 Indoor and outdoor biting behaviour of the two common anophelines

The overall relative abundance of night biting *An. arabiensis* indoors and outdoors varied between villages (Tables 8, 9, 10 & 11). The number indoors in Buse (n=290) and Metehara (n=483) was significantly higher (*Z* test, $P<0.001$) than the corresponding numbers outdoors (n=147) and (n=228) showing that *An. arabiensis* to be more endophagic. In contrast, the outdoor population in the Sugar Estate (n=146) and Gelcha (n=440) was significantly higher (*Z* test, $P<0.001$) than the respective indoor population (n=77) and (n=300). However, the indoor to outdoor biting ratios varied between seasons. In Buse the ratio was highest in July (4.34:1) and lowest in October and November (0.33:1). In Metehara, the indoor biting population was higher than the outdoor throughout the study period being highest in August (7.5:1) and lowest in September (1.1:1). In the

Sugar Estate and Gelcha where ratios were generally smaller, the highest ratios recorded for the Sugar Estate in August (1.35:1) and for Gelcha was in July (1.1:1) while the lowest ratios for the respective sites were in December and March (0) and September (0.57:1).

The second important man-biting species in the area, *An. pharoensis* preferred to feed predominantly outdoors in all sites, comprising 82.4% (14/17) in Buse, 53.8% (7/13) in Metehara, 82.4% (29/36) in Gelcha and 68.5% (37/57) in the Sugar Estate of the total population (Tables 8, 9, 10 & 11).

Table 8. Species and number of monthly indoor and outdoor human bait collections of anophelines in Buse (July 1999- March 2000).

Species	Month									
	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Overall
An. arabiensis										
No. (%) indoor	178(81.3)	53(46.1)	44(66.7)	11(40.7)	2(25.0)	1(100)	1(100)	0	0	290(66.4)
No. (%) outdoor	41(18.7)	62(53.9)	22(33.3)	16(59.3)	6(75.0)	0	0	0	0	147(33.6)
Indoor/outdoor	4.34	0.85	2	0.33	0.33	0	0	0	0	1.97
An. pharoensis										
No. (%) indoor	2(66.7)	0	0	1(50.0)	0	0	0	0	0	3(17.6)
No. (%) outdoor	1(33.3)	6(100)	5(100)	1(50.0)	1(100)	0	0	0	0	14(82.4)
Indoor/outdoor	2	0	0	1.0	0	0	0	0	0	0.2
An. ardensis										
Out door	1	0	0	0	0	0	0	0	0	1
An. ziemanni										
Outdoor	0	1	1	0	0	0	0	0	0	2
An. natalensis										
Outdoor	1	0	0	0	0	0	0	0	0	1
An. coustani										
Outdoor	0	4	0	0	0	0	0	0	0	4
Total	224	126	72	36	9	1	1	0	0	469
(%)	(47.8)	(26.9)	(15.3)	(7.7)	(1.9)	(0.2)	(0.2)			(100)

Table 9. Species and number of monthly human bait collections of anophelines in Metehara town (October 1999-March 2000, July-September 2000).

Species	Month									
	Oct	Nov	Dec	Jan	Feb	Mar	July	Aug	Sept	Overall
An. arabiensis										
No.(%) indoor	185(72.5)	40(80.0)	0	0	0	0	110(74.8)	30(88.2)	118(52.4)	483(67.9)
No. (%) outdoor	70(27.5)	10(20.0)	0	0	0	0	37(25.2)	4(11.8)	107(47.6)	228(32.1)
Indoor/outdoor	2.64	4.0	0	0	0	0	2.97	7.5	1.1	2.12
An. pharoensis										
Indoor	1 (50)	1 (50)	0	0	0	0	2 (100)	0	2 (40)	6 (46.2)
Outdoor	1 (50)	1 (50)	0	0	0	0	0	2 (100)	3 (60)	7 (53.8)
In/out	1	1	0	0	0	0	0	0	0.67	0.86
Anopheles. sp										
Indoor	-	0	0	0	0	0	1 (50)	0	0	1 (33.3)
Outdoor	1 (100)	0	0	0	0	0	1 (50)	0	0	2 (66.7)
Total	258	52	0	0	0	0	151	36	230	727
(%)	(35.4)	(7.2)	0	0	0	0	(20.8)	(5.0)	(31.6)	(100)

Table 10. Species and number of monthly indoor and outdoor anopheline collections on human baits in the Sugar Estate (October 1999 - March 2000, July - September 2000).

Species	Month									
	Oct	Nov	Dec	Jan	Feb	Mar	Jul	Aug	Sept	Overall
An. arabiensis										
No. (%) indoor	5(17.9)	5(50.0)	0	1(11.0)	1(14.3)	0	28(37.8)	31(57.4)	6(20.7)	77(34.5)
No. (%) outdoor	23(82.1)	5(50.0)	8(100)	8(88.9)	6(85.7)	4(100)	46(62.2)	23(42.6)	23(79.3)	146(65.5)
Indoor/outdoor	0.38	1	0	0.13	0.17	0	0.61	1.35	0.26	0.53
An. pharoensis										
No. (%) indoor	1(20.0)	0	0	0	1(14.3)	9(42.9)	5(38.5)	0	1(25.0)	17(31.5)
No. (%) outdoor	4(80.0)	2(100)	1(100)	1(100)	6(85.7)	12(57.1)	8(61.5)	0	3(75.0)	37(68.5)
Indoor/outdoor	0.25	0	0	0	0.17	0.75	0.63	0	0.33	0.46
An. tenebrosus										
No. (%) indoor	0	0	1(33.3)	0	1(25.0)	0	0	0	0	2(18.2)
No. (%) outdoor	1(100)	0	2(66.7)	0	3(75.0)	1(100)	2(100)	0	0	9(81.8)
Indoor/outdoor	0	0	0.5	0	0.33	0	0	0	0	0.22
Total	34	12	12	10	18	26	89	54	33	228
(%)	(11.8)	(4.2)	(4.2)	(3.5)	(6.3)	(9.0)	(30.9)	(18.7)	(11.4)	(100)

Table 11. Species and number of monthly indoor and outdoor human bait collections of anophelines in Gelcha (July 1999- March 2000).

Species	Months									
	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Overal
An. arabiensis										
No. (%) indoor	53(52.5)	88(37.4)	69(36.1)	61(39.9)	15(41.7)	1(14.3)	7(63.6)	5(100)	1(100)	300(40.5)
No.(%) outdoor	48(47.5)	147(62.6)	122(63.9)	92(60.1)	21(58.3)	6(85.7)	4(36.4)	0	0	440(59.5)
Indoor/outdoor	1.1	0.6	0.57	0.66	0.71	0.17	1.75	0	0	0.75
An. pharoensis										
No. (%) indoor	1(33.3)	0	0	0	2(66.7)	1(50.0)	0	3(100)	0	7(19.0)
No. (%) outdoor	2(66.6)	3(100)	9(100)	7(100)	1(33.3)	1(50.0)	6(100)	0	0	29(81.0)
Indoor/outdoor	0.5	0	0	0	2	1	0	0	0	0.24
An. coustani										
outdoor	0	0	0	2	0	0	0	0	0	2
An tenobrosus										
outdoor	0	0	0	0	0	0	1	0	0	1
An. ziemanni										
outdoor	0	0	0	1	0	0	0	0	0	1
Total	102	238	200	163	39	9	18	8	1	778
(%)	(13.1)	(30.6)	(25.7)	(21.0)	(5.0)	(1.2)	(2.3)	(1.0)	(0.1)	(100)

4.4.2 Seasonal trends in the biting rate of *An. arabiensis* and *An. pharoensis*

The seasonal variation of *An. arabiensis* in MBR both indoors and outdoors in all the study sites is depicted in Figs. 2, 3, 4 and 5. In Buse the species was found biting humans between July and January both indoors and outdoors, but higher MBRs were recorded during the wet months (July-October) with a preponderance of indoor MBR (Fig. 2). Similarly in Metehara, biting activity was restricted to the wet months (July- October), with a pronounced indoor biting activity (Fig. 3). In Gelcha and the Sugar Estate there was higher biting activity at outdoors during most of the observation period with peaks in the wet months (July- October).

Although the seasonal trend on all sites was similar, there were variations in the man biting rate of *An. arabiensis* between the four sites. Of all sites, Metehara had the highest average MBR both indoors 53.7 (0-185) and outdoors 25.3 (0-107), with a combined (indoor and outdoor) average MBR of 39.5 (Fig. 3). The next higher biting rate was recorded for Gelcha where the average indoor and outdoor MBRs were 11.5 (0.5-22) and 16.9 (0-36.8) respectively and the average of the two was 14.2 bites (Fig.4). The Sugar Estate and Buse had the lowest MBR. The average man biting rate in indoor and outdoor locations in the Sugar Estate was estimated to be 8.6 (0-31) and 16.2 (1-46), respectively, with an average of 12.4 (Fig.5). For Buse the average MBR indoors was 11.2 (0-44.5) and outdoors 5.7 (0-15.5) with an average of 8.4 (Fig.2)

However, in all sites, intense biting occurred during the wet season, but the highest MBR for this period was recorded for Metehara both indoors 110.8 (30-185) and outdoors 54.5 (4-107) with a combined average MBR of 82.6. The lowest MBR for the same period was observed in Buse with 14.5 (2.5-44.5) and 7.3 (2.3-15.5) indoors and outdoors,

respectively. The combined average MBR was 10.9. In Gelcha the indoor and outdoor average bites were respectively 16.9 (13.3-22) and 27.8 (21-36.8), the combined average being 22.4. The corresponding MBRs in the Sugar Estate were 17.5 (5-31), 28.8 (23-46) and 23.1.

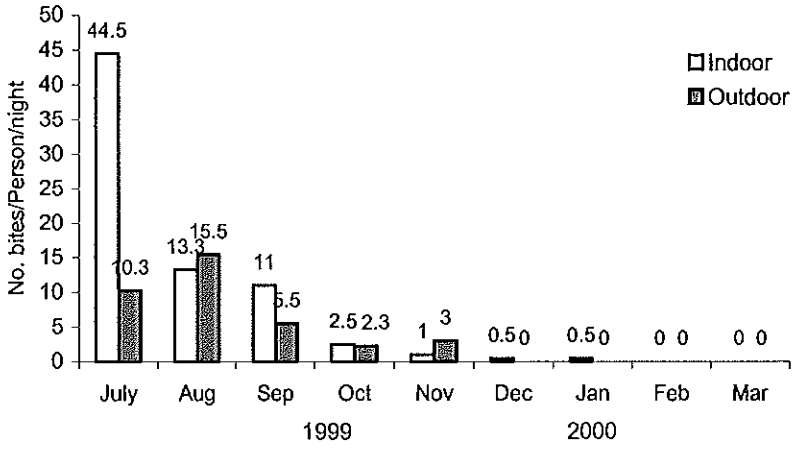

Fig. 2. Seasonal variation in the human biting rate of *An. arabiensis* in Buse (July 1999 - Mar. 2000)

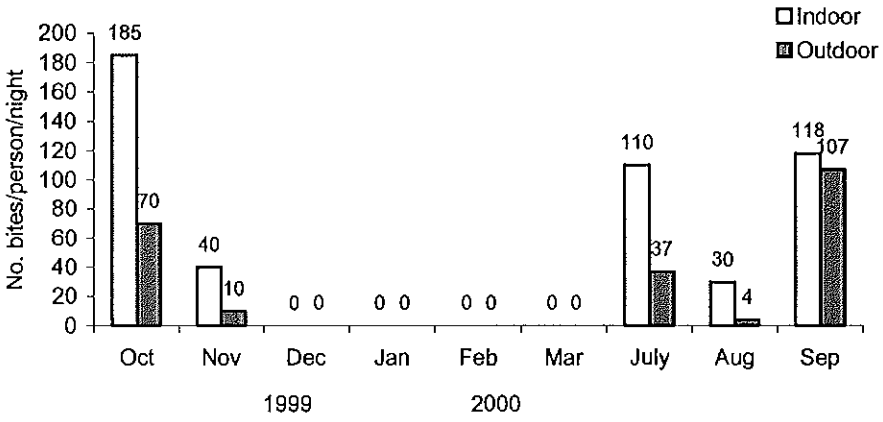

Fig. 3. Seasonal variation in the biting rate of *An. arabiensis* in Metehara town (Oct. 1999- Mar. 2000, July - Sep. 2000)

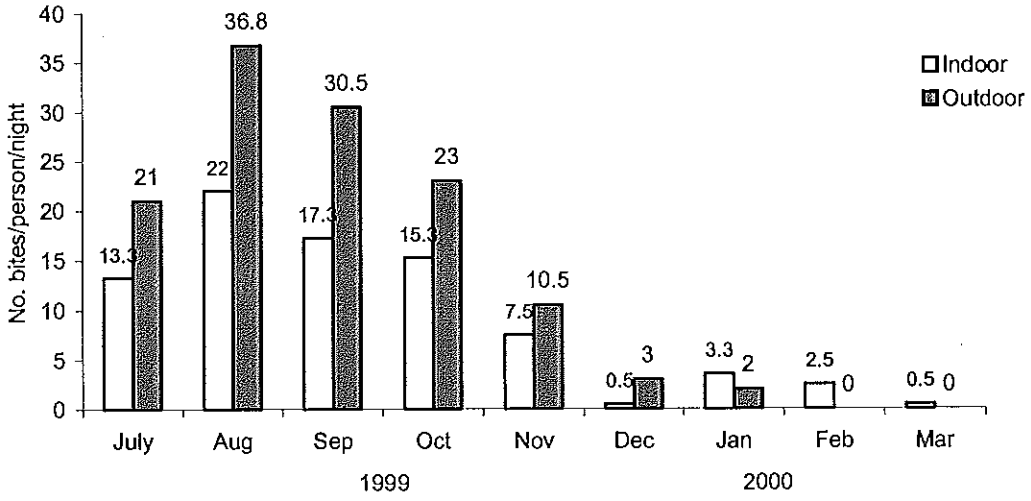

Fig. 4. Seasonal variation in the human biting rate of *An. arabiensis* in Gelcha (July 1999 - Mar. 2000)

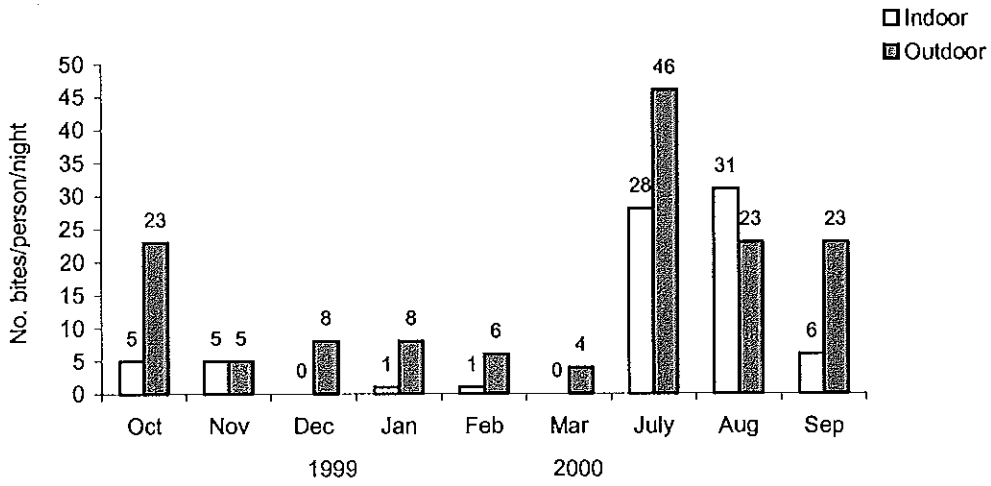

Fig. 5. Seasonal variation in the biting rate of An. arabiensis in the Sugar Estate (Oct. 1999 - Mar. 2000, July - Sept. 2000)

The seasonal man biting rate of *An. pharoensis* indoors and outdoors is shown on Table 12. However, the number of caught flies was small. The density in Buse varied between 0 and 0.5 indoor and 0 and 1.3 (mean 0.8) outdoors in the wet months. This

species was totally absent in night biting collections in the dry months. However, in Buse, it occurred until February, while in the Sugar Estate it occurred during most of the study period. In Metehara town it occurred in small numbers during the rainy season and shortly after. In Gelcha, the highest night biting density was observed in January in the outdoor biting population. The mean biting rate at this site in all the study period indoors and outdoors was 0.3 and 1.0, respectively. The corresponding rate in the Sugar Estate was 1.9 and 4.1. The average in both locations was 3.0. The mean biting rates indoors and outdoors respectively, was 0.7 and 0.8 with an average of 0.7. In general, the density of this species in all the sites appeared to be low.

Table 12. Seasonal man biting rate (no.bites/person/night) of *An. pharoensis* in indoor and outdoor collection sites (July 1999-March 2000, July- September 2000 in Metchara and the Sugar Estate).

Year	Month	Study/collection sites							
		Buse		Gelcha		Sugar Estate		Metchara	
		Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
1999	July	0.5	0.3	0.3	0.5	-	-	-	-
	Aug.	0	1.5	0	0.8	-	-	-	-
	Sept.	0	1.3	0	2.3	-	-	-	-
	Oct.	0.3	0.3	0	1.8	1	4	1	1
	Nov.	0	0.5	1	0.5	0	2	1	1
	Dec.	0	0	0.5	0.5	0	1	0	0
2000	Jan	0	0	0	3.0	0	1	0	0
	Feb.	0	0	1.0	-	1	6	0	0
	Mar.	0	0	0	-	9	12	0	0
	July	-	-	-	-	5	8	2	0
	Aug.	-	-	-	-	0	0	0	2
	Sept.	-	-	-	-	1	3	2	3

4.5 Parous rates of *An. arabienis* and *An. pharoensis*

The results of dissections of *An. arabiensis* from collections of human baits showing parity rates on monthly basis in the four sites are depicted in Table 13. Different parous rates in females caught from indoors and outdoors were recorded. In Buse the overall parous rate was 53.8% with higher average parous rates indoors 56.6% (0-100%) than

outdoors 50.6% (0-60.5%). Similarly, average parous rates of 49.8, 42.5 and 28.6% were recorded in Gelcha, Metehara town and the Sugar Estate, respectively. As in Buse, parous rates in Gelcha and Metehara sites were higher indoors than outdoors but not in the Sugar Estate. The difference in parity between the indoor and outdoor populations in each of the sites was compared using Z-test. Accordingly, the differences in the proportions in Buse and the Sugar Estate were not significant ($P > 0.4157$ and $P > 0.7909$, respectively). In Gelcha it was marginally significant ($P < 0.0478$) while these differences in Metehara was significant ($P<0.005$). The difference in the mean parity index among sites could not be related to insecticide application since higher rates were recorded in both treated (Buse) and untreated (Gelcha) sites.

The result of parous rates of *An. arabiensis* from CDC collections is shown in Table 14. Eventhough, the number of flies dissected was small, the parous rates in Buse and Gelcha were relatively higher, and similar to the rates observed in biting catches. As in human bait catches, the lowest parous rate (42.9%) was recorded in Metehara town. However in all cases, the rates were higher than the rates observed in biting collections.

The parous rate of *An. pharoensis* from human bait catches and CDC traps are shown in Tables 13 and 14. The parous rates of human bait catches of *An. pharoensis* in all sites was much lower than that of *An. arabiensis,* ranging from 0-33.3%. A single female caught in light traps in Metehara town was also parous.

Table 13. Monthly parity rates of anophelines dissected from collections at the four sites in the study area (July 1999- March 2000, July-September 2000).

Year and month	Site, species and locations															
	Buse				Gelcha				Sugar Estate				Metehara			
	An. arab.		An. phar.		An. arab.		An. phar.		An. arab.		An. phar.		An. arab.		An. phar.	
	in	out	in	out	in	out	in	out	in	out	in	out	in	out	in	out
July 1999	33 (51.5)	21 (38.1)	1 (0)	1 (0)	7 (57)	14 (64.3)	0	2 (0)	-	-	-	-	-	-	-	-
Aug.	29 (58.6)	38 (60.5)	0	3 (66.7)	19 (57.9)	52 (36.5)	0	2 (0)	-	-	-	-	-	-	-	-
Sept.	32 (62.5)	15 (46.7)	0	5 (20)	11 (54.5)	59 (55.9)	0	3 (33.3)	-	-	-	-	-	-	-	-
Oct.	1 (100)	7 (57.1)	0	0	26 (61.5)	33 (33.3)	0	3 (33.3)	5 (100)	17 (76.5)	0	4 (50)	28 (39.3)	2 (0)	0	0
Nov.	2 (0)	4 (25)	0	1 (0)	4 (75)	14 (57)	0	0	2 (50)	0	0	2 (50)	24 (58.3)	4 (0)	0	0
Dec.	1 (100)	0	0	0	1 (0)	3 (33.3)	1 (0)	1 (0)	0	4 (0)	0	1 (0)	0	0	0	0
Jan. 2000	1 (0)	0	0	0	3 (33.3)	2 (0)	0	4 (0)	0	4 (0)	0	1 (0)	0	0	0	0
Feb.	0	0	0	0	2 (100)	1 (0)	1 (0)	0	0	2 (0)	1 (0)	2 (0)	0	0	0	0
Mar.	0	0	0	0	1 (100)	0	0	0	0	4 (0)	8 (50)	1 (0)	0	0	0	0
July	-	-	-	-	-	-	-	-	18 (0)	35 (42.9)	3 (33.3)	4 (75)	36 (22.2)	20 (0)	0	2 (0)
Aug.	-	-	-	-	-	-	-	-	27 (55.6)	13 (53.8)	0	0	19 (26.3)	5 (0)	0	0
Sept.	-	-	-	-	-	-	-	-	4 (50)	18 (38.9)	1 (100)	3 (0)	46 (45.7)	55 (32.7)	1 (100)	0
Total	99 (56.6)	85 (50.6)	1 (0)	10 (30)	74 (59.5)	177 (45.8)	2 (0)	15 (13.3)	56 (41.1)	97 (43.3)	13 (46.2)	18 (33.3)	153 (38.6)	86 (20.9)	1 (100)	2 (0)
Overall	184 (53.8)		11 (27.3)		251 (49.8)		17 (11.8)		153 (42.5)		31 (38.7)		239 (28.6)		3 (33.3)	

( ) = % parous An. phar. = *An. Pharoensis* An. arab. = *An. arabiensis* in = indoors out=outdoors

Table 14. Parous rates in anophelines dissected from collections of indoor light trap collections in three sites (July 1999-March 2000, July-September 2000).

	Site and species			
	Buse	Gelcha	Metehara town	
	An. arab.	An. arab.	An. arab	*An. phar.*
Total dissected	21	9	7	1
Parous (%)	76.2	55.6	42.9	100

An. arab. = *An. arabiensis* An. phar. = *An. pharoensis*

4.6 Biting rhythms of *An. arabiensis* and *An. pharoensis*

The night biting activities of *An. arabiensis* populations both indoors and outdoors in the four study sites are depicted in Figs. 6, 7, 8 and 9. The species was found to bite on humans from dusk to dawn in all sites with variations in biting densities between hours and sites.

In Buse, three peaks of biting activity occurred indoors during 21:00-22:00 (early evening), 24:00-2:00 (around midnight) and 3:00-4:00 (after midnight) hours, but the first and last peaks were the most pronounced. Reduced biting activity occurred outdoors with two peaks in the early evening (21:00-22:00 hour) and after midnight (3:00-4:00 hour) (Fig. 5). The biting activity of parous flies also followed a similar trend both indoors and outdoors.

In the Sugar Estate (Fig. 7), there was a higher outdoor biting activity than indoors

with three peaks at 19:00-21:00, 23:00-24:00 and 3:00-5:00 hours, the most important being around midnight. The indoor peak hours were between 21:00-23:00 and 2:00-5:00 hours, the latter being the most pronounced. At outdoor more parous flies activity was observed in the first half of the night between 19:00- 21:00 and 23:00-24:00 hours. Indoors, most biting activity of parous females took place between 21:00-24:00 hours.

In Gelcha, peak outdoor biting activities were observed in the early hours of 19:00-21:00, 22:00-23:00 and later at 24:00-2:00 hours (Fig. 8). There was no distinct activity pattern in the parous population.

In Metehara town two major indoor biting activities, one before midnight (21:00-22:00 hour) and another around midnight (24:00-01:00 hour) were observed (Fig. 9). At outdoors a prolonged biting activity took place between 20:00-01:00 hours with no apparent peaks. The parous population exhibited no distinct biting activity pattern.

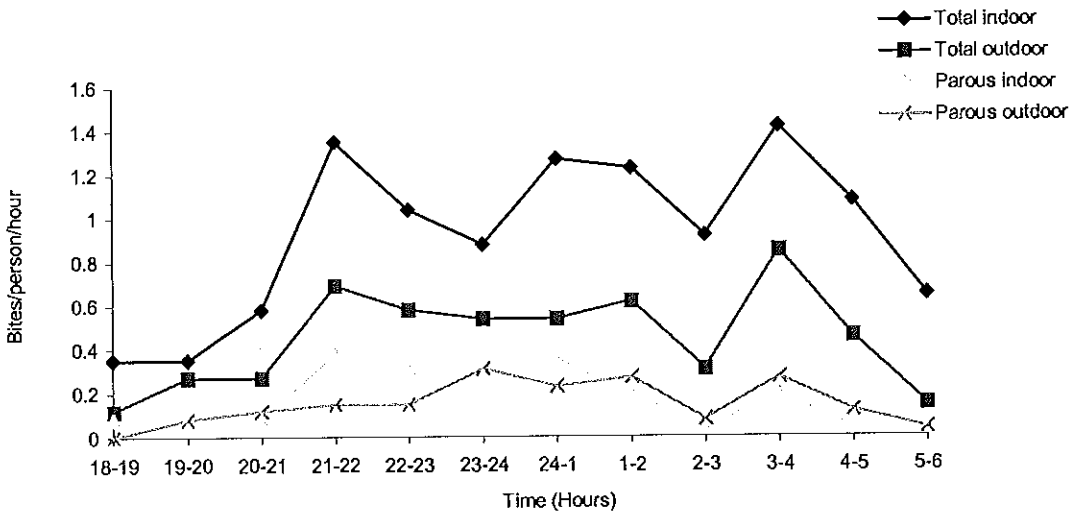

Fig. 6 Showing biting rhythm in total and parous population of *An. arabiensis* in Buse (July 1999 - March 2000)

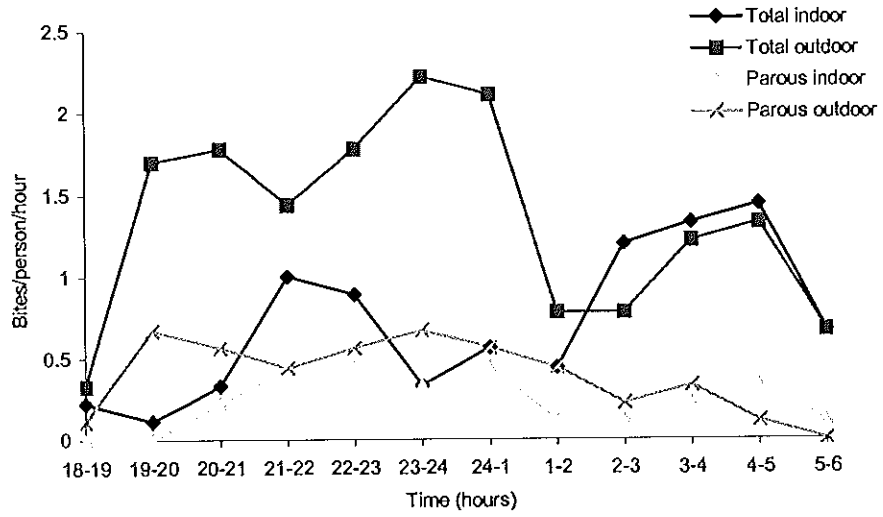

Fig. 7 Showing biting rhythm in total and parous population of *An. arabiensis* in the Sugar Estate (Oct. 1999 - March 2000, July-Sept. 2000)

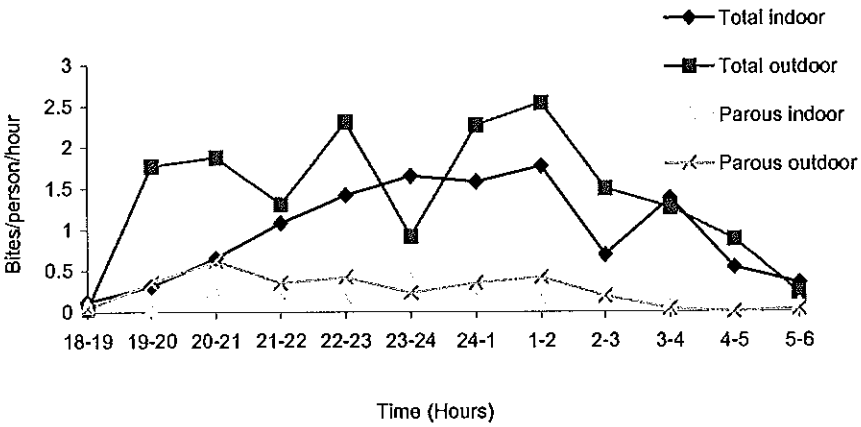

Fig. 8 Showing biting rhythm in total and parous population of *An. arabiensis* in Gelcha (July 1999 - march 2000)

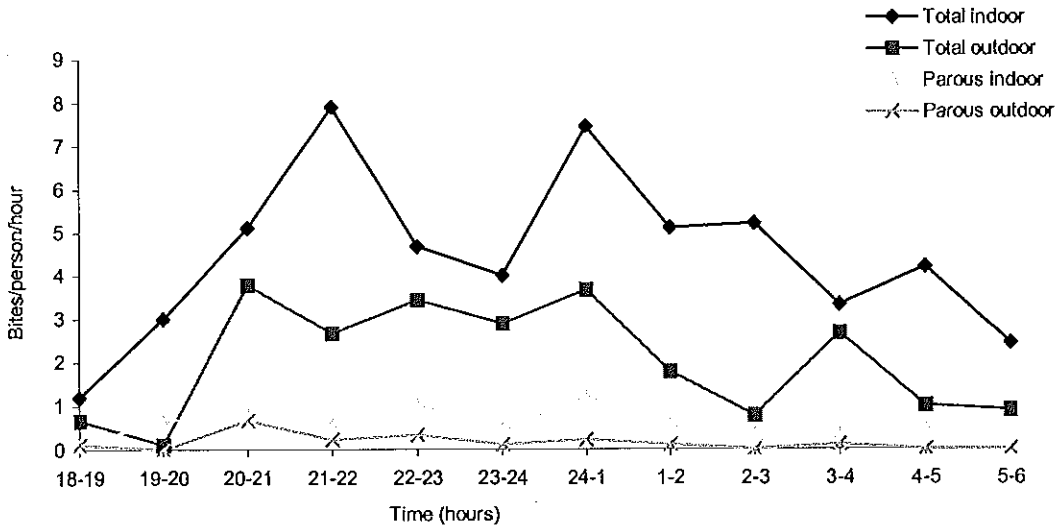

Fig. 9 Showing biting rhythm in total and parous population of *An. arabiensis* in Metehara town (Oct. 1999 - March 2000, July - Sep. 2000)

Calculations were made to see any difference in the biting activity of the total and parous proportions of *An. arabiensis* population before and after midnight the results of which are shown in Tables 15 and 16. It can be seen that in all the sites, the total indoor population active before midnight was low forming 41.0%, 45.3%, 33.8% and 48.2% of the total collections in Buse, Gelcha, the Sugar Estate and Metehara town, respectively (Table 15). The rate was much lower in the insecticide sprayed sites (the Sugar Estate and Buse). In these two sites, the parous population comprised 68.8% and 64.7% of the indoor active females in the same period (Table 16). In Gelcha and Metehara town, 55.3% and 36.4% of the populations active before midnight constituted parous females. In Metehara, the activity of parous females indoors after midnight was either low or equal to the nulliparous females.

The outdoor biting activity before midnight comprised 46.3%, 48.6%, 57.5% and 57.5% in Buse, Gelcha, the Sugar Estate and Metehara town, respectively (Table 16). In these respective sites, the outdoor parous night biting flies in this period were 48.8%, 49.5%, 47.4% and 22.6% (Table 20). The activity of parous flies outdoors declined after midnight in Gelcha, the Sugar Estate and Metehara town.

Table 15. Biting density of *An. arabiensis* before and after midnight. ( )=%

Location of Collection	Time (hours)	Biting density (%) at study site			
		Buse	Gelcha	Sugar Estate	Metehara
Indoor	18:00-24:00	119 (41.0)	136 (45.3)	26 (33.8)	233 (48.2)
	24:00-6:00	171 (59.0)	164 (54.7)	51 (66.2)	250 (51.8)
Outdoor	18:00-24:00	68 (46.3)	214 (48.6)	84 (57.5)	131 (57.5)
	24:00-6:00	79 (53.7)	226 (51.4)	62 (42.5)	97 (42.5)

Table 16. Parous rates of *An. arabiensis* before and after midnight.

Location of Collection	Time (hours)	Parity rates			
		Buse	Gelcha	Sugar Estate	Metehara
Indoor	18:00-24:00	64.7% (33/51)	55.3% (26/47)	68.8% (11/16)	36.4% (32/88)
	24:00-6:00	50.0% (24/48)	59.3% (16/27)	30% (12/40)	40% (26/65)
Outdoor	18:00-24:00	48.8% (21/43)	49.5% (52/105)	47.4% (27/57)	22.6% (12/53)
	24:00-6:00	57.1% (24/42)	37.5% (27/72)	37.5% (15/40)	15.2% (5/33)

Fig. 10 shows the night biting activity of *An. pharoensis*. In all cases, host seeking activity of *An. pharoensis* was distinctly dominant at outdoors in the hours before midnight in the bimodals in Buse (19:00-20:00 and 22:00-24:00 hours) and Gelcha (20:00-21:00 and 24:00-01:00 hours) and unimodal at the Sugar Estate (19:00-20:00 hours) and Metehara (24:00-01:00). In general, similar trends appeared to be followed by indoor biting population of *An. pharoensis*.

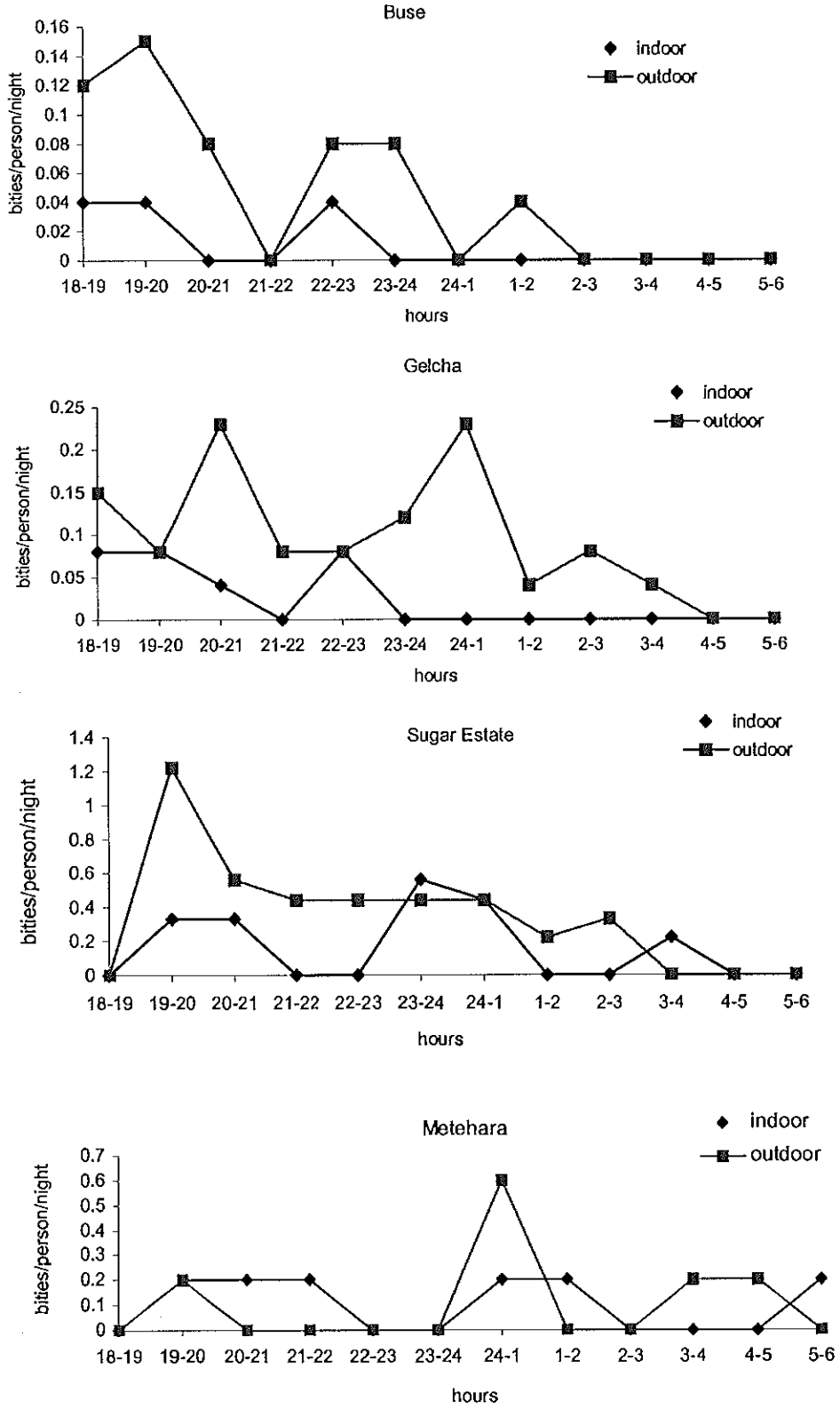

Fig. 10 Showing the biting rhythm of total population of *An. pharoensis* in the four sites (July 1999 – March 2000, July – September 2000).

4.7 Sporozoite infection rates in *An. arabiensis* and *An. pharoensis*

4.7.1 Infection rates in *An. arabiensis*

The results of dissections of *An. arabiensis* collected from human baits and CDC traps from the four sites to detect *Plasmodium* infections are shown in Table 17. Of the total 864 *An. arabiensis* dissected, 390 (45.1%) were determined to be parous. Of these, 3 parous (0.77%) and a single nulliparous (0.21%) were found harbouring sporozoites in their salivary glands giving an overall sporozoite rate of 0.46%. As the number of flies caught in light traps was small, most of the dissections were made from human bait catches. Consequently, 3 of the total infected flies were from human bait catches, 2 of

Table 17. Sporozoite and entomological inoculation rates (EIR) of *An. arabiensis* collected on human baits and CDC light traps in the Metehara area (July 1999- March 2000, July-September 2000).

Parameter	Site								Total
	Buse		Gelcha		Sugar Estate		Metehara		
	HB	CDC	HB	CDC	HB	CDC	HB	CDC	
No. dissected	184	21	251	9	153	0	239	7	864
No. parous	99	16	125	5	65	0	77	3	390
(%)	(53.8)	(76.2)	(49.8)	(55.6)	(42.5)		(32.2)	(42.9)	(45.1)
Parous infected	0	1	1	0	0	0	1	0	3
(%)		(6.25)	(0.80)				(1.30)		(0.77)
Nulliparous inf.	0	0	0	0	0	0	1	0	1
(%)							(0.62)		(0.21)
Total infected	0	1	1	0	0	0	2	0	4
(%)		(4.76)	(0.40)				(0.84)		(0.46)
Man biting rate*	8.4	-	14.2	-	12.4	-	39.5	-	15.1
EIR	-	-	0.06	-	-	-	0.33	-	0.05

EIR = bites/person/night x sporozoite rate HB= human bait

*= The sporozoite rate in mosquitoes caught by human bait was 0.36% and this figure was used to estimate the overall EIR.

them collected in November 1999 and the other in September 2000. Furthermore, the infected flies were found in all villages except the Sugar Estate. However, none of the 184 mosquitoes from human bait collections in Buse were infected, although 1 of 21 CDC light catches indoors was infected. The specimen was trapped in December 1999. All the other infected flies from human bait catches (Gelcha and Metehara) were from indoor collections.

4.7.2 Infection rates in *An. pharoensis*

The results of dissection of *An. pharoensis* collected by human baits and CDC light traps from the four sites are shown in Table 18.

An. pharoensis being less abundant than *An. arabiensis*, only 63 females caught predominantly by human baits (62 human bait vs 1 from CDC) were dissected. Of these, 19 (30.2) were parous, the majority being nulliparous. None of the parous flies from human bait catches was found infected; infection was detected in only a nullipar from the Sugar Estate. The single specimen caught in light traps in Metehara was parous and was found to harbour sporozoites in the salivary glands, giving a total parous sporozoite rate of 5.3% for the study area and 100% for the site.

The overall sporozoite rate of *An. pharoensis* as determined by dissection in the parous and nulliparous population was 3.2% (2/63).

Table 18. Sporozoite and entomological inoculation rates of *An. Pharoensis* collected on human baits and CDC light traps in the Metchara area (July 1999-March 2000, July- September 2000).

Parameter	Site								Total
	Buse		Gelcha		Sugar Estate		Metehara		
	HB	CDC	HB	CDC	HB	CDC	HB	CDC	
No. dissected	11	0	17	0	31	0	3	1	63
No. parous (%)	3	0	2	0	12	0	1	1	19
	(27.2)		(11.8)		(38.7)		(33.3)	(100)	(30.2)
Parous infected (%)	0	0	0	0	0	0	0	1	1
								(100)	(5.3)
Nulliparous	0	0	0	0	1	0	0	0	1
infected (%)					(5.26)				(2.3)
Total infected (%)	0	0	0	0	1	0	0	1	2
					(3.23)			(100)	(3.2)*
Man biting rate	0.33	-	0.69	-	3.0	-	0.72	-	0.86
EIR	-	-	-	-	0.10	-	-	-	0.01

* =The sporozoite rate in mosquitoes caught by human bait was 1.6% and this figure was used to estimate the overall EIR.

4.8 Entomological inoculation rates (EIR)

The entomological inoculation rate (EIR) for *An. arabiensis* and *An. pharoensis* in two specific sites and the whole area was estimated by multiplying the mean human biting rate with the sporozoite rate (Tables 17 and 18).

The EIR due to the bite of *An. arabiensis* on the residents of Gelcha was estimated to be 0.06 infective bites/person/night. In Metehara town, the EIR was estimated to be 0.33. In the Metehara area, on average each resident was exposed to 0.05 *An. arabiensis* infective bites per night. The EIR estimate was made from the average human biting rates in the four sites and sporozoite rates of positive flies collected from human baits.

Similarly, the EIR for *An. pharoensis* was estimated for the whole area and the

Sugar Estate only (Table 18). Parasite infected *An. pharoensis* was collected from the human bait in the Sugar Estate. The EIR was therfore estimated to be 0.10 and 0.01 for the Sugar Estate and for the whole Metehara area, respectively.

4.9 Blood meal identification and HBI

4.9.1 Blood meal collections

During the study period a total of 453 blood meals of *An. arabiensis* was collected from the four study sites from human habitations, mixed dwellings, and animal shelters (Table 19). In addition, blood meals of 2 *An. pharoensis* (1 from Buse and the other from the Sugar Estate) and *An. ziemanni* (both from Buse) were collected.

4.9.2 Blood meal identifications

All of the blood meals were analysed by the double immuno-diffusion technique and the results of the blood meals of *An. arabiensis* are summarized in Table 19.

The human blood index of *An. arabiensis* in Buse resting in human dwellings and mixed dwellings as well as animal shelters was respectively 0.89, 0.73 and 0.33. The index in Gelcha in the three types of dwellings was 0.57, 0.42 and 0. Almost all of the specimens tested for their blood meal in the Sugar Estate and Metehara were positive for humans. The HBI in the study area by dwelling types was recorded as 0.78, 0.48 and 0.13. The overall HBI for the area was about 0.65.

Table 19. Results of blood meal analysis of *An. arabiensis* by double immuno-diffusion

Collection Sites	Total blood meal tested	Blood meal source and HBI	Dwelling types		
			human	mixed	animal shelter
Buse	192	human	135	27	1
		cattle	1	2	0
		mixed	6	3	0
		unidentified	10	5	2
		HBI	*0.89*	*0.73*	*0.33*
Gelcha	240	human	56	57	0
		cattle	14	26	2
		mixed	11	3	0
		unidentified	17	51	3
		HBI	*0.57*	*0.42*	*0*
Sugar Estate	15	human	14	0	0
		cattle	0	0	0
		mixed	0	0	0
		unidentified	1	0	*0*
		HBI	*0.93*	*0*	0
Metehara	6	human	6	0	0
		cattle	0	0	0
		mixed	0	0	0
		unidentified	0	0	*0*
		HBI	*1*	*0*	*0*
Overall	453	human	211	84	1
		cattle	15	28	2
		mixed	17	6	0
		unidentified	28	56	5
		HBI	*0.78*	*0.48*	*0.13*

One of the blood meal of *An. pharoensis* (caught from the Sugar Estate) was that of human. The blood meal of the other specimen of *An. pharoensis* collected from Buse could not be sufficiently identified. The abdomen of the two *An. ziemanni* was found to contain cattle blood.

4.10 Level of insecticide resistance in *An. arabiensis*

In all the tests, the field situations were similar and suitable. In most cases the room temperature ranged from 20^0C to 23^0C.

The results of the insecticide susceptibility tests are summarized on Table 20. Results obtained in this study revealed that the population of *An. arabiensis* was about 70% susceptible and 30% resistant to DDT. Similarly about 75% was susceptible and 25% resistant to permethrin. On the other hand, the study showed that *An. arabiensis* was highly susceptible to propoxur although the test was limited to two replicates due to shortage of impregnated papers. No control mortality was observed in all the tests.

Repeated attempts to test the progenies from the resistant females failed because the females could not oviposit eggs although they were repeatedly fed on human (self) and laboratory animals (guinea pigs and rabbits).

Table 20. Susceptibility status of *An. arabiensis* against three insecticides in the Sugar Estate, in the Metehara area.

Insecticide	WHO discriminating dose (%)	No. of replicates	Total no. exposed	No.dead	% susceptible	% resistant
DDT	4	4	80	56	70	30
Control	-	4	80	0	0	0
Permethrin	0.75	4	80	60	75	25
Control	-	4	80	0	0	0
Propoxur	0.1	2	40	38	95	5
Control	-	2	40	0	0	0

5. DISCUSSION

Malaria in the Metehara area prevails both in the wet and dry months showing the perennial transmission of the disease. But there was variation in the transmission intensity between the two seasons. The intensity was more aggravated in the wet months between June and October. A large number of malaria cases were reported in November. 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.

As in so many other parts of Ethiopia, malaria in Metehara was predominantly caused by *P. falciparum* followed by *P. vivax*. The two malarial parasites are known to be the major causes of mortality and morbidity, respectively. The overall faciparum cases constituted 19.2% of all malaria patients and vivax cases were 17.2%. The proportion of *P. falciparum* was 52.7% while that of P. vivax was 47.3%, showing the difference to be unaccountable. The prevalence of the two malarial parasites in the population was not determined. In Ethiopia it is known that 60% malaria is caused by *P. falciparum* and P. vivax account for 40% of cases (Tulu, 1993a). Ameneshewa (1995) noted that the prevalence of *P. vivax* in Gergedi in the Upper Awash Valley was low because of the high transmission of malaria in the wet months, which in effect raised the incidence of *P. falciparum*. In general, Ethiopia is reported to contain the highest proportion of *P. vivax* in the Afrotropical Region (WHO, 1991).

The appearance of vivax malaria in the dry months might be related to either relapses and or new infections emanating from the bite of infective mosquitoes. Similarly, the presence of falciparum infections in the dry season is an indication of the continuation of active transmission during this period. 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 Metehara was associated with irrigation practices in the sugar cane, orchard and vegetable farms owned by state and private enterprises. The existence of permanent vector 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. In the Sugar Estate and Gelcha, anophelines survived the dry months by colonizing permanent breeding sites. Adult vectors besides spreading malaria in the two sites, could migrate to nearby villages transmitting the disease. The vectors in the town, however, seemed to depend on rainwater pools and margins of Lake Beseka, which is normally, flooded in the rainy months. At this site, there was not any breeding habitats to support vectors in the dry months.

Of all the eight species, *An. arabiensis* was found in appreciable density. It was highly anthropophilic and infected with *Plasmodium*. The species bit humans both indoors and outdoors more frequently in the wet than the dry season. Although, it was caught biting humans in the dry months, its density was very low. The frequency of feeding in the two locations varied between the four study sites. Catches were made in light traps in large numbers in the wet months. The immature forms of the species invaded various breeding habitats in both seasons, and it mainly occurred during the dry season in the Sugar Estate and the villages around it.

Looking at the evidences from the present data, no other malaria vector is important than *An. arabiensis* in the Metehara area. 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 Coetze, 1987; Fontenille and Lochouran, 1999;

Coetze *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 infected with malarial parasites, but the biting and resting density of this species was very low in both seasons. This species can therefore be regarded as a secondary vector of malaria in the Metehara area as in other parts of Ethiopia (Nigatu *et al.*, 1992; 1994; Ameneshewa, 1995; Abose *et al.*, 1998b). The rest of the anophelines found in the area might have little or no role in the transmission of malaria. But, they remain as biting nuisances as they tend to feed on humans.

There was seasonal variation in the abundance of *An. arabiensis* both as larval and adult forms. This is influenced by climatological factors such as amount of rain, temperature and relative humidity. Aquatic larval habitats were suitable when there was deficiency rather than excess rain, which allows the formation of pools on the ground. Flooding of habitats in rainy months particularly in August dramatically decreased the density of both immatures and adults.

The larval breeding sites in the Metehara area included rain pools, irrigation canals, wastewater pools, pools of water from spillage of canals and shore of Lake Beseka. In the wet months, the species at the shore of the lake co-bred with the unidentified *Anopheles* species. Except the collection of few larvae during December at the shore site at the periphery of Metehara town (very near to a fuel station), *An. arabiensis* was absent at that breeding site during the dry months, while the other species continued to survive. But in March, immatures of *An. arabiensis* were collected from the southern side of the lake when a one-day rain probably caused flooding of the shore. Its' breeding in the wet season, therefore could be associated with the dilution of the water in the shore. In the dry season, the concentration of salt in the lake might increase to such an extent that, the species finds it difficult to survive. However this species is known to withstand some amount of salt

concentrations in The Gambia, where it is noted to tolerate water salinity of 3 g/lt (Lindsay *et al.*, 1993). It is therefore possible for *An. arabiensis* to tolerate certain degrees of salinity in Lake Beseka for which an extensive and systemic investigation around the lake is required. Some members of the *An. gambiae* complex are known to be salt-water species occurring in coastal areas of East Africa (*An. merus*) and West Africa *(An. melas*) (Service, 1993a).

The breeding habitats of *An. arabiensis* in the Sugar Estate particularly in the dry season are the sequel of human activities rather than environmental causes. Water accumulated from overflow of large canals, interrupted canals, marshy areas and sewerage remain the most important breeding sites created by human negligence. Apart from surviving the harsh environment, the dry season population might be the source of mosquitoes colonizing several sites in and around the Sugar Estate in the wet months. In Gelcha, marshy breeding habitats, formed from the spillage of canals equally served as breeding sites.

The larval habitats of *An. arabiensis* in the other parts of Ethiopia constituted sunlit rain pools, receding river beds, discarded tyres and containers, edges of lakes, side pools of rivers, marshs, 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 favour 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, and on humans attempting to bite or while they are landing. The largest catch was made on human baits; the contribution of the other methods was comparatively low. The human bait method 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 (Githeko *et al.,* 1996a). Moreover, the method is tedious, labour intensive, expensive and unacceptable as the intervention of collectors disturbs the privacy of residents (Mbogo *et al.,* 1993). This method is not far from bias arising from variations in human attractiveness to mosquitoes (Service, 1993c). Some people are more attracive to vectors than others (Knols *et al.*, 1995). 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 labour (Lines *et al.,* 1991; Costantini, *et al.*, 1998). On the other hand, reports indicated that catches from CDC traps are biased to contain more older females infected with malarial parasites (Lines *et al.,* 1991) and in some cases the efficiency of light traps was shown to depend on the density of vector species (Mbogo *et al.*, 1993). But the use of human bait protected by an insecticide free bed net and the position of the traps by the leg direction has greatly improved the catching efficiency of traps (Githeko *et al.*, 1994a; Costantini, 1998: Mboera *et al.*, 1998). At Gergedi, CDC traps were as efficient as the other methods and collected mosquitoes even in low densities (Ameneshewa, 1995). When light trap collections are used to estimate the human biting rate, it is vital to calibrate the trap catches with human bait catches (Lines *et al.,* 1991).

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. However, the types of chemicals used and the behaviour of the

mosquito influence the number of catches by this method (Service, 1993c). Endophagic but exophilic vectors are obviously underestimated by pyrethrum space sprays (Krafsur, 1977; Githeko *et al.*, 1996b).

In the study area, the density of mosquitoes in light traps in the wet season was fairly high. In the dry months, however mosquitoes entering traps were small, showing the density dependence of traps in Metehara. In addition, light traps were inefficient to catch outdoor frequenting mosquitoes including *An. arabiensis*. In Ethiopia, the capacity of light traps in the presence of bed net protected human bait to serve as a replacement to the human bait method requires further evaluation in selected areas with high and low mosquito densities.

An. arabiensis was caught from indoor resting sites by both space sprays and mouth aspirations, but there was variation in its density between the four study sites. In general, the resting density of *An. arabiensis* indoors in the study area, was lower in both insecticide untreated and treated villages particularly in the wet season compared to other localities in Ethiopia (Fontaine *et al.*, 1961; White *et al.*, 1980; Ameneshewa and Service, 1996). Eventhough, the species was caught indoors, the most productive months were August and September, and even in these months the average density was less than 45 flies/house/day in Metehara town. In the dry months, the density fell to zero inspite of the existence of the species as evidenced from larval and adult collections. In Ziway, the highest density from hand capture was estimated to be 7.23 flies per hut per day. The highest density (100-150 females per house per day) in Ethiopia was documented in the malaria epidemic year of 1958 (Fontaine *et al.*, 1961). White *et al.* (1980) reported that the indoor resting *An. arabiensis* reached to 100 per hut. In Kenya, more than 197 females of the same species per house were reported in the main mosquito season (Githeko *et al.*, 1996b).

An. arabiensis in Gelcha and Buse 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. In treated huts, in Buse, it preferred to rest on the untreated part of the wall, household utensils and clothes. In the Sugar Estate, the species preferred mud to brick houses for its resting site because the rough features of the former were more favourable than the smooth surfaces of walls and roofs of the latter. The brick houses were well sprayed by insecticides in which case mosquitoes find it difficult to rest on. Moreover, the paints of these types of dwelling walls exposed the mosquitoes to frequent disturbances by the act of cleaning the walls with sweeps and brush. As a result resting mosquitoes were low on such walls. Mosquitoes were even absent in brick houses where some inhabitants deliberately washed the insecticide. Some houses are also well illuminated.

The average hut density resting in the insecticide treated villages of Buse and the Sugar Estate was lower than the density in the untreated Metehara town and Gelcha. The difference can be attributed to either the insecticide or to the types of human dwellings. *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 (1996) 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 are practiced 37.5% of the population showed exophily (Ameneshwa and Service, 1996). In our study area, the exophilic population varied from 18% in Gelcha to 69.4% in the Sugar Estate, showing higher exophily in insecticide treated villages. The increased exophilic habit of

this species is a constraint to control it by indoor application of chemicals (Coetzee *et al.*, 2000) or by the use of impregnated bed nets indoors.

Fresh fed females of *An. arabiensis* comprised the highest number in space spray 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 54.5% in sprayed huts.

Despite the high exophilic rate of *An. arabiensis* in the study sites, few outdoor resting sites were located. Animal shelters in Buse and Gelcha were the main resting sites. Tree holes and excavated burrows represented outdoor resting sites in the Sugar Estate. The species in Metehara town might rest among rocks, crevices and small caves in volcanic rocks. It was, nevertheless difficult to search mosquitoes at these sites. There were no pit latrines, ditches and burrows made by animals and humans in Buse and Gelcha that could be potential resting outdoor sites. The vegetation could serve as one but inspite 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), where as, in Kenya granaries were noted to be major outdoor resting sites (Githeko *et al.*, 1996b).

An. arabiensis demonstrated behavioural variation in its indoor and outdoor human biting activities in the study sites. The density of the endophagic population was higher than the exophagic in Buse and Metehara town, while the reverse was true for the Sugar Estate and Gelcha. The results from Buse were not expected in view of the insecticide sprays. In Metehara town there was increased indoor feeders and in Gelcha the exophagic population was higher than the endophagic inspite of the interruption of insecticide sprays for the last

four years. A study in Kenya confirmed that individuals of this species carry indoor and outdoor genetical traits (Githeko *et al.*, 1996b) implying the role of inherent genes in the indoor and outdoor behaviour.

Indoor insecticide sprays have an excito-repellency effect on the indoor biting mosquitoes (Pant *et al.*, 1981). Mosquitoes tend to develop behavioural avoidance of human dwellings sprayed with insecticides. As the result, the density of the outdoor feeders exceed that of the indoor. But, in Buse the situation was different, the endophagic population was higher. This could be attributed to the inefficient coverage of insecticide sprays at this site. In the Sugar Estate, the effect of insecticide sprays have been demonstrated by decreasing the indoor resting and biting densities of *An. arabiensis* and *An. pharoensis*.

Even though data is not available on the biting density of this species before and after insecticide sprays in Gelcha to compare with the results produced in this study, it can be speculated that the four-year interruption of the spray operation, has no or little effect in the density of indoor feeding population. It is obvious that the endophagic population out number those of the exophagic before the operation of insecticide sprays. The fact that increased biting rate indoors in Metehara town where large scale insecticide sprays for mosquito control have never been performed, support the above statement. In this site, the proportion of the indoor biting population was higher than that of the outdoor. The sprays in Buse were not sufficient enough to induce changes in the biting cycle and indoor feeding habit.

The resultant effect of frequent indoor application of insecticides is to reduce the abundance of endophagic and endophilic species. The sequel in some cases is the colonization by exophilic anophelines, which have secondary roles in the transmission of malaria. The reduction in abundance or elimination of the major vector permits secondary

vectors to maintain infection efficiently (Wilkes *et al.*, 1996). In East Africa, the control of *An. funestus* led to the upsurge of one of the *funestus* group, *An. rivolurum* Leeson (Gillies and Smith, 1960). Recently, this species was found to carry malaria infections in Tanzania (Wilkes *et al.*, 1996). In West Africa, the population density of *An. rufipes* was noted to increase during the period of chemical interventions, while that of *An. gambiae* s.l and *An. funestus* decreased appreciably (Molineaux and Gramaccia, 1980). In Metehara, despite insecticide sprays for a number of years, *An. arabiensis* still remained dominant in abundance and biting rate. The second abundant anopheline was *An. pharoensis*, but it has never outnumbered *An. arabiensis*, even in the dry months as it has been documented in the Zeway area (Abose *et al.*, 1998b; Aklilu Seyoum *et al.*, IPB unpublished data). Agricultural pesticides or altitude could have affected the survival of *An. pharoensis* outdoors in Metehara. There are altitudinal differences between Metehara and Zeway.

The density of man biting *An. arabiensis* varied between the major seasons, being higher in the wet months between July and September. Higher biting rates also recorded in the unsprayed sites (Metehara and Gelcha). In contrast, higher man biting rates of *An. pharoensis* in outdoor locations was observed in the Sugar Estate. Malaria transmission took place both in the months of higher vector densities and in the months following them despite the reduced number of the man biting population.

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 dilatation counts are made based on the number of ovipositions completed, and age of the individual mosquito is determined by the number of dilatations as 1-parous, 2-parous, etc. The number of ovipositions so far recorded for *An. gambiae* s.l is six (Detinova and Gillies, 1964). Faced by the difficulty in ovarian dissection, the oldest female detected in the Metehara area in this study was three-parous. Parous females are epidemiologically

dangerous because these are the mosst important to carry malaria parasites to humans. They acquire malaria infections from repeated blood meals.

The composition of parous females of *An. arabiensis* in the four sites showed seasonal as well as site variation, but the seasonal difference was not significant. Lower parous rates (28.6%) were observed in Metehara Town and higher (53.8) in Buse. However, the overall proportion was low. In Metehara town, there was a significant difference in the rate of parity in the population of *An. arabiensisa* active indoors from that of the population found outdoors. The occurrence of such difference is not clear. Parous rates in other localities are reported to be low (Abose *et al.*, 1998b). Ameneshewa (1995) found that the proportion of parous females of this species exhibited seasonal variations being greater in the wet season than in the dry season.

In all the study sites the parous proportions of *An. pharoensis* was low similar to other observations (Rishkesh, 1966; Mekuria, 1983; Ameneshewa; 1995; Abose *et al.*, 1998b). The lower parous ratio in this species is an indicative of the short life. Such flies are less dangerous than with longer survival rates.

In its night biting habit in the study site, *An. arabiensis* was observed to be active all night, with two to three peaks. In the early hours before 21: 00, the number biting both indoors and outdoors was low, and increased progressively there after. But still there were oscillations, at times the biting population increased and other times it decreased. Most activity was shown to be between 22:00-2:00, and a small increased activity also took place between 03:00-04:00 hours. In Zeway the species was found to feed at the early evenings indoors and between 22:00 and midnight outdoors. Small peaks were also observed before dawn (Abose *et al.*, 1998b). At Gergedi, the species frequented outdoors at the early hours of the night (Ameneshewa, 1995). In contrast to these two reports, the species in Sudan has

its peak biting between 04:00 and 05:00 (Mustafa and Dukeen, 1986) and in Senegal between 03:00 and 06:00 (Fontenille *et al.*, 1997).

Mosquitoes tend to shift their feeding time due to behavioural changes induced by exposure to interdomicillary residual sprays and insecticide impregnated mosquito nets. The impact of the latter was shown in *An. farauti* in New Guinea (Charlwood and Graves, 1987 cited in Magesa *et al.*, 1991) and *An. gambiae* s.l in Tanzania (Magesa *et al.*, 1991). *An. arabiensis* in Tigray, north Ethiopia was noted to feed in large numbers at the early hours outdoors to avoid contact of insecticide treated walls (Dr. Lindsay, Durham University, UK, personal communication). Ameneshewa (1995) argues that insecticide sprays at Gergedi are responsible for the early shift of biting habit of *An. arabiensis*. All such observations seem to lead to the conclusion that, the Ethiopian *An. arabiensis* selected by insecticide pressure has shifted its biting activity. But earlier studies in Sodere showed that the species was more active before midnight (Jolivet, 1959). At the time of the report from Sodere, the selective pressure of indoor sprays was comparatively small. Our findings in all the sites showed more *An. arabiensis* being active indoors after midnight. Outdoors, in the Sugar Estate and Metehara town the number was higher before midnight. In Buse and the Sugar Estate, higher number of parous females indoors was recorded before midnight suggesting the tendency of older flies to feed more frequently at the early ours. But this needs further observation. There was no significant difference in outdoor sites in the frequency of parous females before and after midnight in all the sites. Thus the data does not support the effect of insecticide sprays in changing the biting time of *An. arabiensis* in the Metehara area.

Humans were exposed to the bites of *An. arabiensis* in all times of the night. The people including children in the study area were active until midnight, playing out in the environs of their houses and also engaged in other activities. In the Sugar Estate people

watch TV in groups in the open until midnight. Most of the residents in Buse and Gelcha sleep outdoors either in the open or in small circular shelters open from the top. Similarly, it is common to sleep outdoors on vehicles and other locations in Metehara town. Even if people in Buse and Gelcha rest indoors, these locations are risky since the residents were exposed to a number of bites. The openings of the walls and doors are less effective barriers for the entrance of mosquitoes. Farmers also spent nights in their farms scaring animal pests. At times in the middle of the night, people were also active irrigating their farms. Obviously, such activities also expose people to mosquito bites. There is evidence that indicate *An. arabiensis* adjusting itself with the changing habits of its human hosts (Shidrawi, 1970). This might be cited as the cause in the increment of the frequency of the outdoor feeders in Gelcha in addition to the previous effect of insecticide sprays as well as its inherent partial exophagic behaviour.

Furthermore, keeping animals in separate shades and human habitations might expose residents of Gelcha and Buse to frequent bites. In the presence of both cattle and humans, the species could be diverted to feed on humans. In Zeway, *An. arabiensis* was found to be attracted more to humans in mixed dwellings, eventhough both humans and cattle are equally available (Aklilu Seyoum *et al.*, IPB unpublished data). Ameneshewa (1995) also reported similar finding in Gergedi. In Pakistan, cattle deployment near or in human habitations exposed humans to increased bites of *An. stephensi* and other zoophilic mosquitoes (Hewitt *et al.*, 1994), and the number of malaria infections was higher in people who allowed cattle to live in close proximity to human dwellings (Bouma and Rowland, 1995). In west Africa, the frequency of animals was higher in the compounds of severe malaria cases (Adiamah *et al.*, 1993). In contrast, based on the blood meal analysis from mixed dwellings from five localities in south, east and west Ethiopia, Hadis *et al.* (1998)

argued that the presence of cattle near humans protect people from mosquito bites and malaria infections.

The sprozoite rates detected in *An. arabiensis* in the three sites (0.4-0.84%) and in the whole study area (0.46%) were low, in agreement with other studies in Ethiopia (e.g. Nigatu *et al.*, 1992; Ameneshewa, 1995; Abose *et al.*, 1998b). Similarly, 3.2% *An. pharoensis* was found infected. Besides undermining the number of infected mosquitoes, the dissection method, does not allow the identification of the species of infective *Plasmodium*. In Ethiopia, the application of ELISA in some studies, has improved detection of the *Plasmodium* species, but the frequency of the sporozoite rate is not increased by using this method (Nigatu *et al.*, 1992; Ameneshewa, 1995; Abose *et al.*, 1998b; Hunt *et al.*, 1998).

Sporozoite infected *An. arabiensis* were originated from indoor collections. Even though the number of positives was small, our observation agrees with the results of Molineaux and Gramaccia (1980) in which sporozoite rates in *An. gambiae* s.l and *An. funestus* in indoor collections was shown to exceed those of the outdoor. In Metehara, two of the three positive specimens from night biting collections were parous females caught after midnight, indicating the tendency of infected flies to bite late at night. This also goes in agreement with the report of Bockarie *et al.* (1996) which showed *P. falciparum* sporozoite laden *An. punctulatus* in Papua New Guinea and *An. gambiae* in Sierra Leone parous females to bite humans at later hour than nulliparous ones. In this report, however the majority of *P. vivax* infected anophelines appeared to bite between 18:00 and 21:00 hours. An earlier study by Hamon *et al.* (1961) on *An. gambiae* complex in Burkina Faso also indicated the preference of parous flies to feed later than nulliparous females. These observations show that humans active outdoors during pre-bed time are less likely to be exposed to infected mosquitoes. Nevertheless, in the late hours, humans can protect

themselves from mosquitoes by using insecticide impregnated bed nets (Bockarie *et al.*, 1996). This justifies the protective values of bed nets against malaria in endemic localities.

Sporozoite infection was also detected in a nullipar *An. arabiensis* in Metehara. Eventhough one may not expect nulliparous females to be infected, many studies have described infections in these age groups. In Tanzania, 3.7% infection rates in nulliparous females of *An. gambiae*, *An. arabiensis*, *An. merus* and *An. funestus* was reported (Temu *et al.*, 1998). Under natural conditions infection in nulliparous females occurs when females require more than one blood meal to develop eggs (Gillies and De Meillion, 1968). The term 'gonotrophic discordance' is coined to females, which take repeated blood meals before oviposition (Service, 1993c). This situation is common in afrotropical malaria vectors and other old world mosquitoes (Service, 1993c) and is associated with endophily and the availability of human blood as one source of nutrient (Lyimo and Takken, 1993). Gonotrophic discordance occurred in 20% *An. gambiae* in Tanzania (Lyimo and Takken, 1993) and 42% in west Africa (Brengues and Coz, 1973 cited in Lounibos *et al.*, 1998). In addition, 63% of populations of *An. funestus* in west Africa were composed of nullipars that seek multiple blood meals. In that region, the occurrence of gonotrophic discordance is more common in the mosquito population in the dry than in the wet seasons (Molineaux and Grammicia, 1980).

In our laboratory, repeated attempts to establish a thriving colony of *An. arabiensis* from Metehara has failed because F_1 and subsequent females failed to oviposit eggs even after 3-5 full blood meals on either human or laboratory animals at intervals of 3-4 days. The reasons were not immediately clear in the presence of an apparently suitable condition in our insectary or in the presence of an established colony of the same species in an insectary in Nazareth of the Ministry of Health. However, the observation in our laboratory confirms that such phenomenon is also not uncommon in the field and that nullipars can

carry sporozoites in their salivary glands and be epidemiological important, although to lesser extent.

The entomological inoculation rates estimated for *An. arabiensis* in the main transmission season as well as the whole period for Gelcha and Metehara town were different; being lower in Gelcha than Metehara. The difference is related to the MBR and sporozoite rates, which were higher in the latter than the former. The MBR is affected by vector abundance and vector- human contact while the sporozoite rate is influenced by vector survival rate and the length of extrinsic incubation period of the parasite (Onori and Grab, 1980). These important factors are themselves influenced by meteorological variables such as rainfall, temperature and humidity (Onori and Grab, 1980). The meteorological variations that existed between Gelcha and Metehara town are not considered important to bring changes in aspects of vector abundance, survival and extrinsic incubation. In fact, the presence of productive breeding sites in the latter site particularly at the edges of the lake can be attributed to the higher abundance of the vector during the wet seasons.

The absence of infected *An. arabiensis* from human bait catches in Buse and the Sugar Estate made it impossible to estimate EIR. It would have been interesting to compare the EIR of these two sites (where control intervention is undergoing) with the other two sites (with no major control measures), so that the effect of insecticide sprays on the malaria transmission intensity can be correlated.

The daily EIR for *An. pharoensis* in the Sugar Estate was as low as 0.1 infective bites. There were no infected females from the other sites to estimate EIR.

In general, estimates of the EIR for both *An. arabiensis* and *An. pharoensis* in the Metehara area were less than 1 infective bites per person per day and are lower than the infective bites received by residents of Gambela (Krafsur, 1977), suggesting low malaria transmission intensity. However, the figures might not represent the actual picture in view

of the high number of malaria cases in Metehara. EIR estimates are affected by the catch of the biting population and the detection of parasite infected mosquitoes in that population. The method of detection influences the number of sporozoite infected flies. In our study, the dissection method would obviously be expected to underestimate this number, thereby decreasing the rate of the EIR.

The association of EIR and malaria transmission intensity in a locality can be shown by entomological studies coupled with parasitological studies in children less than 5 years (Bier *et al.*, 1994). Due to limitations in the capacity of the researcher, incidence of the disease among children was not studied and hence it was not possible to correlate the EIR with malaria transmission intensity in Metehara.

In Africa, few studies demonstrated the relationship of EIR with mortality due to malaria in children less than five (Snow and Marsh, 1995). The data showed that the relationship between the two was not strictly linear. In one study relating EIR with disease severity, Mbogo *et al.* (1995) in Kilifi, Kenya showed little association between the two. However, in another study in the same country but in a different locality a close relationship between the incidence of infection and EIR per day was found (Bier *et al.*, 1994). In general, many studies suggest the occurrence of lower incidence of disease in communities with low EIR compared to communities with higher EIR (Greenwood, 1997).

The goal of vector control efforts is to decrease vector abundance and man-vector contact, which have a direct effect on the level of malaria transmission (WHO, 1995). In many African countries the implementation of impregnated bed nets resulted in the reduction of the frequency of EIR as low as less than one infective bite per person per night (malaria transmission intensity workshop document, Ouagadugou). The intensity of human-vector contact can be indicated by the human blood index from tests of mosquito blood

meals. Knowledge in this aspect is essential for effective control of malaria vectors and ultimate decrease of the EIR.

The blood meals were detected by the double immuno-diffusion. As a blood meal testing technique, the double immuno-diffusion is found affordable (WHO, 1995) because it demands few equipment, chemicals and reagents. Moreover, its simplicity made it suitable and attractive for routine application, and therefore, can be used alternatively with ELISA and other methods currently in use for mosquito blood meal identifications. With the aid of this method, Crans (1969) efficiently tested 10,000 blood meals of mosquitoes.

As elsewhere in Ethiopia and other countries, the blood meal profiles of *An. arabiensis* in Metehara showed the propensity of the species to feed largely on both humans and cattle (White, 1974). To some extent other animals whose blood was not identified also served as sources. There was variation in proportion of mosquitoes containing human blood in time and place. Where there are humans only or in large number as compared to the number of bovids in Metehara town, the Sugar Estate, and to some extent in Buse, most of the blood meals of *An. arabienis* were of human origins. Krafsur (1971) documented 100% HBI in *An. gambiae* s.l (latter reported as *An. arabiensis* (Krafsur and Armstrong, 1978)) from collections of human dwellings in a region where there were no animals except chickens and dogs. In contrast, there were several cattle in Gelcha and the HBI was comparatively lower.

The results of various studies in Ethiopia indicated that *An. arabiensis* collected from human dwellings, had considerably fed on humans, and those from mixed and cattle sheds largely took blood of cattle and other animals. In a previous study in Jimma area, the highest human fed mosquitoes originated from human habitations followed by mixed dwellings, animal sheds and pit shelters (Garrett-Jones and Boreham (1980). Similarly, *An. gambiae* s.l (where both *An. arabiensis* and *An. quadriannulatus* are known to co-exist)

from a similar ecotype was reported to contain 100% HBI in human dwellings and nil in animal shelters and outdoors (White *et al.*, 1980). The same authors indicated 46 % of the mosquitoes in mixed dwellings fed on humans. In contrast, Adugna and Petros (1996) documented highest HBI proportions in *An. arabiensis* caught in mixed dwellings. Other studies by Hadis *et al.* (1997) and Lulu *et al.* (1998) showed lesser rates of HBI in mixed dwellings compared to specimens collected from human dwellings similar to the present finding. Similarly, the HBI from specimens in animal shelters, though few in number were lower as was also observed by Hadis *et al.* (1997), Adugna and Petros (1996) and Lulu *et al.* (1998).

There were higher proportions of mosquitoes fed on bovine in Gelcha compared to Buse, and this is related to the larger number of cattle in the former site. This shows *An. arabiensis* to be opportunistic in its feeding behaviour depending on the availability of host.

Some of the tested blood meals could not be identified either because of the poor quality of the blood meals (may have aged) or might belong to animals other than man and cattle. *An. arabiensis* does not restrict itself to humans and bovine, rarely it is known to feed on horses, donkeys, sheep, goats and birds (White *et al.*, 1972; Githeko *et al.*, 1994b).

Catches of human fed mosquitoes from mixed dwellings and bovine fed ones in human dwellings have been made and this is associated with the mosquitoes' habit of seeking resting shelter at different biotopes. Mosquitoes that have fed at one place might rest in another. The feeding and resting sites are subject to change depending on the behaviour of the mosquito and the existing environmental condition that has a direct influence on the general activity of the mosquito. This means that the blood meal analysis results based on mosquitoes collected from different dwelling types might not exclusively correspond with the type of host found in the collection site, but rather show the relative number compared to other sites.

The other important component of this study was the evaluation of the level of susceptibility/resistance of *An. arabiensis* of the study area to three representative insecticides in the three major groups of chemical insecticides, notably chlorinated hydrocarbons (DDT), pyrethroids (permethrin) and carbamates (propoxur). In four replicate tests, the mortality rate of *An. arabiensis* after one hour of exposure to 4% DDT impregnated papers and 24 hours of recovery was 70%. In a similar test with 0.75% permethrin, the mortality rate recorded was 75%. Thus the study indicates that part of *An. arabiensis* in the area had already developed physiological resistance to both insecticides. From the observations, it may be concluded that 30% of *An. arabiensis* is resistant to DDT and 25% resistant to permethrin. However, the levels of resistance detected to both insecticides is epidemiologically acceptable, despite several years of indoor spraying of DDT in the area. The levels of DDT resistance detected in other parts of the country are also moderately low (Ameneshewa, 1995; Abose *et al.*, 1998b) except in ArbaMinch and Gambela, where high levels of DDT resistance (60-76%) were detected (Abose *et al.*, 1998b). A few years ago 16 % resistance to DDT was reported in Metehara (Abose *et al.*, 1998b).

On the other hand, the test with the third insecticide, propoxur produced a very high level of mortality rate (95%), showing *An. arabiensis* to be highly susceptible to this insecticide.

No information is available on the application of pyrethroids or carbamates for agricultural and indoor vector control purposes in the Sugar Estate and in the Metehara area in general. There are also no published or unpublished records of susceptibility level studies with either any pyrethroids or carbamates elsewhere in Ethiopia to compare the results obtained here. Actually, susceptibility tests with propoxur might evaluate indirectly the level of resistance to malathion, an organophosphate, since the two groups are known to

share similar mode of action on the nervous system of insects. Malathion is an alternative choice of insecticide in some localities where DDT resistance is recorded (Abose *et al.*, 1998b). The high record of mortality due to propoxur is thus indicative of susceptibility to malathion in the Sugar Estate. Else where in Ethiopia, the species is reported to be 100% susceptible to malathion (Ameneshewa, 1995). Hence, this insecticide is recommended to replace DDT where mosquitoes develop resistance to DDT (Abose *et al.*, 1998b). The moderate level of resistance to DDT in the population of *An. arabiensis* in the Sugar Estate still warrants the further utilization of the chemical in the area as long as the negative attitude of the residents to the chemical is altered and the monitoring of the susceptibility level is undertaken from time to time. The benefits can extend to the other villages in the vicinity. There is yet no strong ground to disregard this chemical for vector control in Metehara area.

However, in the global context, DDT is a point of discussion between environmentalists and vector control scientists, the former aggressively seeking its ban in all countries following the footsteps of the United States of America, some European countries and Latin America. But concerned scientists argue against this option on the ground that developing countries need the chemical to reduce the burden of malaria, since vector control by indoor spraying of DDT is more successful and relatively cheaper than other control methods (Curtis, 1994; Curtis and Lines, 2000). Cost comparative studies showed that indoor spraying of DDT is cheaper and more affordable by most developing countries (Curtis and Lines, 2000). It is usually stated that DDT affects life in water, soil and air, and it is more persistent in the environment and also gets into food webs. But the reviews by Curtis (1994) and Curtis and Lines (2000) indicated that the evidences presented to ban the chemical are not convincing. Moreover, its impact on human health due to indoor sprays is not sufficiently confirmed (Curtis, 1994). Even if its removal is highly desired,

there should be alternative chemicals in the market, which are equally effective and affordable (Curtis and Lines, 2000).

Of particular interest in this study is the appearance of permethrin resistant *An. arabiensis* population in the Upper Awash Valley. The resistant group accounted for only 25%, and this may not be a serious problem at the moment, but poses a serious concern for a country that has never used any of the pyrethroids in large scale vector or agricultural pest control. Resistance to the pyrethroids also remains a serious challenge in view of the desire to impregnate bed nets with pyrethroids. Thus, further evaluations of these insecticides in other parts of the country are highly required.

For malaria vector control, pyrethroids are mainly used to impregnate bed nets, curtains and other fabrics. Concerned with the appearance of resistant vectors to these chemicals, some workers suggest making use of other compounds in mixtures safe to the user (Curtis *et al.*, 1998).

Some reports show that the west African members of the *An. gambiae* complex are developing resistance to pyrerhroids (Chandre *et al.*, 1999). Inaddition, the reviews by Malcolm (1988) and Curtis *et al.* (1998) noted several other anophelines acquiring resistance to this group of chemical. The appearance of pyrethroid resistant vectors is a great concern in view of the absence of alternative insecticides with similar/different mode of action.

In West Africa, pyrethroid resistance in *An. gambiae* s.s is associated with cross-resistance to DDT, various pyrethroids and etofenprox (Curtis *et al.*, 1998). *kdr* gene mutations that are results of selections of insecticides are responsible for the reduced knock down and lethal effects of pyrethroids (Miller, 1988; Martinez-Torres *et al.*, 1998). So far, the genes are not found in *An. arabiensis* (Chandre *et al.*, 1999). Pyrethroid resistance in

other anophelines such as *An. sacharovi* in Turkey and Syria also arises from selections of organophosphates (Curtis *et al.*, 1998).

The appearance of pyrethroid resistance in our study area can be attributable to the use of DDT which can selectively induce the resistance in *An. arabiensis*, or the resistance may arise from household aerosol usage as shown elsewhere (Elissa *et al.*, 1993 quoted by Chandre *et al.*, 1999). Aerosols are intensively used in Metehara and elsewhere in Ethiopia to eliminate household pests including mosquitoes, and perhaps one or both factor(s) stated above played significant roles for the development of resistance to permethrin. Further investigation is required in other areas specifically where higher DDT resistance are reported, such as in ArbaMinch and Gambela (Abose *et al.*, 1998b). Such areas would probably provide information on the inheritance of pyrethroid resistance. It calls also to apply molecular techniques to detect genes that confer resistance (*kdr*), and find means to manage resistance in this important vector of malaria in Ethiopia.

Indoor application of DDT and other insecticides have been the mainstay vector control approach in tropical Africa. Much interest is now centered on the use of impregnated bed nets and curtains (e.g. Snow *et al.*, 1988; Alonso *et al.*, 1991) and have been successful in decreasing morbidity and mortality. Nevertheless, this method has not effectively interrupted transmission.

6. SUMMARY AND RECOMMENDATION

1) Existing knowledge of malaria and the anopheline vectors in Ethiopia is reviewed. Malaria is highly prevalent in the lowlands but occasionally it occurs as an epidemic in the highland regions. The disease is mainly caused by *P. falciparum* followed by *P. vivax*. Two members of the *An. gambiae* complex occur in Ethiopia, of which *An. arabiensis* is the principal vector. Other species, *An. pharoensis, An. funestus* and *An. nili* are secondary vectors in different foci. Information on distribution, vectorial status, host preference, behaviour and insecticide susceptibility of the anophelines in the economically important areas of the country is limited.

2) The present study was carried to observe the ecology, behavior vectorial capacity, host preferences and insecticide susceptibility of anophelines which have significance in the transmission and control of malaria in Metehara. The study also included review of malaria records in the local health centers.

3) The parasitological data showed that *P. falciparum* and *P. vivax* were the two important plasmodia causing malaria in the Metehara area in all months of the year.

4) Larval collections from rain pools, shore of Lake Beseka, canal spills, sewerage, canal and swampy areas revealed the presence of four species (*An. arabiensis*, *An. pharoensis, An. coustani* group and other *Anopheles* species not yet identified). The predominant species was *An. arabiensis* followed by *An. pharoensis*. In some of the breeding sites the two species were found co-existing both in the wet and dry seasons.

5) A total of 3639 adult anophelines representing eight species (An. *arabiensis*, *An. pharoenssis, An. tenobrosus, An. coustani, An. ziemanni, An. ardensis, An. natalensis* and unidentified *Anopheles* species) were collected using human bait, CDC light traps, aspirator

and space spray methods. In all collections *An. arabiensis* remained the dominant species, *An. pharoensis* being the next abundant.

6) The density of *An. arabiensis* resting indoors was higher in the insecticide untreated villages than the treated. There was seasonal variations in the resting density, the highest being between August and October. The degree of exophily of *An. arabiensis* was estimated to be 18%, 21.3%, 66.1% and 69.4% in Gelcha, Metehara town, Buse, and the Sugar Estate, respectively.

7) The night biting catches of *An. arabiensis* showed that this species was more endophagic in Buse and Metehara and more exophagic in the Sugar Estate and Gelcha. There was site as well as monthly variations in the ratio of indoor to outdoor biting population. The highest average man biting rate of *An. arabiensis* was recorded in Metehara and the lowest in Buse. Intense man biting occurred in the wet months. *An. pharoenis* in all the sites was exophagic and its man biting rate in general was low. *An. arabiensis* exhibited two to three peaks of biting activity during the night but most biting took place indoors and after midnight. *An. pharoensis* was more active outdoors before midnight.

8) The parous rate of *An. arabiensis* ranged from 28.6% in Metehara to 53.8% in Buse: the overall was 45.1%. Similarly, the parous rate of *An. pharoensis* was lowest in Gelcha (11.8%) and highest in the Sugar Estate (38.7%), the overall being 30.2%, indicating that this species is short-lived as compared to *An. arabiensis*. Thus, *An. pharoensis* has a lower vectorial capacity than *An. arabiensis*. The sporozoite rate of *An. arabienisis* was 0.77% in parous and 0.21% in nulliparous females; the combined average infection rate being 0.46%. Similarly, infection rates of 5.3 % and 2.3% were detected in parous and nulliparous populations of *An. pharoensis,* respectively, with a total rate of 3.2%.

9) The daily EIR of *An. arabiensis* in Gelcha and Metehara was 0.05 and 0.33,

respectively: no such values could be calculated for the other sites since infection was not detected. The overall EIR was estimated to be 0.05. The EIR of *An. pharoensis* in the Sugar Estate was 0.1 while the overall was 0.01.

10) The HBI of *An. arabiensis* in the four sites was: 0.85 in Buse, 0.47 in Gelcha, 0.93 in the Sugar Estate and 1 in Metehara, the overall being 0.65. This shows that a significant population of *An. arabiensis* also obtains its blood meal from cattle. The HBI of *An. arabiensis* from samples collected in human, mixed and animal shelters was 0.78, 0.48 and 0.13, respectively, showing that the species either feeds on cattle or human depending on availability.

11) Insecticide susceptibility status of *An. arabiensis* indicated that 30% and 25% of the test population of the species was resistant to DDT and permethrin, respectively. The level of resistance is not epidemiologically significant and both insecticides can still be useful for indoor application. In addition, the pyrethroid can also be used for impregnation of bed nets. The species was highly susceptible to the carbamate insecticide, propoxur.

12) The present study has elucidated that *An. arabiensis* and *An. pharoensis* the most important vectors in the Metehara area. Hence all vector control must be targeted against these species.

It is shown that *An. arabiensis* has developed moderate level of resistance to DDT. This justifies the usefulness of this chemical for indoor application to control malaria vectors in Metehara. The chemical can perhaps be used by alternately applying propoxur or malathion to reduce the resistant population. Similarly, pyrethroids can either be used for indoor spraying or for impregnating bed nets, curtains and other fabrics. However, frequent monitoring of the susceptibly levels of the local vectors is required. The health authorities in the Sugar Estate should communicate and convince the residents to cooperate with the Metehara Malaria Control Sector to spray their houses.

The Sugar Estate is considering insecticide impregnated bed nets as one of vector control measure in addition to indoor residual sprays and the Hospital has already started to distribute the materials in some of the camps (Ato Alemu, personal communication). The present data, therefore can serve as a baseline in this venture.

However, the control of vectors has been a challenge in terms of cost, insecticide resistance and availability of suitable control methods applicable to a particular area. Thus, integrated vector control remains the preferred approach. There is a possibility to include environmental management in conjunction with chemical methods in the control of vectors of malaria in Metehara area. Environmental management involves the elimination and control of potential breeding sites. The participation of the community in this aspect through education and training is very crucial. Furthermore, the public should be aware of the side effect of washing insecticides off the walls, plastering of sprayed walls with mud or cattle dung, polythene sheets which otherwise reduce the vector-insecticide contact.

Future research should be geared towards detecting insecticide resistance particularly pyrethroids in the main vectors in the economically important regions. Besides using the WHO discriminating dose, effort should be put to employ molecular techniques to detect genes that confer resistance in mosquitoes.

In Ethiopia it is necessary to correlate EIR and disease intensity with follow up studies in children less than 5 years old. The Metehara area is an idle place to conduct research in this aspect.

Further research is required to evaluate the advantages and disadvantages of the deployment of cattle in and near human dwellings.

Rearing *An. arabiensis* in the laboratory is a constraint to carry out further studies on this important vector. Repeated attempt is necessary to establish the colony.

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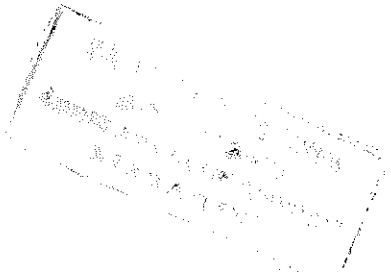

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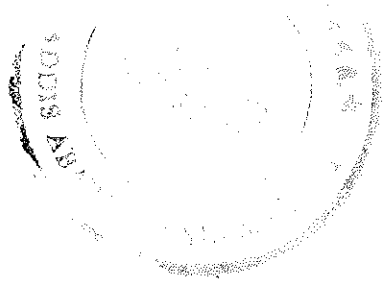

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Appendix I. List of current anopheline species found in Ethiopia.

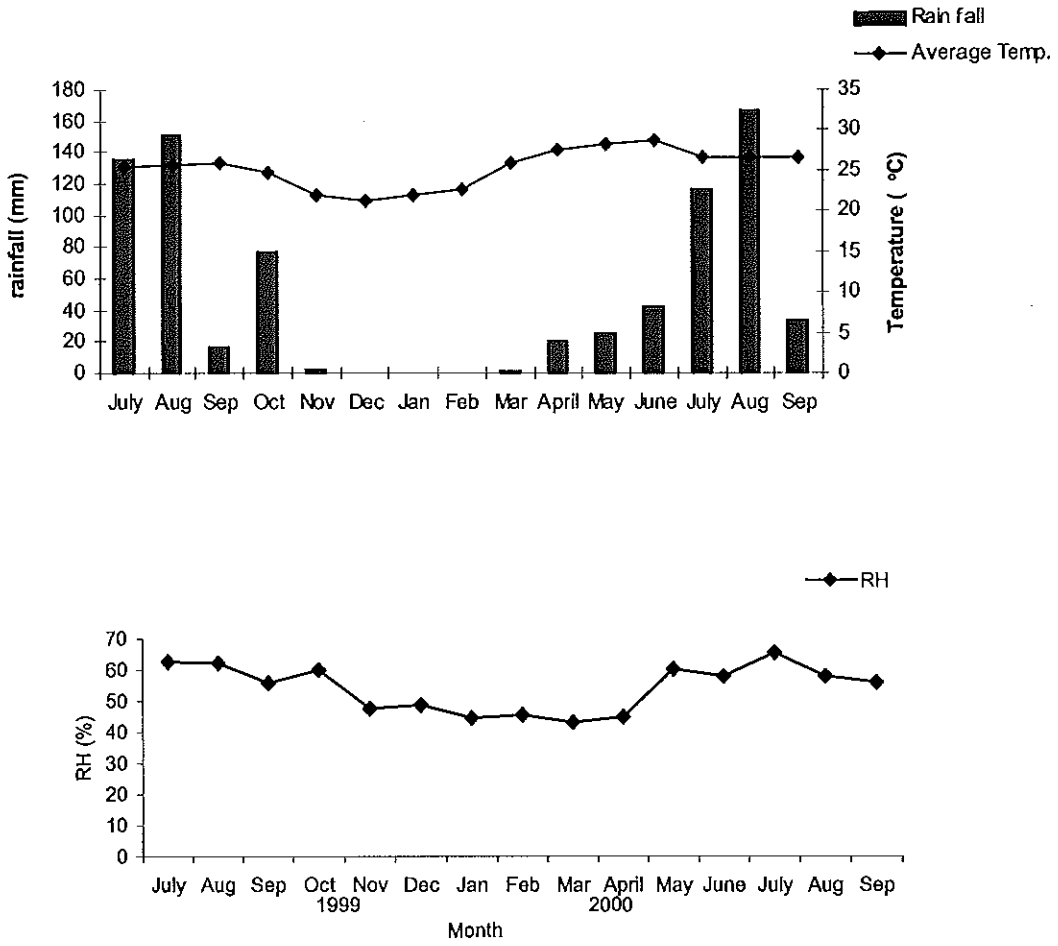
Anopheles arabiensis	*An. gibbinsi*	*An. pretoriensis*
An. quadriannulatus specis B	*An. hargreavsi*	*An. rhodesiensis rhodesiensis*
An.ardensis	*An. harperi*	*An. rhodesiensis rupicolus*
An. christyi	*An. implexus*	*An. rivolurum*
An. cinereus	*An. kingi*	*An. rufipes*
An. confusus	*An. leesoni*	*An. salbii*
An. coustani	*An. longipalpis*	*An. sergenti macmahoni*
An. cydippis	*An. maculipalpis*	*An. seydeli*
A. dancalicus	*An. marshalli*	*An. squamosus*
An. demeilloni	*An. natalensis*	*An. tenobrosus*
An. domiculus	*An. nili*	*An. theilleri*
An. d'thali	*An. obscurus*	*An. turkuhudi*
An. funestus	*An.paludis*	*An. wellcomi*
An. garnhami	*An. pharoensis*	*An. ziemanni*

Appendix II. Sprozoite rates in *Anopheles* mosquitoes in different localities Ethiopia.

Locality and year	Vectors	Techniques	No dissected/ processed	Sporozoite rate (%)	Reference/source
Kobbo-Cherecher, 1959	*An. gambiae* s.l*	Dissection	?	3	Jolivet (1959)
The lake regions, 1964-1965	*An. gambiae* s.l*	Dissection	4573	0.2	Rishikesh (1966)
Different areas in north and central regions, in the years before 1967	*An gambiae* s.l*	Dissection	1921	0-0.15	O'Connor (1967)
Gambela, 1967-1969	*An. gambiae* s.l*	Dissection	8348	1.87	Krafsur (1977)
Gilgil Ghibe Valley, 1973	*An. arabiensis* and *An. quadriannulatus*	Dissection	3000, both species	3 *An. arabiensis* were infected	White *et al.* (1980)
Gambela, 1990	*An. gambiae* s.l*	ELISA	262	0.76	Nigatu *et al.* (1992)
Gergedi, 1992-1994	*An. arabiensis*	ELISA	3626	1.52	Ameneshewa (1995)
Gergedi, 1992-1994	*An. arabiensis*	Dissection	638	0.63	Ameneshewa (1995)
Ziway, 1994	*An. arabiensis*	Dissection	274	0	Abose *et al.* (1998b)
Ziway, 1994	*An. arabiensis*	ELISA	334	0	Abose *et al.* (1998b)
Jimma area, 1998	*An. arabiensis*	ELISA	148	2.7	Hunt *et al*, (1998)
Jimma area, 1998	*An. quadriannulatus* Species B	ELISA	29	0	Hunt *et al*, (1998)

Locality and year	Vectors	Techniques	No dissected/ processed	Sporozoite rate (%)	Reference/source
The lake regions, 1964-65	*An. funestus*	Dissection	339	0	Rishkesh (1966)
Gambela, in the years before 1967	*An. funestus*	Dissection	70	1.4	O'Connor (1967)
Gambela, 1967-1969	*An. funestus*	Dissection	9052	1.23	Krafsur (1977)
Gambela, 1967-1969	*An. nili*	Dissection	619	1.29	Krafsur (1977)
The Lake regions 1964-1965	*An. pharoensis*	Dissection	2577	0	Rishikesh (1966)
Gambela, 1990	*An. pharoensis*	ELISA	462	0.46	Nigatu *et al.* (1992)
Ziway, 1994	*An. pharoensis*	ELISA	272	0	Abose *et al.* (1998b)
Ziway, 1994	*An. pharoensis*	Dissection	624	0	Abose *et al.* (1998b)
Gergedi, 1992-1994	*An. pharoensis*	ELISA	?	0.2	Ameneshewa (1995)

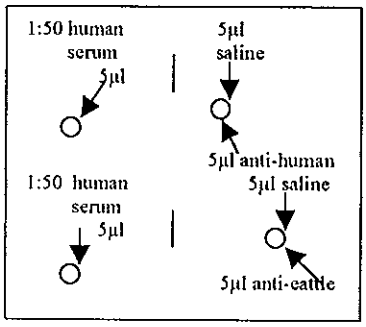
Note. * presumbaly *An. arabiensis*



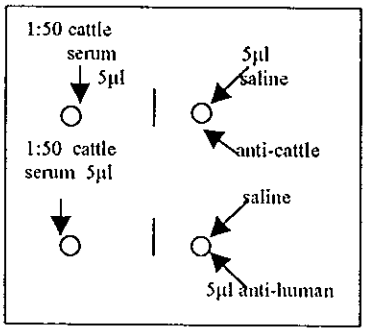
Appendix. III Meteorological Pattern at Metehara (July1999 - Sep. 2000)

Source: Compiled from data obtained from the National Meteorological Services Agency and Metehara Sugar Estate Research Center.

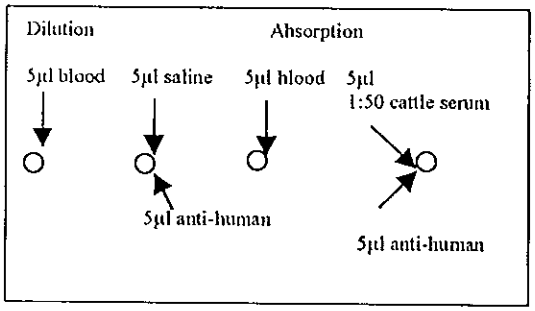
A. Dilution method i) Human serum against anti-human and anti-cattle



B. Dilution method i) cattle serum against anti-cattle & anti-human



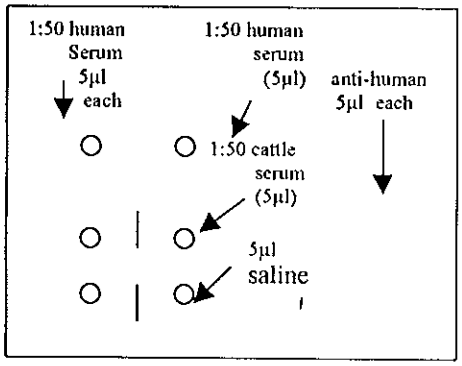
C. Blood meal test i) against anti-human



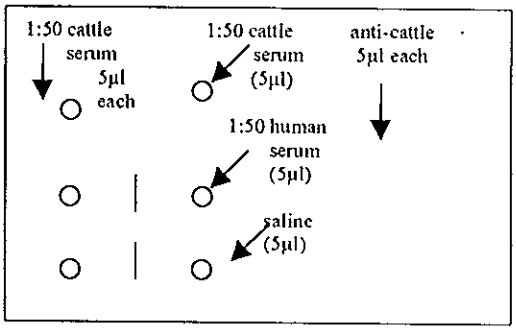
Appendix IV. Diagrammatic presentation showing the method of titer determination, standardization and blood meal analysis by the double immuno-diffusion method.

Note: Lines between wells indicate positive results.

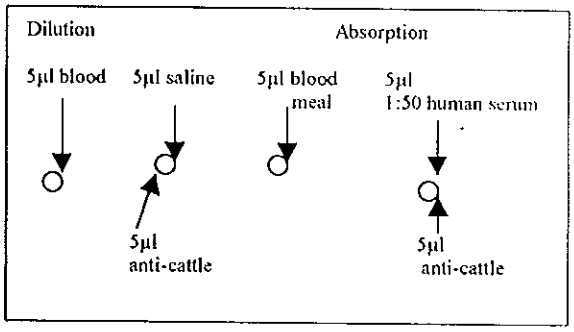
A. Absorption method ii) human serum against anti- human



B. Absorption method ii) cattle serum against anti-cattle



C. Blood meal test ii) against anti-cattle



Appendix V. Microscopieally confirmed cases of malaria recorded among patients attending the Metehara Sugar Estate Hospital and Metehara Malaria control Sector (July 1999- September 2000).

Month	Sugar Estate			Metehara			Gelcha			Buse			Total		
	Ex	P.f	P.v	Ex	P.f	P.v	Ex	P.f	P.v	Ex	P.f	P.v	Ex	P.f	P.v
July	2861	497	909	88	2	17	23	8	3	9	3	0	2981	510	929
Aug.	5209	1027	778	295	85	77	51	22	3	18	5	1	5573	1139	859
Sept.	7018	1777	1710	591	267	139	123	73	12	25	8	4	7757	2125	1865
Oct.	5492	2779	1272	447	231	63	72	48	7	13	5	1	6024	3063	1343
Nov.	4128	1536	1561	226	114	33	18	4	5	9	0	0	4381	1654	1599
Dec.	9772	701	734	103	23	31	12	3	0	1	0	1	9888	727	766
Jan.	6548	354	410	61	7	24	10	2	3	10	3	2	6629	366	439
Feb.	3952	353	500	61	7	18	10	2	2	1	0	1	4024	362	521
Mar.	2212	204	489	83	3	40	10	2	3	0	0	0	2305	209	532
Apr.	2191	44	284	37	3	9	4	0	0	5	0	3	2237	47	296
May	1193	43	247	181	10	45	4	0	1	3	0	0	1381	53	293
Jun.	1232	117	198	358	16	63	8	0	0	7	0	0	1605	133	261
July	2829	187	466	56	9	8	7	0	1	5	0	0	2897	196	475
Aug.	4692	857	796	839	191	153	127	64	11	36	11	4	5694	1123	964
Sept.	3970	1025	478	559	287	98	86	51	7	9	2	2	4624	1365	585
Total	63299	11501	10832	3985	1255	818	565	279	58	151	37	19	68000	13072	11729
Par. (%)		18.2	17.1		31.5	20.5		49.4	10.3		24.5	12.6		19.2	17.2

Ex=total examined P.f=*P. falciparum* P.v= *P.vivax*

Source: Metehara Sugar Estate Hospital and East Shoa Zone Malaria Control Sector, Nazareth.

Appendix VI. Species and number of anophelines based on larval collection and adult breeding from immatures collected from different breeding habitats in the four study sites (July 1999-March 2000, July-Sept.2000)

Year and Month	Study sites, breeding habitats and species of anophelines															Total
	Buse	Gelcha				Sugar Estate								Metehara		
	pool/rain	pool/rain	Marsh			pool/broken pipe	pool/rain	sewerage		canal			canal spillage	edge of Beseka		pool/rain	
	An.ar	*An.ar*	*An.ar*	*An.ph.*	*An.c*	*An.ar*	*An.ar*	*An.ar*	*An.ph.*	*An.ar*	*An.ph.*	*An.c.*	*An.ar*	*An.ar*	*An.sp*	*An.ar*	
July 1999	10	116	0	0	0	24	330	0	0	3	3	0	0	50	-	34	570
Aug.	35	101	0	0	0	192	245	0	0	15	1	0	0	54	14	50	707
Sept.	17	0	0	0	0	80	240	0	0	6	2	0	0	99	5	30	479
Oct.	8	0	15	0	0	12	0	0	0	3	0	0	0	36	7	0	81
Nov.	0	0	14	0	0	0	0	0	0	5	0	0	0	52	10	0	81
Dec.	0	0	20	0	0	0	0	0	0	0	0	2	400	2	6	0	430
Jan. 2000	0	0	25	4	2	0	0	0	0	2	9	14	270	0	10	0	336
Feb.	0	0	14	0	0	0	0	500	150	6	0	0	0	0	21	0	691
March	0	0	12	2	0	0	0	400	136	0	0	0	0	0	19	0	569
July	0	0	0	0	0	0	0	105	70	0	0	0	0	100	14	40	329
Aug.	0	0	0	0	0	0	0	50	50	0	0	0	0	134	8	30	272
Sept.	0	0	0	0	0	0	0	145	0	0	0	0	0	29	6	45	225
Total	70	217	100	6	2	308	815	1200	406	40	15	16	670	556	120	229	4770

Note: *An.ar* = *An. Arabiensis*, *An. ph.* = *An. pharoensis*, *An. c* = *An. coustani* group, *An. sp* = an unidentified *Anopheles* species

Appendix VII. Insecticide susceptibility test results of *An. arabiensis* to DDT, permethrin and propoxur at different replicates in the Sugar Estate, Metehara area (December 1999 and January 2000)

Insecticide	Replicate 1	Replicate 2	Replicate 3	Replicate 4
	Dead (%)	Dead (%)	Dead (%)	Dead (%)
4 % DDT	14 (70%)	15 (75)	15 (75)	12 (60)
Control	0	0	0	0
0.75% permethrin	16 (80)	14 (70)	15 (75)	15 (75)
Control	0	0	0	0
0.1% propoxur	18 (90)	20 (100)	0	0
Control	0	0	0	0