ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF BIOLOGY

Prevalence of Malaria in the Highland Fringes of Butajira Area, Southern Ethiopia: A Retrospective and Cross- Sectional Study.

A Thesis Presented to the School of Graduate Studies, Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (Biomedical Sciences)

By

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Addis Ababa

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Approved by the Examining Board:

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LIST OF ABRREVIATIONS

BB	Blocking buffer
CS	Circumsporozoite
CDC	Center for Disease Control and Prevention
EHNRI	Ethiopia Health and Nutrition Research Institute
ELISA	Enzyme Linked Immno Sorbent Assay
KAP	Knowledge Attitude Practice
MAB	Monoclonal Antibodies
MOH	Ministry of Health
NMSA	National Meteorology Service Agency
PBS	Phosphate Buffer Saline
PBS-TW	Phosphate Buffer Saline Tween 20
PCR	Polymerase Chain Reaction
WHO	World Health Organization

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Abstract

To determine the magnitude of malaria transmission in the highland fringes of Butajira area during the year 2006/07, a cross-sectional data on malaria prevalence was collected using standard parasitological, entomological and KAP study techniques. Retrospective data for the period between 2000/01 and 2005/06 was obtained by reviewing clinical records at Butajira Health Center. The retrospective clinical data showed malaria to be one of the major causes of outpatient consultations in the study area. The domiciles of the patients who received treatment for malaria at the District Health Center were Butajira town and the adjacent highland fringe rural localities. This indicated the transmission of malaria to be both in Butajira town and the adjacent highland fringe rural localities, all of which are higher than 2050m above sea level. In the parasitological survey conducted in October through December 2006, a 4.4% malaria positivity rate was detected. The majority (66.7%) of the infections were due to P. vivax and the rest were due to P. falciparum.The infections were observed in all age groups, with a relatively higher prevalence (3.6%) in adults. Difference in malaria prevalence was observed in the two localities of the study area with Misrak-Meskan, the lower highland fringe area, having a relatively higher peak season prevalence (5.3%) compared to that in Mirab-Meskan (2.7%). However, monthly incidence of malaria for October- November was not significantly different between the higher highland fringe areas (Mirab-Meskan) and the lower (Mirab-Meskan). Larval mosquito collections showed An. christyi was the dominant species during the major peak season (Oct-Dec) and during the small rainy season (Apr-May).However, An. gambiae s.l. was the dominant species in adult collections in both seasons suggesting that the adult mosquito sampling techniques used may have been more suited to its collection. More adult mosquitoes were collected outdoors than indoors in both localities, suggesting the likelihood of outdoor malaria transmission. The KAP survey showed that low proportion of household heads from Mirab-Meskan identified the role of mosquitoes in transmitting malaria as compared to those from Misrak-Meskan. This may be an indication that malaria transmission in Mirab-Meskan is a relatively new introduction, as a result of which the population has poor awareness about the disease. Therefore, health education about the risk of malaria in the upper highland fringes of Buitajira area must be given adequate attention to minimize potential epidemics. Furthermore, in light of its abundance, which coincided with the transmission seasons, the possible role of An. christyi as a secondary vector in the highlands must be investigated.

Key-words: Highland malaria, Butajira, An. gambiae s.l, An. christyi, KAP

1. Introduction

Malaria is one of the world's most serious and complex public health problems. The disease is caused by four distinct species of *Plasmodium* parasites; *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* transmitted by anopheline mosquitoes. Each year, it causes an estimated 300-500 million clinical cases. Of these 90% occur in Africa, which are mostly caused by *P. falciparum* infection. Its death toll is estimated to range between 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, 2007).

Malaria transmission is usually associated with topography, climate and socio-economic conditions. The problem of the disease in Africa is aggravated by climate change (Zhou *et al*., 2004), poverty and the lack of efficient control strategy (Paul, 2005). The emergence of insecticides and drug- resistance, human population growth and movement, land use change, and deteriorating public health infrastructures also contribute to the spread of the disease (Hay et *al.,* 2002).

Because of climate and ecological diversity, there is variation in the epidemiology of transmission. It may be stable or unstable in its form. In the case of stable transmission, the community usually develops herd immunity to the disease, whereas in unstable form immunity is absent and recurrent epidemics are very common (Lindsay and Martens, 1998; Cox *et al.,* 1999).

Due to Ethiopia's extraordinary diverse topography and climatic condition, the epidemiology of malaria in the country is more variable and unstable than any other country in Africa. Highland fringe areas at an altitudinal range of 1500-2500 meters above sea level (masl) (Northern and Central area) and lowland arid areas (Eastern and Southern areas) at altitudinal ranges of below 1500m a.s.l. are prone to malaria epidemics. Some areas in the Western lowland, however, have relatively stable transmissions (MOH, 2004). A cyclic epidemic with a period of five to eight years occurs in most parts of the country following climatic changes (MOH, 2004). All age groups are particularly vulnerable to severe malaria during epidemics.

The conditions were the same in Butajira highland fringe areas in which epidemics were reported several times (Cox *et al.,* 1999). Because of the scantiness of information regarding highland malaria in rural Ethiopia, the study was conducted in the vicinity of Butajira.

1.1 The Parasites

The pathogens causing malaria are parasites belonging to the family *Plasmodidae* within the order Coccididae, suborder Hemosporidiidae, which comprise various parasites found in the blood of reptiles, birds, and mammals (Schmidt and Roberts, 1983).

The life cycle of all species of human malaria is essentially the same (Fig.1.). Infection begins when a female anopheline mosquito inoculates *Plasmodium* sporozoites from its salivary gland during a blood meal. Once in the human blood stream, the sporozoites arrive in the liver and penetrate hepatocytes, where they remain 9-16 days, multiplying within the cells. The sporozoites of *P. vivax* and *P. ovale* differentiate into either hypnozoites or into developing tissue schizonts in varying proportions, depending on the strain. In *P. falciparum and P. malariae* the sporozoites do not form hypnozoites, but develop directly into pre-erythrocytic schizonts. At the end of pre-erythrocytic stage, the swollen liver cell raptures, discharging motile merozoites into the bloodstream. The merozoites released from the tissue schizonts invade the erythrocytes. Some merozoites differentiate sexually and form gametocytes (Gilles and Warrel, 1993). Another mosquito arriving to feed on the blood may suck up these gametocytes into its gut, where ex-flagellation of microgamete occurs, and the macrogametes are fertilized. The resulting ookinite penetrates the wall of the gut in the midgut, where it develops into oocysts. When sporogony is complete, and the oocysts raptures, the sporozoites are relasesed and migrate to the salivary gland, for injection into another host during blood meal visits (Gilles and Warrel, 1993).

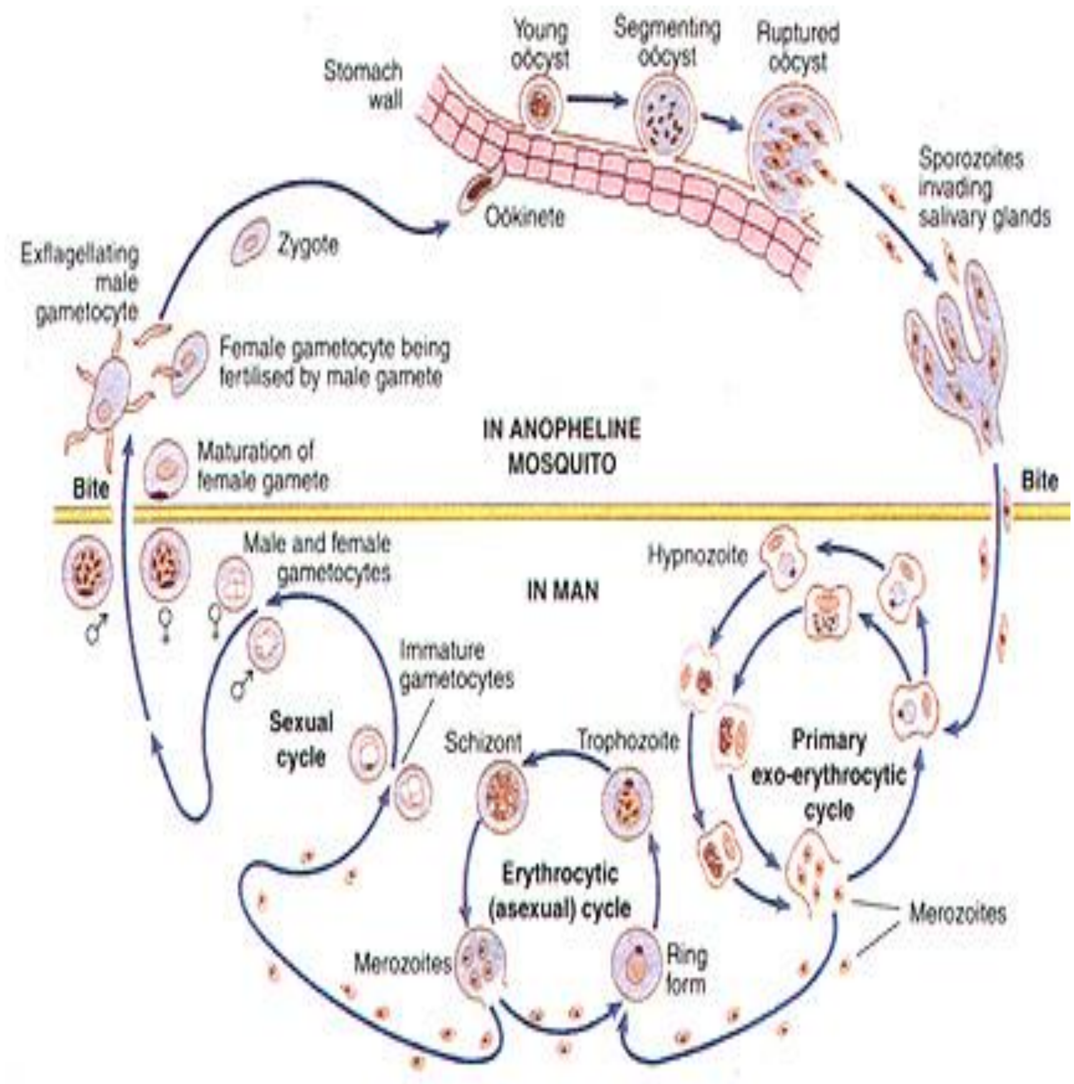

Fig . 1 life cycle of *Plasmodium* species

(**Source**; htpp: www malaria site. com)

The invasion and destruction of red blood cells by the asexual parasite together with the host reaction to the parasite cause malaria symptoms. The malarial paroxysm that coincides with the release of merozoites from ruptured red blood cells(RBCs) starts with malaise, abrupt chills and fever rising to 39^0 C to 41^0 C and increasing head ache and nausea. The fever phase is followed by a profuse sweating that occurs over a period of 2 to 3 hours. Clinical manifestations common to all forms of malaria include anemia, jaundice, splenomegaly, hepatomeglay and malarial paroxysm (Schmidt and Roberts, 1983; Gilles and Warrel, 1993).

Among the four species of malaria parasites that infect human, *P. falciparum* and *vivax* are causes for the greatest prevalence in the world. Of these 2 species, *P. falciparum* is regarded as the greater menace because of the high level of mortality with which it is associated, its wide spread resistance to anti-malarial drugs, and its dominance on the world's most malarious continent, Africa (Mendis *et al.,* 2001). But regarding geographical distribution *P. vivax* has the widest geographical range: it is prevalent in many temperate zones, but also in subtropics and tropics. *Plasmodium falciparum* is the commenst species through-out tropics and subtropics, although it may occur in some areas with a temperate climate. *Plasmodium malariae* is patchily present over the same range as *P. falciparum* but much less common. *Plasmodium ovale* is found chiefly in tropical Africa, but also occasionally in West pacific (Gilles and Warrel, 1993).

Opposite to its wide geographical distribution, *P. vivax* is exceedingly rare in the countries of sub-Saharan West Africa. Here P. *falciparum* predominates although *P. malariae* has been recorded in up to a third of cases (Mendis *et al.,* 2001). In this region *P. vivax* accounts for less than one percent of recorded infections. The extreme rarity of *P. vivax* in West Africa is apparently due to the almost universal prevalence in native West Africans of the Duffy negative trait, an inherited red cell phenotype that lacks red cell receptors for *P. vivax* merozoites invasion (Mendis *et al.,* 2001). However, in some parts of Eastern and Southern Africa and in Madagascar, *P. vivax* has been reported in up to 20% malaria infections (WHO, 2000). Forty percent of cases in Ethiopia are also due to *P. vivax* (MOH, 2001).

1.2 The Vectors

There are about 422 species of *Anopheles* mosquitoes through out the world, but only some 70 species are vectors of malaria under natural condition (Gilles and Warrel, 1993). *Anopheles* mosquitoes are most frequent in tropical and subtropical region, though also found in temperate climate. Anopheline mosquitoes transmitting human malaria are divided into primary and secondary vectors based on their role in transmission.

Anopheles gambiae complex and the composite *An. funestus* having a wide distribution and *An. nili* and *An. moucheti* with limited distribution are considered the primary vectors in tropical Africa (Coetzee, 2004). *Anopheles zeamanni*, *An. rufipes* are generally secondary vectors in this region, although *An. pharoensis* can be a major vector in some riverine ecotones (Coetzee, 2004).

Anopheles gambiae complex which has seven sibling species (*An. gambiae s.s*., *An. arabiensis, An. quadriannulatus,* A and B, *An. merus, An. melas, and An. bwambae*) is uniquely efficient in tropical Africa. Except for *An. melas* and *An. merus* that prefer salt water for breeding, others are fresh water species. *Anopheles gambiae s.s* commonly associated with *An. funestus* is responsible for intense transmission either seasonal or perennial depending on local climate conditions and opportunities for larval breeding (Coeteez et *al*., 2000).

Climatic factors such as precipitation and temperature are important determinants of the range and relative abundance of individual species of the *An. gambiae* complex (Lindsay and Martens, 1998). *Anopheles gambiae* s.s. and *An. arabiensis* are predominant species in moisture saturated environment, though *An. arabiensis* is common in all other areas too. Although climatic conditions are directly associated with elevation, elevation is not a good predicator of malaria vector species distribution (Minakawa *et al.*, 2002).

Anopheles quadriannulatus is markedly exophilic and zoophagic except endophilic tendency reported in mixed cattle stables and human dwellings in Ethiopia (White *et al.*, 1980). This species has negligible malaria vectorial capacity under natural condition, although experimental infections with *P. falciparum* demonstrated its susceptibility (Taken *et al.*, 1991).

Anopheles melas in Western Africa and *Anopheles merus* in East Africa are mostly found in coastal brine waters where they are jointly important vectors especially when associated with *An. gambiae s.s.* (Lindsay and Martens, 1998) . The sixth member of *An. gambiae* complex An. *bwambae* is a much localized malaria vector in Uganda. This species breeds in geothermal waters (Gilles and Warrel, 1993).

Anopheles funestus is capable of producing very high inoculation rates in a wide range of geographical, seasonal, and ecological conditions in Africa (Coluzzi, 1984).

Anopheles pharoensis has very wide distributions and occupies a broad variety of ecological zones. It is a vector that can maintain active transmission of malaria in areas where the main vectors are absent (Kenawy *et al.,* 1987).

Anopheles nili and *Anopheles moucheti* are both associated with running water, their larval stages finding a suitable environment along the river banks. *Anopheles nili* lives in the West and Central African equatorial area, mainly in forest but occasionally in Sahel Savanna, and is more abundant during the rainy season (Gilles and Warrel, 1993). *Anopheles nili* is considered to one of the major vectors, in Gambella, S.W. Ethiopia (Krafsur, 1977).

Early research in South-West Uganda implicated *Anopheles christyi* as a vector of malaria. However, successive study in the area showed no additional sprozoite positive *An. christyi* (Lindblade *et al*., 2000).

1.3 Epidemiology of Malaria

1.3.1 The Global Picture

Malaria is currently endemic in more than100 countries or territories inhabited by about 41% of the world populations.The level of malaria transmission varies in different regions, and within countries. Endemic regions are characterized by warm temperature and high humidity, both suitable for mosquito breeding, and where human population and malaria parasite co-exist (WHO, 2007). Most people at risk of the disease live in areas of relatively stable malaria transmission where infection is common and occurs with sufficient frequency that some level of immunity develops. A smaller proportion of people live in areas where the risk of malaria is more seasonal and less predictable, because of either altitude or rainfall patterns (Breman, 2001).

In areas of stable malaria transmission, very young children and pregnant women are the population groups at highest risk for malaria morbidity and mortality. Most children experience their first malaria infections during the first year or two of life, when they have not yet acquired adequate clinical immunity, which makes these early years particularly dangerous. Ninety percent of all malaria death in Africa occurs in young children. Adult women in areas of stable transmission have a high level of immunity but this is impaired especially in the first pregnancy, resulting in an increased risk of infection. In areas of lower or irregular transmission, all age groups fall ill when infected and are vulnerable to severe disease. Because of the absence of sufficient immunity, all age groups are at risk of death or develop severe complications, especially during epidemics. The populations most at risk of epidemics are those living in the highlands, arid and desert fringe zones (Lindsay and Martens, 1998; Cox *et al.*, 1999).

In tropical Africa, malaria accounts for 10% to 30% of all hospital admissions and is responsible for 15% to 25% of all deaths of children under the age of five. Around 800,000 children under the age of five die from malaria every year, making this disease one of the major causes of infant and juvenile mortality. Pregnant women are also at risk since the disease is responsible for a substantial number of miscarriages and low birth weight babies (WHO, 2005).

Malaria thus has social consequences and is a heavy burden on economic development. It is estimated that a single bout of malaria costs a sum equivalent to over 10 working days in Africa. The cost of treatment is between $US0.08 and $US5.30 according to the type of drugs prescribed as determined by local drug resistance. It also deprives Africa of $US 12 billion every year in the lost Gross Domestic Product (GDP) (Greenwood, 2004).

The significance of malaria as a health problem is increasing in many parts of the world. Epidemics are even occurring around traditionally endemic zones in areas where transmission had been eliminated. These outbreaks are generally associated with deteriorating social and economic conditions (Cox, *et al.*, 1999). In Africa poor people are at increased risk both of becoming infected with malaria and of becoming infected more frequently. Child mortality rates are known to be higher in poorer households and malaria is responsible for a substantial proportion of these deaths (Breman, 2001).

The absence of adequate health services in Africa frequently results in recourse to self-administration of drugs often with incomplete treatment. This is a major factor in the increased resistance of the parasites to previously effective drugs (Alessandro and Buttiens, 2001). Chloroquine resistance, first reported in East Africa in 1978 among non- immune tourists is now verified in all tropical African countries (Hay *et al.*,2002). The widespread insecticide resistant vectors in the world are equally important in the spread of malaria globally (WHO, 2005).

1.3.2 Malaria in Ethiopia

Malaria is believed to be the disease of antiquity in Ethiopia; however malariological studies started only recently, by the Italian and British scientists around world war II.

The distribution of malaria in Ethiopia is affected by altitude, topography and climatic factors. The epidemiology of malaria at higher altitude particularly above 1800 is unstable (Ghebreyesus *et al.*, 2006). In these areas, transmission is highly seasonal that takes place following end of both light rains (February to March) and heavy rains (June to September); in contrast, in humid lowland areas with convenient breeding sites, transmission is usually perennial with slight seasonal variation in magnitude (Tulu, 1993). In general the length of transmission is believed to progressively decrease with increasing altitude and with no transmission, generally at 2000m though with few exceptions of epidemics above this altitude (Melville et *al.,* 1945).

Because the nature of malaria transmission in Ethiopia is unstable, the country experiences moderate to severe epidemics periodically (Fontaine *et al.,* 1961). Extensive epidemics showing similar but less characteristics to the 1958 epidemic occurred in many parts of Ethiopia in 1965, 1973 and 1981/1982 (Ghebreyesus *et al.*, 2006). Recent epidemics with cyclical patterns of variable magnitude were at sites elsewhere in 1990, 1992, 1997/98 and 2002 (MOH, 2004). Most of the epidemics of Ethiopia was followed a period of exceptional meteorological condition in which unusually high temperature and humidity to create conditions conducive to very high vector concentration (Covell, 1957; MOH, 2004).

In addition to annual changes in climate, the ecological upheavals in the late 1980s and early 1990s has an immense role in changing the epidemiology of malaria in the country, for instance the Pawie settlement scheme and development activities in the area of the township of Arbaminh (Nega and Haile-Meskal, 1991). Small scale irrigation dams in Tigray increased the level of malaria transmissions (Ghebreyesus *et al.*, 1999).

All of the four human malaria parasites species occur in Ethiopia. However, *P. falciparum* is the dominant species accounting for 60% to 70% of the cases followed by *P. vivax*, which is responsible for 30% to 40% of cases. *Plasmodium malariae* and *P. ovale* are rare, accounting far less than 1% of the cases. *Plasmodium malariae* is mainly reported from the Arbaminch area, while *P. ovale* is identified from a few patients who live or lived in Humera, Gambella, and Gamugofa (Ghebreyesus *et al.*, 2006).

In Ethiopia *P. vivax* is more common during dry season, and weather this is due to active transmission or relapses has not been clearly determined. Concerning ethnic groups, Nilotic Ethiopians have been found to be more resistant to *P. vivax* than Semitic and Cushitic speaking populations due to the absence of Duffy antigen in the Nilotic speakers (Ghebreyesus *et al.*, 2006).

1.3.2.1 The vectors

Most of the vector distribution of Ethiopia was studied by the Italian and the British expatriates at the beginning of twentieth century. The works of Giaquinto-Mira (1950), Covell (1957), Verrone (1962a and 1962b), and O'Connor (1967) have contributed a lot in the identification and generating information on the distribution of malaria vectors in Ethiopia.

A total of 42 species of anopheline mosquitoes have been documented in the country. *Anopheles arabiensis* and *An. quadriannulatus spp* B are the only two species in *an. gambaie* complex that are found in Ethiopia (Hunt, *et al.,* 1998). *Anopheles quadriannulatus* distribution was shown to be in the highlands of South-Western and Northern regions, co-existing with *An. arabiensis* (White *et al.*, 1980).

Anopheles arabiensis is the most important vector of Ethiopia irrespective of the highly diversified vectors of the country (White *et al.* 1980; Tulu, 1993; Amenshewa, 1995; Abose *et al.,* 1998a). This species breeds in small, temporary, sunlit water collections.

Anopheles funestus, *An. pharoensis* and An. *nili* are regarded as secondary vectors of malaria in Ethiopia (Krafsur, 1977; Mekuria, 1983; Tulu, 1993; Abose *et al*., 1998b).

Anopheles funestus was regarded as the most important secondary vectors of malaria after An. *arabiensis* (Mekuria, 1983) but countrywide collection had shown *An. pharoensis* to be 6 fold greater than *An. funestus* (Tulu, 1993).

Anopheles nili is the least common species, and it is more localized, to Southwestern, Western and Northwestern parts of the country (Krafsur,1970).This species is an important malaria vector in Gambella (Krafsur,1970).

Both *An. funestus* and *An. pharoensis* prefer large, permant, and shaded water bodies with emergent vegetation. For instance, the swamps in Baro River in Western Ethiopia provide an environment that permits *An. funestus* to exceed *An. gambiae* in number throughout most of the year (O'Connor, 1967).

The co-existence of secondary vectors with *An. arabiensis* has been reported in Gambella (Krafsur, 1970), Gergedi (Amenshewa, 1995) and Ziway (Abose *et al.*, 1998a).The co- existence is much more frequent with *An. pharoensis* than with the others.

Anopheles pharoensis might be responsible for the transmission of malaria in the absence or low number of *An. arabiensis*, particularly in dry season (Nigatu *et al.*, 1992; Abose *et al.*, 1998b; Kibert, 2008).

Anopheles arabiensis and *An. funestus* occur in all administrative regions while *An. nili* is reported from the regions of Amhara, Western Oromiya, Gambella and Southern Nations and Nationalities and People Administrative Region. *Anopheles pharoensis* is found in all regions except the Bale zone of Oromiya (Ghebreyesus *et al.*, 2006).

Anopheles christyi is regarded as harmless with no epidemiological importance in malaria transmission in Ethiopia. This species was recorded in highland such as Gullele Torrent (2800masl) Addis Ababa (Giaquinto-Mira, 1950). Although the species is generally regarded as zoophliic (Gilles and De-Mellion, 1968), a blood meal analysis showed its anthropophlic behavior (Adugna and Petros, 1996).

The sporozoite rate is the proportion of *Plasmodium* infected mosquitoes in a population of local vector species. It can be determined by dissecting the salivary glands and detecting sprozoites under microscope. There is also modern technique like enzyme linked immonosorbant assay (ELISA) and polymerase chain reaction (PCR) methods that estimate the rate more precisely. The ELISA is easy to perform, utilizes stable reagents, requires less expensive equipment and is relatively sensitive and specific (Writz *et al.*, 1987). This technique sufficiently detects circumsporozoite proteins from as few as 100 sporozoites.

Sporozoites infection rate in Ethiopia is very low and a large number of specimens need to be tested in order to be able to determine the actual infection rate (Abose *et al.*, 1998a).

Krafsur, (1971) recorded 1.87% sporozoite rate in which 156 positives out of 8348 dissected *An. gambiae mosquitoes*. In that study a relatively high sporozoite rate was recorded for *An. gambiae* in October (4.97%), and in November (5.43%). Nigatu *et al.,* (1992) reported 2 positives out of 262 mosquitoes by using ELISA both for *P. falciparum* and *P. vivax*. Amenshewa (1995) reported 1.52% infection rate by using ELISA on 3626 *An. arabiensis* specimens in Gergdi but the dissection result was 0.63% from 638 specimens.

A recent sporozoite detection of *An. arabiensis* in Southern Ethiopia Sille gives 0.5 % (4/796) infection rate for *P. falciparum* and 1.76 %(14/790) for *P. vivax* by ELISA method (Taye et *al.*, 2006). A study conducted in Ziway, by ELISA method also give five specimens positive for giving the sporozoite rate of 1.18 %(Kibert, 2008).

Anopheles pharoensis has been naturally infected in Egypt, Nigeria, Kenya and Ethiopia. Nigatu *et al.,* (1992) detected 0.46% infection rate (2 positives out of 436 for *P. vivax*) in this species by ELISA method. Kibert, (2008) also found 0.59 % infection rate for this species in Ziway area for *Plasmodium falciparum*. The USAAID team dissected 70 *An. funestus* and found 1 positive to sporozoite (O'Connor, 1967). Dissection of 619 *An. nili* in Gambella area yielded 8 positives (Krafsur, 1971).

The reason for such low rates consider as due to relatively low malaria endemicity prevailing in Ethiopia compared to highly endemic regions of tropical Africa, and the possibility that non–vector species are included in the study.

1.4 Factors Associated with Highland Malaria Transmission

Highland malaria is epidemic or unstable malaria that occur, at a local altitudinal limit (Cox *et al.,* 1999). It is characterstied by the monthly and yearly fluctuation in the incidence of the disease. The fluctuations are also observed from place to place with high variation in localities of the same zone. The unstable transmission can lead to high prevalence rate and high epidemic potential due to none or little immunity (Cox *et al.*, 1999). In Africa areas of unstable transmission are usually in semi arid zone, and areas of high altitudes particularly in the East and Southern Africa (Lindsay and Martens, 1998).

European settlers in Africa sought refuge from the heat and the disease of the plains by moving to the cool highlands (Lindsay and Martens, 1998). This picture appears to be changing, with recent evidence indicating an increase in the number of malaria epidemics occurring in the highland areas, as well as increasing stability of transmission in highland fringes. Meteorological and non-meteorological factors are responsible for this apparent change in epidemiology (Cox *et al.,* 1999).

1.4.1 Meteorological Factors and Highland Malaria

Although altitude has long been recognized as an important determinant of malaria endemicity, transmission factors, which are directly or indirectly affected by altitude, are of epidemiological significance rather than altitude *pe rse* (Hay *et al.,* 2002). The most important of these is environmental temperature, which affects the development and survival of vectors and, more significantly, the duration of *Plasmodium* parasite development within *Anopheles* mosquitoes (Cox *et al.,* 1999). Thus, the minimum temperature for parasite development of *P. falciparum* and *P. vivax* approximates 18^0 C and 15^0 C, respectively, limiting the spread of malaria at higher altitudes (Shanks *et al.,* 2005; Patz and Olson, 2006). But, small increase in temperature near the limit for parasite and mosquito development would probably produce greater mosquito densities, higher biting rates and more rapid parasite development in the mosquito. For instance, Lindsay and Birely, (1996) observed a reduced extrinsic incubation period by 17.3 days (from 55.5 days to 38.2 days) when the temperature is increased from 18^0 C to 18.9^0 C. At 22^0C, sporogony is completed in less than three weeks, and mosquito survival is sufficiently high (15%) for transmission cycle to be completed. The potential number of infective mosquitoes reaches a peak at 30.6^0 C (after which it decreases rapidly) (Shanks *et al.,* 2005).

Many studies demonstrated the association between *An. gambiae* s.l abundance and rainfall, but a direct, predictable relationship does not exist (Koenraadat *et al.,* 2003).

Anopheles gambiae s.l. can breed prolifically in temporary, turbid water bodies such as hoof prints or rain puddles, while *An. funestus* prefers permant water bodies, however, both temporary and permant waters are dependent on adequate rainfall in suitable altitudinal range. Therefore, there is good reason for using rainfall to indicate the probable presence of vectors, their survival and the potential for malaria transmission (Koenraadat *et al.,* 2003).

Although continuous and heavy rainfall disturbs mosquito breeding, however, as soon as the frequency and intensity of rainfall decreases, it is likely that numerous mosquito–breeding sites will be created (Cox *et al.,* 1999). On the other hand, when the number of rainy days in a specified period becomes few (and there is an intermittent rainfall) fast created pools become favorable breeding sites (MOH, 2004). Malaria transmission is also common, when the amount of rainfall is much below normal or if there is drought because water bodies such as streams and rivers will create small intermittent pools in river beds, which are favorable for anopheline breeding. When such phenomena are coupled with high air temperature, unusual epidemic may occur in the highlands and/or highland fringe areas (MOH, 2004).For instance, the drought in Ethiopia, which occurred in 1958, 1965, 1973-1974 and 1983-1985 overlapped with subsequent malaria epidemics in respective years (Mengesha *et al.,* 1998).

Apart from creating mosquito-breeding sites, rainfall also affects malaria transmission through increasing humidity, which in turn will help increase the longevity of adult vectors (Hay *et al.,* 2002). In general, relative humidity of 60% or more is deemed necessary for effective malaria transmission in hyper/holoendemic areas (Koenraadat *et al.,* 2003).

Zones of unstable malaria, such as the East African highlands are more sensitive to climate variability (Mouchet *et al.,* 1999). Temperature and precipitation in highlands are expected to rise above the minimum temperature and precipitation thresholds of malaria transmission at cyclical patterns (Githeko and Ndegwa, 2001). Rainfall peaks in East Africa corresponds to ELNINO Southern Oscillation (ENSO) years (Kovats, 2000). ENSO is metrological phenomenon that occurs every 2-10 years and tends to exaggerate the extremes of climate in a specific region of the world (Kovats, 2000). For instance, the 1958 catastrophic malaria epidemic in Ethiopia was associated with unusually heavy and prolonged rains combined with abnormally high temperature and humidity (Fontaine *et al.,* 1961).

There is a considerable debate between two groups concerning the role of climate as driving force for malaria epidemics in the highlands of East Africa (Malakooti *et al*., 1998; Hay *et al.,* 2002; Shanks *et al.,* 2002; Zhou *et al.,* 2004). According to Hay *et al.,* (2002) mean temperature and rainfall was not changed significantly at four locations in their study sites where malaria incidence has been increasing. As a result they concluded that recent increase in malaria in East African highlands cannot be attributed to global warming; instead an increase in drug-resistance is more likely explanation for the observed increase in malaria. Similar studies in Kericho Tea Plantations by Shanks *et al*., (2002) in Western Kenya supported the position of Hay *et al*., (2002) conclusions.

On the other hand, Zhou *et al*., (2004) unlike Hay *et al., (*2002) found a significant temperature increase in their study sites and concluded that the increased malaria epidemic in 1990s compared to those in 1980s in East African highland is associated with climate variability.

Zhou *et al,* (2004) emphasized the importance of simultaneous analysis on the long-term time series of meteorological and parasitological data to demonstrate the effect of climate on malaria prevalence. Moreover, they hold that climate variability (short term fluctuations around the mean climate state on fine time scale) may be epidemiologically more relevant than mean temperature increase.

Pascual *et al.,* (2006) agree with the conclusion of Zhou *et al.,* (2004) because they found a half degree Celsius rise in temperature in the last 50 years by analyzing the same site, in which Hay *et al.,* (2002) analyzed and found no evidence for warming.

According to Pascual *et al.,* (2006) a half-degree Celsius rise in temperature would raise the mosquito number between 30% and 100% due to the effect of temperature on their time of development. But this increase had a marginal effect in lowland areas, where mosquitoes are abundant whereas in the highland areas, where the insects are much scarcer, it is likely to be affecting transmission rates.

1.4.2 Non-Meteorological Factors and Highland Malaria

Besides climate, other factors are also responsible for the distribution of malaria and its level of transmission. Among the main are local climate changes due to land use change, anti-malarial drug resistance, population movement, degradation of health care infrastructure (Lindblade *et al.,* 1998; Malakooti *et al.,* 1998; Shanks *et al.,* 2005).

Changing landscapes can significantly affect local whether more acutely than the long-term change (Patz and Olson, 2006). Land cover change can influence microclimate conditions, including temperature, evapotranspiration and surface runoff (Snow *et al.,* 1998), which are key to determining mosquito abundance and survivorship.

In Kenya, Malakooti *et al.,* (1998), found that open treeless habitats experience warmer midday temperature than forested habitats and also affect indoor hut temperature. As a result, the gonotrophic cycle of female *An. gambiae* s.l was found to be shortened by 2.6 days and 2.9 days during the dry and rainy season, respectively, compared with forestry sites. Similar findings have been documented in Uganda where higher temperature has been measured in communities bordering cultivated fields compared with those adjacent to natural wet lands, and the number of *An. gambiae* s.l. per house increased along with minimum temperature after adjustment for potential confounding variables (Lindblade *et al.,* 2000).

Increased canopy cover in Western Kenya is negatively associated with the presence of *An. gambiae* complex and *An. funetus* larvae in natural aquatic habitats (Minakawa *et al.,* 2002). In artificial pools, survivorship of *An. gambiae* larvae in sunlit open areas was 50 times higher than the survivorship in forested areas and is also related to the assemblages of predatory species (Lindblade *et al.,* 2000).

The expanding cultivation of maize on small farms in many highland rural villages of Ethiopia increases malaria infection rates (Kebede *et al.,* 2005). This is attributed to the proximity of mosquito breeding sites to maize pollen source (i.e., within 10 meters) (Ye-Ebiyo *et al*., 2000; 2003). Maize pollen increase vectorial capacity by allowing more larvae to survive to adulthood, and to develop more quickly into larger and long lived vectors (Ye-Ebiyo *et al*., 2000; 2003). Maize pollen release also coincides with the period near the end of the rainy season when mosquito-breeding sites are most stable and abundant, optimizing its effect on larvae; thus growing of maize near households increases biting rate and malaria transmission (Kebede *et al.,* 2005).

Drug resistance aggravates malaria induced morbidity and mortality (Malakooti *et al.,* 1999). It is known that drug resistant *P. falciparum* produces more gametocytes and infects more mosquitoes than drug sensitive strains. Therefore, small changes in the number of persons carrying infective gametocytes can initiate malaria epidemics through increased mosquito transmission (Shanks *et al.,* 2005).

Different studies in East African highlands confirmed drug resistance as the main factors for the increased transmission of malaria (Shanks *et al.,* 2002; Hay *et a*l., 2002). For instance, studies in Tea State in Kericho District in Western highland Kenya showed that renewed epidemic activities to have coincided with the emergence of chloroquine–resistant *falciparum* malaria where climate, the environment, the human population and its structure, health care provision and malaria control are known not to have changed (Shanks *et al.,* 2002). These workers observed a decrease in the rate of *faliciparum* gametocytes from 5% to 1% in young children when the first line malaria treatment was changed from choloroquine to sulphadoxine pyrimetamine in 1999 at Tea Plantation of Kericho. Likewise, the malaria resurgence in the Usambara Mountains in Tanzania has been linked to the rise in anti-malarial drug resistance (Bodker *et al*., 2000). The key point is to cure enough infection so that the remaining parasites cannot rapidly expand during seasonal transmission (Shanks *et al.,* 2002).

The movement of infected people from areas where malaria is still endemic to areas where the disease is rare may increase the incidence of the disease (Martens and Hall, 2000). They also acquire the disease through the technology they introduce, for example, through deforestation and irrigation systems (Service, 1991). Such activities can create favorable habit for *An. gambiae* s.l. to breed in the highlands. In addition to slow upland encroachment, *An. gambiae* s.l. probably colonizes the highlands through a process of passive dispersal, as well, being transported there by car, train, or ox wagon (Lindsay and Martens, 1998).

When basic health services and malaria control activities decline, increase in malaria transmission has been occurring resulting in irregular epidemics (Cox *et al.,* 1999). The condition may be aggravated by emerging drug resistance problems (Shanks *et al.,* 2005) and the lack of immunity in the highland population (Cot *et al.,* 2002).

Highlands have traditionally been regarded as areas of little or no malaria transmission, primarily because of the negative impact of low temperature on parasite sporogony and vector development. This picture appears to be changing, with recent increase in the number of malaria epidemics occurring in the highland areas, as well as increasing stability of transmission in highland fringes. Various factors have been identified as responsible for this apparent change, that is, increase population movement from malaria-endemic area to highlands and vice versa, increasing degradation of health care infrastructure, emergence of anti–malarial drug resistance, increased malaria transmission as a consequence of land use changes and global warming (Cox *et al.*, 1999).

Studying the magnitude of malaria prevalence in the highland areas of different communities would be necessary to implement effective control measures in highland fringe areas of Ethiopia. Because repeated epidemics were reported in the highland fringe areas of Butajira, the study hypothesized autochthonous transmission in the highland fringe areas.

2. Objective of the Study

2.1 General Objective

To determine the presence and magnitude of malaria transmission in the highland fringes of Butajira area.

2.2 Specific Objectives

To review retrospective data on malaria situation by using clinical data from Butajira Health Center.

To determine the periodic prevalence and incidence of malaria by specific parasite and age structure of the population.

To survey the mosquito breeding sites, sporozoites rate, and the *Anopheles* species associated with malaria endemicity in the study area.

3. Materials and Methods

3.1 The Study Area and Population

Butajira District is located in Gurage zone, in Southern Nations, Nationalities, and Peoples Regional State (SNNPR). The District borders the Siliti Zone to the South, the Mareko District to the East, the Muhur and Akillil District to the West and the Sodo District to the North. The estimated size of the District is 56,200 hectares. The District lies at an average altitude of 1900m a.s.l. ranging from 1750m a.s.l. in the lowlands to 3400m a.s.l. in the mountains.

Annual rainfall ranges between 700 to 1870mm. The main rainy season is from June to September, with "small rains" common around March and April. The warmest months are between January and June with maximum average temperature of 27.6^0C and 27^oC in February and March in the last six years (National Meteorology Service Agency). In 2005 the total amount of rainfall was recorded to be 1869.4mm, the highest of the last three decades.

The area has rich soil and farming is the main mode of living. Teff, maize, millet, barely and legumes are the main crops. Pepper and khat (a mild stimulant drug) are also growing as cash crops. Enset (false banana) cultivation is very common and gives the main staple food in the area. Extensive crop cultivation and relatively high population density leave little room for animal husbandry.

The population of the District is estimated to be 216,968 in 2005/06 (Regional statistical office). The District has one Hospital, three-Health Centers, 32 functional Health Posts, 11 registered non-governmental health facilities, and among these is the Gelen Hansen Memorial Primary Hospital located in the highland of Yetebon area. No systematic malaria control program has been known to be undertaken in Butajira town and the highland rural locality in recent years. But there is control program in the lowland area (Meskan District Health Office, unpublished report).

The District is characterized by seasonal malaria, with fluctuations in the number of cases from year to year. A large peak in the number of malaria cases is seen from September to December, after heavy rainfall during June to August. This is followed by a second, less pronounced, peak in April and May. Between these peak transmission periods, the level of transmission is more or less constant and low (Meskan District Health Office, unpublished report).

Malaria is a number one cause of morbidity and mortality in the district. *Plasmodium faliciparum* and *vivax* are the two species responsible for malaria. During periods of peak transmission *P. faliciparum* is the most common cause of malaria while during periods of low transmission, *P. vivax* is as frequent a cause as *P. falciparum* (Meskan District Health Office unpublished report). The highest cases were recorded in October 2003/04 in the last six years (Butajira Health Center monthly morbidity report).

The survey was conducted in two Peasant Associations in Butajira District, which have altitudes higher than 2000m a.s.l. The selection of the two Peasant Associations was not random but based on the prevalence of malaria which was obtained from Butajira Health Center. From all highland localities Misrak-Meskan Peasant Association had high prevalence of malaria, while the neighboring Peasant Association Mirab-Meskan was chosen to determine the altitudinal limit at which malaria is prevalent. In addition to a topographical map, a handheld GPS unit was used to record the elevation of the two-peasant associations. Based on this, the average altitude of Misrak-Meskan Peasant Association is 2100m a.s.l., while the average altitude of neighboring locality is 2600m a.s.l., but two localities from Mirab-Meskan was not included in the study because they are at an altitude higher than 2500m a.s.l. and were considered to be beyond anopheline occurrence.

The two-peasant associations have numerous water bodies, such as swamps, rivers, and streams. The Rivers Auffaro and Erinzaaf being the Major ones. The overflow of

Auffaro River is common during the rainy season; due to which numerous breeding sites created during the summer season near village 2 of Misrak-Meskan locality. Misrak-Meskan is swampy and collects water pools during the summer (Ato Taye Tesfaye,Pers.Comm. Meskan District soil and water resource expert).

3.2 Sample Size Determinitation

Sample size of the study area was determined based on malaria prevalence obtained from Butajira Health Center and consideration of cases out of the scope of the Health Center. Taking as an acceptable error 0.04 the sample size was calculated using the formula for estimating single proportion with a level of significance α=0.05(Getu and Fasil, 2003).

3.3 Sampling Technique and KAPs Study

The lists of households of 2-selected localities were used as a sampling frame, with an assumption of similar exposure of houses and random malaria infections among inhabitants. A total of 100 households were selected systematically based on the calculated proportion form both localities to sample 366 individuals. The calculated proportion was based on the total population of the two localities obtained from Meskan District Administrative Office

If the occupants refused to participate in the survey, the household nearest to it was selected. After obtaining consent, blood samples were taken from all selected family members of the household by fingerpick, and thick and thin smears were prepared. This was done monthly on the same individuals from October through December. In addition, the heads of household were interviewed about the family‘s socio-demographic conditions and knowledge of malaria by using a questionnaire. If the male households were absent at a time of interview, the next responsible person was interviewed.

3.4 Review and Documentation of Malaria Cases in Butajira area

Data on malaria cases were collected from monthly outpatient morbidity report of Butajira Health Center between 2000/01 to 2005/06. Outpatient morbidity data of Butajira Hospital was rejected because most of malaria patient came from the lowland area and the data was incomplete, whereas malaria data of Butajira Health Center comprise cases from Butajira town and the highland localities of the District. Due to this, the Health Center data was used to document the malaria situation in Butajira area.

3.5 Blood Sample Collection in the Study Area

Blood film collection was performed in peak malaria transmission season of the country (Tulu, 1993; Fontaine *et al.,* 1961; Covell, 1957) from October through December 2006 after cessation of the major rainy season. A finger prick was done by trained professionals from randomly selected houses by house-to-house visit. Thin and thick blood smears were prepared on the same slide and allowed to air dry. This was transported to the laboratory where it was stained with Giemsa. Thick and thin films were examined by using 100X magnification (oil immersion) for the presence of plasmodia and species identification respectively.

3.6 Entomological Studies

Both immature and adult collections were conducted to determine the species composition. Female adult *Anopheles* mosquitoes were used to determine the sporozoites infection rate. Samples were collected from resting sites and also from breeding sites. The collected specimens were identified to species level on morphological basis using Verone (1962a and 1962b) for adults and larvae.

3.6.1 Collections of Larvae

Larval collection was performed from suspected breeding sites such as artificial ponds, broken pipes, stream margins, swamps, excavation ditches and rain pools using standard dippers (350ml) twice a month in major malaria transmission season(Oct- Dec, 2006) and minor rain season(Apr- May, 2007). The surface area of each potential mosquito breeding site was estimated in square meter (m^2) and sampling was made at a rate of 6 dips/m^2 (4 dips at the margin and 2 from the middle). One 'sample' was defined as 30 dips (or lesser, in smaller sites) taken over a surface area of 5m^2. For sites in the range of 5-10 m^2, one sample was taken, whereas two samples were taken from sites in the range of 11-20 m^2 and so forth (Minakawa et *al.,* 1999).Larval anophelines sampled from each type of breeding habitat were transferred to separate vials by direct pipetting. Larvae were killed gently by heating and preserved in 70% alcohol for later species identification.

3.6.2 Adult Mosquito Collection

3.6.2.1 Indoor Collections

Adult *Anopheles* mosquitoes, resting inside habitations were collected from 5 randomly selected houses near marshy areas. Collection was done during major malaria transmission period through October to December, 2006 and the minor peak in April/ May, 2007.

The collections were done by space spray (knock down collection), CDC (Center for Disease Control) light trap catches and mouth operated aspirators.

Space spray collections were conducted early in the morning from 06; 00 to 08:00 twice a month in 5 selected houses. A white sheet of cloth and aerosol were used to perform space – spray (knock down) collection.

After consent from the inhabitants, all occupants, removable house utensils, foods and drinks were removed from the sprayed room. All opening that allowed mosquito-escaping, doors and windows were closed: the entire floor was covered with the white cloth sheet. The houses were then sprayed with aerosol for about 10 minutes. After waiting for about 10 minutes, the sheet was brought outside the room to inspect and collect the fallen mosquitoes. The mosquitoes were picked using forceps and put in box for later identification and for sporozoites rate determinitation.

Four dry cell battery-operated CDC light traps were placed indoors near the bed from 18:00 in the evening to 06:00 in the morning to collect endophagic *Anopheles* mosquitoes. The collected mosquitos were then killed with chloroform.

Mosquitoes were also collected indoors using aspirator from their resting sites. The collection was done from 5 houses for about 20 minutes each by two persons for two days per month. A flash light was used in locating the mosquitoes in relatively dark parts of the interior of a dwelling. The collected specimens were transferred to a box and killed with chloroform vapor.

3.6.2.2 Outdoor Collections

Mosquitoes were surveyed and collected from outdoors resting place among vegetation (bush, tall grass), in animal sheds, in pits, on the ground or under logs of wood by the help of aspirator. Battery operated CDC light traps were also used to collect exophagic mosquitoes. The CDC light traps were hung in vicinity of sampled houses from 18: 00 in the evening to 06:00 in the morning.

3.6.2.3 Sporozoite Detection by ELISA

The collected female *Anopheles* mosquitoes were dry-stored on silica gel before running the sporozoites ELISA (Writz *et al.,* 1987). Two separate 96-well, micro titer plates were coated with 50µl of the appropriate monoclonal antibodies (MAB) solutions. For *P. falciparum* 0.2µgMAB per 50µl PBS was used and for *P. vivax* 0.025µgMAB per 50µl PBS was used. The plates were covered and incubated for 30 minutes after which well contents were dump emptied and filled 200µl BB buffer for one hour for further incubation. At the same time mosquitoes were ground with 50µl BB; Igepal 630 with pestle. To avoid contamination between mosquitoes the pestles were cleaned with tissue paper. After the removal of the buffer, 50µl mosquitoes triturate were added to wells of both *P. falciparum* and *P. vivax* duplicate test wells. The test was controlled using positive and negative controls. The controls procedures were the same as that of the test samples. Then the plates were covered and incubated for 2 hours and washed 2 times with PBS-tween 20. fifty µl aliquots of the homologous peroxidase- conjugated MAB (0.05µg/50µlBB) were dispensed to each well of the respective plates. After 1 hour incubation the plates were emptied and washed with PBS-tween 20 three times. Hundred µl substrates were added to each well after washing. Finally the plates were read visually or by 405-415nm ELISA reader after adding the substrate. Samples were considered positive if the absorbance values exceeded the mean absorbance of seven negative controls (Writz *et al.,* 1987).

3.7 Meteorological Data

Meteorological data that include monthly minimum and maximum temperature and rainfall data were obtained from the National Meteorological Service Agency. The climatic change of the last three decades expressed as change from the mean (anomalies) of Butajira area was complied in appendix one.

3.8 Data Analysis

Data collected on parasitological surveys were managed and analyzed using a statistical computer Program SPSS version 13.0. Chi-square tests were used to assess different variables.

3.9 Ethical Consideration

After discussing the objective and application of the study to Meskan District Health Office and elders of the study area, the inhabitants were contacted to secure their consent in the same way. Blood smear was obtained with finger prick using disposable blood lancet and cotton immersed in 75% alcohol.

Treatment of malaria to confirmed cases was given in collaboration with Misra-Meskan Health Post freely. The study obtained ethical approval from the ethical committees of Biology department, Addis Ababa University.

4. Results

4.1. Malaria cases in Butajira Area from 2000/01 to 2005/06

Monthly outpatient morbidity report of malaria cases treated based on clinical and laboratory diagnosis in Butajira Health Center from 2000/1 to 2005/6 is complied in Table 1. An increased treand of malaria cases was observed except in 2001/2 and 2004/5.Adults aged 15 years and above (52.9%) were more affected compared to other age groups. Moreover, children aged 0 and 4 (25.8%) and 5-14 (21.3%) were also treated for malaria.

Table1. Age related prevalence of malaria based on clinical and laboratory diagnosis at Butajira Health Center between 2000/1 and 2005/6 (**Source**: Butajira Health Center monthly outpatient morbidity report)

Age group				
Year	0-4 n (%)	5-14 n(%)	15+ n(%)	Total n(%)
2000/1	151(17.7)	180(21.1)	521(61.1)	852(5.1)
2001/2	135(20.4)	163(24.6)	365(55)	663(3.9)
2002/3	542(23.6)	432(18.8)	1321(57.6)	2295(13.5)
2003/4	1051(21.6)	1090(22.3)	2736(56.1)	4877(28.7)
2004/5	1183(34.5)	710(20.7)	1538(44.8)	3431(20.2)
2005/6	1318(27.1)	1049(21.6)	2492(51.3)	4859(28.6)
Total	4380(25.8)	3624(21.3)	8973(52.9)	16977(100)

The Prevalence of *P. falciparum* and *P. vivax* in different age categories are given in Table 2 and Table 3, respectively. The highest numbers of *P. falciparum* infections, 59.6% were confirmed in age categories of 15 and above from 2000/01 to 2005/06. (18.7%) and (21.7%) was also seen in children aged between 0 to 4 and 5 to 14 respectively. The maximum rate of infection by *P. vivax (*51.5%) was observed in age groups of 15 and above. Increment of *Plasmodium falciparum* infection was observed except in 2004/05 compared to *P. vivax.* In addition, *Plasmodium falciparum* was the dominant species accounting for 54.4% of the cases.

Table 2. Age related prevalence of *Plasmodium falciparum* recorded in Butajira Health Center between 2000/01 and 2005/06 (**Source:** Butajira Health Center outpatient monthly morbidity report)

Age group				
Year	0-4 n(%)	5-14 n(%)	15+ n(%)	Total n(%)
2000/1	21(15)	26(18.6)	93(66.4)	140(6.2)
2001/2	50(31.3)	46(28.7)	64(40)	160(7.1)
2002/3	85(18.8)	109(24.1)	258(57.1)	452(20.2)
2003/4	133(17.0)	169(21.5)	482(61.5)	784(34.7)
2004/5	16(13.7)	24(20.5)	77 (65.8)	117(5.2)
2005/6	117(19.3)	116(19.1)	373(61.6)	606(26.8)
Total	422(18.7)	490(21.7)	1347(59.6)	2259(100)

Table 3. Age related Prevalence of *Plasmodium vivax* recorded in Butajira Health Center between 2000/01 and 2005/06 **(Source:** Butajira Health Center outpatient monthly morbidity report)

	Age group			
Year	0-4 n(%)	5-14 n(%)	15+ n(%)	Total n(%)
2000/01	45(25.7)	49(28)	81(46.3)	175(9.2)
2001/02	21(21.0)	29(29)	50(50)	100(5.3)
2002/03	32(18.9)	51(30.2)	86(50.9)	169(9.0)
2003/04	81(22.4)	74(20.4)	207(57.2)	362(19.2)
2004/05	35(25.5)	32(23.4)	70(51.1)	137(7.2)
2005/06	240(25.3)	230(24.3)	477(50.4)	947(50.1)
Total	454(24.0)	465(24.6)	971(51.4)	1890(100)

4.2 The Study Population and Malaria Prevalence in Highland Fringes of Butajira area.

4.2.1 The Study Population

The age and sex distribution of the study population are presented in Table 4. A total 366 study participants were enrolled in the study but in the last survey which was conducted in December, 2006 fifteen individuals missed due to different reasons. The study population is composed of mainly of individual above 15 years old (67.8). The age groups vary from 9 months to 88 years. Of the total study population, 188(51.4%) were males and 178 (48.6%) females.

Table 4. Age and sex distribution of the study participants in Misrak- Meskan and Mirab- Meskan localities in highland fringes of Butajira area from October to December, 2006

Age groups	*Study locality*	*Average Altitude(a.s.l)*	*No exam n(%)*		
			Male	*Female*	*Total (%)*
0-4	Misrak	2100m	16(57.1)	12(42.9)	28(7.6)
	Mirab	2280m	6(46.2)	7(53.8)	13(3.5)
5-14	Misrak	2100m	32(52.5)	29(47.5)	61(16.7)
	Mirab	2280m	6(37.5)	10(62.5)	16(4.4)
	Misrak	2100m	78(51.7)	73(48.3)	151(41.3)
15+	Mirab	2280m	50(51.5)	47(48.5)	97(26.5)
Total			188(51.4)	178(48.6)	366(100)

4.2.2 Malaria Prevalence in the Highland Fringes of Butajira Area.

From three months surveys (October- December, 2006) in the study area malaria prevalence was determined. Out of the total 1082 blood film examined in three surveys 48 (4.4%) were infected. Thirty-two of the 48(66.7%) and 16(33.3%) were due to *P. vivax* and *P. falciparum* respectively. *Plasmodium vivax* was the dominant species in all the three surveys, though; the difference was not statistically significant (X^2 = 5.333 P> 0.05) (Table 5). Of the total 48 individuals infected 25(52.1%) and 23(47.9%) of them were males and females respectively (Table 5).

Table 5. Prevalence of parasite species in relation to the survey period in all age groups in Misrak- Meskan and Mirab-Meskan localities in the highland fringes of Butajira area from October to December, 2006

Infection by Infection

sex (%) by parasite

species (%)

Months	Localites	Total examined	Total positive (%)	Male	Female	Pf	Pv
October	Misrak	240	23(9.6)	13(56.5)	10(43.5)	8(34.8)	15(65.2)
	Mirab	126	5(4.0)	3(60.0)	2(40.0)	2(40.0)	3(60.0)
November	Misrak	240	13(5.4)	4(30.8)	9(69.2)	4(30.8)	9(69.2)
	Mirab	126	5(4.0)	3(60.0)	2(40.0)	2(40.0)	3(60.0)
December	Misrak	231	2(0.9)	2(100)	-	-	2(100)
	Mirab	119	-	-	-	-	-
Total		1082	48(4.4)	25(52.1)	23(47.9)	16(33.3)	32(66.7)

Differences in malaria prevalence were observed in two localities of the study area. Misrak-Meskan (5.3%) had high prevalence of malaria compared to Mirab- Meskan (2.7%). The difference in malaria prevalence was statistically significant in Misrak-Meskan and Mirab-Meskan (X^2=16.333 $P<0.05$). All age groups were infected by malaria with high prevalence in age groups of above 15 years old (3.6%). Similarly the difference in malaria prevalence among the different age groups was statistically significant (X^2 =52.204 $P<0.05$) (Table 6).

Table 6. Malaria prevalence among different age groups in Misrak- Meskan and Mirab-Meskan localities in Butajira highland fringe areas from October to December, 2006

		Age				
Locality	Total examined	0-4 n(%)	5-14 n(%)	15+ n(%)	Total positive n(%)	P value
Misrak	711	1(0.1)	7(1.0)	30(4.2)	38(5.3)	P< 0.05
Mirab	371	1(0.3)	-	9(2.4)	10(2.7)	P<0.05
Total	1082	2(0.2)	7(0.6)	39(3.6)	48(4.4)	P<0.05

In the first survey which was conducted in October 2006, 28(7.7) of 366 individuals examined had malaria. Adult aged 15 and above were more affected, even if, the infections were observed in all age groups (Table 7). In this survey *Plasmodium vivax* was the commonest species (64.3%).The difference in malaria prevalence among different age groups(X^2= 23.214 P< 0.05) and locality (X^2 = 11.571 P< 0.05) in the survey was statistically significant (Table 7).

The incidence of *Plasmodium* species among different age groups conducted in November, 2006 is presented in Table 8. In this survey, 18(4.9%) positive malaria cases were observed. The incidence was higher in adult population in Misrak-Meskan and Mirab-Meskan localities even if, all age groups were affected by malaria like the first survey in both localities. The differences in malaria incidence in both localities is not statistically significant (X^2 =2.00 P>0.05).Like the first survey *Plasmodium vivax* was the commonest species (66.7%) (Table 8).

The prevalence of *Plasmodium* species in December, 2006 is presented in Table 9. In this survey, which was conducted in December 2(0.6) individuals examined were positive for *Plasmodium vivax,* whereas no *Plasmodium falciparum* was observed (Table 9). One of the individual aged between 5-14 in Mirab- Meskan locality was also *Plasmodium vivax* positive in the first survey which was conducted in October. This may be due to relapse of *Plasmodium vivax* or the resistant of the species to chloroquine (Table 9).

In general in all the three survey malaria infection was observed in all age groups. On the other hand, most of the positive cases in all of the survey had no travel history to malaria endemic areas with in the last two weeks.

Table 7. Prevalence of *Plasmodium* species among different age groups in Misrak-Meskan and Mirab-Meskan localities in Butajira highland fringe areas, October 2006

Age groups	*Locality*	*No exam*	*P.falciparm (%)*	*P.vivax (%)*	*Total positive (%)*	P value
0-4	Misrak	28	-	-	-	
	Mirab	13	1(7.7)	-	1(7.7)	
5-14	Misrak	61	1(1.6)	5(8.2)	6(9.8)	
	Mirab	16	-	-	-	
15+	Misrak	151	6(4.0)	11(7.3)	17(11.3)	P<0.05
	Mirab	97	2(2.1)	2(2.1)	4(4.1)	
Total		366	10(35.7)	8(64.3)	28(7.7)	P<0.05

Table 8. Incidence of malaria among different age groups in Misrak-Meskan and Mirab-Meskan Butajira highland fringe areas, in November, 2006

Age groups	*Locality*	*No exam*	*P.falciparm (%)*	*P.vivax (%)*	*Total Positive (%)*	*P value*
0-4	Misrak	28	1(3.6)	-	1(3.6)	
	Mirab	13	-	-	-	
5-14	Misrak	61	-	-	-	
	Misrab	16	-	-	-	
15+	Misrak	151	3(2)	8(5.3)	11(7.3)	P>0.05
	Mirab	97	2(2.1)	4(4.1)	6(6.2)	
Total		366	6(33.3)	12(66.7)	18(4.9)	

Table 9. Prevalence of malaria among different age groups in Misrak- Meskan and Mirab- Meskan Butajira highland fringe areas, December, 2006

Age groups	*Locality*	*No exam*	*P.falciparum (%)*	*P.vivax (%)*	*Total positive (%)*
0-4	Misrak	22	-	-	-
	Mirab	11	-	-	-
5-14	Misrak	61	-	1(1.6)	1(1.6)
	Mirab	13	-	-	-
15+	Misrak	148	-	1(0.7)	1(0.7)
	Mirab	95	-	-	
Total		350	-	2(100)	2(0.6)

4.3 Entomological studies

4.3.1 Larval Mosquito surveys

Expressed as number of larvae per 100 dips, the larva density from the two study localities in different breeding sits are summarized in Table 10. Overall, for *An. gambiae* s.l., excavations ditches were more productive, these were much more abundant in Misrak-Meskan than Mirab-Meskan. However, during the small rainy season, rain pools were more productive for *An. gambiae* and these were also more abundant in Misrak-Meskan. In swampy habitats, *An. chrysti* were most abundant, and these were again true for Misrak-Meskan.

During the dry months small pockets of rain pools and associated collection of water disappeared, due to this the swamps and edges of Aufaaroo River were found to harbor anopheline larva. The swamp that is found in the study area was a typical breeding site to *An. christyi* whereas *An. gambiae* s.l. *larvae* were collected during the light rain season in rain pools and in excavation ditches both in October to December and April to May. The larvae of *An. gambiae* s.l and *An. christyi* were collected in different breeding sites at different localities up to altitude range 2280m a.s.l. More numbers of larvae for both species were collected in Misrak-Meskan than Mirab-Meskan (Table10).

Table 10. Species of mature *Anopheles* larvae collected per hundred dips from various breeding sites in Misrak-Meskan and Mirab-Meskan localities in Butajira highland fringe areas in October-December 2006 and April-May 2007

Breeding sites	Locality	*An. gambiae* s.l.		*An. christyi*		Total
		Oct- Dec	Apr-May	Oct-Dec	Apr-May	
Excavations ditches	Misrak	76.7	70	147	126.7	420.4
	Mirab	16.7	13.3	46.7	23.3	100
Rain pools	Misrak	0	116.7	0	100	216.7
	Mirab	0	93.3	0	23.3	116.6
Stream margins	Misrak	0	0	28.3	0	28.3
	Mirab	0	0	0	0	0
Swamps	Misrak	0	0	476.7	188.3	665
	Mirab	0	0	183.3	220	403.3
Total		93.4	293.3	882	681.6	1950.3

4.3.2 Adult Mosquito surveys

A total of 80 *Anopheles* mosquitoes were collected indoors and outdoors by various methods and the species were identified. The species and the number of *Anopheles* mosquito collected by different methods are presented in Table 11.Of these collected *Anopheles* mosquitoes, An. gambiae s.l. comprise 55% of the collection while the remaining 45% was *An. christyi*. Both species were collected more outdoors, in Misrak-Meskan and Mirab- Meskan (57.5%). The number of *An. christyi* out numbered in Mirab-Meskan than Misrak- Meskan (Table 11).

Table 11. Species and number of female *Anopheles* mosquitoes collected indoors and outdoors in Misrak-Meskan and Mirab- Meskan localities in Butajira highland fringe areas in October-December 2006 and April-May 2007

Species	Locality	Oct-Dec		Apr-May		*Total*
		Indoors (%)	Outdoors (%)	Indoors (%)	Outdoors (%)	*(%)*
An. gambiae s.l.	Misrak	6(21.4)	8(28.6)	8(28.6)	6(21.4)	28(35)
	Mirab	4(25)	3(18.75)	3(18.75)	6(37.5)	16(20)
An. christyi	Misrak	3(20)	2(13.3)	3(20)	7(46.7)	15(18.7)
	Mirab	2(9.5)	5(23.8)	5(23.8)	9(42.9)	21(26.3)
Total		15(18.7)	18(22.5)	19(23.8)	28(35)	80(100)

4.3.3. ELISA Sporozoite Rate Determination

The 44 *An. gambiae* s.l. and 36 *An. christyi* adult mosquitoes were assayed by ELISA to determine the infection rate, but none were found infected with malaria parasites.

4.4 Meteorological Factors

In the past three decades, a significant annual mean temperature rise was observed after 1984 in the area, whereas the pattern of rainfall did not change (appendix one).

The difference from the mean of maximum and minimum temperature relative to the mean of 2000/1 to 2005/6 is presented in Fig 2. Minimum temperature anomaly decrease 2001/2 onwards whereas maximum temperature anomaly fluctuates year to year with higher maximum anomaly was seen in 2005/6. The temperature situations of Butajira area is not in agreement with the higher malaria cases of the area in 2003 and 2005.The annual rainfall and malaria cases in the last six years are presented in fig 3. Annual rainfall in 2003 and 2005 was higher. In these years, high numbers of malaria cases were recorded in Butajira Health Center.

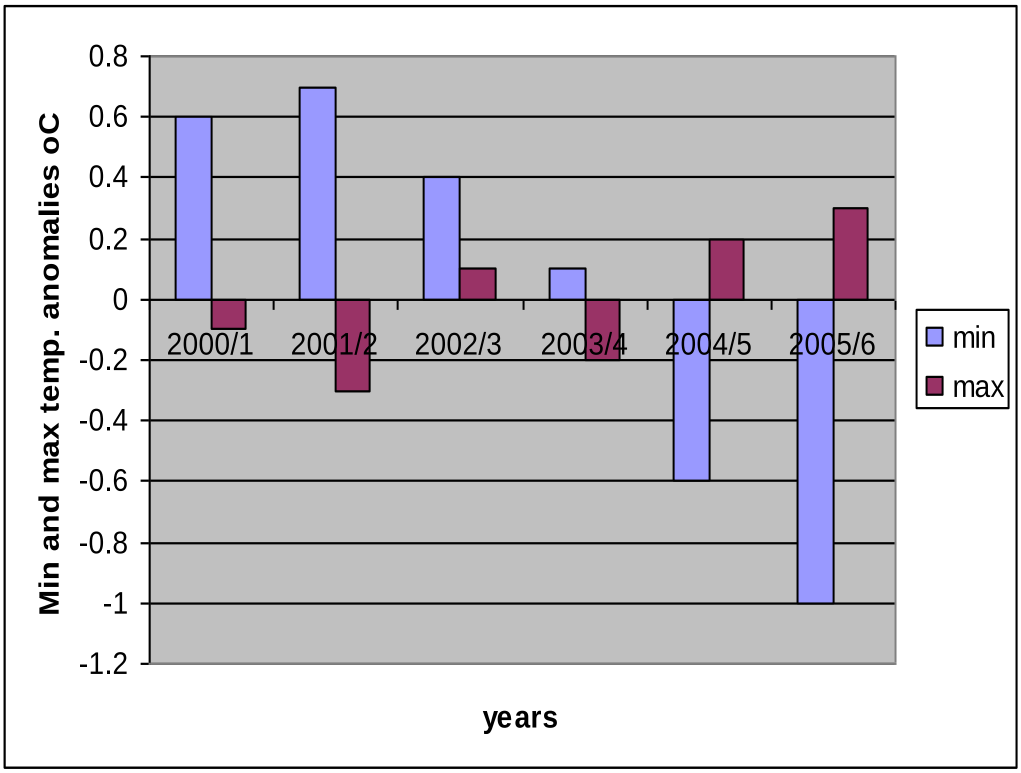

Fig.2 Annual mean minimum and maximum temperature anomalies from year 2000 to 2005, Butajira area (**Source**: NMSA)

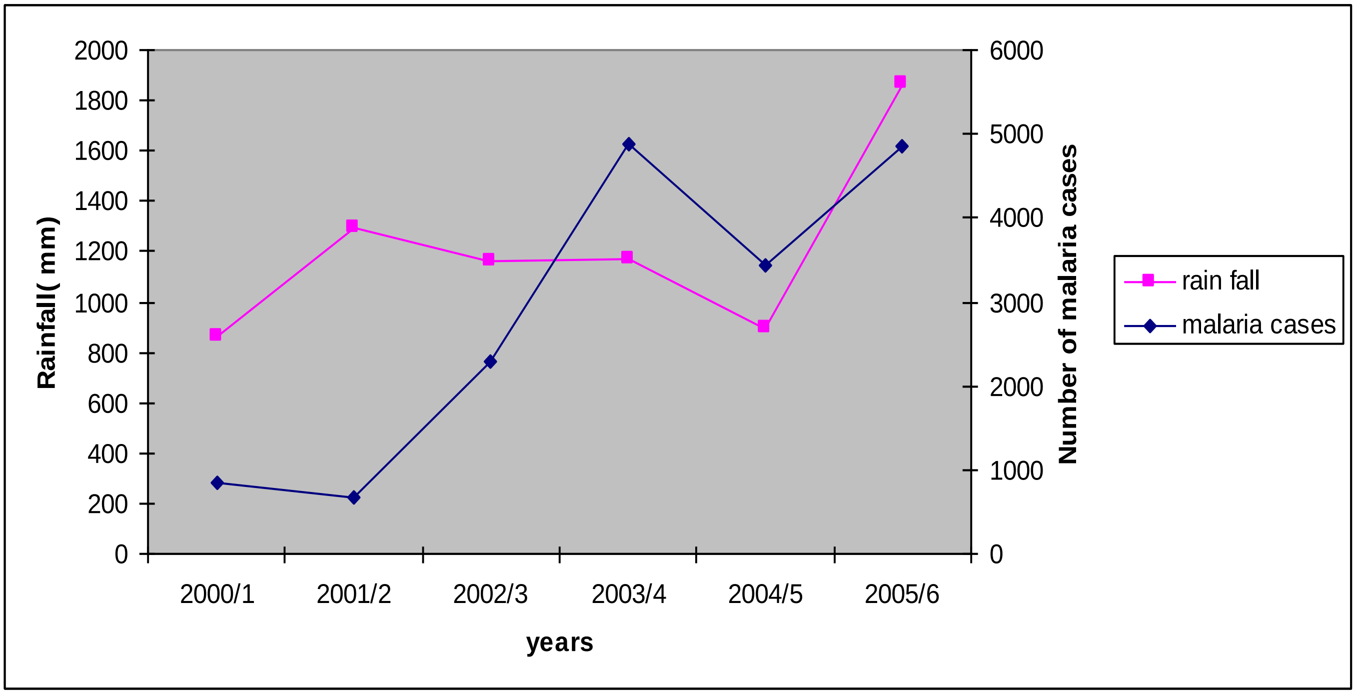

Fig.3 Annual rainfall and number of malaria cases in Butajira area from 2000/01 to 2005/06 (**Source:** NMSA and Butajira Health Center monthly morbidity report)

4.5. KAPs Survey

Concerning the educational status of the interviewees, 39(39%) were illiterate and the rest (61%) literate. Of the total literate (54.1%) said they could read and write only, (37.7%) completed elementary school, (6.6%) high school while the reaming (1.6%) learned theology (Quran).Their occupation consisted of farmer (99%) and government employee (1%).

Regarding malaria transmission, 69(69%) subjects both in Misrak- Meskan and Mirab-Meskan believed that malaria could be transmitted from person to person, 76.8% of them mentioned the bite of mosquitoes in both localities. The awareness in Misrak-Meskan (81.4%) was better than Mirab-Meskan (69.2%). Nearly 12 (17.4 %) of the 69 respondents associated it with un sanitary living condition. This includes unsafe water and eating contaminated food, biting of lice and exposure to bad odour. The other respondent replied; close contact 2(2.9%), by curse of God 1(1.45%) and shortage of food 1(1.45%) respectively (Table, 12).

Table 12. Knowledge and attitude of household heads(n=69) interviewed regarding malaria transmission in Misrak- Meskan and Mirab- Meskan localities in the highland fringe Butajira areas from October to December, 2006

Means of transmission	Frequency		Total
	Misrak-Meskan n (%)	Mirab- Meskan n(%)	n(%)
Bite of infected mosquitoes	35(81.4)	18(69.2)	53(76.8)
Unsafe drinking water and eating contaminated food	5(11.6)	4(15.4)	9(13.05)
Close contact with malaria patient	0	2(7.7)	2(2.9)
Exposure to bad odour	0	2(7.7)	2(2.9)
Lice bite	1(2.3)	0	1(1.45)
Shortage of food	1(2.3)	0	1(1.45)
Curse of God	1(2.3)	0	1(1.45)
Total	43(62.3)	26(37.7)	69(100)

When the heads of households were asked where they obtain treatment for malaria, 76(76%) said government clinics and Health Centers, 12(12%) replied they go to private clinics. The rest replied, 10(10%) self treatment, 2 (2%) using traditional healing practices. Concerning travel history to malaria endemic areas with the last two weeks, 96

(96%) of said no travel history while the remaining 4(4%) had travel history to malarious areas during 15 days of the survey period.

The household heads also interviewed about highest malaria incidence in the family. From 100 households 70(70%) of them said the incidence was common in the entire family member, 14(14%) said my older children greater than 5, whereas 8 (8%) said my self. The remaining 5(5%) and 3 (3%) said my spouse and my younger children less 5 respectively.

The household heads interviewed about the malaria episodes of the area. Of 100 respondent 70(70%) of them said they contracted the disease in the last five years, while the disease was not occurred in 30(30%) of household heads. From 70 of the respondent who contracted malaria in the last five years, 68(97.1%) of them said they become well of malaria symptoms only after drug treatment, while the reaming 2(2.9%) said they become well of the malaria symptoms spontaneously with out drug treatment.

The household heads were also interviewed about the episodes of the disease in there family member. From 100 respondents 80(80%) of them said the family member contracted the disease in the last five years, the reaming 20(20%) said the disease was not appear in the entire family in the last five years. The household heads also said their family became well of the symptoms only after drug treatment.

The movement of the people in the study area was also included in the interview. From 100 household studied 51(51%) was said they travel to malaria endemic area. From this 32(62.7%) of them said they travels to malarious area at least once a year for different purpose,11(21.6%) of them said twice year for many days to work on agriculture,7(13.7%) them said every month for religious purpose, while the reaming one (2.0%) said many times because of his second wife in malaria endemic area.

Cold (86%), shivering (85%), headache (84%), fever (82%), and thirsty and poor appetite (81%) were the most frequently mentioned signs and symptoms when the household heads were interviewed about them and their family member. The household heads in Misrak-Meskan locality was better in recognizing common malaria symptoms compared to Mirab-Meskan locality (Table13). More than 80% of the respondents in both localities recognize common malaria symptoms.

Table 13. Knowledge of household heads (n=100) interviewed about symptoms of malaria in Misrak-Meskan and Mirab-Meskan localities in the highland fringes of Butajira area on October and November, 2006

Symptoms	*Locality*	*Frequency n (%)*[a]
Cold	Misrak	56(56)
	Mirab	30(30)
Shivering	Misrak	57(57)
	Mirab	28(28)
Fever	Misrak	50(50)
	Mirab	32(32)
Thirsty and poor appetite	Mirab	49(49)
	Misrak	32(32)
Head ache	Misrak	52(52)
	Mirab	32(32)

[a] percents total exceed 100% because of multiple responses

The study subjects were interviewed about the history of malaria epidemic and the situation of the disease in the area, almost half of the respondents' remembered the occurrence of epidemic in 1997/98. According to them the epidemic killed many people especially young children aged below 14 years old, this epidemic was reported by malaria control center at that time (Meskan District Health Office, unpublished report).

5. Discussion

According to the Italian and the British malariological studies, the upper limit of malarial transmission was 2000 m a.s.l. in Ethiopia, with few exceptions of frequent epidemics above this limit (Melvill *et al.,* 1945; Giaquinto-Mira, 1950). Based on this information a pilot malaria eradication campaign was undertaken in areas below this elevation (Gebre-Mariam,1988).Contrary to the earlier reports, recent parasitological and entomological investigations in the outskirts of Addis Ababa (Akaki town) indicated an autochthonous malaria transmission at an altitude of 2110m a.s.l. (Woyesa *et al.,* 2004). The present study, conducted in highland fringes (2100-2280m a.s.l.) of Butajira area, has provided additional evidence for the existence of autochthonously contracted malaria in high altitudes.

Clinical data obtained from Butajira Health Center showed *P. falciparum* to be the dominant species accounting for 54.4% of the cases. The higher prevalence of *P. falciparum* in Butajira Health Center (2100m a.s.l.) is not consistent with the report of Melville *et al.,* (1945), because they documented the predominance of *P.vivax* at altitudes higher than 1500m a.s.l. This is also in disagreement with another report that showed *P. vivax* as the predominant species in towns and highland areas (Mendez *et al.,* 2001).However, it is possible that most of the *P. falciparum* cases may have been treatment seeking patients that may have come from the nearby lowland malarious areas because it is in the town of Butajira where treatment services were adequately available. Because of the absence of enough information concerning the addresses of all patients that received treatment in Butajira Health Center, it was not possible to determine the origin of *P. falciparum* patients. Thus, it is not clear weather the high prevalence in the highland fringes is due to the changing *P. falciparum* epidemiology or patients visiting the Health Center from the nearby lowland malaria endemic localities. However, since in recent years the number of cases that come from the lowland malaria endemic areas to Butajira Health Center was highly reduced due to the expanding health facilities in the District (Ato Ahemed Nuri, Pers.Comm. Head of Butajira Health Center), autochtonous *P. falciparum* transmission in the Butajira highlands is a more likely explanation. This is possible explanation supported by the finding in the present study that a considerable number of children aged 0 to 4 years were diagnosed for malaria both clinically and microscopically. As a stable community, the mobility of this age group cannot be adequate to explain the relatively high *P. falciparum* prevalence among them. Therefore, it is more likely a result of an autochthonous malaria which is consistent with the changing epidemiology of malaria reported by many in recent years, in which the replacement of *Plasmodium vivax* by *P. falciparum* was documented in highland areas. For instance, *P. falciparum* malaria was reported in the central highlands of Afghanistan at an altitude of 2250m to 2400m (Rab *et al.,* 2003).

According to clinical records at Butajira District (Meskan District Health Office, Unpublished report) malaria is the major cause of morbidity in the area with the peak malaria transmission occurring in October, after the cessation of the heavy rains. The light rains in March and April also contribute to malaria cases in the area. This is in agreement with the overall malaria epidemiological picture of the country (Tulu, 1993).

In Ethiopia *P. falciparum* is known to be the dominant species during the peak malaria transmission in September and October while *P.vivax* tends to dominate during the dry season (Kitaw *et al.,* 1989).However, the early appearance of dry season in September 2006 may have contributed to the dominance of *P. vivax* during the present survey. Furthermore, the relapsing cases *of P. vivax* may have also increased the prevalence of the species in the area during the survey.

Although malaria was observed in all survey localities in the study area, the high number of cases in Misrak-Meskan was associated with the presence of swamps near the villages and the relatively low altitude (2100masl). The swamps that contained water throughout the study period was determined to support anopholine mosquito vector breeding.

It is also known that in hyper or holoendemic conditions, the occurrence of malaria infection in infants or children in stable communities confirms autochthonous transmission and the standard characterization of the epidemiology of malaria is based on an age-prevalence curve. With the prevalence of infection peaking at an early stage with an increase up to the age of 9 years and showing a sudden fall in the age group 10 to 15 years and then slowly declining with age (WHO, 2000), whereas in populations where malaria is either previously absent or persisted at low or moderate endemic levels, it is characterized by high incidence at all age groups (Rudulf et *al.,* 2007). Such inter age variation in malaria prevalence was reported from Nazareth, Ethiopia (Yohannes and Petros, 1996). However, the finding from Butajira highland fringe areas where age prevalence was not evident would suggest be a relatively recent introduction to the region. Such lack of inter age variation in malaria prevalence in newly introduced malaria has been documented from Jabalpor in Central India (Singh et *al.,* 2006).

In the present study, a relatively high malaria prevalence with statistically significance was observed in individuals above 15 years of age. This suggests the poor development of immunity to the malaria parasites in the populations. Thus, the individuals aged above 15 years would make up the bulk of the population that probably constitutes the main reservoir of infection in the study area. This is supported by the fact that most of the asymptomatic gametocyte carriers, which are considered to be the main source of infection (Gilles and Warrel, 1993), detected in the study area were above 9 years age.

The KAP survey conducted in the study area has provided some information about repeated malaria infection of individuals in the area, which is a feature of hypo-endemic malaria epidemiology (Theander, 1998).

The KAP survey with respect to the cause of malaria showed most study participants from Misrak- Meskan to be aware of malaria transmission by the bite of infected mosquitoes. The level of awareness about malaria transmission by mosquito bite has increased among the population of Butajira area from what was reported (59.5%) by Derressa, *et al., (*2004) compared to the combined Misrak and Mirab determination from the present study (76.8%). However, looked at separately, the level of awareness in Misrak- Meskan(81.4%) is higher than in Mirab- Meskan(69.2%).

The knowledge about malaria symptoms was relatively higher in Misrak- Meskan than, in Mirab, suggesting that the disease may be a recent introduction into the Mirab-Meskan highlands and hence the population may not be adequately familiar with it. The higher awareness in Misrak- Meskan, on the other hand, is an indication that the endemicity of malaria is unstable and hence infection is associated with severe clinical manifestations of the disease. This is unlikely in holoendemic regions of Western Kenya where only 30% awareness was reported (Ongure et *al.,* 1989).

The spread of malaria from one district to another is a common phenomenon associated with population movements and migrations. The present study revealed active population movements between the malarious lowland and the hitherto non-malarious highlands in the Butajira region. The main purpose of the movement included work on agricultural fields and religious and social functions. In case of religious functions known as'leqqa,' adults meet every month by a rotation in different localities, both in the highland and lowland areas. Such occasions may increase the probability of vectors and gametocyte carrying hosts meeting in the highlands thus creating conditions for malaria transmission.

The relationship between climate and malaria transmission is complex and varies according to location (Githeko et *al.,* 2001). The actual cause for the rise in malaria incidence in Butajira highlands can not be established with out detailed study of changes in climate and agro- ecological factors.

The detection of *An. gambiae s.l.* at an altitude of 2280m above sea level is a confirmation of the of the report by Woyessa *et al.*, (2004) from the outskirts of Addis Ababa, at an altitude of 2110m asl. It also is in agreement with the report of Chen et *al.,* (2005) from the Kenyan highlands.

The presence of *Anopheles christyi* in the oily swamps of the study area is in agreement with what was reported from Akaki town where it was reported from water bodies polluted with organic substances (Woyessa et *al.,* 2004). Earlier studies in Ethiopia have also confirmed that larvae of *An. christyi* unusually tolerate relatively high level of pollution (Covell, 1957). On the other hand, the oily swamps may have suppressed the distribution *An.gambiae s.l*, which is the main malaria vector. The abundance of *An. christyi* especially in the highland swamps where malaria is prevalent, makes it suspect as a possible vector of malaria in that locality. However, since *An. christyi* was found to breed in almost all the water collections investigated, its low density in adult collections may be related to its poor anthropophilic behavior (Gilles and De-million, 1968). *Anopheles arebiensis* and *An. chriysti* collected both indoors and outdoors but more were collected outdoors. Abose et al., (1998b), have reported similar observations in Ziway for An. arabiensis. In Gergedi, Amenshewa (1995) reported that biting behavior of *An. arabiensis* depends on the availability of host whether outdoors or indoors.

The absence of sporozoite infection in the mosquito samples assayed may have been because of the very low sporozoite rate in the mosquito populations in the study area, which has a relatively low level of malaria prevalence. A similar finding has been reported by Abose *et al.*, (1998b) where sporozoite detection in An. *arabiensis* in Ziway area, gave negative results in both salivary gland dissection and ELISA detections methods.

6. Conclusions and recommendations

6.1 Conclusions

1. The clinical records at Butajira Health Center showed *P. falciparum* to be the dominant species in the area whereas a relatively higher prevalence of *Plasmodium vivax* was determined during the cross- sectional study indicating a shift in malaria epidemiology of the area. This may have been brought about by changes in agro- ecology of the area.

2. The origin of cases treated in Butajira Health Center was mostly from the town and the highland fringe rural localities around the Butajira town.

3. Two anopheline species were identified in the study area; these were *Anopheles gambiae s.l.* and *Anopheles christyi*. In larval collections *An.* christyi was the dominate species whereas the opposite was true in adult collections.

4. The poor awareness about malaria in the higher altitude (Mirab-Meskan) locality may be an indication of a recent introduction of the disease in the area.

6.2. Recommendations

1. Sero-epidemiological studies in addition to the parasitological and entomological surveys must be done to assess the extent of malaria endemicity in the highland fringes of Butajira area.

2. The possible relevance of *An. christyi* in malaria transmission in highland fringe localities must be studied by including blood meal source detection.

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Appendix A. Meteorological conditions of Butajira area between 1972 and 2005.

		Temperature			
Year	*Rainfall (mm)*	*Maximum °C*	*Minimum °C*	*Mean °C*	Temp Mean Anomaly
1972	1415.1	23.8	11.1	17.5	-1
1973	702	23.7	12.2	17.9	-0.6
1974	892.9	23.7	8.6	16.2	-2.3
1975	1034.3	24.4	8.8	16.6	-1.9
1976	1101.2	24.4	11.1	17.8	-0.7
1977	1113.5	23.7	11.7	18.1	-0.4
1978	730.4	24.4	10.8	17.6	-0.9
1979	1271.9	24.7	11.2	18	-0.5
1980	1180.8	25.6	9.6	17.6	-0.9
1981	X	26.5	8.3	17.4	-1.1
1982	1228.8	25.7	8.6	17.2	-1.3
1983	1419.6	25.8	7.6	16.8	-1.7
1984	796.8	26.1	12.6	19.3	+1.3
1985	885.4	25.4	15	20.2	+1.7
1986	1061	25	4.1	19.6	+1.1
1987	1250.9	25.7	13.8	19.8	+1.3
1988	1118.5	25.9	1.9	18.9	+0.4
1989	1284.4	26.9	11.9	19.4	+0.9
1990	1464.4	26.6	13	19.8	+1.3
1991	X	26.2	12.7	19.5	+1
1992	X	X	X	X	X
1993	X	X	X	X	X
1994	1026.1	25.5	13.2	19.4	+0.9
1995	1150.8	25.7	13.2	19.5	+1
1996	1467.6	23.3	13.2	19.3	+0.8
1997	1237.3	25.8	13.3	19.6	+1.1
1998	1453.8	X	X	X	X
1999	968.8	24.8	11.5	18.2	-0.3
2000	865.4	26	11.6	18.8	+0.3
2001	1291.2	25.8	11.7	18.8	+0.3
2002	1158.3	26.2	11.4	18.8	+0.3
2003	1167.4	25.9	11.1	18.5	0
2004	898.6	26.3	10.4	18.4	-0.1
2005	1869.4	26.4	10	18.2	-0.3

X= Data not obtained

Source : NMSA

Appendix B A questionnaire on malaria prevalence in Misrak- Meskan and Mirab-Meskan localities in the highland fringes of Butajira area.

1. Address: *zone*-------------- *Woreda*------------- locality--------------.

House no-----------

2. Household profile

2.1. Name of the head of the household----------------------------

2.2. Age of the head of the household ( ) years

2.3. Sex of the head household

1=Male 2=Female

2.4. Religion of the household ( )

1=Orthodox 3= protestant

2=catholic 4= Muslim

5 other (specify)

2.5 Occupation of the household

1=Government employee

2=farmer

3=merchant

4= other (specify)

2.6. Educational level of the head of the household

0=Illiterate

1=read and write only

2=elementary school education (Grades 1-8) enter grades completed-----------

3=Secondary school education (Grades 9-12) enter grade completed------------

14=Collage diploma

15= First degree

16=Above First degree

2.7 Family sizes of the house hold

1= male----------- 2=female--------------

2.8. Type of housing

1=Thatched top 2=Corrugated tin top

3. Do you know how malaria transmitted from person to person?

1= Yes 2= No

3.1. If yes, how does malaria transmitted from one person to another person ?

1=by the bite of mosquito

2=by eating and drinking unhygienic food and water

3=by using the same utensils with the patient

4=by the curse of god

5=other (specify)

4. Travel history to malaria endemic areas with last two weeks

1=yes 2= no

5. Where they obtain treatment for malaria

1 = Private Clinics

2 = Government Clinics and Health Center

3= Traditional healing practice

4= Self treatment by purchasing anti malaria drugs.

6. Which family members has the highest malaria incidence

1= My self 2= My spouse

3= My older children greater than 5

4= My younger children less than 5

5= All of the family member

7. Have you contracted malaria in the last 5 years?

1= Yes 2= No

8. If no, how many years ago did you have last malaria___________?

9. If yes, did you get well of the symptoms ___________?

1= Spontaneously

2= only after drug treatment

10. How about your spouse and children? Go to Q 7 and 9 and ask the condition of his spouse and children.

11. Do you have a habit of traveling to lowland malaria endemic area *("Kola")*?

1= yes 2= No

12. If yes, how frequently goes to malaria endemic area *(“Kola*”) and for what purpose?

1= every on month for religious purpose

2= Twice a year for the purpose of agricultural work.

3= At least once a year for different purpose 4= other (specify)

13. Which of the following malarias symptoms are manifested in you and your family members? (Starting from the parents write their names and circle the number under appropriate category, you may circle more than one).

1. --------------------------------------1 2 3 4 5 6 7

2. --------------------------------------1 2 3 4 5 6 7

3. -------------------------------------- 1 2 3 4 5 6 7

4--1 2 3 4 5 6 7

1= cold 2= Shivering 3= Fever 4= vomiting 5= Thirsty and poor appetite 6= head ache 7= joint and body pain

14. History of malaria in the area--

Appendix-C Consent Form

Identification No----------------------- age------------------ sex---------------------

A study will be conducted under the objective of determining the presence and magnitude of malaria transmission in the highland fringes of Butajira area. For this purpose, a blood sample will be taken by finger pricking.

To avoid infections with blood borne pathogens like HIV, one disposable lancet will be used for finger pricking for each study participants. For those who have bleeding problem, special care will be given.

All costs related to microscopic examination and anti- malaria drugs (if the study participants became positive) will be cover by the investigator.

I, who registered in------------------- identifications number, clearly understood the above statement and agree to participate in the study.

Name and signature of the participant / parent/ care taker

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