STUDIES ON THE SPECIES COMPOSITION AND BEHAVIOUR OF *ANOPHELES* MOSQUITOES IN RELATION TO MALARIA TRANSMISSION IN DOUBTI WOREDA (AFAR REGION)

BY

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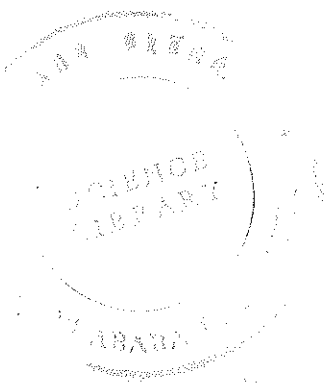

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ABSTRACT

The species composition and behavior of *Anopheles* mosquitoes was studied in three selected agriculturally irrigated villages in Doubti Woreda (Afar region). Information on the malaria cases were also gathered from Afar Regional Health Bureau and Doubti hospital. The results of malaria case data showed that malaria has perennial transmission and its incidence increased from year to year. *Plasmodium falciparum* followed by *Plasmodium vivax* are the most frequently prevalent *Plasmodium* parasites in this area. Although both males and females are infected with malaria, males are more vulnerable. Age groups above 15 years are more affected followed by age groups 5-14 years.

Larvae collected from different breeding habitats throughout the study period showed the presence of two species: *Anopheles arabiensis* and *Anopheles pharoensis,* of which *Anopheles arabiensis* was predominant and encountered in several breeding habitats throughout the study period.

Adult Anophelines collected from different resting places revealed that both *Anopheles arabiensis* and *Anopheles pharoensis* predominantly rest indoors than outdoors. *Anopheles arabiensis* collected indoor by aspirator shows significance difference at $\chi 2 = 5.544$, $P = 0.019$. The biting behavior of these two species was predominantly exophagic. *Anopheles arabiensis* collected by human bait shows significance difference at $\chi 2 = 30.01 P = 0.00$. However, CDC light trap collection of this species shows predominantly indoor density at $\chi 2 = 65.47$, $P = 0.000$. The parous rate of *Anopheles arabiensis* was 23.8% where as that of *Anopheles pharoensis* was 16.6%. The salivary glands dissected for sporozoite rate showed none of which were found infected with sporozoites.

1. INTRODUCTION

Malaria is probably one of the oldest diseases known to human being. The disease was supposed to have originated in the jungles of Africa, where it is still very much widespread. Once it was thought that the disease comes from field marshes, hence the name 'Malaria' (bad air). Later it was discovered as life-threating parasitic disease. Four main species of the parasite: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale* and *Plasmodium malariae* are responsible for human malaria (Boyed, 1949). These are protozoan parasites which invade the red blood corpuscles.

Malaria is an acute and often chronic disease which commonly begins with a brief and indefinite illness, shortly followed by a characteristic shaking, chill, with rapidly rising temperature, usually accompanied by headache and ending with profuse sweating (Goma, 1966). Of the four parasites, *Plasmodium falciparum* is the most severe, causes fatal malaria fever and accounts for 80% of all malaria incidence world wide and is the most common in tropical Africa (WHO, 2000).

Malaria parasites are transmitted from person to person through the bite of female *Anopheles* mosquitoes, which require blood to nurture their eggs (Service, 2000). Both man and the mosquito are essential to the propagation of the parasite (King *et al.*, 1960). In man the parasites live and multiply in the red-blood corpuscles, liver and possibly other organs. The parasites enter the gut of the mosquito along with the blood-meal, undergo series of developmental stages and eventually the sporozoites invade its salivary glands (Foote and Cook, 1959). At this stage the mosquito is capable of infecting its next human host.

Malaria is one of the major diseases recognized by the World Health Organization as serious public health problems. It is a major cause of death, poverty and under development in Africa It

kills around a million people each year (OAU, 1999). Together with other diseases malaria undermines socio-economic development. Malaria is a disease usually associated with topography, climate and socio-economic conditions. The problem of the disease in Africa is aggravated by climatic change, massive ecological changes, and population movements related to developmental schemes, poverty and lack of efficient control strategies. Evolution of drug resistant parasites, insecticide resistant vectors, international travel, global warming and several other factors also contribute to the spread of the disease. (Bouma *et al.,* 1994)

Malaria is a major public health problem in Ethiopia where highland populations are exposed to frequent waves of epidemic (Abose *et al.*, 1998a; MOH, 2004). It has been consistently reported as a leading cause of morbidity and mortality in the past years. The magnitude of the problem in 2002/2003 has been worsened and the disease has been reported as the first cause of morbidity and mortality accounting for 20.4% admissions and 27% in patient deaths. In a non-epidemic year, 5-6 million clinical malaria cases were reported from health facilities (MOH, 2004). However, the number of malaria cases reported by health facilities is only a portion of the actual magnitude (MOH, 2004).

All four species of *Plasmodium* are known to be present in Ethiopia. However, *Plasmodium falciparum* and *Plasmodium vivax* are the two dominant parasite species with relative frequency of 60% and 40% respectively. This proportion varies from place to place and from season to season (MOH, 1999; 2004). About 42 *Anopheles* mosquito species are known in Ethiopia. Of these *Anopheles arabiensis* is the major malaria vector while *Anopheles pharoensis*, *Anopheles funestus*, and *Anopheles nili* are considered as secondary vectors. Children who seem to lack primary immunity and pregnant women are the main victims of the disease (Abose *et al.*, 1998b).

Large state agricultural development enterprise particularly the cotton producing farms as well as small private owned agricultural farms attract labourcres from the highland localities such as Shewa and Wollo to Afar region. Previous studies revealed that malaria is one among the top infectious disease along the Afar region (Regional Health Bureau, 2003). In view of this fact this study was proposed to determine the vector *Anopheles* species responsible for malaria transmission in selected site of irrigated agricultural areas in Doubti.

Objectives of the study

General

This study was proposed to determine vector *Anopheles* species composition in relation to malaria transmission in the study area.

Specific

1. To study the malaria situation of the region (Afar), particularly Doubti Woreda.
2. To investigate thc breeding habitats of the Anopheline species at the study area
3. To determine the *Anopheles* vectors responsible for malaria transmission.
4. To study the resting and feeding behavior as well as the human bitting ratc of the *Anopheles* species.
5. To determine parity rate and sporozoites rates in *Anopheles* mosquitoes at the study area.

2. LITERATURE REVIEW

2.1 Current global malaria situation

Malaria was once wide spread throughout the world. However, it was successfully eliminated from many countries with temperate climates during the mid 20th century (WHO, 2000a; Samba, 2000). Today malaria is found throughout the tropical and sub-tropical regions of the world and cause acute illness and deaths. It is severe infectious disease, ranks second to tuberculosis (WHO, 2000a). It remains an important public health problem, and is endemic in more than 100 countries or territories in the world. Today over 40% of the world's population mostly those living in the world's poorest countries are at risk of malaria, because of the poor performance of health service delivery systems for malaria control has had limited success in highly endemic countries (Breman *et al.*, 2001).

The incidence of malaria world wide is estimated to be 300-500 million clinical cases per year. Of these 90% of the clinical cases are occurring in Africa mostly caused by *P. falciparum* infection. It is thought to kill 1.5 to 2.7 million people world wide each year, of which about 1 million are children under the age of 5 years in Africa, south of the Sahara (WHO, 2000a). Deaths from malaria in countries outside Africa, south of the Sahara, occur principally in areas where diagnosis and treatment are not available (Samba, 2000). The risk of getting malaria attack and its severity has recently increased with the improved transportation facility of modern world that escalated population mobility from non-malarious areas to malarious and the vice-versa.

Malaria epidemics have occurred in practically all continents. Thus, the global malaria situation is worsening, particularly in Africa, where some of the most severe malaria epidemics have taken place. Malaria epidemics usually resulting from climatic changes, human activities and from

failed control policies (Sherman, and Irwin1998). Epidemics occur when the factors limiting transmission are altered as a result of either temporary climatic changes such as abnormal rains, long periods of increase humidity and high temperatures, or more permanent changes of the microclimate such as the development of agricultural irrigation systems (Bouma *et al.*, 1994; Bouma and Vander Kaay, 1996). Global warming may also affect the future pattern of malaria. Estimating the potential impact of climate change on malaria transmission has generated a great deal of interest due, in part to the concern that this debilitating disease may emerge or re-emerge in many parts of the world (Bayoh *et al.*, 2003).

Man-made epidemics are consequences of some form of unstability, after which large numbers of people are displaced. This displacement may be either a consequence of war or the result of natural disasters such as earth quakes, hurricanes and draught. They lead not only to an increase in vector breeding places but of even more concern to increased human-vector contact since, residents are forced to take shelter in partially destroyed homes or temporary camps (Loevinsohn, 1994; Warsame *et al.*, 1995). Military conflicts, civil unrest, and ecological changes favourable to the vectors have greatly contributed to malaria epidemics in countries such as Angola, Somalia, Cambodia, Rwanda and Burundi as large number of unprotected and non-immune refuges moved into malarious areas (Marfin *et al.*, 1994).

Less dramatic but not less important for the spread of malaria are the internal and international movements of populations including economic and environmental refuges and migrants into and out of malarious areas. Such population movements contribute to new out breaks and make epidemic-prone situations more explosive (Meek, 1988). A similar epidemiological pattern accompanies such development projects as railway and road construction and large irrigation

projects that undertaken in highly malarious areas. Even with modern control measures and the wealth of resources available for such projects, malaria epidemics continue to develop and threaten the population (Packard, 1986; Najera *et al.*, 1996).

2.2 The Malaria Vector-*Anopheles* Mosquitoes

Mosquitoes are among the best known groups of insects, because of their importance to man as pests and vectors of some of the most distressing human diseases. They are small, two winged insects belonging to the family culicidae of the order Diptera (two-winged flies). They are easily distinguished from most other flies by a combination of the characters: a long proboscis projecting forwards from the head; a fringe of scales along the posterior margin of the wing and a characteristic wing venation; the second, fourth and fifth longitudinal veins branched (Service, 2000)

Mosquitoes are distributed widely throughout the world and practically no part of the globe that can serve for human existence is free from them. Culicidae are classified into three subfamilies: Anophelinae, Toxorhynchitinae and Culicidae, the last being sub-divided in to two tribes, Sabethini and Culicini (Service 2000).

The sub-family Anophelinae contains three genera *Chagasia, Bironella* and *Anopheles*, of which the genus *Anopheles* contains species of medical importance. This genus comprised about 430 species. Many species of *Anopheles* mosquitoes are important vectors of malaria, filariasis and several arboviruses. About 70 species of *Anopheles* have been definitely incriminated as vectors of human malaria (Service, 2000). Ronald Rose in India in 1895, determined to find the means by

which malaria was transmitted by mosquitoes. During this investigation although, he was able to observe exflagellation in the stomaches of mosquitoes that had ingested crescents from the blood of human volunters infected with malaria, he could get no further (Harrison, 1978).

The distribution and density of the *Anopheles* vectors are determined primarily by the availability of the suitable breeding sites, and such sites often arise through human activities, enhancing a close association between man and the vectors (Muirched-Thomson, 1951). Female mosquitoes lay about 30-300 eggs at any one oviposition. Many mosquitoes lay their eggs directly on the water surface.

2.2.1 The feeding and resting behavior

Females of most species of *Anopheles* mosquitoes require a blood-meal before the eggs can develop, and this is taken either before or more usually after mating. They obtain their blood meals from a wide range of hosts. Many species bite human to obtain their blood meals and a few feed on humans in preference to other animals. However, others prefer feeding on non-human hosts and many species never bite people (Service, 2000).The choice of host varying with the mosquito species and the opportunity available to it (Goma, 1966). Females are attracted to hosts by various stimuli from them, such as body odors, carbon dioxide and heat. Some species feed more or less indiscriminately at any time of the day or night; others are mainly diurnal or nocturnal in their biting habits (Service, 2000).

A few species of *Anopheles* mosquitoes frequently enter houses to feed (endophagic forms) in their feeding habits, where as those that bite their hosts outside houses are exophagic forms (Service, 2000). The biting behavior of female *Anopheles* mosquitoes may be very important in

the epidemiology of disease transmission. As children will be indoors and a sleep at the night time *Anopheles* mosquitoes that feed on people predominantly outdoors and late at night will not bite many young children. During hot and dry periods of the year a number of people may sleep out of doors and as a consequence be bitten more frequently by exophagic mosquitoes (Service, 2000).

After having bitten human or other hosts, mosquitoes seek resting places in which to shelter during digestion of their blood-meals. Some species rests inside houses during the time required for blood digestion and maturation of the ovaries and are called endophilic. In contrast, mosquitoes that rest outdoors are termed to be exophilic (Service, 2000).

The resting and feeding behavior of adult *Anopheles* mosquitoes may be an important consideration in planning control measures. In several malaria control campaigns the interior surfaces of houses, such as walls and ceilings, are sprayed with residual insecticides such as DDT, to kill adult mosquitoes resting on them (Bruce-Chwatt, 1993). However, increment in the numbers of *Anopheles* species resistant behavior to insecticide is equally on the increasing momentum. For instance, in 1946, only two species of *Anopheles* were resistant to DDT, but from two subsequent reports by 1991 and 1997 a total of 55 resistant *Anopheles* species have been recorded, of these 21 were very important vectors of malaria. Nearly, all members of *An. gambiae* complex, that are the potent vectors of malaria in tropical Africa, have shown various degree of resistant to DDT as well as to other organo-chlorine insecticide (Bruce-Chwatt, 1993). *Anopheles* vectors transmitting human malaria are divided in to main and secondary vectors based on their role of transmissions (Jenssen and Wery, 1987).

2.2.2 Parity and sporozoites infection rates

The relative proportions of the different ages of mosquitoes in the populations are a measure of their survival probability and hence daily mortality. One method of aging anophelines evolves dissecting and extracting out the ovarioles from the ovaries. Parous females are those that have taken a blood meal atleast once and oviposited atleast once hence potentially infected with malaria parasites (Service, 2000). Nulli-parous mosquitoes are those that have not taken a blood meal yet and have not laid eggs, hence not transmit malaria parasites. High parity rates imply that mosquito population survives longer, has more ovarial dilations and therefore, more gonotrophic cycles and therefore, capable of transmitting malaria parasites for long (Jensen *et al.*, 1998).

Whether *Anopheles* plays a role in malaria parasite transmission can be demonstrated by dissecting the captured females and investigated the presence of malaria parasites (sporozoites) in the salivary glands and/or in the stomach (Holistein, 1954). Immunological methods like ELISA based investigations, and more recently molecular methods like PCR probes are more effective methods that ean be used to determine the presenee of sporozoites in suspected *Anopheles* mosquito (WHO, 2003).

2.3 Malaria situation in Africa

Malaria together with HIV/AIDS and TB is one of the major public health challenges undermining development in the poorest countries (RBM, 2001). It is one of the most important human disease, but progress in its control has been slow in Africa where approximately 90% of the infection occurs mostly in young children (Collins and Paskewitz, 1995). The vast majority of malaria deaths occur in Africa, south of the Sahara, where also presents major obstacles to socio-economic development (RBM, 2001). Malaria threatens the lives and livelihoods of more than

500 million Africans and exerts such a huge public health burden that it has been incriminated in the continued under development of the continent as a whole (Breman *et al.*, 2001).

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

Africa bears an over whelming proportion of the malaria burden because of several reasons. The region is home to the most efficient, and deadly species of the mosquito which transmit the disease (RBM, 2001). The warmth and moistened climatic condition of the region is conducive for the development of the parasite and the breeding of the vector (Temu *et al.*, 1998). In many parts of the region changes in the environment, population migration and financial problems have led to serious increase in malaria (Collins and Paskewitz, 1995). For example, deforestation can increase human-vector contact and create improved breeding conditions for several vectors species. Water development projects such as poor irrigation systems, impoundments, and dams can also create improved breeding grounds. Furthermore, economic and political stress, often result in population migration, which increase movement of non-immunes into malarious areas and of carries into non-endemic areas (Collins and Paskewitz, 1995).

Although, some countries in southern Africa are successfully applying integrated malaria control, such programs currently cover only a small proportion of those at risk on the continent. One of the greatest challenges facing Africa in the fight against malaria is drug resistance to the parasite. The other factors influenced for control of malaria are the occurrence of insecticide resistant

vector and change in the resting bchavior of mosquitoes (from endophily to exophily) as the result of frequent indoor insecticide sprays (Toure, 1999).

Throughout most of Africa thc parasite species *Plasmodium falciparum* is responsible for approximately 90% of all malaria cases. But, in Ethiopia up to 40% of cases are the result of infection with *P. vivax*, a species which is usually non-fatal (Fortenille and Lochouran, 1999). *Plasmodium falciparum* malaria is the most severe, pernicious and causes most malaria-specific morbidity and mortality (Collins and Paskewitz, 1995).

2.3.1 Population at risk of malaria

In areas where transmission is high such as in wet savanna areas of tropical Africa children under the age of 5 and women in their first pregnancy are most vulnerable to the disease. At the other extreme, all age groups are at risk in areas of low transmission, where epidemics may be common and the immunity of the population is low (Kondrachine, 1994).

Although, older children have developed some degree of immunity, the disease remains one of the most common causes of school absenteeism in Africa (WHO, 1997b). Children are vulnerable to malaria from about 4 months of age. In highly endemic areas during the peak transmission season, approximately 70% of one year-olds have malaria parasites in their blood. Malaria accounts for one in five of all child-hood deaths in Africa (Temu *et al.*, 1998). Anemia, low birth weight, epilepsy and neurological problems are frequent consequences of malaria, compromise the health and development of million of children throughout Africa (Ijumba and Lindsay, 2001; RBM, 2001).

Malaria infection during pregnancy is a major public health problem in tropical regions throughout the world (Temu *et al.,* 1998). In most endemic areas of Africa, pregnant women are the main adult risk group for malaria. The burden of malaria infection during pregnancy is caused chiefly by *Plasmodium falciparum,* the most common species in Africa. The symptoms and complications of malaria during pregnancy differ with the intensity of malaria transmission and thus, with the level of immunity the pregnant women has acquired. In areas of unstable malaria transmission, adult women have not acquired any significant level of immunity and usually become ill when infected with *Plasmodium falciparum* malaria (Fortenille *et al.,* 1997).

2.3.2 Malaria in areas of irrigation

The higher population growth rate of the African continent has led to increased demand for the food. In order to meet this need, many governments have sought ways of improving food production by initiation of large scale irrigation project, involving reclamation of arid and semi-arid areas for cultivation of crops (Herrel *et al.,* 2001). Irrigation development projects have been associated with negative impacts on human health, particularly with respect to vector-borne disease (Oomen *et al.,* 1994). Malaria is one of the major tropical diseases associated with irrigation schemes (Ijumba and Lindsay, 2001).

There are grounds for concern that irrigation may increase the health risk of local communities in enhancing conditions favoring transmission of specific vector mosquitoes, particularly when irrigated rice is cultivated (Bradely, 1988). It has often been assumed that high members of malaria vector *Anopheles* mosquitoes resulting from irrigation schemes lead inevitably to increased malaria in local communities (Ijumba *et al.,* 2002). However, conflicting results have been obtained from studies evaluating the impact of rice irrigation in different parts of Africa,

where malaria transmission is stable. Rather surprisingly, most studies reported similar or reduced prevalence of malaria at rice irrigation schemes compared with adjacent areas without irrigation (Harison and Scalon, 1975).

The introduction of irrigated–rice cultivation results in wealth creation in local communities (Audibert *et al.,* 1990; Boudin *et al.,* 1992). Income and wealth clearly affect the severity of the malaria problem. If the population has the financial resources to build housing in hospitable to mosquitoes, if knowledgeable about the use of personal protection measures and can afford them, understand the importance of seeking effective treatment at the first sign of illness and can pay for health service and drugs . Since, malaria is a major cause of morbidity most rural community's planners of irrigation schemes should take the opportunity to strengthen the health services in these areas (Boudin *et al.,* 1992).

Malaria is potentially dangerous when irrigation is introduced in to dry areas of unstable malaria, sites with generally low levels of transmission. There are a number of possible reasons for this finding, perhaps most important, it may have resulted from the large scale migration of semi-immune or non-immunes moving into a malarious areas. This situation may have been exacerbated by the low income of migrant farmers, who were unable to afford bed nets to protect them selves from malaria mosquitoes and medical facilities in the scheme (Hunter *et al,* 1993).

Unlike rice that is grown under flooded conditions, sugar-cane and cotton crops are never flooded because of its susceptibility to water logging. Thus, a well maintained sugar-cane plantation should not offer breeding places for important malaria vectors, (Packard, 1986). However, poorly maintained irrigation canals may become overgrowing with vegetation, encouraging the breeding

of the malaria vector *An. funestus* and leakages from canals may create pools of standing water suitable for the breeding of *An. Gambiae.*

2.4 *Anopheles* vector of malaria in Africa

Although, there is a great diversity of anopheline species in Africa, members of the *Anopheles gambiae* complex and *Anopheles funestus* are widely distributed and are responsible for the transmission of malaria in the region (White, 1974). Others such as *Anopheles nili* Theobald, *An. moucheti* Evans and *An. pharoensis* Theobald have local importance (Fortenille and Lochouran, 1999).

The most intensive risk of infection was documented in both east and West Africa, with an infective mosquito bite ranging from 200-300 annually (Berier,1998). *Anopheles gambiae* complex, which has seven sibling species (*An. gambiae senseo stricto, An. arabiensis, An. merus, An. melas An. bwambae, An, quadriannulatus* sp.A and *An. quadriannulatus* sp. B) are uniquely effective and efficient vectors of human malaria in Africa, and the most important in the world (Coluzzi, 1984; Bogh *et al.,* 2003). *Anopheles gambiae* complex is the primary vector of malaria parasite in sub-Saharan Africa where almost 90% of the world's malaria specific morbidity and mortality occurs (Emanuel *et al.,* 2004). In large areas of Africa *An. gambiae* s.s and *An. arabiensis* occur in sympatry.

Anopheles gambiae complex first discovered in 1899 by Ross and co-workers in 1900 as they are efficient vectors of malaria in Africa (White, 1974). *Anopheles gambiae* complex was considered to be a single biological species, and then it has been constitute several kinds of scientific problems. However, in the early literature there were many reports of variation in the larval

habitats and adult female resting behavior (Coetzee *et al.*, 2000). Their wide ecological range in Africa, north western Arabia and oceanic islands from Cape Verde to Mauritius helped to create nomenclatural confusion during the early 20th century when these mosquitoes were extensively documented under different names (Carlo *et al.*, 1996).

Pronounced ecological and behavioral diversity of the members of *An. gambiae* complex coupled with some morphological variation has always sustained suspicious that more than just a single biological species of mosquito might be involved (Coetzee *et al.*, 2000). Various sorts of biological races and genetic strains have been also postulated to account for the short fall of control measures thwarted by the renewed versatility and opportunism of *An. gambiae* where by populations seem to vary their to vicissitudes of the movement (White, 1974).

The two principal vectors of human malaria, *An. gambiae* s.s. and *An. arabiensis* are widely distributed through out the tropical Africa. They are with both endophilic and exophilic resting as well as endophagic and exophagic biting behavior (Gilles and Coetzee, 1987). *Anopheles gambiae* s.s. breeds in temporary collection, permanent water bodies and along the extensive alluvial areas along the river flooded areas (Bogh *et al.*, 2003) where as *An. arabiensis* breeds in a wide variety of water bodies.

Anopheles quadriannulatus is limited in distribution to east and south Afriea, and Ethiopia, where it is thought to be mainly zoophilic and there for not a vector of human malaria (Bayoh *et al.*, 2003).

Anopheles merus and *Anopheles melas* are salt water species which breeds in a large variety of water bodies including swamps, ponds and small pools, all of which are saline (Gilles and

Coetzee, 1987). *Anopheles melas* was the dominant species which breeds in the flooded areas followed by *An. gambiae* s.s. and *An. arabiensis* (Bogh *et al.*, 2003). *Anopheles melas* and *Anopheles merus* are found to costal region of west Africa and in east Africa, respectively. They are regarded as secondary vectors of malaria and exophagic, zoophagic and referred as sole members of the *Anopheles gambiae* complex (White, 1973).

Anopheles bwambae breeds in geothermal mineral water. It is known to occur in ten km radius of the geothermal spring located in Bwambae country, Uganda (White, 1973). It is less efficient vector of malaria, bites and rests in near by houses and forest (Service, 1993c).

Sympatry of two or more sibling species is a common phenomenon among members of the *Anopheles gambiae* complex (Lindsay *et al.*, 1998). *Anopheles gambiae* complex sibling species are identified by different techniques such as reproductive isolation, biochemical techniques (cuticular hydrocarbon analysis and enzyme electrophoresis), cytogenetic technique and molecular techniques.

2.5 Malaria situation in Ethiopia

Geographically Ethiopia is located between arid Asia and humid central Africa. It is particularly situated in the tropical zone, physio-geographically constituting a mass of central high lands grinded by low-lying, hot and generally arid regions (Wold–Mariam, 1972). Thus, the physio-geographic diversity of the country is pertinent to a wide variation for climatic conditions. Therefore, the country experiences three locally known climatic zones i.e., cold, temperate and warm climatic zones with elevations of above 2500m, 1500m-2500m and below 1500m respectively (Tulu, 1993a). European travelers in the 19thc attested the presence of malaria in the

low lands of the country, i.e. Lake Tana, Awash valley, Lake Ziway and Gambella. Malaria was reported to be endemic in Ethiopia first and for most by the Italian and British scientists from the mid 1930s to the late 1950s (Brambella, 1940; Covell, 1957).

According to Federal Democratic Republic of Ethiopia Ministry of Health malaria control profile (2000) malaria affects about 4-5 million people annually and is prevalent in 75% of the country putting over 40 million people at risk. Individuals and communities living in rural areas are more likely to be at increased risk of exposure to malaria infection due to the proximity of mosquito breeding sites, poor quality of housing and inability to afford prevcntive measures and treatment proportionally higher for low income house (Tulu, 1993b; MOH, 2000).

Malaria surveillance data in Ethiopia indicated that the mean number of reported cases is increased from year to year for instance, during the year 1980-1984, the mean parasite *Plasmodium* rate increased from 8.9% to 33.1% per annum that is a 3.7 fold increase (Wondwossen, 1991). Large scale migration due to resettlement, instability to conflict areas and spread of chloroquine resistant *falciparum* malaria were thought to be reasons for the increase of transmission in the low land (Mengesha and Mekonnen, 1999).

The distribution of malaria in Ethiopia is related to variation in altitude, topography and climate, (Gebre–Mariam, 1984; Tulu, 1993b). In Ethiopia, there is no administrative region free of the disease. In fact some region for example, Afar and Somali are 100% malarious (MOVBDCU, 1999). Malaria, is common in areas lying below 2000m, but highly prevalent below 1500m. Areas lying at altitudes of 1500 -2000 are prone to occasional malaria epidemic (Nega and Haile-Meskel, 1991). It 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. In addition to

annual changes in climate, the ecological up-heavals in the late 1980s and early 1990s has an immense role in changing the epidemiology of malaria. For instance, the Pawie settlement scheme and development activities in the area of the town ship of Arabaminich are worth mentioning (Nega and Haile-Meskel, 1991).

A study carried in the year 1960 in Gambella revealed that malaria was caused mainly by *P. falciparum* and *P. malariae*. The role of *P. vivax* and *P. ovale* was small. But a similar study 22 years later showed *P. falciparum* and *P. vivax* to be the major causes of malaria in the region (Nigatu *et al.*, 1992). Tulu (1993a) suggested that the over crowded population settlement to high land areas of the country for centuries by partly considered to be due to malaria endemicity in the fertile low lands.

Ethiopia because of its heterogeneous physio-geographic features and climatic variability suffers mostly of the epidemic form (Tulu, 1993b). Unstable malaria occurs in most parts of the county particularly in the high land fringes when climatic conditions are conductive for its transmission. In areas of higher altitudes malaria is occurring immediately after the light rainy seasons of March and April as well as after the long rains of June through September. Its transmission continues less intensively in the wet seasons (Nega and Haile-Meskel, 1991). Several epidemics in these high land fringe areas caused innumerable deaths (Tulu, 1993a).

Normally, the upper limit for malaria transmission was considered as 2000m a.s.l. but periodic epidemics were recorded above this level (Covell, 1957, Chand, 1965). Consequently, moderate to sever malaria epidemics was known to occur in the country (Fontaine *et al.*, 1961; Gebre-Mariam *et al.*, 1988). In 1953, Malaria 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). In 1958, there was an epidemic in several regions affecting about 3 million people, out of which 150,000 died (Fontaine *et al.*, 1961). Moreover, the epidemics in Ethiopia can be substantiated by its repeated events with similar feature but of lesser intensity in 1965, 1973, 1981 and 1982 (Gebre-Mariam *et al.*, 1988). Recently, frequent malaria epidemics of cyclical patterns of variable magnitude were recorded for example, the epidemics of the year 1988, 1991, 1995 and 1998 in different parts of the county (MOH, 1999).

Regional and global events, notably draught and famine as well as climatic phenomenon attribute to the recent malaria Epidemics (Trigg and Kondrachine, 1998; Mengesha *et al.*, 1998). In the epidemic situation, communities that lack protective immunity are severely attached.

In Law land areas, malaria transmission is usually perennial with slight variation in magnitude; this is common in areas such as Gambella, Mekele and Setit-Humera districts. In such localities the environmental and climatological situations permit the continual breeding of vectors in permanent breeding sites (Tulu, 1993a).

The introduction of more than half a million non-immune high Landers into the malaria endemic low lands has elevated malaria prevalence of the country from year 1984 to 1989. In general, the problem of malaria in the country is aggravated by population movements to the low lands associated with agricultural and agro-developmental projects, urban developments as well as settlement operations (Nega and Haile-Meskel, 1991). Malaria is a number one disease because large state and private owned agro-industrial schemes in the Awash valley in the eastern part of the country attract labourers from all corners of the country which have no immunity (Mekuria *et al.*, 1992).

2.6 Malaria vectors in Ethiopia

Observation 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. 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*. Giaqinto-Mira (1950) carried the first systematic study on the distribution of mosquitoes, in which a list of 13 species was given with details on their biology and locality. Covell (1957) collected *Anopheles* mosquito from the Lake Tana region in the north, among them, *An. gambiae*, *An. funestus* and *An. pharoensis* were found.

Following the changes made on the taxonomy of *Anopheles* mosquito, a total of 42 *Anopheles* species have so far been recorded in Ethiopia (White *et al.*, 1980). Among the *Anopheles gambiae* complex members *Anopheles arabiensis* and *An. quadriannulatus* sp.B are the only two species that are found in Ethiopia (Gebere-Mariam 1984). *Anopheles pharoensis*, *An. funestus* and *An. nili* are regarded as secondary vectors of malaria in Ethiopia (Gebre-Mariam *et al.*, 1988; Abose *et al.*, 1998b). One or more of the secondary vector occur sympatrically with *An. arabiensis* as has been reported in Gambella (Nigatu *et al.*, 1992).

The results of early cytogenetic studies on the *An. gambiae* complex species in Ethiopia revealed the presence of only *An. arabiensis* in all study areas. However, later cytogenetic works have proved the occurrence of *An. arabiensis* and *An. quadriannulatus* in the mid-western part of the country (White *et al.*, 1980).

Anopheles arabiensis is incriminated as the principal vector of epidemic malaria in all administrative regions of Ethiopia (Ameneshewa, 1995). It is usually the vector of epidemic malaria. Mosquito of this species breeds in small, temporary, sun-lit water collections created during the rain such as rain pools. It can also breed in a wide variety of other types of water bodies (Mekuria, 1983). Recent field data indicate that larvae of this species prefer sun-lit water bodies with emergent vegetation. Some breeding habitats observed are discarded tires, construction pits such as those used for plastering of houses, hoof prints of animals, tractors and other vehicles in agricultural development areas; small water collections in riverbeds during the dry seasons; and irrigation canals. It is known that one or more of the secondary vectors are sympatric with *An. arabiensis* more frequently with *An. pharoensis* than *An. funestus* and *An. nili* (Abose *et al.*, 1998 a). Mekuria *et al.*, (1992) have shown the presence of only *An. arabiensis* in the Awash valley. The biology and behavior of *An. arabiensis* in relation to the epidemiology and control of malaria has been studied in the Awash River Basin and central Ethiopia. The result of the study revealed that *An. arabiensis* was the only species important in malaria transmission (Amenshewa, 1995).

Adults of *An. arabiensis* in Ethiopia inhabit in and around human dwellings as well as in areas where human being are absent (Jolivet, 1959). It rests in human dwellings, animal shelters and also outdoors in ditches, vegetation, tree holes and others. The larvae of this species were collected about 30 km away from human dwellings (Jolivet, 1959). *Anopheles arabiensis* species exhibits partial zoophily, feeds and rests both indoor and outdoor (White, 1974).

The host preference of *An. arabiensis* depends on the availability of the host. Where the available hosts are only humans, it drives its blood meal from humans, in areas where both humans and cattle are found, it feeds on both of the hosts with variable proportions (White, 1974). The

feeding and resting behavior of *An. arabiensis* are affected by insecticide sprays either by reducing the number or altering the behavior.

Anopheles quadriannulatus in Ethiopia was reported for the first time by Turner (1972) and its distribution was shown to be in the high lands of south western and northern region; co-existing with *An. arabiensis* (White 1974; White *et al.*, 1980). Hunt *et al.*, (1998) to date reported the occurrence of *An. quadriannulatus* species B in Jimma area, Ethiopia which differs from the South-western Africa Population designated as *An. quadriannulatus* sp. A. Polymerase chain reaction (PCR) method has been enabled to distinguishes *An. quadrianulatus* sp. A from sp. B (Fettene *et al.*, 2001).

Anopheles quadriannulatus exhibits endophilic behavior resting in animal shades. It is zoophilic with a negligible role in malaria transmission. This species is not normally found in human dwellings but to a lesser extent in mixed dwellings (White, 1974). The collections from the Awash valley included samples from human dwellings in which goats and sheep were also sheltered at night. It was also reported to feed on man either indoors or outdoors in the presence of cattle (White, 1974). Recently, Coetzee *et al.*, (2000) stated that further work should be done on *An. quadriannulatus* species B. regarding its vectorial capacity and behavior.

Anopheles pharoensis is found in all regions except Bale Zone of Oromiya region (Gebre-Mariam *et al.*, 1988). In contrast to *An. arabiensis*, *An. pharoensis* prefers large, permanent and shaded water bodies with emergent vegetation, including irrigation canals, rice fields and lake shores. It is widely spread in the riverine areas of the Baro and Awash in Lake Tana, in the Lake region of Shewa, Sidamo and Gamo Gofa. *Anopheles pharoensis* is the second most frequent and widely distributed vector of malaria in Ethiopia. It might be responsible for the transmission of

malaria in the absence or low density of *An. arabiensis* particularly in the dry seasons (Amenshewa, 1995). It was considered as a secondary and not an efficient vector. In the previous studies the contribution of *An. pharoensis* in malaria transmission was considered to be insignificant (Gilles and De Meillion, 1968). However, current reports have confirmed that *An. pharoensis* are responsible for the transmission of malaria in Ethiopia. *Anopheles pharoensis* feeds predominantly outdoors but rest mostly indoors (O'Conner, 1967; Krasfur, 1971). It is relatively short-lived as compared to *An. arabiensis* (Abose *et al.*, 1998b).

Anopheles funestus, which is not a member of *An. gambiae* complex, is also capable of producing very high inoculation rates in a wide range of geographic, seasonal and ecological conditions in Africa (Coluzzi, 1984). It occurs in all administrative regions of Ethiopia. Four members of the *An. funestus* groups which comprise seven species that are not morphologically distinctive in the larval or adult stages occur. While all four can be specifically identified in the larval stage, it is very difficult to differentiate them in the adult stage (Mekuria, 1983). It breeds more or less in large permanent water, especially with vegetation, such as marshes, rivers edges and ditches and rice fields with matured plants providing shade. *Anopheles funestus* bites human predominantly, but also domestic animals (Service, 2000). *Anopheles funestus* was regarded as the most important secondary vector of malaria, associated with endemic malaria in the western part of Ethiopia (Jolivet, 1959). It is the third most common vector of malaria in the country. It feeds primarily outdoor and also resets outdoors (Tulu, 1993b). Although, being anthrophilic and endophilic, this potentially good vector usually has a low sporozoites index (Mekuria, 1983). Indoor insecticide sprays might have eliminated the indoor frequenting *An. funestus*, which is reported to be highly sensitive to such control measures (Gilles and De Mellion, 1968).

Anopheles nili is the least common species, and it is more localized being confined to the south western and north western parts of Ethiopia (Giaquinto-Mira, 1950; Gebre-Mariam *et al.*, 1988). It is sparsely distributed in the northern regions of Amhara, western Oromiya, Gambella, and Southern Nations Nationalities and People Administrative Region (Gebre-Mariam *et al.*, 1988). Krasfur (1970 b) reported that *An. nili* is an important malaria vector in Gambella and the surrounding areas. It is more endophagic but also exophilic. Due to the fact that there is cross border movement of population to and from southern Sudan, a possibility of transmission remains even under conditions of total insecticidal coverage. (Krasfur, 1970b)

Sporozoites infection rates are very low in Ethiopia and a large numbers of specimens need to be tested by dissection in order to be able to determine the actual infection rate.(Abose *et al.*, 1998a) Insecticide resistance is a problem in Ethiopia. In the past ten years, the WHO insecticide test kit has been used to evaluate the susceptibility or resistance levels of the species to DDT and other insecticides. In some selected areas of the country levels of resistance for DDT varying from 30% to 70% were detected. Higher resistance accounting 60-70% was detected in some localities, such as in Arba-Minch in the south and Gambella in the west (Abose *et al.*, 1998a). However, recent study conducted in Metahara sugar estate and Melka-Warer Agricultural development enterprise revealed that in average *Anopheles arabiensis* was resistant to DDT and permethin 63.75% and 87.5 respectively (Meshesa *et al.*, 2003). In places where higher DDT resistance is detected, malathion, an organophosphate, is being taken as the alternative insecticide for indoor applications (Abose *et al.*, 1998 a).

3. MATERIALS AND METHODS

3.1. Description of the study area

Afar Region is located in North-east Ethiopia between 8^0 40' to 14^0 27' N latitude and 39^0 51' to 42^0 23'E longitude with its major portion lying towards west of the Rift valley. It has a large geographical area which is estimated to 270,000 square kilometers (Industrial project service: IPS, 1998).

According to 1994 population and housing census, the total population of the region was 1, 106, 383, of which 56.1% are males and 43.9% females. The proportion of population living in rural area is 91.9% while that of urban is only 8.1 %. The dominant ethnic groups in the region are the Afar which constitutes 91.8% followed by Amahara (4.5%), Argoba (0.9%), Tigre (0.8%), Oromo (0.8%) and others (1.2%) (Central Statistic Authority,1994).

The region has some important rivers such as Awash, Mille, Awdae, Awra, Dewale, Telalk besides other numerous seasonal rivers. The river Awash dominated the region, the flow of which is highly seasonal and depends largely on the rainfall in the highland regions. Only during the rainy season is the outflow of the water so high to adjacent areas (Regional Health Bureau: RHB, 2000).

The region consists of mainly lowlands ranging from 100 meters below sea level and highly escarpments in the west and south ranging from 1000-1500 meters above sea level. The Great Rift Valley also interseets the region east-west. The climate of the region is arid and semi-arid (IPS, 1998). It is one of the hottest regions in Ethiopia. It becomes hottest as one goes from

the middle to the lower Awash valley. The hottest months are June, July and August and during these months the temperature reaches up to 45^0c and some times up to 50^0c in the Dallol depression (RHB, 2000). The minimum temperature is about 18^0c. The region is characterized by an extremely variable and low some times very scarce rain fall ranging from 200mm on the lava plains to the east and 520 mm on the western edge of the escarpment during the summer. The area gets its main rainfall in July to September and little rains in March to April. The severest dry season occurs in May and June and a less severe dry season is from November to March (IPS, 1998; RHB, 2002).

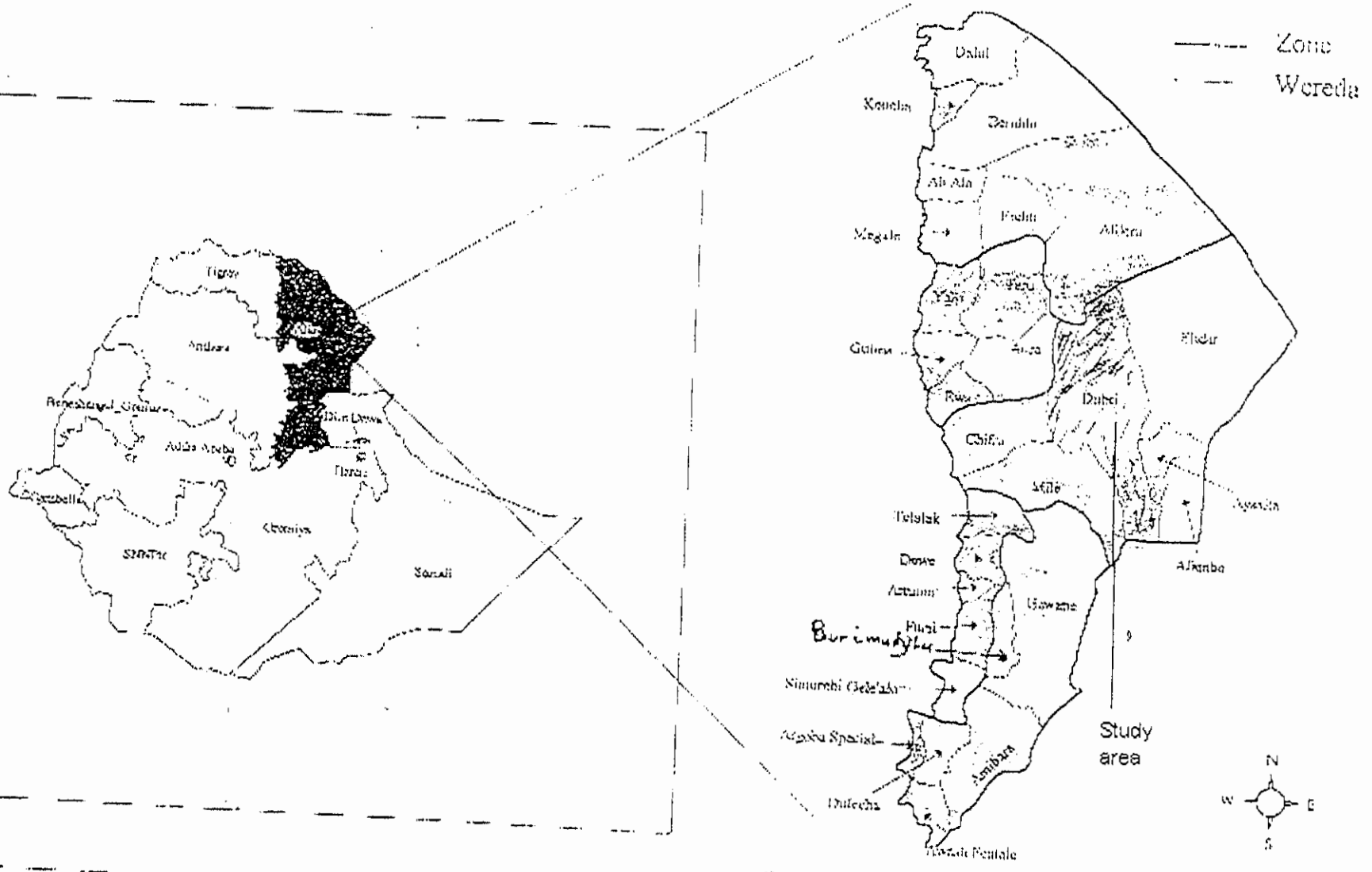
The arid environmental condition and the scarcity of rain fall have shaped the life of the Afar people to lead nomadic mode of life. The main stream of the regional economy is livestock husbandry. It is found that more than 97% of the regional populations are pastoralists, heavily dependent in live stock rearing. Infrastructure and social services such as roads, communication, water supply, education and health services are generally inadequate in the region (Ayalew *et al.*, 2002).

Over 80% of the populations have no access to basic health services. Of the 20% population which have access to basic health services about 87% of them have clinics, 5% have pharmacies and only 0.5% have hospital service. The most common infectious diseases in the region are tuberculosis and malaria (RHB, 2003).

Along the bank of the Awash River and all the perennial rivers have potential for irrigation to cultivate cotton and maize in large scale state and small private farms. One of the largest agro-industry developments in the region is Tendaho agricultural development enterprise in Doubti Woreda which is selected for this study (Fig.1). Doubti wereda is located in the eastern part of the

region between 11° 45'N latitude and 41° 23'E longitude. Temperature varies between 25 to 48°C. and altitude of the wereda is 495m. The rain fall ranges from 220 to 460mm. This wereda comprises about 15 Kebeles with a total population of 67398. The health facilities in this wereda are only one hospital and seven clinics. This entomological study covered three villages (camps) of Tendaho agricultural development enterprise: Farm-1, Farm-2 and Farm-3 camps. Farm-1 is a site located about 6 km North from Dobuti town with a population of about 1,500 permanently settled. Farm-2 is located about 7 km North-east from Dobuti towns and comprises about 2000 permanent population. Farm -3 is located about 6 km North-west from Doubti town with about 1700 permanently settled population. In addition to these permanently settled populations about 3,000 to 3,500 laboureres are coming from the high land regions to these camps during cotton harvesting seasons (RHB, 2003).

Fig.1. Afar National regional State Wereda Level Map



All Administrative borders shown are unofficial and approximate.

3.2 Malaria Case Studies

Parasitological studies were employed by gathering information on the malaria case distribution in different age groups of human population and prevalent malaria parasites. Data on all malaria cases confirmed from 1999/2000 to 2003/2004 at different health service units throughout the region were compiled from Afar Regional State Health Bureau annual reports. Monthly data about malaria cases of Doubti wereda including Tendaho agricultural development enterprise from September 2004 to April 2005 were collected from Doubti hospital.

3.3 Entomological Studies

Both immature and adult *Anopheles* collections were conducted to determine the species composition, and behavior of mosquitoes. Samples were collected from feeding and/or resting sites and also from breeding sites. The collected specimens were identified to species on morphological bases using Verone (1962a) and Verone (1962b) for adults and larvae respectively. Female adult *Anopheles arabiensis* and *Anopheles pharoensis* caught were also dissected for extracting salivary glands and ovaries to determine the sporozoites infection rate and parity rates respectively.

3.3.1 Collection of immatures

Larval collections were conducted to study the different breeding places preferred by the *Anopheles* species and to supplement the data of adult female collections regarding to the study of species composition. If a water body was located, sampling was purposely carried out at each site. Therefore, larvae sampling was carried out in a variety of aquatic habitats of the study area.

Anopheles larva collections were undertaken for 6 hours per day, two days per month by two collectors for 8 months in each of the three study sites by using sampling techniques recommended by WHO (2003). Thus, larvae of *Anopheles* were collected from permanent and temporary breeding habitats such as along the edges of Awash river, artificial canals, broken pipes, marshyareas, sewerage, drainage, pool, discarded tires, hoof prints of animals etc. Appropriate pipettes, scoops, dipper, and vials were used for larval collection.

Larvae found in small bodies of water were sampled by stirring the water briskly with a finger and wait quietly. Larvae surfacing for air were picked up directly with the pipette. Larvae collected in vials and killed by a flame of a burning cotton wool soaked with
alcohol (placed on piece of rock) then preserved in 75% ethyl alcohol. The 3rd and 4th instar larvae then, mounted on slides and identified by Verone (1962 b) identification key using microscope.

3.3. 2. Adult Mosquito Collections

3.3.2.1 Indoor collections

Adult *Anopheles* mosquitoes which were resting and tempted to bite human inside habitations and associated structure were collected from randomly selected houses. Collections were employed in each month throughout the study period by using space spray (knock-down collection), CDC (Center for disease control) light trap catches and mouth operated aspirators. Collected *Anopheles* specimens were identified to species and freshly caught unfed females were dissected to determine sporozoite and parity rates according to WHO (2003).

Space-spray collections were used to estimate indoor resting density of female *Anopheles* mosquitoes. It was conducted early in the morning from 06:00 to 08:00 twice a month in 6 selected houses which were very compact with fitting doors and windows. A white sheet of cloth and aerosol were used to conduct this collection.

After permission was granted from the inhabitants all occupants, removable house utensils, food and drinks were removed from the sprayed room. All openings that were allowed mosquito escaping, doors and windows were closed; the entire floor was covered with the white cloth sheet. An aerosol containing 0.2% pyrethrin, and 1% piperonylbutoxide was sprayed for about 5 minutes on walls and roofs. After the spray operation, the sprayer waited out side and the rooms were closed for about 15 minutes to produce a knock-down effect. After 15 minutes all knocked down mosquitoes were collected from white sheet cloth using forceps and torch light and collected in paper cups for later studies of identification and dissection.

Dry cell battery-operated CDC light traps were positioned indoor besides a bed with a mosquitonet from 18:00 in the evening to 06:00 in the morning to collect *Anopheles* mosquitoes tempted to feed blood from human indoors. Mosquitoes were collected and killed with chloroform. They were pinned immediately before they get dried by using non-corrosive entomological pins.

Mosquitoes were collected by aspirating indoors from their resting sites. Mouth-operated aspirators collections were carried from 16:00 - 18:00 and 05:00 -07:00 at the early morning. Aspirator collection was carried by three collectors for 4 days per month in the three study sites for 8 months. Each collector spent about 20 minutes in a house. A flash light was used in locating the mosquitoes resting in the relatively dark parts of the interior of a

dwelling. Collected specimens were transferred to a paper cup and killed with chloroform vapour. During aspiration, while four or five mosquitoes were collected inside the tube they were transferred to the paper cup to avoid injuring the specimens according to WHO (2003).

3.3.2.2. Outdoor Collections

Mosquitoes were surveyed and collected from their outdoors resting places among vegetation (bush, tall grass), in pits, on the ground or under logs of wood etc. Outdoor resting collections were conducted by using aspirator. Outdoor resting mosquitoes were collected by aspirator and a flash light is used in locating the mosquitoes. Aspirator collection however, is too tedious, time consuming and relatively unproductive owing to the fact that there were innumerable place where mosquitoes were find shelters even in a small area. Battery operated CDC light traps were suspended out side houses (outdoors) beside beds where people sleep.

3.3.2.3 Night biting collections

Night biting collections of the Anopheline mosquitoes were conducted to determine man-biting rate, the degree of exophagic and endophagic, and hourly biting cycles.

In this study, night biting catches were carried out both indoors and outdoors at 19: 00 - 23:00 before people goes to bed. Two human volunteers (collectors) in each of the three study sites took prophylaxis and they were acted as collectors bait. Collectors were sat on chair with their body usually their legs and arms exposed to mosquitoes biting. The mosquitoes which were located as they bite the collectors were caught by using a flash light and a suction tube and collected in paper cups. Collectors were rotated monthly between indoors and outdoors to compensate for individual differences in their relative attractiveness and also their skill in catching the biting mosquitoes. Both outdoors and indoors hourly collected mosquitoes were kept in separate

containers and properly labeled. Collected specimens were identified and unfed females were dissected. Moreover, bites per man per hour were computed (compared) for the commonly encountered and collected species.

3.3.3 Dissection of female *Anopheles* mosquitoes

Dissection of female *Anopheles* mosquitoes is necessary to determine parity rate and sporozoite infection rates (Service, 1993). Mosquitoes dissections were employed from mosquitoes collected indoor, outdoor and night biting catches. Mosquitoes were killed

with chloroform vapor and identified to species level. To dissect the mosquitoes and extracting salivary glands and ovaries, dissecting microscope (stereoscopic microscope), compound microscope, dissecting needles, fine forceps, microscope slides, dropper, saline solution and distilled water were used.

3.3.3.1 Parity rate determination

Blood digestion stages of mosquitoes were determined according to abdominal condition. Only females which were unfed or freshly fed were used for parity rate determination. *Anopheles* mosquitoes, with ovaries of coiled (enlarged) tracheolar skeins were identified as nulliparous, where as those with ovaries in which the tracheolar skeins had become stretched out were identified as Parous as recommended by WHO (2003).

During dissection of mosquito and extraction of ovaries to determine parity rate, mosquitoes were killed, arranged on a slide with the head pointing towards the right, legs and wings were removed (WHO, 2003). A drop of distilled water was placed a little behind the posterior tip. Ovaries were come out of the abdomen by holding one needle gently on the thorax and the tip of the abdomen

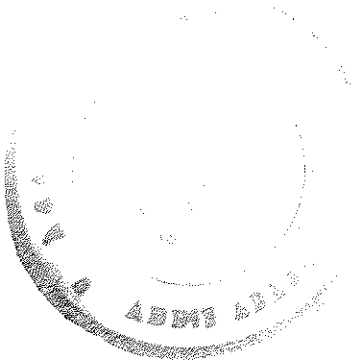
was pull away from the rest of the body with another needle held in the right hand. Ovaries were separated from the rest of the body by cutting through the common oviduct. The ovaries were transferred to a drop of distilled water solution on another slide and allowed them to dry. Dried ovaries were examined under a microscope using 10x objectives and confirmed by 40 x objectives. Determinations of the ovarial dilations were made by spreading and examining ovarioles according to the recommendations of Detinova (1962).

3.3.3.2 Sporozoite rate determination

In dissection and extraction of salivary glands a parous mosquito was arranged on clear slide with the head position to the right, its appendages were trimmed properly to avoid its interference during the dissection. A drop of saline solution (0.9%NaCl) was placed close to the thorax. Dissection needles were cleaned and free from rust or grease. The needle was placed in the left hand obliquely across the thorax. The head was pulled gently from the thorax; glands were extracted by pulling from the thorax, attached to the head. While the glands did not come out with the head, thorax was gently squeezed. Glands were separated with the other needle and placed on a slide in a drop of saline solution. Small micro-cover slips (18x18 mm diameter) were used to cover the glands, as it was easier to locate the glands. While the glands have not been crushed by the cover slip, it was gently pressed with a dissecting needle so that the glands break and sporozites are released recommended by WHO (2003). Fatty tissues, which surrounded the glands, were removed from the vicinity of the glands before spacing the cover slip. The glands examined under microscope in a high power 40 x objective.

3.4 Data Analysis

Data collected for entomological studies were analyzed using SPSS statistical soft ware program version 10 for windows. Larval density among months and among the most frequently available breeding habitats were computed by one way ANOVA. Hourly human biting rate of adult Anopheline were also analyzed by one way ANOVA (Gomaz and Gomaz, 1984). Indoors and outdoors resting density as well as biting density was compared using chi-square test.



4. RESULTS

4.1 The malaria cases and *Plasmodium* infection rates

Data of malaria cases confirmed in different health facilities of Afar region were compiled from the regional health bureau (RHB) malaria and other vector borne diseases control and prevention department (Appendix 1). There was an increment in the overall confirmed malaria cases for the past five successive years except in the case of 1999/2000 -2000/2001. The highest increment rate 10.1 percent was observed during 2002/2003 -2003/2004. The data showed that males were more affected by malaria infection than females throughout the past five years. On the average, of the total examined 531,115 febrile cases, 271278 (51.07%) were confirmed as malaria positive.

Confirmed malaria cases in different age categories are shown in Appendix 2. Although all age groups were vulnerable to malaria infection, a maximum 43.46 percent of the malaria confirmed cases were seen in age categories of 15 and above followed by 25.02 percent of 5-14 age categories. The prevalence of malaria parasites in the region for the past five years is shown in Appendix 3. The infection rate by *Plasmodium falciparum* shows increment in each successive year except, in the case of 2001/2002. The highest increment rate 9.2 percent was recorded from 2001/2002 to 2002/2003. Of the total malaria confirmed, 271278 cases throughout the past five years, 66.22 percent was *falciparum* malaria. Contrary to that of *Plasmodium falciparum*, infection rate of *Plasmodium vivax* shows declined from year to year. The maximum rate 44.75 percent of infection by *Plasmodium vivax* was recorded in 2001/2002, but the overall five years average prevalence rate of *Plasmodium vivax* was 33.78 percent.

4 .2 Species composition of *Anopheles* mosquitoes and other entomological studies

4.2.1. Anophelines based on larval collections.

Anophelein larvae were collected and identified from the three study sites in Doubti wereda. During the study period a total of 939 larvae were collected from different breeding habitats. The most abundant species recorded from larval collections was *Anopheles arabiensis* which comprised 91.8 percent. Larvae of *Anopheles pharoensis* comprised 8.2 percent of the total larvae collected and identified during the entire study period. During the dry months small pockets of rain pools and associated collection of water disappeared. Therefore, breeding sites associated with irrigation and along the edges of the Awash River, and large collected water pools were found to harbour immature stages of *Anopheles* mosquitoes. The important breeding sites and mean number of Anopheline larvae collected are summarized in Table 1.

Larvae of *Anopheles arabiensis* were collected from a variety of small collected water bodies where as *Anopheles pharoensis* collected in specific breeding habitats such as sewerage and irrigation canals. Table 2 shows mean of monthly variation significantly in the density of *Anopheles arabiensis* and *Anopheles pharoensis* larvae. Although, the density varies from month to month, *Anopheles arabiensis* breeds throughout the study periods. The relative maximum number of *Anopheles pharoensis* larvae was sampled during the dry months than the wet months during the study period.

Table 1 Mean of Anopheline larvae collected from different breeding habitats.

Breeding habitats	**Mean ± SE**	
	Anopheles arabiensis	***Anopheles pharoensis***
Construction pits, and discarded tires	23.33 ± 3.75 ab	1± 1a
Broken pipe/pool	17.33 ± 9.82 ab	0 ± 0a
Rain pool water and flooded patched water	19.33 ± 9.73 ab	0 ± 0a
Edges of River Awash and large pools	40.00 ± 8.38 ab	8± 4.35ab
Small irrigation canals	108.66 ± 16.89 c	14.66 ± 4.05ab
Drainage and sewerages	69.00 ± 17.38 bc	2 ± 2a
Undisturbed swamp	9.66 ± 6.48a	0 ± 0a
Total	41.04 ± 8.23	3.66 ± 1.63

Means within a column followed by a different letters are significantly different from each other (at $P < 0.05$) Tukey's studentized test.

Table 2 Mean of Monthly collected Anopheline larvae from the three study sites (Septembers 2004 - April 2005)

Month	Mean ± SE	
	Anopheles arabiensis	*Anopheles pharoensis*
September	65.66 ± 4.66 b	0.00 ± 0.00a
October	61.00 ± 18.24 b	1.33 ± 0.88 ab
November	25.66 ± 4.05 ab	3.66 ± 1.20 abc
December	13.66 ± 2.96 ab	5.00 ± 1.15 b
January	7.33 ± 7.33 a	4.33 ± 1.2 abc
February	14.33 ± 6.35 ab	7.33 ± 0.88 c
March	55.66 ± 7.85 ab	1.33 ± 0.33 ab
April	44.00 ± 20.25 ab	2.66 ± 1.45 abc
Total	35.91 ± 5.58	3.20 ± 0.54

Means within a column followed by a different letters are significantly different from each other (at $P < 0.05$) Tukey's studentized test.

4.2.2 Feeding and resting behavior of *Anopheles* mosquitoes

The number of adult female Anopheline collected indoors and outdoors in the three study sites (Farm-1, Farm-2, Farm-3) and monthly collected are summarized in Table 3 and 4 respectively. A total of 2419 adult female Anophelines were collected indoor and outdoor and identified to species during the study period. Of the total collected adult anopheline, *Anopheles arabiensis* were the most abundant which comprised, 93.35 percent followed by 6.4 percent of *Anopheles pharoensis*. The third speeies, *Anopheles coustani* were found rarely and comprise only 0.25 percent of the total collection.

Anopheles arabiensis was the predominant species in all the three study sites both indoor and outdoors. Data on the indoors and outdoors density of *Anopheles arabiensis* collected by aspirator and light trap were computed and presented in Table 5. Indoor resting density of *Anopheles arabiensis* collected by aspirator significantly outnumbered its own outdoor resting density ($\chi 2 = 5.544$; $P = 0.019$). This species collected indoors by light trap while tempted to bite human has highly significant difference from its outdoor density ($\chi 2 = 65.474$; $P = 0.000$). *Anopheles pharoensis* density collected resting indoors collected by aspirator also show significant difference from its outdoors resting density ($\chi 2 = 6.25$; $P = 0.012$) (Appendix 10). Relatively high density of both *Anopheles arabiensis* and *Anopheles Pharoensis* were collected in Farm-1.

Table 3 Summary of indoor and outdoor collected adult female Anopheline from the three study sites (September 2004 – April 2005).

Species	Farm 1	Farm 2	Farm – 3	Total
Anopheles arabiensis				
Indoor	552 (68.14%)	539 (73.43%)	492 (68.9%)	1583 (70.1%)
Outdoor	258 (31.86%)	195 (26.57%)	222 (31.1%)	675 (29.89%)
Total	810(100%)	734 (100%)	714(100%)	2258(100%)
Anopheles pharoensis				
Indoor	36 (59.1%)	22(56.41%)	40 (72.7%)	98 (63.23%)
Outdoor	25 (40.9%)	17 (43.59%)	15 (27.3%)	57 (36.37%)
Total	61 (100%)	39 (100%)	55 (100%)	155 (100%)
Anopheles coustani				
Indoor	1(33.33%)	1(100%)	0 (0%)	2 (33.33%)
Outdoor	2 (66.67%)	0 (0%)	2 (100%)	4 (66.67%)
Total	3 (100%)	1 (100%)	2 (100%)	6 (100%)

Table 4 Summary of monthly indoor and outdoor collected adult Anopheline from the three study sites (September 2004 – April 2005)

Species	Sep	Oct	Nov	Dec	Jan	Feb	Mar	April	Total
Anopheles arabiensis									
Indoors	217	337	244	135	176	80	225	169	1583 (70.16%)
outdoors	113	180	106	54	73	50	79	20	675 (22.89%)
Total	330	517	350	189	249	130	304	189	2258 (100%)
Anopheles pharoensis									
Indoors	8	12	16	17	22	19	0	4	98 (63.23%)
Outdoors	5	8	0	6	9	17	9	3	57 (36.77%)
Total	13	20	16	23	31	36	9	7	155 (100%)
Anopheles coustani									
Indoors	1	1	0	0	0	0	0	0	2(33.33%)
Outdoors	0	2	1	1	0	0	0	0	4 (66.67%)
Total	2	3	1	0	0	0	0	0	6 (100%)

Table 5 Total indoors and outdoors collected *Anopheles arabiensis* from the three study sites by different techniques (September 2004-April 2005).

Collecting techniques	Farm-1	Farm-2	Farm-3	Total	Test Statistics	
					χ^2	P-value
Aspirator						
Indoor	110	179	162	451		
Outdoor	148	111	124	383	5.544	0.019
Total	258	290	286	834		
Light Trap						
Indoor	200	193	130	523		
Outdoor	110	84	98	292	65.474	0.000
Total	310	277	228	815		
Space Spray						
Indoor	206	173	230	609		
Outdoor	--	--	--	--		
Total	206	173	230	609		

4.2.3. Biting density of *Anopheles* mosquitoes based on night biting caches.

The results of night biting caught *Anopheles* with the human baits both indoors and outdoors once a month during the study period are summarized in Tables 6 and 7. A total of 955 female *Anopheles* mosquitoes were collected and identified as *Anopheles arabiensis* and *Anopheles pharoensis*. *Anopheles arabiensis* was the predominant species comprised 96.12 percent of the total biting catches. Monthly collected data indicated that the peak night biting rates of *Anopheles arabiensis* were observed in October and April, where as the peak biting rate for *Anopheles pharoensis* was recorded during the dry months especially in February.

The overall outdoor night biting rate collected in 8 months for both *Anopheles arabiensis* and A*nopheles pharoensis* exceed their indoor biting densities. Of the total collected 918 *Anopheles arabiensis,* 542 (59.04%) were collected biting humans outdoor and 376 (40.96%) were biting indoor, which shows significant difference ($\chi 2 = 30.017$; $P = 0.000$). Similarly outdoor biting *Anopheles pharoensis* exceeds indoor night biting densities, significantly ($\chi 2 = 4.56$; $P = 0.033$). The highest night biting catches of *Anopheles arabiensis* and *Anopheles pharoensis* were collected in Farm- 1 and Farm-3 respectively.

Hourly biting rates of *Anopheles arabiensis* per human are given in Table 8. The peak biting hour of this species was recorded from 20:00- 21:00 and 21:00 – 22:00 for indoor and outdoor respectively. The density of biting catches of *Anopheles pharoensis* were expressed as the average hourly biting rate per human is very low (Table 9).

Table 6 Monthly indoors and outdoors night biting collected *Anopheles arabiensis* per day from the three study sites

Study Sites	Months									Test Statistics	
	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Total	$\chi 2$	P-value
Farm-1											
Indoor	18	20	22	20	14	16	14	28	152		
Outdoor	34	46	30	14	6	12	4	36	182	2.695	0.101
Total	52	66	52	34	20	28	18	64	334		
Farm-2											
Indoor	18	26	6	12	12	4	12	16	106		
outdoor	28	44	12	14	16	10	12	32	168	14.029	0.000
Total	46	70	18	26	28	14	24	48	274		
Farm-3											
Indoor	16	32	16	14	6	4	12	18	118		
Outdoor	56	40	26	18	8	8	4	32	192	17.665	0.000
Total	72	72	42	32	14	12	16	50	310		
Over all total											
Indoor	52	78	44	46	32	24	38	62	376		
Outdoor	118	130	68	46	30	30	20	100	542	30.017	0.000
Total	170	208	112	92	62	52	58	162	918		

Table 7 Monthly collected indoors and outdoors night biting *Anopheles pharoensis* from the three study sites

Study Sites	Months									Test Statistics	
	Sept.	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**Total**	$\chi 2$	P-value
Farm-1											
Indoor	0	0	1	0	1	0	0	0	2(15.38%)		
Outdoor	1	0	0	2	1	3	2	2	11(84.62%)		
Total	1	0	1	2	2	3	2	2	13 (100%)		
Farm-2											
Indoor	0	0	0	1	0	3	1	1	6 (75%)		
outdoor	0	0	0	0	0	1	1	0	2(25%)		
Total	0	0	0	1	0	4	2	1	8(100%)		
Farm-3											
Indoor	0	0	0	1	1	2	0	0	4(25%)		
Outdoor	0	1	0	4	3	0	2	2	12(75%)		
Total	0	1	0	5	4	2	2	2	16(100%)		
Over all total											
Indoor	0	0	1	2	2	5	1	1	12(32.43%)		
Outdoor	1	1	0	6	4	4	5	4	25(67.57%)	4.568	0.033
Total	1	1	1	8	6	9	6	5	37(100%)		

Table 8 Mean of hourly collected indoors and outdoors *Anopheles arabiensis* from the three study sites per human

Collecting hour	Mean ± SE	
	Indoors	**Outdoors**
19:00 – 20:00	4.87 ± 0.76 a	6.00 ± 1.42 a
20:00 – 21:00	18.00 ± 1.61 ab	15.37 ± 4.14 ab
21:00 – 22:00	9.37 ± 2.36 c	26. 87 ± 6.08 b
22:00 – 23:00	14.00 ± 1.87 bc	22.62 ± 4.72 ab
Total	11.71 ± 1.22	17.71 ± 2.54

Means within a column followed by a different letters are significantly different from each other (at $P < 0.05$) Tukey's studentized test.

Table 9 Average hourly indoors and outdoors biting rate of *Anopheles pharoensis* per human in different months

Sites	Months								
	Sep.	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Apr.**	Average
Farm -1									
Indoors	0	0	0.25	0	0.25	0	0	0	0.09
Outdoors	0.25	0	0	0.5	0.25	0.35	0.5	0 .5	0.34
Farm -2									
Indoors	0	0	0	0.25	0	0.75	0.25	0.25	0.19
Outdoors	0	0	0	0	0	0.25	0.25	0	0.09
Farm-3									
Indoors	0	0	0	0.25	0.25	0.5	0	0	0.13
Outdoors	0	0.25	0	0.5	0.75	0.5	0.5	0.5	0.38

4.2.4 Parity and sporozoite rates

The results of dissection of *Anopheles arabiensis* and *Anopheles pharoensis* are shown in Table10. During the study period, a total of 214 female *Anopheles arabiensis* and 41 *Anopheles pharoensis* were dissected from human bait collected and resting collected samples to determine parity and sporozoite infection rates. The highest parous rate 34.1% of *An. arabiensis* collected indoor from resting places and from night biting was recorded from Farm 3. Over all total indoor collected *An. arabiensis* and *An. pharoensis* showed relatively grater parous rate than outdoor collected in the area. Of the over all total 214 dissected *Anopheles arabiensis* only 51 were parous, of these none were ineriminated with sporozoite, where as among 41 dissected *anopheles pharoensis* only 7 were parous but none of them were sporozoitc positive.

Table 10 Female Anopheline dissected for parity rate and sporozoites rate determination (October 2004- March 2005)

Study sites	*Anopheles arabiensis*			*Anopheles pharoensis*		
	No. dissected	**No Parous (%)**	**Sporozoites positive**	**No dissected**	**No Parous (%)**	**Sporozoites positive**
Farm-1						
Indoor	35	11 (31.4)	0	9	2 (22.2)	0
Outdoor	22	5 (22.7)	0	6	1 (16.6)	0
Total	57	16 (28.07)	0	15	3 (20)	0
Farm-2						
Indoor	32	4 (12.5)	0	3	0 (0)	0
Outdoor	37	8 (21.6)	0	3	0 (0)	0
Total	69	12 (17.4)	0	6	0(0)	0
Farm-3						
Indoor	41	14 (34.1)	0	12	3 (25)	0
Outdoor	47	9 (19.2)	0	8	1 (12.5)	0
Total	88	23 (26.1)	0	20	4 (20)	0
Over all total						
Indoors	108	29 (26.85)	0	24	5 (20.83)	0
Outdoors	106	22 (20.75)	0	17	2 (11.76)	0
Total	214	51 (23.80)	0	41	7 (16.60)	0

5. DISCUSSION

Malaria in Afar region is a serious public health problem. Information obtained on malaria cases from the regional health bureau annual reports and eight months malaria case of Doubti wereda showed that malaria has been highly prevalent throughout the year. Malaria transmission in this area prevails perennial both in the dry and wet months with slight variation in magnitude from month to month but it shows increment from year to year.

The intensity was more aggravated after the rainy months in September and October as well as on March, while malaria transmission reached to its peak. Even in the dry months, while the availability of habitats for the vector breeding is reduced transmission of malaria is not inconsiderable but showed less intensity as compared to the wet months. In general malaria remained to be a major cause of morbidity in this area. According to the regional health bureau (2002) almost 100% of the total land mass is regarded as malarious and all people are at risk to malaria infection. About 28% of disease burdean of the region is attributed to be malaria.

In some areas of the region especially; in agricultural development areas such as Doubti wereda malaria strikes during planting and harvesting seasons; cutting down the productive capacity of the people at a time when there is the greatest need for agricultural work. The disease also associated with loss of earnings, low school attendance and high treatment cost (RHB,2003).

According to Gebre-Mariam (1984), the distribution of malaria in Ethiopia is related to variation in altitude, topography and climate. Malaria is highly prevalent in warm climatic Zones. Thus, this area (Afar region) experiences warm climatic zones of 18^0c to 48^0c temperature with an elevation below 1500m. In addition to these factors the presence of several perennial and

seasonal rivers sum up with the application of poor irrigation system makes the area endemic to malaria. This is because the availability of permanent water creates year round favorable breeding places for the malaria vector Anopheline.

Studies conducted in many parts of Ethiopia by Fontaine *et al.*, (1961) and Gebre- Mariam (1984) revealed that *Plasmodium falciparum* was reported to be the predominant species. Therefore, as many parts of Ethiopia malaria in Afar particular reference to Doubti is predominantly caused by *Plasmodium falciparum* followed by *Plasmodium vivax.* Previously, *Plasmodium malariae* was commonly reported in Ethiopia from south, South-west and in the Awash valley (MOH, 2000).

In general previous studies revealed that *Plasmodium falciparum* accounts 80% of all malaria patients world wide (WHO, 2000). It is only in Ethiopia that *Plasmodium vivax* accounts the highest proportion 40%. Fontaine *et al.* (1961) reported from country wide surveillance that 60% of malaria was caused by *Plasmodium falciparum* in the country while that of *Plasmodium vivax* 25% and *Plasmodium malariae* to be 15%.

The rate of *Plasmodium falciparum* infection in Afar showed increasment from year to year for the last five years. In the year 2000 it was 65.4% of the total confirmed cases but four years later in 2004, it was increased to 72.9% of the total malaria infection. The overall average five years *falciparum* confirmed cases in this area were 66.22%. In contrast to *Plasmodium falciparum* infection, the rate of *Plasmodium vivax* has been decreased from year to year. In the year 1999/2000 *Plasmodium vivax* infection was about 34.6% but in the year 2004, it was declined to 27.1% and the overall average *Plasmodium vivax* infection rate throughout the past five years

was 33.78%, which is very low prevalence rate even from previous studies of country wide surveillance.

The endemicity of malaria in Afar especially in Doubti wereda is associated with poor irrigation practices in the large state cotton farms and small private subsistence farms which may attract none-immune population from none-endemic areas. Both males and females of all age groups are affected by malaria every year owing to the seasonality and low or moderate level of transmission. Over all males are more affected by malaria than females. The phenomenon may partly to be explained by the fact that males especially adults are more likely to spend the evening hours out of doors and therefore, are bitten more often. Age groups 15 and above is very much affected may be due to the labour and trade movements as well as other household activities.

Anopheles mosquitoes breed in a variety of water collections. Larval habitats vary from large permanent to small temporary water collections (Service, 2000). Quantitative observations on collected and identified larvae in this study area showed variation in density of Anophelines in different water bodies (breeding habitats) and from month to month. Anopheline mosquitoes in the study area were found to breed permanently throughout the study period in a variety of water collections mainly associated with irrigation and along the edges of Awash river.

The existence of permanent vector breeding habitats which was created by poor management of water supported the continual existence of the *Anopheles* species in a good density throughout the year. Although, there was no considerable rain during the study period, the little rainfall on September and March played a role to create large number of breeding habitats and enable to collect the highest number of larvae. The rain fall on the high land regions play significant role to flood the Awash River which creates several patched water, breeding habitats in the study area.

Anopheles larvae were rarely encountered in constructing pits and none is found in bath room drainage pits. Since the three study sites far apart 6-7 km one another, people in these study sites shares similar climatological factors, the same water management system, living and housing condition. As a result almost similar types of breeding habitats were encountered in the study sites irrespective of density.

Previous studies conducted by (Jolivet, 1959; Tulu, 1993b) revealed that, *Anopheles arabiensis* in many parts of Ethiopia breeds in sun-lit rain pools, discarded tires and artificial containers, edges of lakes, side pools of rivers, marshes, swamps and irrigation ditches. In this study area larvae of *Anopheles arabiensis* were collected in a variety of small temporary collected water, such as small canals, small pools along the Awash river with emergent vegetations and sun-lit rain pools. Larvae of this species were also collected in broken pipe pools of water and discarded tires. Tulu (1993b) discussed that *Anopheles arabiensis* was known to exhibit in a wide ecological range as its breeding habitat. Generally, densities of the *Anopheles arabiensis* larvae were closely associated with the availability of temporary suitable breeding places in different months. There was monthly variation in the density of *Anopheles* larvae due to human activity (time of irrigation), and climatological factors such as temperature and rainfall.

Anopheles pharoensis preferred to breed in irrigation canals and drainage. The larvae of this species were also collected at the edges of the Awash River which had vegetation shades as well as in the large pool water.

Adult anophelines were collected from their resting places, while they tempted to bite human and from human baits by landing collections. In Ethiopia variety of species composition were

reported in different localities. In general about 42 species of Anophelines were identified (White *et al.,* 1980). In this study area, Doubti, Tendaho Agricultural development enterprise only three species of adult *Anopheles* were encountered and identified, namely *Anopheles arabiensis*, *Anopheles pharoensis* and *Anopheles coustani*. *Anopheles arabiensis* is the most abundantly encountered species followed by *Anopheles pharoensis*. *Anopheles coustani* which has no importance for malaria transmission was encountered and identified rarely.

Anopheles arabiensis was identified by its morphological feature (diagnostic characteristics), spacklings on the hind leg femur, absence of laterally projecting tuffs of scales on abdomen, absence of scales on the dorsum of abdomen and by its smooth three pale bands of palp (Verone, 1962). *Anopheles pharoensis* was identified by its diagnostic
morphological features of their abdomen with laterally projecting tufts of scales and by its pale colored fifth tarsus of the hind leg, which looks "a white soak".

Of the four *Anopheles* species which have medical importance for malaria transmission in Ethiopia (i.e. *Anopheles arabiensis*, *Anopheles pharoensis, Anopheles funestus* and *Anopheles nili)* only the first two are encountered in this area may be because of the difference in the breeding habitat preference of each species. *Anopheles arabiensis* was found in appreciable density in the study area. *Anopheles arabiensis* collected resting both indoors and outdoors out numbered the *Anopheles pharoensis* may be because of the variety of breeding habitat preference of the former species than the latter one.

Both indoors and outdoors densities of *Anopheles arabiensis* varied in different months. However, its maximum density caught from their resting places was in September and October at

the end of the rainy months because of the presence of rain water pools and flooded patched water bodies in addition to the regular breeding habitats associated with irrigation.

The higher density of *Anopheles arabiensis* was collected indoors than outdoors. Of the total collected 2258 *Anopheles arabiensis* only 675 (29.89%) were outdoors, the rest 1583 (70.1%) from indoor. Similarly, *Anopheles pharoensis* were collected mostly indoors than outdoors. Of the total 155 *Anopheles pharoensis* collected on different sites, 98 (63.22%) were sampled indoors and 57 (36.77%) outdoor. The maximum density of this species collected from their resting places was in January and February when the indoor density of *Anopheles arabiensis* was relatively declined.

In general, both *Anopheles arabiensis* and *Anopheles pharoensis* showed higher density of resting indoors than outdoors. This may agree with that of Service (1995) comparatively few species of *Anopheles* mosquito regularly rest in human habitation. Hence, in this area both *Anopheles arabiensis* and *Anopheles pharoensis* preferred resting mostly indoors than outdoors may be because of lack of adequate shaded and humid resting places out of doors. The other assumption to this density difference is that to find mosquitoes resting outdoors are very difficult due to great diversity of outdoors hiding places that are not easily identified.

Previous studies by Toure (1999) also revealed that *Anopheles* mosquitoes change their resting behavior from indoor to outdoor as a result of frequent indoor insecticide spray. However, in this study area, DDT were sprayed in August by the regional health bureau in both the three study sites, but large number of *Anopheles* mosquitoes were sampled in September, after 15 days of spraying indoors from resting on the residual of DDT, which may indicate that they may have high resistance level to DDT.

During the study period both *Anopheles arabiensis* and *Anopheles pharoensis* were collected indoor on the wall, roof, on furniture and hanged on spider web, as well as in the shaded and moistened side of the water pot and barrel. Considerable numbers of *Anopheles* were collected in bath room which were shaded and moistened. Outdoor resting mosquitoes were collected on walls, vegetation, cattle shades, and tree holes. Most of the outdoor resting *Anopheles arabiensis* were collected from walls outdoor and cattle enclosures at the early morning.

Female *Anopheles* mosquitoes are attracted to humans and/ or animals to obtain blood meals (to bite). The number of vectors biting humans is therefore, a major determinant of malaria transmission, and it is important to indicate which Anopheline species bite humans, which of those that bite humans are vectors of malaria, whether the vectors bite indoors or outdoors, their peak biting time and the seasonal variations in the numbers of mosquitoes biting humans (WHO, 2003).

In this study both *Anopheles arabiensis* and *Anopheles pharoensis* were showed to land on and bite human baits. By giving equal time, four hours indoor and outdoors for human biting collection, *Anopheles arabiensis* showed higher exophagic behavior than endophagic in Farm-2 and Farm-3, but insignificant in Farm-1. Over all outdoor biting density of *Anopheles arabiensis* exceeds significantly the indoor biting density. However *Anopheles arabiensis* collected by CDC light trap when they tempted to bite human showed predominately indoors. *Anopheles pharoensis* also showed greater exophagic behavior in Farm-1 and Farm-3. Of the total collected 37 *Anopheles pharoensis* 25(67.56%) were caught while they tempted to bite human outdoors and 12 (32.43%) were collected to bite human indoors. This phenomenon may agree with the

previous studies of Krasfur *et al.* (1971) which discussed that *Anopheles pharoensis* feeds predominantly outdoors.

According to Service (2000) the night time biting of Anopheline is associated with the night time habits of the local people. Observation on this study area confirmed that most of the people spend almost the overall night time outdoors in several of the months especially during the hottest months. People feed, drink and sleep out of doors because of the higher temperature of indoor than outdoors during the night time. Studies that have been conducted on odor-mediated host-seeking behavior of *Anopheles* mosquitoes also indicated that host odors and carbon dioxide play a major role in feeding behavior (Coluzzi *et al.*, 1979). Hence, in this area *Anopheles arabiensis* and *Anopheles pharoensis* bite predominantly outdoors may be because of the human activities during the night and they are attracted to carbon dioxide, sweat and heat of human out of doors.

The highest human biting densities of *Anopheles arabiensis* was recorded on September and October. This rise was partly due to the occurrence of additional breeding habitats as mentioned in larval collection and the total indoor and outdoor resting densities in these months during the study period. The rate of both outdoor and indoor biting density of this species declined from November to March then rise again on April.

The highest biting rate of *Anopheles pharoensis* was shown in the dry months while the density of *Anopheles arabiensis* was relatively decreased. In September and October the human biting rate of *Anopheles pharoensis* was declined. This phenomenon may agree with the discussion that *Anopheles pharoensis* is responsible for malaria transmission in low density of *Anopheles arabiensis* particularly in the dry months (Ameneshewa, 1995).

In this study the peak-biting hour of *Anopheles arabiensis* was recorded from 20:00-21:00 and 21:00-22:00 indoors and outdoors respectively. The early morning biting rate and the mid night biting rate were not conducted in this study, hence the average daily biting rate of Anopheline is not determined due to the limited hour collection. Therefore, there is a need of further work to evaluate the overnight time biting rate of Anopheline (biting rate per day per person).

According to studies conducted so far in Ethiopia and in other parts of Africa, *Anopheles arabiensis* is the principal vector of malaria (Abose *et al.,* 1998a; Fortenille and Lochouran, 1999; Coetzee *et al.,* 2000). The number of vectors biting human is a major determinant of malaria transmission (WHO, 2003). Thus, in this study the data obtained from human bait collection, *Anopheles arabiensis* is the major vector for malaria transmission because of its high rate and density of night biting catches. This may be duc to the possibility of vector-human contact (bite) increase as the density of the vector *Anopheles* increases.

The abundance of *Anopheles arabiensis* in a remarkable density throughout the study period, its frequently encountered in human bait collection as well as the malaria case data together confirmed the presence of perennial (uninterrupted) malaria transmission in this area. Other studies in Ethiopia example by Tulu (1993b) reported that *Anopheles pharoensis* serves as the second most frequent and widely distributed vector of malaria. Thus, in this study although its density is less the frequent occurrence of this species in the study area and their encountered in human biting indicate that *Anopheles pharoensis* to be the second important malaria vector. However, further advanced work such as sporozoites test by enzyme linked immuno sorbent assay (ELISA) and blood analysis are required to confirm its actual rate of infection and vectorial capacity of these species in this area.

Determining the abdominal condition or blood digestion stages (unfed, freshly feed, half gravid and gravid) is important component in vector incrimination studies (WHO, 2003). By determining the abdominal condition or blood digestion stages unfed *Anopheles arabiensis* and *Anopheles pharoensis* were dissected to determine parity rate. During the study period a total of 214 *Anopheles arabiensis* and 41 *Anopheles pharoensis* were dissected and extracted their ovaries. The parity rate for the two species was 23.83% and 13.72% respectively.

Parity is an entomological indicator used to determine if malaria transmission has been reduced or not. Parous females are epidemiologically dangerous because they have potentially infective malaria parasites (Service, 1993). Where as, nulliparous mosquito may not transmit malaria. However, not all parous *Anopheles* mosquitoes transmit malaria because; the rate of parity may determine the ability to transmit the parasite. A parous female that had laid eggs once (or twice) may not yet to old enough to transmit malaria parasites because the gonotrophic cycle, the time from the first blood seeking to the second blood seeking averages only three days but sporozoite development takes 10-12 days (WHO, 2003). Therefore, a mosquito may needs at least three gonotrophic cycles before it is able to transmit malaria.

The physiological age determination of mosquito is therefore, an important activity to determine the parity rate as well as their potential to transmit the malaria parasite. An ovarial dilation count described by Detinova (1962) is the method to determine ages of mosquitoes. WHO (2003) also recommended that, determination of physiological age by counting the number of scares (ovarial dilation) and to multiply this number by the gonotrophic cycle usually done only in special research projects.

During the study period parous *Anopheles arabiensis* and *Anopheles pharoensis* were tested for the presence of sporozoites of either *Plasmodium falciparum* or *Plasmodium vivax* in the head thorax by dissecting method. Of all dissected parous mosquitoes none were found to be sporozoite positive. Actually sporozoite test with dissection method has been complained because it is too tedious and inefficient. The application of ELISA in some studies improved detection of sporozoite rates (Nigatu *et al.*, 1992; Abose *et al.*, 1998). Therefore, study by using ELISA is required to determine the sporozoite rates of Anophlines in this area.

6. CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Background knowledge of malaria and the Anopheline vectors in Africa as well as in Ethiopia is reviewed. Malaria in Ethiopia is a major public health problem, highly prevalent in the low lands but occasionally it occurs as an epidemic in the high land regions. The disease is mainly caused by *Plasmodium falciparum* followed by *Plasmodium vivax*. Members of the *Anopheles gambiae* complex are refereed as the main vector of malaria in Africa. Particularly *Anopheles arabiensis* is the major vector of malaria in Ethiopia. *Anopheles funestus*, *Anopheles pharoensis*, and *Anopheles nili* are referred as secondary vector which occurs sympatrically with *Anopheles arabiensis*.

Entomological data were collected by using different techniques to determine the species composition, breeding habitats and behavior of *Anopheles* mosquitoes in relation to the malaria transmission in Doubit Woreda. The study also included informations (data) of malaria confirmed cases in different health facilities from the regional health bureau and Doubti hospital.

The malaria case records showed that malaria has perennial transmission and also showed increment from year to year. Of the four *Plasmodium* parasites *Plasmodium falciparum* accounts the highest prevalent rate followed by *Plasmodium vivax*. Both males and females of all age groups are vulnerable to malaria.

In this area Anopheline mosquitoes breed in collected water, rain pools, flooded patched water, edges of river Awash, ponds, canal spillages, sewerages, drainages and artificial containers such as discarded tires as well as broken pipe pools.

Anopheles arabiensis and *Anopheles pharoensis* are the most important malaria vectors in this area. *Anopheles arabiensis* is the most abundantly encountered and the major vector, where as *Anopheles pharoensis* is the secondary one.

Anopheles arabiensis and *Anopheles pharoensis* in this area rest predominantly indoors. Both of these two species collected by human bait mostly encountered to bite human outdoors than indoors. The over all parous rate of *Anopheles arabiensis* determined by tracheolar skeins showed 23.8% where as the parous rate of *Anopheles pharoensis* is 16.6%.

6.2 Recommendation

1. Detailed and frequent entomological studies are required especially on the insecticide resistance level, host preference and sporozoite rates are required to elucidate the vectorial status of the Anopheline vectors in this area.
2. In line with the need to have the active involvement of the community in the control programme, social mobilization will be necessary to reduce the breeding habitats such as the broken pipe pool, discarded tires and small patches of flooded water and so on.
3. More impute is expected form Tendaho Agricultural development interprise in environmental management.
4. Use of insecticide treated nets (ITNS) should be encouraged.

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Appendix 8. Summary of Analysis of Variance of Anophliene larvae collected in different breeding habitats.

Source of Variation	Sum of square	Df	Mean square	F	Sig
Anopheles arabiensis					
Between groups	23061.619	6	3843.603	9.904	0.000*
With in groups	5433.333	14	388		
Total	28494.952	20			
Anopheles Pharoensis					
Between groups	570.00	6	95	5.481	0.004 †
With in groups	242.667	14	17		
Total	812.67	20			

* denotes significance at 5% level of significance (P< 0.05)

† denotes significance at 5% level of significance (P< 0.05)

Appendix 9. Summary of Analysis of Variance of hourly collected night biting *Anopheles arabiensis*

Variation	Sum of square	df	Mean square	F	Sig
Indoors					
Between groups	801.844	3	267.281	10.868	0.000*
With in groups	688.625	28	24.594		
Total	1490.469	31			
Ontdoors					
Between groups	2005.844	3	668.615	4.252	0.014†
With in groups	4402.625	28	157.237		
Total	6408.469	31			

* denotes significance at 5% level of significance (P< 0.05)

† denotes significance at 5% level of significance (P< 0.05)

Appendix 10 Over all Chi-square value of *Anopheles pharoensis* collected from their night biting behavior at the three study sites

	Observed N	Expected N	Residual	Test statistics	
Indoor biting	87	77	30	Chi-square	6.25
Outdoor biting	55	77	-30	df	1
Total	144			P-value	0.012