Abstract

Despite more than six decades of continued control effort, malaria remains a major public health problem in Dembia District. In order to determine the causes for the persistence of malaria to the control measures, retrospective and longitudinal prospective studies were conducted in the District. A retrospective data on monthly malaria incidence, malaria control interventions and meteorological variables were collected for the period 2001 to 2015. For the prospective parasitological surveys, all consenting family members selected randomly from four rural Kebeles were examined for the presence of malaria parasites following standard parasitological procedures. Knowledge, attitude and practice survey was conducted in parallel with parasitological surveys. Adult and larval mosquito surveys were undertaken in four selected Kebeles following standard entomological methods. Adult mosquitoes were collected indoors and outdoors and identified into species morphologically based on keys; their host preference and sporozoite infection rates were determined by enzyme linked immuno sorbent assay. The identification of Anopheles arabiensis was confirmed by polymerase chain reactions. In spite of increased indoor residual spraying coverge, retrospective data showed high malaria incidence in most years, except a sharp decline in 2006, 2007, 2008 and 2014. The incidence of monthly total malaria due to Plasmodium falciparum significantly ($P<0.05$) correlated with rainfall at one to four months lag. In the six month prospective parasitological surveys, a total of 4568 samples were examined microscopically. This showed an average of 7.4% positivity for malaria, of which 74.3% were due to P. falciparum and 20.4% P. vivax, whereas the remaining 4.4% and 0.9% were P. falciparum/P. vivax co-infections and P. ovale, respectively. Lack of consistency in indoor residual spraying practice in lower altitude Kebeles and not including malarious mid-altitude Kebeles in indoor residual spraying program explain the highest malaria incidence

observed in the major malaria transmsion season (Sepember-December) in the reterospective and prospective studies. The prevalence of Plasmodium falciparum infections during the dry season appears to be the consequence of river edge pools created mainly by irrigation activities. The study also provided evidence that frequent population movements in and out of malarious areas in the region and outdoor human activities at night are common features that would impede the efficiency of malaria control measures. In addition, poor utilization of long lasting insecticidal net was determined as a factor contributing to the persistence of malaria in the region. The detection of one Plasmodium falciparum infected Anopheles arabiensis from outdoor collection in a sprayed (September) low altitude Kebele is an indication that outdoor transmsion may be taking place. This is another possible reason for the ineffective malaria control. One evidence for the wrong timing of indoor residual spraying operations for malaria control in the District was 50% P. falciparum sprozoite infected An. arabiensis collected during the wet season (June and August 2015). Therefore, the failure to target May to August malaria transmsion season by the malaria control activities as well as the disregard for malaria control in the mid-altitude localities appear to be the main weaknesses of the malaria control program in the District. In addition, increasing human outdoor activities which will expose them to the outdoor biting and resting behavior of Anopheles arabiensis, population movement and irrigation activities are important contributors for the persistence of malaria in Dembia District.

Keywords: *Dembia, malaria persistence, malaria incidence, malaria control interventions, meteorological variables, population movment, Sprozoite rate.*

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Dedication

To my father Tesfaye Sime and my mother Lubaba Hussien

who are always in my heart.

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ACTs Artimisinin-Based Combinations Therapies

AL Artemether-Lumefantrine

An *Anopheles*

CDC Centers for Disease Control

CI Confidence Interval

CSP Circum Sporozoite Protein

DDT DichloroDiphenyl Trichloroethane

DVS Dominant Vector Species

EIR Entomological Inoculation Rate

ELISA Enzyme-Linked Immuno Sorbent Assay

ENSO *El Nino* Southern Oscillation

FMOH Federal Ministry of Health

HBR Human Biting Rate

IB/P/year Infective Bites Per Person Per Year

IRS Indoor Residual Spraying

ITN Insecticide Treated Net

KAP Knowledge, Attitude, Practice

KDT Knock Down Time

LLIN Long Lasting Insecticide Net

Masl Meter above Sea Level

MIS Malaria Indicator Survey

OR Odd Ratio

P *Plasmodium*

PCR Polymerase Chain Reaction

PBS Phosphate-Buffered Saline

PSC Pyrethrum Spray Catch

RDTs Rapid Diagnostic Tests

s.l. sensu lato

s.s. sensu stricto

SP Sulfadoxine-Pyrimethamine

SR Sporozoite Rate

WHO World Health Organization

1. Introduction

1.1.1. The global malaria situation

In 2015, malaria was endemic in 91 countries and territories, causing 212 million clinical cases and 429,000 deaths where majority of them was reported from Africa south of the Sahara, mainly due to *Plasmodium falciparum* infections. While *P. vivax* accounts for 4% malaria cases and were distributed more in Southeast Asia, Eastern Mediterranean and the horn of Africa, although only four countries, Ethiopia, India, Pakistan and Indonesia account for 78 % of *P. vivax* cases (WHO, 2016).

Regarding geographical distribution, *P. vivax* has the widest geographical range in temperate, tropics and subtropics zones (Gething *et al.*, 2012). Unlike its wide geographical distribution, *P.* vivax is exceedingly rare in sub-Saharan West Africa due to the absence of Duffy receptor in red blood cells where the merozoites of *P. vivax* use this receptor for the invasion of red blood cells (Howes *et al.*, 2011). Contrary to West African countries, 33% and 25% of malaria cases in Ethiopia and Eritrea are due to *P. vivax* (WHO, 2016). *P. falciparum* is a tropical and sub-tropical species but sometimes found in temperate climates (Gething *et al.*, 2011). *P. malariae* is patchily present over the same range as *P. falciparum* but much less common whereas *P. ovale* is found in tropical Africa, but also occasionally found in West Pacific (Collins and Jeffery, 2005; Mueller *et al.*, 2007). *P. knowlesi,* a lately confirmed human pathogen is important in a small geographical range in Oceania (Jongwutiwes *et al.*, 2004).

Based on parasite / spleen rates, malaria endemic areas can be classified as holo, hyper, meso and hypo endemic. In holo-endemic areas, the transmission is intense and year round, whereas if the transmission is intense, but there is a period of lower prevalence during the dry season, the area is called hyper-endemic. On the other hand, if transmission is seasonal and regular, the area is considered as meso-endemic and if the transmission is low and intermittent, the area is called hypo-endemic (Baird *et al.*, 2004). Transmissions in holo- and hyper-endemic areas are stable in which adults develop partial immunity to clinical malaria due to high and frequent transmission. In areas of stable transmissions, very young children and pregnant women are the population groups at highest risk of malaria morbidity and mortality. On the hand, transmissions in meso- and hypo-endemic areas are unstable where all age groups are at risk of clinical malaria (Bruce-Chwatt, 1993).

As Rieter, (2008) reviewed, the introduction of DichloroDiphenyl trichloroethane (DDT) in vector control, wider use of quinine in malaria treatment, the adoption of new farming methods, urbanization, improvements in house construction were the factors that contributed to malaria eradication in Europe and America. This shows eradication of malaria in America and Europe in the past was not only due to efficient malaria control interventions, but also the real development activities that change the living standard of the people.

Due to scaling up of malaria control intervations (long lasting insecticidal nets, insecticidal residual spary and artimisinin-based treatment), there is a remarkable progress in the control of malaria worldwide in recent years (WHO, 2016).

1.2. Malaria parasites

The life cycles of the five species of malaria that infect human are essentially the same (Fig 1). Infection begins when an infected female *Anopheles* mosquito inoculates sporozoites in a human host during blood meals (1) and carried around the body until they invade liver hepatocytes where (2) they undergo a phase of asexual multiplication (exoerythrocytic schizogony) resulting in the production of many uninucleate merozoites. The sporozoites of *P. vivax* and *P. ovale* differentiate into either hypnozoites (3) or into developing tissue schizonts in varying proportions. The merozoites invade red blood cells where (4) they initiate a second phase of asexual multiplication (erythrocytic schizogony) resulting in the production of many merozoites which invade new red blood cells. This process is repeated almost indefinitely and is responsible for the disease, malaria. Some merozoites differentiate into sexual erythrocytic stage to produce male and female gametocytes (5). The female *Anopheles* mosquito ingests the gametocytes which further develop into male and female gametes which fuse to form a diploid zygote. The zygote differentiates to an invasive ookinetes that penetrates the gut wall and attaches to the outer portion of the mosquito gut and develops into oocysts. When sprogony completes, the oocyst raptures, the sporozoites are released and migrate to salivary gland for injection into another host during blood meal visits.

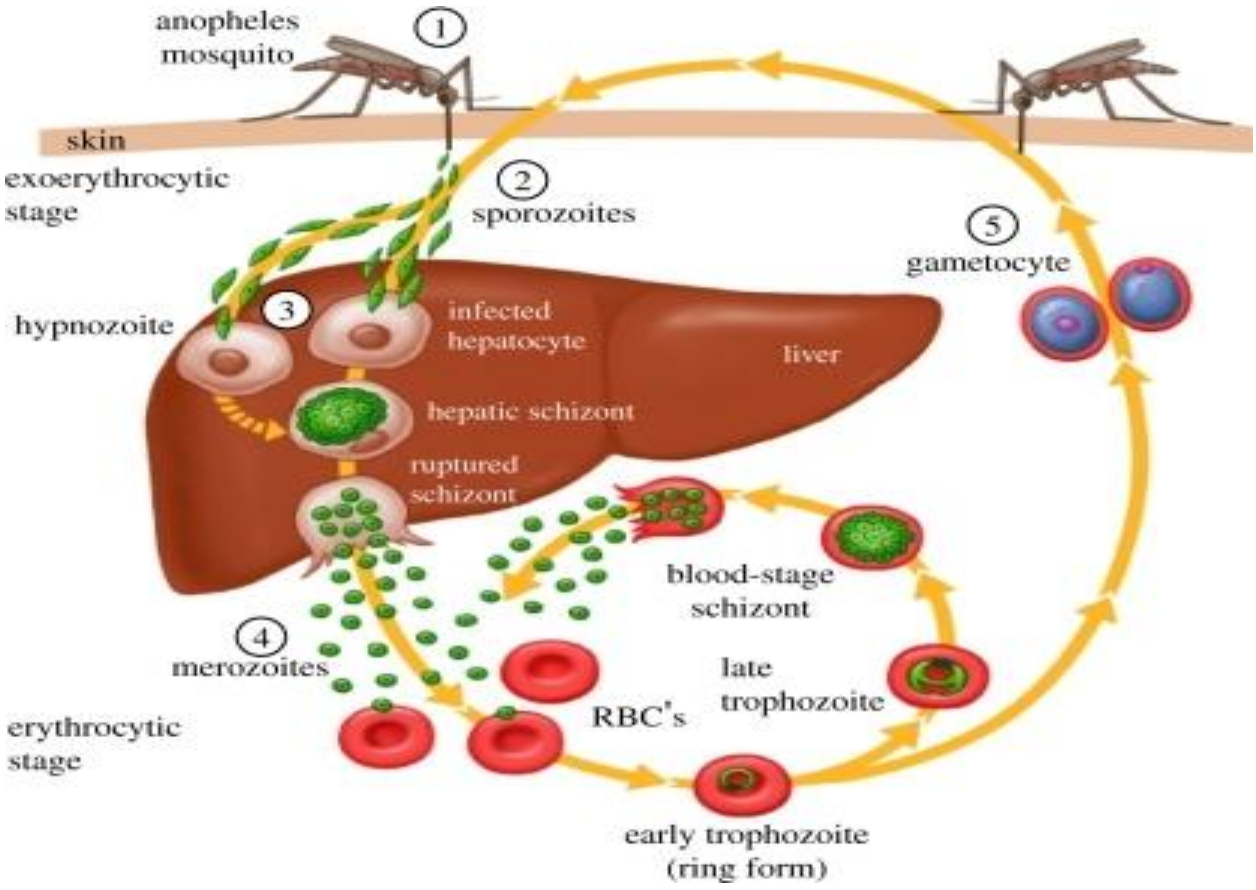


Figure 1. Generlized life cycle of malaria parasite

(Source;http://lhncbc.nlm.nih.gov/system/files/tr2010002, retrieved, 2011), (NB: *P.vivax* and *P.ovale* have a dormant liver stage, the hypnozoites) which are released into the blood later).

1.3. Malaria vectors

There are about 465 formally recognized species of *Anopheles* mosquitoes and more than 50 unnamed member of species complex worldwide (Harbach, 2013). Among these, 70 species are vectors of malaria under natural conditions and 41 of them are the dominant vector species/

species complex (DVS) transmitting malaria at a level of major public health problem (Hay *et al.,* 2010).

Despite higher diversity of *Anopheles* mosquitoes in Africa (140 species), only 8 species are considered as DVS in the continent (Harbach, 2013). From these species, *An. gambiae, An. coluzzii* and *An. arabiensis* (from *An. gambiae* complex) and *An. funestus* are considered as dominant primary vector species whereas *An. melas* and *An. merus* (from *An. gambiae* complex) and *An. moucheti and An. nili s.l.* are considered as secondary dominant vector species with respect to distribution and efficiency in transmitting malaria in the continent (Sinka *et al.,* 2012).

An. gambiae complex has 8 sibling species; *An. gambiae* Gilles, *An. coluzzii* Coetzee and Wilkerson, *An. arabiensis* Patton, *An. bwambae* White, *An. melas* Theobald, *An. merus* Dönitz, *An. quadriannulatus* Theobald, *An. amharicus* Hunt,Wilkerson, Coetzee and Fettene (Coetzee *et al.,* 2013). Due to their highly anthropophilic, endophilic and endophagic behaviors, *An. gambiae* and *An. coluzzii* were associated with intense malaria transmission (Harbach, 2013), however, due to mass distribution of LLINs and IRS spraying, the changing in species composition from anthropophilic and endophagic species (*An. gambiae* and *An. coluzzii*) into opportunistic species (*An. arabiensis*) was observed in some parts of Kenya and Tanzania (Russell *et al.,* 2010; Mutuku *et al*., 2011). But, in some areas populations of *An. gambiae* and *An. coluzzii* continue to dominant because of insecticide resistance (Corbel *et al.,* 2007; N'Guessan *et al.,* 2007) and adjusting its behavior (Sinka *et al.,* 2012).

The wide range of in the behaviors of *An. arabiensis* is associated with the distribution of the species in large geographical areas compared to *An. gambiae, An. coluzzii* and *An. funestus*. For instance, the distribution of *An. arabiensis* towards Sahel and desert of Namibia and Botswana showed that the species tolerate a drier environment than *An. gambiae, An. coluzzii* and *An. funestus*. However, *An. arabiensis* is absent from the humid, forested areas of western Africa (Sinka *et al.,* 2012). The adaptability and plasticity of *An. arabiensis* to feed and rest outdoor on cattle or human make it difficult to control by traditional control methods such as LLIN and IRS that favors the species to become the dominate vector in areas previously considers as secondary vectors (Mwangangi *et al.,* 2013; Lwetoijera *et al.,* 2014). *An. quadriannulatus,* the species in South Africa has negligible malaria vectorial capacity under natural condition, although experimental infection with *P. falciparum* demonstrated their susceptibilities (Takken *et al.,* 1999).

An. gambiae and *An. coluzzii* have different behaviors with respect to the preference of larval habitat where the *An. coluzzii* preferred temporary pools or puddles that only occur after rain whereas the *An. gambiae* is found in more permanent sites such as rice fields or flooded areas (Caputo *et al.,* 2008;Costantini *et al*., 2009). *An. melas* in western Africa and *An. merus* in East Africa is mostly found in the coastal brine waters, whereas the other member of *An. gambiae s.l., An. bwambae* is a much localized malaria vector in Uganda, where it breeds in geothermal waters (Coetzee *et al.,* 2000).

The *An. funestus* Giles group consists of 9 species that are difficult to distinguish morphologically in the adult stage with of which, *An. funestus*, *An. vaneedeni*, *An. parensis* and *An. aruni* having identical morphology at all life stages and are known as the *Funestus* sub-group. On the other hand, the other species in the group are identified by difference in egg and larval stage, larval characteristics and chromosomal banding (Gillies and Coetzee, 1987; Coetzee and Fontenille, 2004). All the species in *An. funestus* group is mainly zoophilic, while *An. funestus* is highly anthropophilic (Gillies and Coetzee, 1987; Coetzee and Fontenille, 2004) and probably the first species to adapt it itself to take a blood meal from a human (Charlwood *et al.*, 1995).

An. funestus is an efficient vector of human malaria throughout its distribution and in some cases exceeding the role of the other vectors in malaria transmission because of its highly anthropophilic and endophilic behavior, longer longevity and late night biting behavior (Gillies and De Meillon, 1968; Coetzee and Fontenille, 2004; Awolola *et al.*, 2005). However, extensive use of the IRS was successful in controlling *An. funestus* in an area where the species was primary vector, by taking advantage of its highly anthrophilic and endophilic behavior (Gillies and Smith, 1960). Similarly, the introduction of ITNs highly reduced *An. funestus* s.s. in the intensive malaria transmission area in western Kenya (Gimnig *et al.*, 2003). However, re-emergence of anthropophilic, endophagic *P. falciparum* infected *An. funestus s.s.* was observed after a long period of losing its major role in transmitting malaria due to the introduction of insecticide treated nets in Asembo, Western Kenya and Kilombero Valley, in Tanzania (McCann *et al.*, 2014: Lwetoijera *et al.*, 2014). Although study about the status of *An. funestus*

group as a vector of malaria is highly reduced in the last 50 years (Coetzee and Fontenille, 2004), the species is considered as the dominant primary vector of Africa (Sinka *et al.,* 2012).

An. nilli and *An. moucheti* are both associated with running water, their larval stages finding a suitable environment along the river banks. *An. nili* lives in the west and central Africa equatorial area, mainly in the forest, but occasionally in the Savanna and is more abundant during the rainy season (Sinka *et al.,* 2012). *An. melas*, *An. merus* and *An. moucheti* considered as secondary dominant vectors with respect to their importance as a public health problem but *An. nili* and *An. mouchete* covers a wide area compared to *An. merus* and *An. melas* (Sinka *et al.,* 2012).

An. pharoensis has a very wide distribution and occupies a broad variety of ecological zones and associated with active transmission of malaria in areas where main vectors are absent (Afrane *et al.,* 2016).

1.4. Malaria in Ethiopia

Malaria is believed to be the disease of antiquity in Ethiopia. For instance, severe epidemic probably due to *P. falciparum* infection occurred in 1618 during the reign of emperor Suseneyos around Gorgora in the north Gondar zone, in North West Ethiopia, which was responsible for the shifting of the seat of the emperor from Gorgora to Guzaraa (Haile, 2000). However, malarological studies were started by Italian and British scientists during World War II (Cox *et al.,* 1999).

Malaria is a major public health problem in Ethiopia, where 75% of the country's landmass comprising 68% of the country's total population are vulnerable to malaria transmission (WHO, 2016). Due to extraordinary diverse topography and climatic condition, the epidemiology of malaria in Ethiopia is more variable and unstable than any other country in Africa (FMOH, 2012). The peak of malaria transmission in Ethiopia coincides with rainfall distribution, and the highest malaria prevalence is observed between September and December, immediately after the major rainy season. Some areas that have short rainy season experience a second "minor" malaria transmission period, from April to June. However, malaria transmission is generally low or absent in most areas in the dry season (MOP, 2016).

They are five main malaria eco-epidemiological areas in Ethiopia (Figure 2). In the humid lowland areas with convenient vector breeding sites, a relatively long, but seasonally intense transmission, occur. However, areas that lack the convenient breeding habitat, intense transmissions occur during and immediately after the major rainy season (June-September). These areas are located in the southwest, northeast and Rift Valley regions with altitude less than 1500 masl. Those areas located from 1,500- 1,750 masl are considered as epidemic prone high malaria transmission areas. Malaria transmission in epidemic prone highland fringe areas in the altitude range of 1750-2000 masl are low compared to the highland fringe area in the altitude range of 1500-1750 masl. Occasional epidemics also occur in the highland areas in the altitude range of 2000-2500masl. Highland areas greater than 2500masl and semi-arid areas that lack water are considered malaria free in Ethiopia.

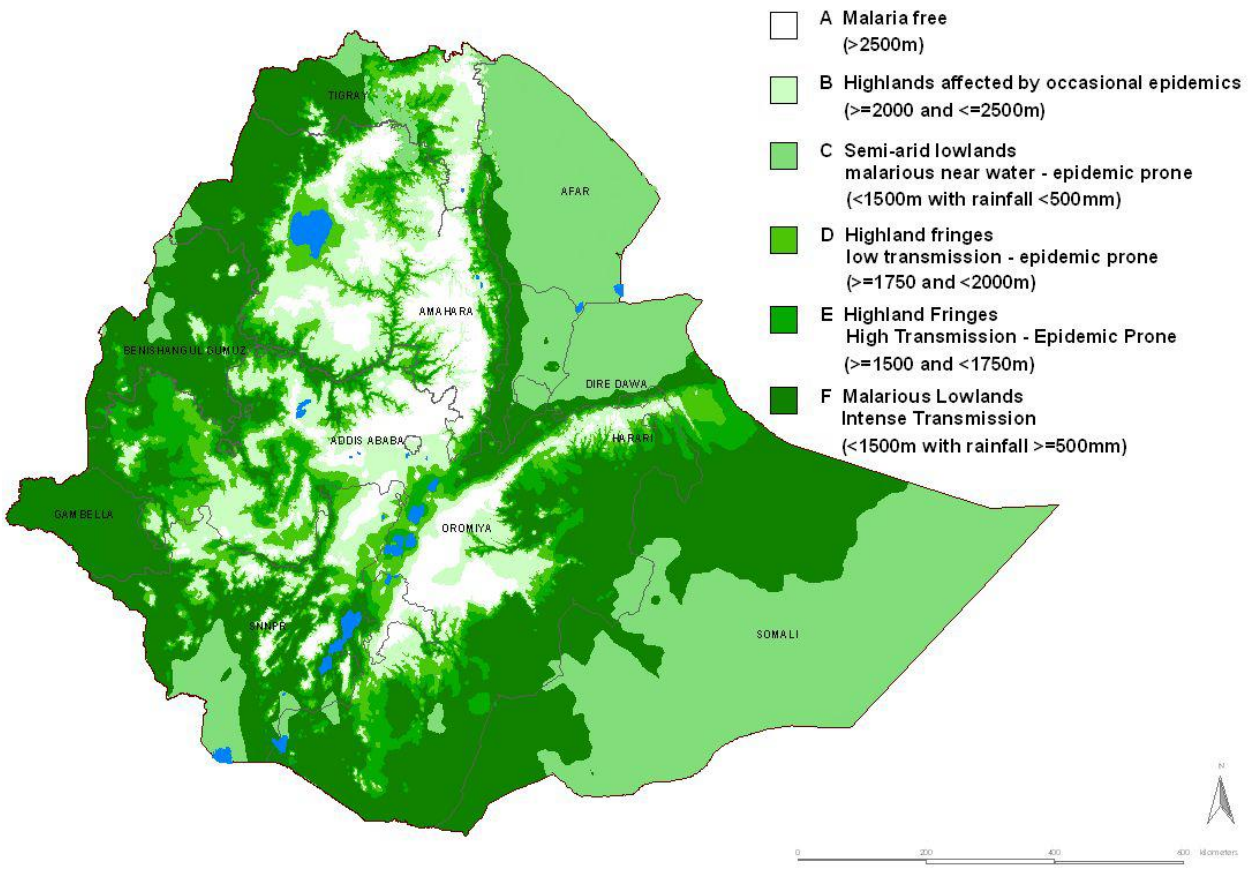


Figure 2. Malaria risk stratification, Ethiopia (Source: adopted from MIS, 2015).

Because the nature of malaria transmission in Ethiopia is unstable, the country experiences moderate to severe epidemics periodically. For instance, the epidemic of 1958 is estimated to have caused 3 million cases and resulted in 150,000 deaths throughout the country (Fountaine *et al.*, 1961). Extensive epidemics showing similar but less characteristic to the 1958 epidemic occurred in many parts of Ethiopia in 1965, 1973 and 1981/1982 (Ghebreyesus *et al.*, 2006). It is estimated that, 48 epidemic episodes occurred between 1986 and 2005, with severe outbreaks occurring in 1988, 1991, 1992, 1998, 2003, 2004 and 2005 (MOP, 2008). Most of the epidemics of Ethiopia occurred after a period of exceptional meteorological conditions in which unusually

high temperature and humidity to create conditions conducive to very high vector concentration (Covel, 1957; MOP, 2008). Although historically Ethiopia has been prone to periodic focal and widespread malaria epidemics that occurred five to eight years, malaria epidemics have been largely absent since 2005, after the scale up of malaria control intervention (MOP, 2008; FMOH, 2012).

All members of the population are at risk of severe disease due to lack of immunity, unlike other countries of Africa in which adults develop partial clinical immunity to the parasite (FMOH, 2014). Since peak malaria transmission often coincides with the planting and harvesting season, and the majority of malaria burden is among older children and working adults in rural agricultural areas, there is a heavy economic burden in Ethiopia (MOP, 2016).

1.4.1. The parasites

All of the four human malaria parasite species occur in Ethiopia. However, *P. falciparum* is the dominant species, accounting for 64% of the cases followed by *P. vivax* responsible for 36% of the cases (WHO, 2016). *P. malariae* and *P. ovale* are rare, accounting far less than 1% of cases. Mis-diagnosis of *P. malariae* and *P. ovale* with *P. falciparum* and *P. vivax* in the area where the species suspected might under-estimate these two species in Ethiopia. For instance, a study conducted in Dembia District in Northwest Ethiopia showed a high discrepancy between microscopy and real-time PCR with no *P. ovale* was reported in microscopy, but more *P. ovale* compared to *P. vivax* was detected by real-time PCR (Tajebe *et al.,* 2014). *P. malariae* is mainly

reported from Arambaminch area, while *P. ovale* is identified from a few patients who live or lived in Humera, Metemma, Gambella and Gamugofa (Ghebreyesus *et al.,* 2006). In Ethiopia, *P. vivax* is more dominant during the dry season (Tesfaye *et al.,* 2012; Woyessa *et al.,* 2012) and weather this is due to active transmission or relapses has not been clearly determined.

1.4.2. Distribution of *Anopheles* species and their vector status in Ethiopia

Like parasitological studies, the distribution of *Anopheles* species and their status as vector were studied by Italian and British expatriates at the beginning of twenty centuries. The works of Covell (1957), Verrone (1962a and 1962b) and O'Connor (1967) have contributed a lot in the identification and distribution of malaria vector in Ethiopia.

A total of 45 *Anopheles* mosquitoes has been documented in Ethiopia (O'Connor, 1967), among which *An. gambiae s.l.* is the most prevalent. *An. arabiensis* Patton and *An. amharicus* Hunt, Wilkerson, Coetzee and Fettene, previously known as *An. quadriannulatus B* are the only two species in *An. gambiae* complex that is still reported in Ethiopia (FMOH, 2012). Similar to *An. quadriannulatus, An. amharicus* Hunt, Wilkerson Coetzee and Fettene (Coetzee *et al.,* 2013) is zoophagic and has no role in malaria transmission (Fettene *et al.,* 2004). *An. amharicus* is distributed in the highland of South-Western and Northern regions co-existing with *An. arabiensis* which exhibits endophilic behavior resting in animal shades and mixed dwellings. (White *et al.,* 1980).

An. arabiensis Patton is the most important vector of malaria in Ethiopia, despite highly diversified vectors of the country (O'Connor, 1967; Kibret *et al.,* 2010; Animut *et al.,* 2013; Yewhalaw *et al.,* 2014). *An. arabiensis* occurs in most parts of the country and breeds in different types of water collections from small sunlit temporary breeding habitat produced after rain to permanent habitat found in river margin and on the shores of lakes (O'Connor, 1967; Animut *et al.,* 2012; Gone *et al.,* 2014).

An. pharoensis Theobald is the secondary malaria vector of Ethiopia (O'Connor, 1967; Kibret *et al.,* 2010; Animut *et al.,* 2013). *An. pharoensis* might be responsible for the transmission of malaria in the absence or low number of *An. arabiensis* particularly in the dry season (Kibret *et al.,* 2010). The co-existence of infected *An. pharoensis* with *An. arabiensis* was reported in the low and mid-altitude localities of Butajira area and in the central rift valley, Ziway area (Kibret *et al.,* 2010; Animut *et al*., 2013).

Previous studies showed that *An. funestus s.l.* and *An. nili* is important malaria vectors in Gambella (Krafsur, 1970; Krafsur, 1977) but currently, the role of *An. funestus* s.l. (Massebo *et al.,* 2013b; Jaleta *et al.,* 2013; Gone *et al.,* 2014) and *An. nili* (Jaleta *et al.,* 2013), as a vector of malaria is uncertain, because *An. funestus* s.l. are reported rarely and none of them were positive for *Plasmodium* species. Both *An. funestus* and *An. pharoensis* prefer large, permanent water bodies with emergent vegetation. For instance, the swamps in the Baro River in western Ethiopia provide an environment that permits *An. funestus* to exceed *An. gambiae s.l.* in number throughout most of the year. Similarly, higher number *An. funestus* were collected in Bahar Dar in permanent papyrus swamps in the southern edge of Lake Tana compared to *An. gambiae s.l.*

and other five species present in sympatry (O'Connor, 1967). The co-existence of infected *An. gambiae s.l.* with *An. funestus* and *An. nili* was reported in earlier study in Gambella (Krafsur, 1970).

An. cinereus, *An. coustani*, *An. rhodesiensis*, *An. d'thali*, *An. maculipalpis* and *An. paludis* are indicated to be susceptible to malaria parasites in other parts of Africa (O'Connor, 1967) but the vector status and their importance in the transmission of malaria is not yet known except one study reported *P. vivax* infected *An. coustani* in southwest Ethiopia (Yewhalaw *et al.,* 2014).

An. christyi was regarded as harmless with no epidemiological importance in malaria transmission in the highland fringes and highland areas in Ethiopia (Tesfaye *et al.,* 2011; Gone *et al.,* 2014). Study on the status of *P. falciparum* and *P. vivax* sporozoite rate in *An. garnahami* in southern Ethiopia showed negative results (Massebo *et al.,* 2013b).

The sporozoite infection rate (SIR) is the proportion of *Plasmodium* infected mosquitoes in the population of local vector species. SIR is determined by three methods; dissecting the salivary glands, ELISA and PCR (WHO, 1975; Wirtz *et al.,* 1987; Bass *et al.,* 2008). The dissection method is the gold standard method where the sporozoite can be determined by dissecting the salivary glands and detecting sporozoite under microscope (WHO, 1975). The dissection method is labour intensive, requires trained expertise and difficult to differentiate between the *Plasmodium* species (Wirtz *et al.,* 1987). ELISA detects *Plasmodium* circumsporozoite protein (CSP) from sporozoites in the thoracic or salivary glands or mature oocysts in the midgut

(Burkot *et al.,* 1984). ELISA is species-specific and can detect precisely the four or mixed species easily. It is possible to perform a single specimen separately or pooled mosquito specimens of the same species (Wirtz *et al.,* 1987).

The limitation of the ELISA is it overestimates the true salivary gland infection because the method detects CSP in other mosquito tissue or detecting the CSP from the oocysts bursting, which occurs two to three days before the sporozoites actually reach the salivary glands (Fontenille *et al.,* 2001). The other limitation of the CSP ELISA is the lack of sensitivity to very low-level infections (Arez *et al.,* 2000). Due to the limitation of microscopy and ELISA, molecular tools are adopted to determine SIR with better sensitivity than microscopy and ELISA, although the method has its own limitation. Multiplex PCR, real-time PCR and duplex real-time PCR uses primers designed against species specific regions in the sequences encoding the small subunit ribosomal RNA (ssrRNA) to detect all *Plasmodium* species (Bass *et al.,* 2008).

SIR in Ethiopia is very low and a large number of specimens need to be tested in order to determine the actual infection rate. For instance, Krafsur, (1971), dissected 8348 *An. gambiae s.l.* collected from Gambella and he found 156 infected mosquitoes with SIR of 1.87%. However, seasonal variation with the highest SIRs in October (4.97%) and November (5.43%) were detected (Krafsur, 1971). Nigatu *et al.* (1992), reported SIR of 0.8% (2/262) by using ELISA both for *P. falciparum* and *P. vivax* in Gambella. *An. arabiensis* SIR of 0.24% and absence of sporozoite in *An. amharicus* was reported in southwest Ethiopia (Fettene *et al.,* 2004). A study conducted in different altitudinal transects of Butajira showed, *P. falciparum* and *P. vivax* SIR of

0.2% and 1.7% in *An. arabiensis*, respectively (Animut *et al.*, 2013). Similar to the highland fringe of Butajira area, *P. falciparum* sporozoite infected *An. arabiensis* was 0.3% in the relatively higher transmission area in South west Ethiopia (Massebo *et al.*, 2013b). All of *An. arabiensis* tested for *Plasmodium* sporzoites found to be negative in south central and south west Ethiopia (Gone *et al.*, 2014; Gari *et al*., 2016; Kenae *et al.*, 2016). However, a higher sporozoite infected *An. arabiensis* (4.6%) was reported in the large scale irrigation area in Western Ethiopia (Jaleta *et al.*, 2013).

An. pharoensis has been naturally infected in Egypt, Nigeria and Kenya (O'Connor, 1976) but enough infection was not found throughout Ethiopia. Nigatu *et al.* (1992) detected 0.46 % (2/436) *P. falciparum* and *P. vivax* sporozoite infected *An. pharoensis* in Gambella. A study conducted in south-central Ethiopia, Ziway area detected *P. falciparum* SIR of 0.59 % (3/509) (Kibret *et al*., 2010). In a study conducted in the highland fringe of Butajira area, the SIR of *P. vivax* was 2.5% (2/79) in *An. pharoensis* (Animut *et al.*, 2013). However, all of *An. pharoensis* tested for *Plasmodium* sporozoite were negative in the studies conducted in Ziway (Kenae *et al.*, 2016) and in the southwestern part of Ethiopia (Massebo *et al*., 2013b; Gone *et al.*, 2014).

The reason for such low rates might be due the relatively low malaria endemicity prevailing in Ethiopia compared to the highly endemic regions of tropical Africa (WHO, 2016). Compared to the earlier studies (Krafsur, 1971; 1978), lower sporozoites rate in *An. arabiensis* in the recent studies (Massebo *et al.*, 2013b; Animut *et al.*, 2013; Gari *et al.*, 2016; Kenae *et al*., 2016) might be the decrement of malaria cases due to scaling up of control interventions (WHO, 2016). The other possible reason for the low sporozoite rate in Ethiopia is the presence *An. arabiensis* as a

major malaria vector in different parts of the country. Due to its opportunistic host preference (feeding both on human and cattle) (Gari *et al.,* 2016; Anemut *et al.,* 2013; Massebo *et al.,* 2013a), feeding behavior (endophagy and exophagy) (Kenae *et al.,* 2016) and resting behavior (endophily and autophily) (Gari *et al.,* 2016; Massebo *et al.,* 2013b) contact between human and the vector is highly reduced unlike the highly anthropophilic and endophagic *An. gambiae* or *An. funestus* (Sinka *et al.,* 2012). For instance, a lower sporozoite rate was reported in *An. arabiensis* compared to *An. gambiae s.s.* and *An. funestus* in a study conducted in western Kenya (McCann *et al.,* 2014).

The entomological inoculation rate (EIR) is the number of infectious bites per person per unit time, which is expressed per year most of the time. EIR is the product of the human biting rate (HBR) and the sporozoite rate (SR). EIR measures the endemicity of malaria and risk of epidemic development and most favored methods to assess the level of malaria endemicity (Shaukat *et al.,* 2010). Several alternative methods measure the HBR, including human landing catch (HLC), light traps (CDC miniature light trap) with or without bed net and indoor resting catches by PSC. Human landing catch (HLC) is the most frequently used methods and considered as the gold standard to determine HBR (WHO, 1975). It is the most reliable measure of human-vector contact for evaluating malaria transmission. However, it is difficult to replicate the technique and raises the ethical question for the possible risk of transmission of malaria and other vector borne diseases (Shaukat *et al.,* 2010). The method overestimates the result and bias might arise due to the variation of attractiveness between individuals for mosquito bite (Mukabana *et al.,* 2002). Like HLC, each method is subjected to bias and shortcomings and, therefore, influences the result of the EIR. A CDC light trap hanged near sleeping people under

untreated bed nets can be used to estimate HBR, as it catches mosquitoes that attempt to feed on humans. However, light traps mostly collect the anthropophilic and endophagous specimens that enter into houses, although the presence of light attracts other species that are not anthropophilic. Specimens sampled by pyrethrum spray catches (PSC) are mostly feed females resting indoors, so the morning EIR obtained from PSC may underestimate the result because the mosquitoes leave the house before or during spraying (WHO, 2013a). Therefore, it is important to consider *Anopheles* behavior and their ecological niches into account, as their vectorial role varies greatly depending on different factors (Shaukat *et al.,* 2010).

Blood meal source identification was performed by precipitin test, ELISA and PCR (WHO, 1975; Beier *et al.,* 1998; Kent and Norris, 2005). Earlier studies used precipitin test in principal that when the serum of the blood antigen in the mosquito is put in contact with a specific antiserum, a precipitation occurs in place of contact (WHO, 1975). ELISA is more rapid, sensitive, specific, automated, quantified and easy to operate compared to precipitin test (Beier *et al.,* 1998). PCR identifies mammalian meal directly with increase speed and cost-effectiveness (Kent and Norris, 2005).

There is a genetic difference on host preference of different species or sibling species in species complex. However, host availability plays a dominate role in host preference (WHO, 1975; Tirados *et al.,* 2006). The identification of blood meal source is important in order to understand the host preference of the mosquito. The role of *Anopheles* mosquitoes in transmitting the disease depends on their preference to feed on man, which in turn affected by different factors such as availability of control interventions (IRS and ITNs) (Nzovu *et al.,* 2013; Ratovonjato *et*

al., 2014) and environmental factors (Lefevre *et al.,* 2008) which is an important parameter in transmission of the parasite.

Studies on host preference of *An. arabiensis* from different parts Ethiopia showed the opportunistic behavior of the species feeding both on human and cattle. For instance, studies showed the anthtropophilic behavior of *An. arabiensis* (HBI= 0.78) in Ziway area, in central rift valley (Kibret *et al.* 2010; Gari *et al.*, 2016). However, the zoophilic tendency of *An. arabiensis* was observed in southwest Ethiopia (Massebo *et al.,* 2013; Yewhalaw *et al.,* 2014; Gone *et al.,* 2014). The similar tendency for human and cattle blood was observed in Butajira and Konso in South Ethiopia (Tirados *et al.,* 2006; Animut *et al.,* 2013).

Studies conducted in Ziway area showed, the antropophilic behaviour of *An. pharoensis* (Kibret *et al.,* 2010; Gari *et al.,* 2016). However, different studies from different parts of Ethiopia indicated the zoophilic behavior of *An. pharoensis* (Habtewold *et al.,* 2004; Massebo *et al.,* 2013; Animut *et al.,* 2013; Gone *et al.,* 2014).

Unlike the antropophagic, endophagic and sporozoite infected *An. funestus* studied earlier in Gambella (Krasur, 1970; Krasur, 1977), recent entomological studies showed negative sporozoite and the zoophagic and exophagic tendency of species (Massebo *et al.,* 2013a; Gone *et al.,* 2014; Kenea *et al.,* 2016). PCR identifications of *Funestus* Group in Ethiopia showed, the presence non-vector species, *An. parensis* (Weeto *et al.,* 2004) instead of the highly effective vector *An. funestus s.s.* indicating the species might be replaced by the non-vector species due to

the IRS spraying and ITN utilization as seen from some parts of Africa (Hargreaves *et al.,* 2000 Mwangangi *et al.,* 2013).

1.5. Factors associated with malaria transmission

A number of interrelated factors are associated with malaria transmission in malaria endemic areas. Factors such as climate change, socioeconomic and scio-demograpic factors, population movement, lack of effective malaria control intervention, insecticide-resistant vectors, drug resistant parasites and human behavior have been cited as some of the contributors of malaria transmission variation (Bodker *et al.,* 2000; Cohen *et al.,* 2008; Yeshiwondim *et al.,* 2009; Coleman *et al.,* 2010).

1.5.1. Environmental factors

1.5.1.1. Meteorological factors

Meteorological factors are important drivers of malaria transmission. Temperature, rainfall and humidity have been associated with the dynamics of malaria vector population and, therefore with the spread of the disease. Ambient temperature plays a major role in the life cycle of the malaria vector. The development of the parasite within the mosquito (sporogonic cycle) is also dependent on temperature. It has been shown that the optimum range for parasite development in the vector is 25-30 °C. The minimum temperature observed for survival for *P.*

falciparum and *P. vivax* is 18 °C and 15 °C, respectively, and the maximum has been reported at 40 °C (Shanks *et al.,* 2005; Cohen *et al.,* 2008).

The sporogonic cycle of *P. falciparum* takes about only 12 days at a temperature of 25 °C but at a temperature of 20 °C, over 30 days are needed to complete the cycle. However, a small increase in temperature near the limit of the parasite and vector 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 °C to 18.9 °C. At 22^{O}C, sporogony is completed in less than three weeks, and mosquito survival is sufficiently high (15%) for the transmission cycle to be completed. The potential number of infective mosquitoes reaches a peak at the 30.6^{O}C (after which it decreases rapidly) (Shanks *et al.,* 2005).

The daily survival of the vector is dependent on temperature as well. At temperatures between 16°C and 36°C, the daily survival is about 90%, while the survival drops rapidly at temperature above 36°C. The highest proportion of vectors surviving the incubation period is observed in temperatures between 28°C and 32°C (Craig *et al.,* 1999).

Changes in rainfall have always affected malaria transmission because the major malaria vector *An. gambiae s.l.* can breed prolifically in temporary water bodies such as hoof prints or rain puddles (Bomblies, 2012). Permanent breeding habitats also depend on adequate rainfall in the

suitable altitudinal range. However, the impact of rainfall varies based on its amount and the topography of the area. Continuous and heavy rains cause severe flooding associated with temporary flushing of breeding places. Consequently, the breeding of vector population is greatly reduced, but it will be soon be re-established when the states are restored. Moderately frequent rainfall, but with fairly long periods of sunshine will increase the opportunity for prolific breeding (WHO, 1975). Drought may increase or decrease malaria transmission depending on local conditions. For instance, in areas where permanent water bodies (river or stream) are absent malaria transmission is highly reduced due to the absence of vector breeding habitat. However, in areas with river and stream, the drought creates small intermittent pools in river beds, which are favorable for anopheline breeding. The associations of drought in Ethiopia, which occurred in 1958, 1965, 1973-1974 and 1983-1985 with subsequent malaria epidemics in respective years might be due to the creation of small intermittent pools in river beds (Mengesha *et al.,* 1998). Apart from creating mosquito-breeding sites, rainfall also affects transmission, though increasing humidity, which in turn will help to 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 (WHO, 1975).

High mosquito density is associated with high concurrent monthly rainfall in a study conducted in Tanzania, where the principal malaria vector is *An. arabiensis* (Oesterholt *et al.,* 2006). However, the peak of *Anopheles* abundance was not coinciding with the peak of precipitation month in a study conducted in the ArbaMinch Zuriya District and Adami Tullu-Judo-Kombolcha in south Ethiopia (Massebo *et al.,* 2013b; Gari *et al.,* 2016).

Inter-annual climate variability associated with El Niño southern oscillation (ENSO) have been strongly correlated with increases in malaria incidence in some parts of southern Asia, South America and East Africa (Kovats *et al.,* 2003). The eastern Africa highlands are more sensitive to climate variability due to the unstable nature of malaria (Bodker *et al.,* 2000). Temperature and precipitations in the highlands are expected to rise above minimum temperature and precipitation at cyclic pattern. Rainfall peaks in east Africa correspond ENSO years. ENSO – a periodic warming and cooling of the Pacific Ocean coupled with changes in air pressure – leads to changes in precipitation, temperature and extreme rainfall events. For instance, the 1958 catastrophic malaria epidemic in Ethiopia was associated with unusually heavy and prolonged rains combined with abnormally high temperature and humidity (Cox *et al.,* 1999). However, Abeku *et al.* (2003) reported abnormal high minimum temperature was associated with most of the epidemics occurred in Ethiopia between 1986 and 1993.

1.5.1.2. Spatial and temporal variations of malaria transmsion

There are large among-site variations in the abundance and temporal dynamics of malaria vector populations, indicating that the risk of parasite transmission differs among sites. Even in one topographic area, mosquito vectors and malaria infections may not be distributed homogeneously, and some households within the same area have a higher malaria incidence than others (Brooker *et al*., 2004). For instance, significant spatio-temporal variations were observed in malaria incidence by sex, age, and village in Ethiopia (Yeshiwondim *et al.,* 2009). Similarly SIR and EIR varied in three villages in rural communities of western Nigeria (Oduola *et al.,* 2012) and in rural and urban areas in Cameron (Bigoga *et al*., 2012). High EIR in the dry season

than rainy season was observed in perennial transmission areas of Cameron (Bigoga *et al*., 2012), whereas EIR and malaria cases were higher in the rainy season in a low endemicity area in northern Tanzania (Oesterholt *et al*., 2006). The presence of highest indoor densities of *An. gambiae s.l.* was observed in studies conducted in Ethiopia and Kenya during the wet season (Amek *et al.,* 2012; Massebo *et al.,* 2013b; Gari *et al*., 2016).

Proximity to water bodies was also associated with increased incidence of malaria (Pullen *et al*., 2010; Peterson *et al*., 2009). Living further away from the river and use anti-insect window screens were independent protective factors for the risk of malaria infection in Tanzania (Oesterholt *et al*., 2006). A case control study in western Kenyan highland showed higher malaria risk with living <250 meter of a forest, 250 meter of a swamp, <200 meter of maize fields, in the absence of trees <200 meter and on flat land (Ernst *et al.,* 2009). In western Kenya distance to water bodies was associated with increased density of *An. gambiae s.l.* (Amek *et al.,* 2012).

A study in Ethiopia showed malaria prevalence was higher in the irrigated village than the non-irrigated village in dry season while the reverse occurred in the non-irrigated village during the rainy season. However, larval and adult abundance of the malaria vectors, *An. arabiensis* and *An. pharoensis*, was higher in the irrigated than non-irrigated village throughout the study period (Kibert *et al*., 2010). Similarly, earlier study in Tigray, Northern Ethiopia, showed introduction of small scale irrigation increased malaria incidence in Children (Ghebreyesus *et al.,* 1999). Introduction of maize cultivation increases malaria transmission in Ethiopia (Kebede *et al*.,

2005) because maize pollen increases vectorial capacity by allowing more larvae to develop quickly into larger and long-lived adults (Ye-Ebiyo *et al*., 2000).

A statistically significant difference was observed between malaria infection with altitude and distance from administrative centre in Papua New Guinea (Myers *et al.,* 2009). Similarly 17 times greater EIR were observed in lowland area than highland area in Tanzania although the incidence of infection was differed only 2.5 times (Maxwell *et al.,* 2003). Higher malaria transmission due to a relatively high temperature in low-altitude localities compared to highland localities was reported in Kenya (Brooker *et al.,* 2004). High density of *An. arabiensis* was reported in low altitude in Butajira area, central Ethiopia during the dry season (Animut *et al.,* 2013). Similarly densities of *Anopheles* mosquitoes generally decreases when the altitude increase in highland of northeastern Tanzania (Kulkarni *et al.,* 2006).

In areas with seasonal transmission, immunity develops slowly that individual in all age groups are affected by malaria (Luxemburger *et al.,* 1997), whereas in high transmission areas children in age range of 1-5 years were more affected by malaria (Snow *et al.,* 1997) but children between the ages of 5 and 10 develop immunity to severe disease while continuing to suffer from mild disease (Pierce and Miller, 2009). Sterile immunity to malaria infection is never acquired but due to partial immunity adult living in hyperendemic areas rarely suffer from clinical disease even though they often carry the parasite (Schofield and Mueller, 2010). A shift in malaria incidence towards older children was observed in Botswana and Tanzania due to a decline in malaria transmission and prevalence through control effort (Chirebvu *et al.,* 2016: Winskill *et al.,* 2011).

1.5.2. Socio-demographic and socio-economic factors

Contrary results were reported in studies that associate incidence of malaria with poverty at household and community level. For instance, two studies that used the same types of approach to construct wealth index in Ethiopia found different results on the association of malaria incidence and wealth of households, where one of the study reported the incidence was highest in poor (Loha and Lindtjørn, 2012) whereas, in other study significant association were not observed between incidence of malaria and wealth index (Gari *et al.,* 2016). Similarly study conducted in holoendemic area of Nigeria found no significant difference in malaria morbidity patterns between different socioeconomic groups (Somi *et al.,* 2007), whereas case-control study conducted in hypoendemic area of Mpumalanga Province in South Africa found a significant inverse association between household wealth and malaria incidence (Coleman *et al.,* 2010).

Except for the biological evidence for the susceptibility of pregnant women, which have an increased risk of *falciparum* malaria (Desai *et al.,* 2007) due to the ability of the parasite to sequester in the placenta; higher malaria incidence in male adults in most studies (Loha and Lindtjørn, 2012; Alemu *et al.,* 2014) are associated with occupational risks. However, study in the murine model clearly established increasing susceptibility of malaria in male as result of testosterone hormone (Cernetich *et al.,* 2006).

A study in Kenya showed malaria vector abundance was influenced by type of house construction and the presence of domestic animals in the household. In regions where the malaria

mosquito vectors feed on animals and humans, the presence of farm animals close to the household may also affect the risk of malaria transmission to humans (Mutuku *et al*., 2011).

Population movement is believed to have initiated malaria transmission in African highlands. Study in Kenya showed, movement of people associated with joining of military posts and construction of railway from highland to lowland area introduced malaria into highlands (Shanks *et al.,* 2005). Population movement is also one of the factors that have been incriminated for the spread of anti-malaria drug resistance (Shanks *et al*., 2005).

Labor-related seasonal human population movement from the highland to malaria endemic large scale agriculrural areas found in the lowland is common in Ethiopia although its impact on malaria transmission in highland is not known. Case-control health facility based studies conducted in highland fringe of Dabat in Northwest Ethiopia (Alemu *et al.,* 2014), and malaria endemic area of South Ethiopia (Yukich *et al.,* 2012) showed recent travel associated with increased risk of malaria infection.

1.5.3. Malaria control and prevention methods

Malaria transmission is much more difficult to control in Africa than most other places because the presence of efficient long-lived anthropophagic vectors, ability of vectors to avoid many domestic insecticide interventions (Trape *et al.,* 2014), inter-species differences in behavior of

the vector (Sinka *et al.,* 2012), poor health infrastructure, the potential for resistance among mosquito vectors and the parasite (WHO, 2016). The most commonly methods used to control and prevent malaria transmissions are those interventions that prevent host vectors contact and anti-malaria drugs once the infection is occurred in the host (WHO, 2016).

1.5.3.1 Insecticide treated nets

An insecticide-treated net (ITN) is a mosquito net that repels, disables and/or kills mosquitoes coming into contact with insecticide on the netting material. The widely distributed net currently in Ethiopia is a long-lasting insecticidal net (LLINs) (FMOH, 2012). The netting materials of LLIN has insecticide incorporated within or bound around the fibers and retain its effective biological activity without re-treatment for at least 20 WHO standard washes under laboratory conditions and three years of recommended use under field conditions (WHO, 2008).

LLIN act as a physical barrier preventing access by vector mosquitoes and thus providing personal protection against malaria to the individual(s) using the nets. Insecticides, which are used to treat nets, have an excito-repellent effect that adds a chemical barrier to the physical one, further reducing human–vector contact and increasing the protective efficacy of the mosquito nets. Pyrethroid such as permethrin, deltamethrin and lamdacyhalothrin, are used commonly because of their low toxicity hazard and good residual effect (WHO, 2008). A low level of ITN usage (WHO, 2016), shifting from indoor to outdoor biting (Russel *et al.,* 2010), early biting immediately after sun set and before sunrise (Fornadel *et al.,* 2010) and wide spread resistant

for pyrethroid insecticide (Wiebe *et al.,* 2017) are among the major problems in using ITNs as a malaria vector control strategy. LLINs are considered to be effective for controlling of *An. gambiae s.s.* and *An. funestus* because of their anthropophagic, endophagic and endophilic characteristics, which prefer to bite at night when people are in bed (Sinka *et al.,* 2010). However, behavioral change and insecticide resistant in these two species make LLINs ineffective (Sougoufara *et al.,* 2014).

Many of the risk factors for malaria are related to access to interventions. For instance, a study in Kenya showed mosquito density, human biting rate and EIR of indoor resting mosquitoes were reduced by more than 75% for *An. gambiae s.l.* and 92% for *An. funestus* when the bed net coverage reached 60-86%. This study also confirmed feeding choice of both vectors shifted more toward non-human vertebrates (Mutuku *et al.,* 2011). A significant reduction in the abundance of *An. gambiae s.l.* over the last 20 years due to increase coverage of IRS and LLINs were also reported along Kenyan coast (Mwangangi *et al.,* 2013). However, in Senegal ITNs quickly selected resistance mosquitoes with long lifespan and unchanged feeding behavior (Ndiath *et al.,* 2014), so the density was not impacted by ITN utilization. Statistically insignificant reduction of *An. arabiensis* in ITNs used households was also reported in Zambia (Chanda *et al*., 2008). Similar to entomological studies different parasitological studies also reported sleeping under LLINs reduced malaria infection (Graves *et al.,* 2009: Pullan *et al.,* 2010; Loha and Lindtjørn, 2012; Ayele *et al*., 2012) provided that they are use properly and regularly. However, some studies reported no differences in malaria prevalence between household that possess and utilize LLINs and those that are not (Roberts and Matthews, 2016).

It is reported that ITNs provide protection to individual users (Graves *et al.,* 2009), what is less obvious is the impact of widespread ITN use at the community level. ITNs are able to reduce the density, feeding frequency and survival of mosquitoes (Gimnig *et al.,* 2003: Bekele *et al.,* 2012) and wide-scale use can mediate protection of all community members, including the vulnerable portion without a bed net (Maxwell *et al.,* 2002; Hawley *et al.,* 2003). On the other hand, it has been suggested that ITN use could increase malaria risk for unprotected people by diverting mosquitoes away from users and concentrating their host-seeking efforts upon them (Pullen *et al.,* 2010). Protection of LLINs at individual level but the lack protection of LLINs at a community level was reported in longitudinal study conducted in Chano-Mille in South Ethiopia (Loha and Lindtjørn, 2012). Prolonged usage of ITNs and IRS has been linked to a shift of human exposure from occurring indoors during hours when most people are sleep, toward occurring outdoors in the evenings and mornings for the two important malaria vectors in Africa, *An. gambiae s.l.* and *An. funestus* (Russel *et al*., 2011). However, a study in Zambia showed human exposure to anopheline mosquitoes occurs primarily indoors in the presence of ITNs (Seyoum *et al*., 2012).

Ethiopia had a very good experience in malaria vector control through indoor residual spraying (IRS). However, malaria vector control with insecticidal impregnated net was started early compared to IRS (FMOH, 2012). For instance, mass distribution of ITNs first implemented in returnee and resettlement sites in the Western part of the Tigray Region, in 1997 through a cost recovery scheme at a subsidized price of Birr 40 paid in four installments. In 1997-1998, ITNs were also distributed in Oromia, Amhara and SNNPR regional states with the support of WHO and Italian Co-operation. A total of around 45,000 nets were distributed in this early phase

(FMOH, 2004). The coverage of net utilization reached 1.5% in 2000. Following small scale ITNs distributions between 2000 and 2003 large scale ITN distribution was started in 2004 (FMOH, 2004). The malaria indicator survey (MIS) of 2007 showed 65.6% households participated in the study owned at least one ITN. The overall net utilization was 53.2% in all household member but better utilization was reported in children <5 years of age (60.1%) and women 15-49 years of age (65.7%) (Jima *et al.*, 2010). Malaria indicator survey of 2015 indicated net possession was almost similar (64%) with 2007 MIS but the overall net utilization in all household member (40%), in children less than 5 years old (45%) and pregnant women (44%) was much more less in 2015 MIS compared to 2007 MIS of Ethiopia (Jima *et al.*, 2010; MIS, 2015). Both the 2007 and 2015 malaria indicater survey showed Ethiopia is below set target both in coverage and utilization in order to achieve effective vector control since the effectiveness of ITNs/LLIns are depends on high coverage and effective utilization (WHO, 2016).

1.5.3.2 Indoor residual spraying

The primary effects of IRS are to reduce the life span of vector mosquitoes so that they can no longer transmit malaria parasites from one person to another and to reduce the density of the vector mosquitoes. The number of mosquitoes entering the sprayed room and human-vector contact are reduced due to the repellant effect of some insecticides. During malaria eradication era (1955–1969), DDT spraying significantly reduced the global malaria burden, particularly in Asia, Latin America and Southern Africa. These efforts, combined with other measures, led to malaria eradication from Europe, the former USSR, and several countries in Asia and the

Caribbean. About 700 million people, or more than half of the previously exposed populations, were freed from malaria risk (WHO, 2006).

The consistent application of IRS has changed the distribution of vector and the epidemiological pattern of malaria in Botswana, Namibia, South Africa, Swaziland and Zimbabwe. The major vector, *An. funestus* has been eliminated or reduced to negligible levels (Smith, 1966). *An. gambiae* is effectively controlled in some areas because the species rest and bite mostly indoors. Another vector, *An. arabiensis*, which does not rest indoors as much as *An. gambiae* is less affected by IRS, even at high coverage levels and is responsible for low levels of transmission and seasonal increases and outbreaks (Mwangangi *et al.*, 2013).

Reducing risk of malaria infection with insecticide residual spraying was reported in different studies conducted at different parts of Ethiopia (Ayele *et al*., 2012; Bekele *et al.*, 2012) and Uganda (Roberts and Matthews 2016). In Madagascar, highest malaria prevalence in children with untreated houses, intermediate prevalence in children with houses sprayed with DDT and lowest prevalence in children with house sprayed with pyrethroid (Ratovonjato *et al.*, 2014). Similar to Madagascar study, spraying of IRS with deletamethrin reduced malaria infection while reduction was not observed in DDT spraying in a cohort longitudinal study conducted in Araba-Minch area, south Ethiopia (Loha and Lindtjørn, 2012). Statistically significant reduction of malaria morbidity for three months after IRS was sprayed but reduction of residual effect and increasing of slide positivity rate after four months was observed in retrospective study conducted in northern Uganda (Tukei *et al.*, 2017).

A demonstration control projects with the objectives of testing the effectiveness of residual house spraying in a range of transmission settings was carried out in Kobo Chercher (1955), the upper Awash Valley (1956), Dembia Plain (1957) and Gambela (1959). However, none of the projects achieved complete interruption of transmission but considerable reduction in malaria transmission was observed in all of pilot studies. For instance, in all of IRS demonstration sites the 1958 epidemic was not occurred whereas devastating epidemic was common in most parts of country even in localities near demonstrations sites (Cox *et al.,* 1999). The success of IRS spraying in demonstration sites led to the establishment of the National Malaria Eradication Service in 1959 and spraying continued on the demonstration sites and other areas of economic significance but a comprehensive spraying programme was initiated in 1966 though out the country (Cox *et al.,* 1999).

In Ethiopia areas below 2000 masl are the target of IRS spraying and the operation is carried out around the month of June. The timing of IRS operation is usually determined by the residual efficacy period of the insecticide used and the length of the malaria transmission period. So until wide spread resistance to DDT was reported, DDT (75% WP) was in use until 2007. Then pyrethroids insecticide (i.e. deltamethrin) have been in use for short period of time but discontinued in most parts due to increasing resistance (FMOH, 2014).

The efficacy of IRS and ITNs depends, among other things, on the proportion of the vectors that resist to the insecticide used. It is, therefore, important to monitor the development and extent of insecticide resistance in a vector population (WHO, 2012). The Global Malaria Eradication

Programme of the 1950s and 1960s failed in part due to the resistance of malaria vectos to DDT (WHO, 2006**)**.

Insecticide resistance refers the ability of the disease vector that survives a dose of insecticide that would normally have killed or manage to avoid coming into contact with insecticide (WHO, 2013b). Insecticide resistance is a genetically inherited characteristic developed due to selective pressure of the insecticide. The selection of the inherent characteristics of the insecticide trait is dependent on the amount and frequency of insecticides used (Nauen *et al.,* 2006). Information on insecticide resistance is important for effective vector control, so detection and monitoring of insecticide resistance in malaria vectors is crucial and has to be conducted together with other entomological indices (Coleman and Hemingway, 2007). Insecticide resistance can be detected at phenotype and genotype level. Measuring phenotypic resistance using bio-assay is the recommended initial step in establishing resistance levels before genotyping for target-site and metabolic resistance and biochemical assays (WHO, 2013b).

Two biochemical mechanisms, target-site and metabolic resistance are known to cause resistance. Target-site resistance occurs when an insecticide fails to bind to its target. The four classes of insecticides most commonly used for contemporary malaria vector control include organochlorines, organophosphorus, carbamates, and pyrethroids have cover only two target sites. For instance, DDT and pyrethroid insecticides are insect neurotoxins that interfere with ion flow regulation across the sodium ion channels. Ion channel modification via an amino-acid substitution leads to reduced target site sensitivity known as knockdown resistance (*kdr*) (WHO,

2013b). The substitution of leucine at position 1014 for either a phenylalanine or a serine has been reported in *An gambiae s.s.* and *An. arabiensis* from East Africa (Ranson *et al.,* 2000; Verhaeghen *et al.*, 2006).

Resistance to DDT with varying level of mortality and knockdown were reported in different parts of Ethiopia (Balkew *et al.,* 2010; Abate and Hadis, 2011: Massebo *et al.,* 2013). Resistance also significantly high for deltamethrin, permethrin, lambdacyhalothrin and alphacypermethrin in different regions of Ethiopia (Balkew *et al.,* 2010; Massebo *et al.,* 2013; Gari *et al.,* 2016). However, *An. gambie s.l.* was susceptible for carbamates (propoxur and bendiocarp) in south central Ethiopia (Gari *et al,,* 2016) and in a further 12 localities (Balkew *et al.,* 2012).

Because the insecticides used are small in number, WHO and its partners have developed a Global Plan for Insecticide Resistance Management to minimize the increasing trend of insecticide resistance (WHO, 2012). Rotating insecticides with different mode of action year to year and using two or more insecticide-based vector control interventions in a house (e.g. pyrethroids on nets and an insecticide of a different class on the walls) are the major components of insecticide resistance management. Based on evidence, using one compound in one geographic area and a different compound in neighboring areas and using a mixture of two or more compounds of different insecticide classes in a single product or formulation is the other alternatives for insecticide resistance management so that the mosquito is guaranteed to come into contact with the two classes at the same time (WHO, 2012).

1.5.3.3 Malaria chemotherapy

Besides vector control, treatment of malaria by effective chemotherapy is the core stone of malaria control in malaria endemic countries of the world. WHO recommends that every suspected malaria case should be confirmed by microscopy or an RDT before treatment (WHO, 2014). Chloroquine has been used for the first line treatment of uncomplicated malaria and prophaylaxis for decades but the emergence of chloroquine resistance in 1960's for the first time in southeast Asia (Verdrager, 1986) and its worldwide expansion within short period time necessitated the replacement of chloroquine by sulphadoxin-premethamine (SP) in some *P. falciparum* endemic countries but SP resistance was also reported first in Southeast Asia and then in Africa (Vinayak *et al*., 2010).

Anti-malarial drug resistance is considered as one of the greatest challenges facing malaria control in the future (WHO, 2016). Some authors credited increase drug resistance is the main reason for the resurgences and emergence of malaria in east African highlands in 80's and 90's (Hay *et al.,* 2002; Shanks *et al.,* 2005). For instance, according to Shanks *et al.,* (2005) drug resistance to chloroquine remains the leading causes for the emergence of highland malaria in the Kerchio tea plantation. Similarly the malaria resurgence in the Usambara Mountains in Tanzania has been linked to rise in anti-malaria drug resistance (Bodker *et al.,* 2000).

Due to drug resistance problem, all *P. falciparum* endemic countries updated their treatment policy from use of mono-therapes with drugs such as chloroquine, amodiquine, to currently recommended artimisinin-based combinations therapies (ACT) (WHO, 2014). Introduction of ACTs as treatment of uncomplicated *P. falciparum* substantially reduced malaria morbidity and mortality (WHO, 2016). The most important features of ACTs are their gametocytocidal properties. For instance, Bousema *et al.* (2011) reported ACT can shorten the duration of gametocyte carriage by approximately four times. In addition, a study conducted in Kenyan highland showed treatment of children with AL combined with IRS spray significantly reduced gametocyte carriage and density compared with period befor intervention (John *et al.*, 2009).

A national wide survey carried out in Ethiopia in 1997 for the efficacy of chloroquine against uncomplicated *P. falciparum* malaria showed the resistance of *P. falciparum* for chloroquine which prompt a treatment policy change that substitute chloroquine with sulfadoxine-pyrimethamine (SP) as the first-line drug for the treatment of uncomplicated *P. falciparum* malaria and retaining chloroquine for the treatment of *P. vivax* malaria (FMOH, 2014). However, treatment failure study conducted in late 2003 at a national level initiated the replacement of SP into artimisinin-based combinations therapies (ACT) in 2004 (FMOH, 2014). In vivo efficacy of Artemether/ lumefantrine (Coartem) showed rapid clearance of parasitemia within three days in 96.3% and 95% of the study participants, in Bahir Dar and Enfranze Districts, in North West Ethiopia (Ebstie *et al.*, 2015; Getnet *et al.*, 2015). Similarly, the efficacy of Coartem was high in a study conducted in Dembia District (Deressa *et al.*, 2017). Although the efficacy of the drug is high at a moment in Ethiopia, artemisinins resistance *P. falciparum* were reported in many countries in South-East Asia in the same geographical locations where choloroquine resistant

first reported. Since no other anti-malaria medicines are available at present with the same level of efficacy and tolerability as ACT, the major obstacles in the control of malaria in the future is the spread of artemisinins resistant *P. falciparum* (WHO, 2014).

1.6. Justification of the study and its Significance

Malaria epidemics killing thousands of people were reported in Dembia District during the previous years. For instance, the 1953 epidemics killed 7,000 people in Dembia plain (Covell, 1957). Due to successive epidemics with high mortality, malaria control pilot program (DDT spraying) was started in 1957 (Cox *et al.,* 1999) with estabilishing the first health center in Ethiopia in Koladeba, the capital town of Dembia District. However, regardless of over 60 years of continued control efforts, malaria is a number one cause of morbidity in outpatient services in Dembia District (Alemu *et al.,* 2012). The occurrence of *Anopheles* species and their association with malaria transmission was not studied in Dembia District, although the District is affected by epidemics many times. In this regard, the present study provides baseline data on the species composition, the distribution of the *Anopheles* species and the entomological risk factors in relation to the risk of malaria transmission. Except for few health center based parasitological studies (Alemu *et al.,* 2012; Tajebe *et al.,* 2014; Deressa *et al.,* 2017), population based descriptive epidemiological studies were not carried out in the District.

Contradictory results were reported on the existence of the link between malaria transmission and meteorological factors (Hay *et al.,* 2002: Zhou *et al.,* 2004). Therefore, in order to

understand the impact of meteorological variables on transmsion of malaria, assessing other non-meteorological factors (e.g. application of malaria control intervention) influencing malaria transmission is important to link between local meteorological factors and the incidence of malaria. Variation in the risk of malaria infection was observed among different sites and within a micro-environment (Yeshiwondim *et al.,* 2009). This information allows malaria control interventions to specifically target hot spots. The impact of currently available interventions (LLINS and IRS) on transmisions of malaria should be known since the information will assess the effectiveness of the interventions and guide policy. The impact of human activities such as internal population movement and outdoor occupation at night on malaria persistence should be known, since these activities influence the efficiency and effectiveness of the major malaria control interventions (IRS and LLINs) which are effective agianist indoor biting and resting mosquitoes.

Studying the impact of developmental activities (small scale irrigation and construction of terrecing canals) on larval habitat productivity and larval abundance is important since the larvae drive adult vector populations and malaria transmsion. In order to provide evidence based vector control and disease management strategies, understanding the feeding and resting behaviors of *Anopheles* mosquitoes with respect to seasonality, locality, and control interventions are important. Due to this, integrated entomologic and epidemiologic studies are necessary in order to understand the casuses of malaria persistence in Dembia District.

Hypothesis of the study

Persistence of malaria to control measures in Dembia District is due to ineffective malaria control interventions, human population movement, increasing human outdoor activities, expansion of irrigation activities and outdoor biting and resting behavior of *An. arabiensis*, with some influence of meterological variables.

2. Objectives of the study

2.1. General objective

The overall objective of the study is to determine the causes of malaria persistence to the control measures in Dembia District.

2.2. Specific objectives

1. To assess wheither persistence of malaria was due to ineffective IRS, meterological variability and insecticide resistance in Dembia District using reterospective data.

2. To determine periodic prevalence of malaria and assess the effect of IRS, possession and utilization LLINs, population movement, increasing human outdoor activities and other individual and household level risk factors on malaria persistence in the study area.

3. To assess the effect development activities (irrigation and construction of terracing canal), IRS and local meteorological conditions on larval abundance of primary and secondary vectors and malaria persistence in the study area.

4. To determine the effect of IRS, possession and utilization LLIN, local meteorological conditions on indoor biting density, indoor and outdoor resting density, host preference, sprozoite rate and entomological inoculation rate of *An. arabiensis*.

3. Materials and Methods

3.1. Study area and population

Dembia District is located in North Gondar Zone, Amhara Regional state 729 km northwest of Addis Ababa. The District shares border with Gondar City and Lay Armachoho in the north, Gondar Zuriya District in east, Chilga and Alefa in the west and part of Lake Tana in the south. The topography of the District makes up plains (89%), mountains (5%), valleys (5%) and swamps (3%). The altitude of the District ranges between 1750 and 2200 masl and covers an area of 1490km^2(Dembia District Agricultural and Rural Development Office). Dembia District is located at 12^{O}17'N latitude and 37^{O}26'E longitude (Figure 3). The population of the District is estimated to be 310,361 in 2015 (estimated from 2007 housing and population census).

The annual rainfall ranges from 811 to 1765 mm with average annual rainfall of about 1071 mm during the last 15 years in the District. Dembia District is characterized by mono-modal rainfall pattern. The average onset of rainfall in District is mid May but it varies from early May to the end of May. However, the main rainy season in the District is from June to September. The warmest months are between January and May with maximum temperature recorded in March in most years in the past 15 years. During the last 15 years, the annual mean maximum temperature was 29.6 ^{O}C and minimum mean temperature was 13.2 ^{O}C in the District (National Meteorology Agency).

The main cereals produced in the District include, Teff, Sorghum, Maize, Wheat, Barely, Finger Millet and Rice. Spice (White cumin, Black cumin, Fenugreek and Hot pepper) and oil crops (Neger seed, Safflower, Linseed and Gomen zer) are the major cash crops produce in the District (Dembia District Agricultural and Rural Development Office).

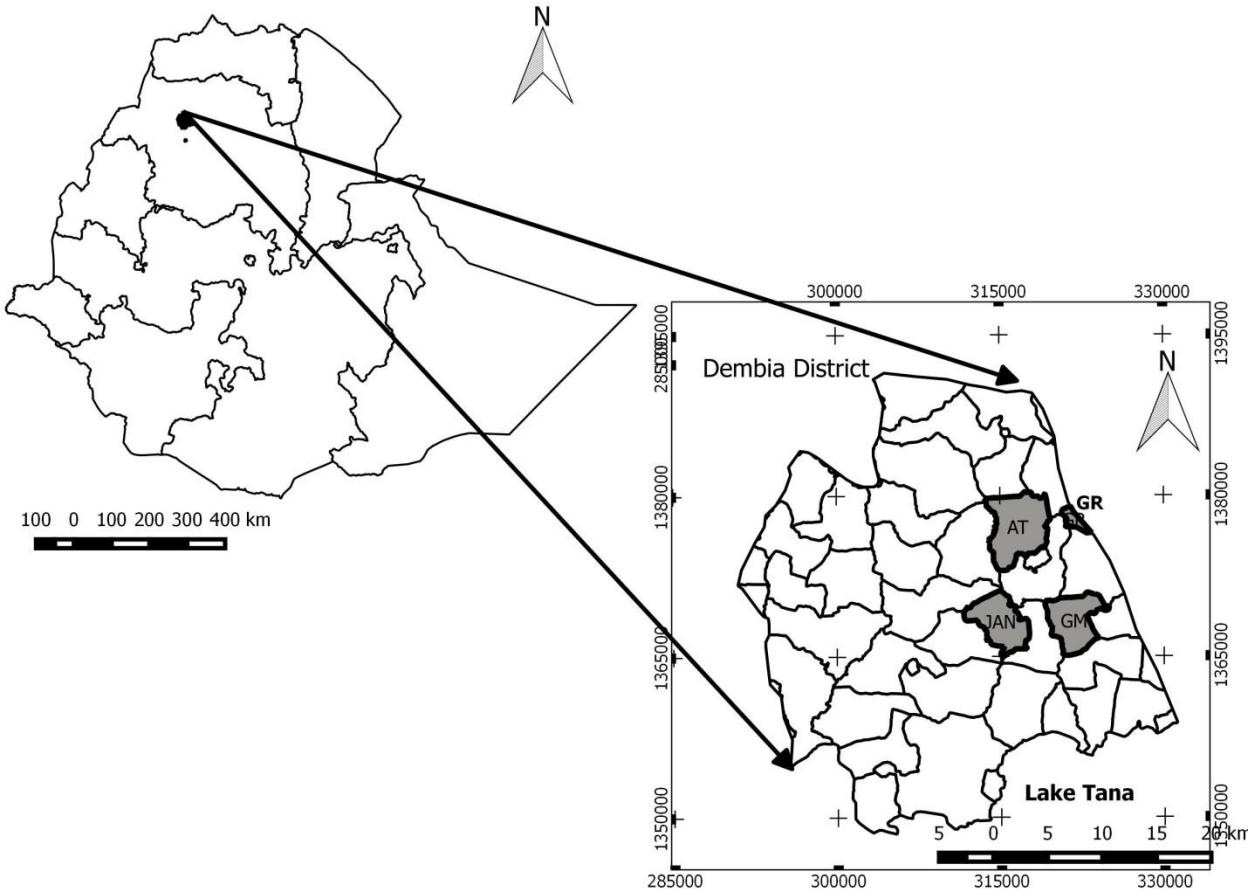


Figure 3. Map showing the location of Dembia District in Ethiopia and the location of the four selected *Kebeles* in Dembia District (AT, Atkelit-Telifit; GR, Girargii; JAN, Jangua; GM; Guramba-Mickael) (Source: Ethiopian Mapping Agency, 2009).

The District has 40 functional health posts (one for each locality), 10 health centers and District level Hospital (but not functional at time of data collection). Malaria transmission occurs on an annual basis in Dembia District, although the peak of the transmission is typically limited to October and November. IRS is carried out in selected localities situated in plain lowland areas. The numbers of localities sprayed by IRS depend on the available resource allocated to the District. But LLINs are distributed to all rural and urban localities of the District starting from 2005 (Dembia District Health Office Report).

Cultivation of onion, cabbage, tomato and some cereal crops during the dry season in localities near Ayenekura, Dirma and Megech Rivers and many other streams are a source of income for farmers in recent years. The water is pumped from the river and released directly into the farm through surface canals (Dembia District Agriculture and Rural Development Office). Dirma and Megech rivers are two of the seven perennial rivers that drain Lake Tana. In some localities, farmers use ground water for cultivation of vegetables.

3.2. Study design

- Community-based longitudinal prospective study that included parasitological, entomological and KAP surveys from four rural *Kebeles.*
- Retrospective study on malaria incidence, IRS spray activities and meteorological condition in the District were documented between 2001 and 2015.

3.3. Retrospective data collection

A retrospective data on monthly malaria cases and malaria control interventions for periods of 2001 to 2015 were collected from monthly outpatient morbidity report of Dembia District health office. For the same period monthly cumulative rainfall, minimum and maximum temperatures were compiled from Ayenba, Azezo, Koladeba, Chuahite and Gorgora weather stations obtained from the National Meteorological Service Agency, Bahir Dar Branch. Similarly populations of Dembia District of the same periods (2001-2015) were estimated from 2007 population and housing census. Hence annual incidences of malaria per 1000 populations were calculated as:-

Annual Parasitic Incidence Rate (A.I.R) =Total number positives (slides+RDT) for parasite in a year × 1000/ total population (Baird *et al.*, 2002; Nkurunziza *et al.*, 2010).

Data on the type and amount of insecticide sprayed, date (period) of spray operation, the number of people and localities covered by IRS spray were obtained from Dembia District Health Office. Based on the information, proportion of population and localities at risk protected by IRS was calculated. Residual efficacy of the insecticide use was based on WHO recommendation on mud wall and the maximum wall efficacy was used (WHO, 2013a). A well prepared checklist was prepared to collect data on type and doses of agricultural fertilizer and pest used and implementation of the new drug artemether/lumefantrine (Coartem) as first-line treatment and uncomplicated *P. falciparum* malaria in the study area (Dembia District Health and Agricultural Offices).

3.4. Parasitological data collection

3.4.1. Sample size calculation for parasitological survey

Estimation of the sample size for the assessment of malaria prevalence was based based on Alemu *et al*. (2012). Employing assumptions of expected prevalence = 9.0%, margin of error = 3%, α = 5% (95% confidence level), design effect = 2 and 10% non-response rate, a sample size of 770 persons were included in the study by using the formula for estimating single proportion.

$$n = \frac{(z\alpha/2)^2 * p\,(1-p)}{d^2}$$

Where:- n= sample size

zα/2= 1.96 (Z=score corresponds to 95% confidence interval)

p= 0.09 (Prevalence of the study area based on Alemu *et al*., 2012)

d= 0.03 (margin error)

$$n = \frac{(1.96)^2 * (0.09)\,(0.91) = 350}{(0.03)}$$

When multiplied by design effect (2) the total sample become= 2*350= 700

10% contingency considered = 70 which give a total of 770 study participants

3.4.2. Study area selection and sampling procedure

Girargii and Atkelit-Telifit from non-sprayed relatively high altitude *Kebeles* (lowest administrative unit in Ethiopia); and Jangua and Guramba-Mickael from sprayed low altitude *Kebeles* were purposely selected from 40 rural *Kebeles* of the District. Accessibility for transport and IRS status were considered during the selection of the *Kebeles*. Atkelit-Telifit is located at average altitude of 1872 masl and the *Kebele* was not sprayed for the last five years whereas Gerargii is situated at average altitude of 1960 masl and was not sprayed for more than 10 years. Although Guramba-Mickael (altitude, 1802 masl), was situated on commonly sprayed low altitude areas it was fully covered by IRS in Sept, 2015 whereas only half of the households were covered by IRS in Sept, 2013 and the *Kebele* was not sprayed at all in Sept, 2014. On other hand, IRS was carriedout on the other low altitude *Kebele*, Jangua (Altitude, 1810 masl) during data collection periods (Dembia District Health office report).

The lists of households of the four selected *Kebeles* were used as a sampling frame. The number of targeted households residing in 4 selected *Kebeles* numbered 4230. From all villages of the selected *Kebeles*, 196 households were selected randomly using probability proportion to size (PPS) sampling methods. After the households are identified, 770 individuals were selected randomly from the selected households.

3.4.3. Blood sample collection

Malaria prevalence surveys were carried out six times in the study population during Dec, 2013, Nov, 2014 and Oct, 2015 during main transmission season and in May, 2014, Feb, 2015 and July, 2015 in minor, dry and wet seasons, respectively. Finger prick blood collection was done by health post workers from randomly selected houses by house-to-house visits. The thick and thin blood smears were prepared on the same slide side by side, properly labeled, air dried and then the thin blood smears were fixed with methanol at the sites. Both thin and thick films were stained with 10% Giemsa for 10 minutes. A microscopic examination was done at 1000x magnification. During a microscopic examination, a slide was regarded as negative after 100 fields had been examined (Woyessa *et al.*, 2012). The thick smears were examined for the presence of malaria parasites by two laboratory technologist one in the field and the other in University of Gondar, Microbiology lab. A third reader re-examined the discrepant results. Those positive for *P. falciparum* but, with no evidence of severe illness were treated with a 6-dose regimen of Coartem (20 mg artemether/120 mg lumefantrine) in the health posts in accordance with national guidelines (FMOH, 2012). Likewise, chloroquine was given to patients, positive for *P. vivax* and malaria species other than *P. falciparum.*

3.5. Data collection by questionnaire

A questionnaire was administered to 196 household heads during Dec, 2013 parasitological survey to record demographic, socio-economic characteristics, knowledge about malaria and its

control intervention methods. Information about travel, LLINs possession and utilization, IRS spray status and outdoor work practice were collected concurrently with parasitological surveys. The survey questionnaire was adopted from the malaria indicator survey household questionnaire of 2011 (MIR, 2011), partly modified for local conditions.

3.6. Entomological data collection

Both immature and adult collections were made to determine the species composition. Female adult *Anopheles* mosquitoes were used to determine indoor biting density, indoor and outdoor resting density, host preference, sporozoite infection rate and entomological inoculation rate. Samples were collected from resting sites and also from breeding sites. The collected specimens were identified to species level on morphological basis using Gillies and Coetzee (1987) and Verrone (1962a and b) for adults and larvae, respectively.

3.6.1. Collections of larvae and pupae

A monthly mosquito larvae collection was undertaken from suspected breeding sites for 14 months between Dec 2013 and Dec, 2015 in two IRS sprayed *Kebeles* (Jangua and Guramba-Mickael) and two non-sprayed *Kebeles* (Atkelit-Telifit and Girargii). Megech in Guramba-Mickael and Dirma in Atkelit-Telifit are relatively large rivers surveyed for the presence of anopheline larvae during the dry season. On the other hand, Ayenekura and Tantikura rivers in

Girargii and Jangua were surveyed in most months except in July and August, 2015 surveys. However, edge of small streams such as Kench Wuha, Bata Begude, Gurate in Atkelit-Telifit, Galaye in Girargii, and Ye Aheya Hod in Guramba-Mickael were surveyed both in dry and wet seasons. Anopheline larvae were also surveyed in swamps present in Atkelit-Telifit and Jangua localities. All hand pipes were not functional in Girargii during data collection period. Temporary (rain pools, terracing canals and erosion pits) and permanent (water leaked from hand pipes, river edges and swamps) were surveyed in all localities for the presence of larvae (Figure 4).

A. Swamp (Huruta, Atkelit-Telifit)

B. River edge(Ayenekura River)

C. Water leaked from hand pipe D. Erosion pit

B. Rainpool F. Terrcing canal

Figure 4. Different types of larval habitats searched for the presence of anopheline larvae in four *Kebeles* of Dembia District, 2013-2015.

Each temporary and permanent breeding site such as swamps, erosion-pits, terracing canals, rain pools, river and stream margins, water leaked from hand pipes, were searched for the presence of

anopheline larvae. If the breeding habitat contains larvae, sampling was done by dipping method by standard dippers (11.5 cm diam and 350 ml capacity). Larval density in each breeding site was calculated as the number of 3rd and 4th instars larvae collected per 100 dips. The numbers of dips were dependent upon the size of the habitat. The surface area of each potential mosquito breeding site was estimated in square meter (m^2) and six dips were taken per m^2. Depending on the size of the larval habitats, 3-30 dips were taken at the edge of larval habitat (Amerasinghe and Munasinha, 1988). Sampling was done by the same person in the morning (0900- 1200) during the survey period. Both anopheline and culicine larvae were sampled and the stage of larval development was recorded as early (1st and 2nd instars) and late (3rd and 4th instars) after transferring the larvae from sampling dipper into white plastic tray. All culicine larvae were discarded after counting. All 3rd and 4th instars anophelines larvae were transferred to separate vials and preserved in 90% absolute alcohol and transported to Gondar university general entomology lab. A code was given for each vial and accordingly information about the larval habitat was properly recorded in pre prepared cheek list. In the laboratory, anopheline larvae were mounted in Gum- chloral and identified under a compound microscope according to Gillies and Coetze, (1987). First and second instar larvae and pupae collected during data collection period were transported safely to laboratory and reared in an insectary until adult stage emerges. The emerged adults were identified using standard keys (Gillies and Coetze, 1987).

3.6.2. Adult Mosquito Collection

Adult mosquitoes were collected using CDC light trap (BioQuip Products, Inc, CA, USA) and pyrethroid space spray collections (PSCs) from indoors and artificial pit sheleters from outdoors for 12 months between Dec, 2013 and Oct, 2015.

3.6.2.1. Indoor-biting mosquito collection

Eight houses were selected from each *Kebele* for indoor biting mosquito collection. Before the CDC light trap collections carried out, household heads were informed about the purpose of the collections, the CDC light traps schedule and what the households were expected to do. Family size, size of house, number of livestock, house construction material, presence of holes in wall, possession and utilization of LLINs and distance to breeding habitat were considered in order to select similar household characterstics. A CDC light traps was hung 1 m to 1.5 m from the floor and 50 cm from occupied untreated bed nets if the household did not possess LLINs and the CDC light trap was run from sunset (6:00 pm) to dawn (6:00 am) (WHO, 1975). However, if the household possessed LLINs, the CDC light trap was hung near the occupied LLINs in the same manner with untreated bed net (Kilama *et al.*, 2014). Mosquitoes collected by CDC light trap were collected in the morning by mouth aspirator and identified us freshly fed, unfed, half gravid and fully gravid based on abdominal conditions. Culicines and male *Anopheles* mosquitoes were discarded after counting, and the females were stored in properly labeled plastic cups for later use.

3.6.2.2. Indoor-resting mosquito collection

Indoor-resting mosquito collection was conducted in another 8 selected houses near CDC light trap catch houses in each village using the PSC method. Houses surveyed with PSC were similar characteristics with CDC light trap catches houses. Size of the house, house construction material, family size, number of livestock, presence of hole in wall, distance to nearest breeding site were considered during PSC.

PSC were carried out early in the morning from 7:00 am to 9:00 am once per month in each study *Kebele.* A white sheet of plastic and aerosol were used to perform PSC (WHO, 1975). Prior to spraying, occupants and all domestic animals left the house. In addition, all food covered and small furniture was removed from the room before the spraying took place. All openings in the room that would allow mosquito-escaping were closed and the entire floor was covered with plastic sheet. Two (2m ×1m, 2m×2m, 2m×3m) plastic sheets were prepared and used according to the size of the room. Spraying with Baygon (Egypt) insecticide was carried out in clockwise direction towards the ceiling until the room was filled with a fine mist (WHO, 2013a). The operator leaves the room rapidly and doors and windows were closed. After 15 minutes the sheets were taken outside the house and all knockdown mosquitoes were collected in the daylight with forceps and placed in a labeled plastic cup with silica gel. The abdominal condition of the mosquitoes was recorded according to WHO, (2013a.)

3.6.2.3. Outdoor-resting mosquito collection

Three artificial pits were used for outdoor-resting mosquito collection in each locality per month during data collection period. Pits with 1.5–2 m deep, 1.2–1.5 m long and 1 m wide were dug in shaded area within a distance of 20-50 m from the nearest house. Four small cavities were then hollowed out to a depth of roughly 0.3 m in the sides of the pit from 0.5 to 0.6 m above the bottom. Mosquitoes were collected from inside the cavities or on the sides of the pit by a mouth-held aspirator using a torch as a light source. During the time of collection, each pit was covered with a transparent net to prevent mosquitoes from escaping. After abdominal condition was identified the female *Anopheles* were preserved in a labeled plastic cup.

3.6.3. Entomological parameters

3.6.3.1. Species composition

All mosquitoes collected from all houses of selected localities by all collection methods were sorted, counted, categorized as unfed, fed, half gravid, and gravid and finally placed separately in labeled plastic cups for identification. All collected female mosquitoes using different methods were transported to University of Gondar entomology lab, and by using a compound microscope species identification was carried out morphologically by appropriate keys provided by Gillies and Coetzee (1987) and Verrone (1962a). Polymerase chain reaction (PCR) techniques were performed on 6.5% randomly selected *An. gambiae s.l.* by using species-specific PCR (Scott *et al.,* 1993). The legs of each specimen were removed from each mosquito and mixed with 12.5 µl PCR master mix that consists of 10x dNTPs, MgCl2 solution, QD primer, UN Primer, GA

primer, ME primer, AR primer, deionized water and RTag in a 0.2ml PCR tube and centrifuged for 20s-20min at 16 K r.p.m. and amplified in thermocycler (PTC-100™, Thermo cycler, MJ Research, Inc., USA). The PCR cycle conditions were (95°C/5 min × 1 cycle; (95°C/30s, 50°C/30s,72°C/30s) × 30 cycles; 72°C/5 min × 1 cycle; 4°C hold). After mixing 5 µl of PCR product with 2 µl of loading dye and 4 µl of DNA ladder the mixture was electrophoresed through a 2% agarose-tris-borate-EDTA containing ethidium bromide gel (with a 100 V and 150 mA power source) and visualized under a UV light box (Alpha Innotech, MultiImage™, Light Cabinet, Taiwan).

3.6.3.2. Indoor-biting density and indoor and outdoor resting density

Monthly indoor biting density (host seeking density) was obtained by dividing the total number of mosquito collected by CDC light trap in each house per total number of CDC trap nights (WHO, 2013a).

On the other hand, monthly indoor resting density was obtained by diving total number mosquito collected by PSC per total number pyrethrum spray catch per 45 minutes in a day. Similarly, monthly outdoor resting density was calculated dividing total number of mosquito per total number of pit searched for anopheline mosquito in 30 minutes (WHO, 1975).

3.6.3.3. Blood meal source identification

The abdomens of freshly feed mosquitoes collected by different methods were crushed in 50µl phosphate buffer saline (PBS) and the volume was brought to 200 µl with PBS buffer. Fifty micro-liters of mosquito triturate was added to two separate 96-well micro-titer plate (for human and bovine blood) simultaneously, and incubated overnight at room temperature. Each well was washed twice with PBS-Tween 20 and 50 µl peroxidase-conjugated anti-humans IgG and peroxidase-conjugated anti-bovine IgG was added in each well of the first and the second plate then incubated for one hour. After one hour, each well was washed three times with a PBS–Tween-20. Finally, 100 µl of ABTS peroxidase substrate was added to each well and incubated at room temperature for 30 minutes and the absorbance at 405 nm was recorded with an ELISA plate reader (Dynex Technologies, USA). Each blood meal sample was considered positive if the absorbance value exceeded the mean plus three times the standard deviation of the four negative controls. Negative controls from a laboratory colony of *An. arabiensis* adults not fed with blood and positive controls contained human and bovine blood were included in the plates (Beier *et al*., 1988). Human blood index (HBI) and bovine blood index (BBI) of each anopheline species was determined by dividing human fed and cattle fed anophelines, respectively to the total tested. In addition, mixed (human + bovine) blood meals were added to the number of human and bovine blood meals when computing HBI and BBI (WHO, 2013a).

3.6.3.4. Sprozoite rate (SR) determination by ELISA

Unfed, freshly feed, half-gravid and fully gravid females were dry-preserved on silica gel before running the sporozoite ELISA (Writz *et al.,* 1987). The dried head and thorax of unfed, gravid and freshly fed (the abdomen kept for blood meal analysis) mosquitoes were carefully separated from the abdomen and simultaneously tested for the presence of *P. falciparum* and *P. vivax* CSPs. Three separate 96-well micro titer plates were coated with 50 µl capture mAb of *P. falciparum*, *P.vivax*-210 and *P.vivax*-247 respectively, and incubated overnight at room temperature. The well contents were dumped and emptied, washed three times with PBS-tween 20, filled with 200 µl blocking buffer (BB) and incubated at room temperature for one hour. At same time mosquitoes were ground individually in 50 µl grinding buffer (25 mlBB:125 µl Igepal CA-630) and the grinding pestle were rinsed 2 times with 100 µl grinding buffer to bring the final volume 250 µl. To avoid contamination between mosquitoes, the pestle was rinsed with PBS-Tween twice and the pestle was cleaned with tissue paper. After one hour incubation, the BB was aspirated from the well and 50 µl mosquitoes triturates were loaded to each of the three test wells. Similarly 50 µl CSP positive sample (Well A1) as positive control and laboratory breed *An. arabiensis* (Well B1-H1) as negative controls were loaded into three test wells. Plates were incubated for two hours and washed with PBS-Tween 20 twice. 50 µl peroxidase mAB (0.05µg/10µlBB) were added to each triplicate well in plates and incubated for 1 hour. The plates were aspirated and washed three times with 200 µl PBS-Tween 20. After the enzyme activity was checked, 100 µl ABTS substrate were added per well and incubated for 30 minutes. Plates were visually observed for green color and 405 nm micro plate reader. Samples were considered positives if the absorbance value (OD) greater than 2 times mean OD of negative

samples. Positives samples were retested for confirmation by quantitative testing (Writz *et al.*, 1987). Sporozoite rate was determined dividing CSPs infected *Anopheles* per total number of mosquitoes tested. Sporozoite rate were determined for each localities and collection methods separately.

3.6.3.5. Human biting rate

Human biting rate was calculated as the average number of bites received per person per night of collection. Since human landing caches were not performed, human biting rate was indirectly obtained from CDC light trap catches. Therefore, the factor determined for *An. arabiensis* in Zambia by Fornadel *et al.* (2010) where a CDC represents 1.91 of an HLC indoors was used for human biting rate determination.

Monthly human biting rate= 1.91 * Number of mosquito collected by CDC/No CDC trap night (Kilama *et al.*, 2014).

3.6.3.6. Annual Entomological Inoculation Rate (EIR)

The annual *P. falciparum* EIR was calculated from CDC light traps by using the standard method 1.91 *(number of sporozoite positive ELISA results from CDC light trap/no. mosquitoes tested) *(no. mosquitoes collected from CDC light ptrap/no. catches) *365 days, and monthly EIR was determined as a product of the EIRs for each day of the month (Fornadel *et al.*, 2010).

3.6.3.7. Insecticide susceptibility tests

Insecticide susceptibility tests were performed following World Health Organization (WHO) protocol and using insecticide susceptibility test kits and insecticide impregnated papers (WHO, 2013b). Mosquito larvae and pupae collected from various breeding sites of the selected localities were reared into adults in the University of Gondar entomology lab. For each replicate 20 to 25 five non-blood fed two to three days old *An. arabiensis* were exposed to discriminating dosages of DDT (4%), Deltamethrin (0.05%), Malathion(5%), Fenitrothion (1%), Propoxur (0.1%) and Bendiocarp (0.1%) for 1 hour. Number of mosquitoes knocked down during exposure time was recorded at intervals of 5 minutes and the proportions of survivors and dead mosquitoes were recorded 24 hours post exposure. Mosquitoes were also exposed to insecticide free papers as controls. Mosquitoes were then transferred to a recovery tube, supplied with sterilized 10% sucrose solution and kept in insectery with room temperature of 26 °C and relative humidity 70%. Four to five replicates of the tests and two to three replicates of controls were carried out for each insecticide tested. Mortality was calculated as follows:-

$$\text{Exposure mortality (E)} = \frac{\text{Number of dead mosquitoes}}{\textit{Total number of Mosqutoes in expermental tube}}$$

$$\text{Control mortality (C)} = \frac{\text{Number of dead mosquitoes}}{\textit{Total number of mosqutoes in control tube}}$$

If control mortality is greater than or equal to 5% and less than or equal to 20%, the value for exposure mortality E was corrected by using Abbott's formula (Abbott, 1925).

$$\text{Corrected exposure mortality (\%)} = \frac{E - C * 100}{100 - C}$$

3.7. Meteorological Data

For resterospective study monthly cumulative rainfall, minimum and maximum temperatures were compiled from Azezo, Koladeba, and Chuahite and Gorgora weather stations for the period 2001 to 2015. Meteorological data were obtained from National Meteorological Service Agency, Bahir Dar branch.

For prospective parasitological and entomological studies, daily rainfall and temperature data up to 6 weeks lag were compiled from the nearest weather station for each study locality. Daily rainfall data of Azezo weather station was used for Gerargii *Kebele* (5 km from Azezo air port and found in similar altitude). Rainfall data of Koladeba weather station was used for Guramba-Mickael and Atkelit-Telifit *Kebeles*. The daily rainfall of Chauit weather station was used for Jangua *Kebele*. In addition, IRS spray status was recorded for the sprayed locality. By considering 6 months wall life for Bendiocarp and Propoxur (FMOH, 2012), the locality and the survey month was categorized as sprayed (if it is less 6 months) and non-sprayed (if it greater than 6 months).

3.8. Data analysis

Statistical analysis was conducted using SPSS statistical software package version 20 (SPSS Inc., Chicago. IL). Descriptive analysis was made to present the frequency of variables and presented

in Tables in retrospective, parasitological, KAPS and entomological studies. Graphes were also used to show trends and associations.

Spearman correlation coefficient was used to quantify the strength of linear relationships between meteorological variables, proportion of population and localities at risk protected by IRS and malaria incidences for data collection period (2001-2015). To assess the effect of meteorological variables with zero to four months lag on malaria cases, the monthly malaria incidences were considered as the dependent variables, while meteorological variables such as monthly maximum, minimum, average temperature and total monthly rainfall were considered as independent variables. To observe the effect of each meteorological variable on monthly total malaria incidence, multiple linear regression was used. Yearly and monthly variations of incidence of total malaria, *P. falciparum* and *P. vivax,* were tested by one sample t-test.

Chi-square(X^2) tests were carried out to test significance difference in malaria prevalence between localities, gender and survey seasons. Because a generalized estimating equation (GEE) model is appropriate to examine repeated measurements in longitudinal study, the model was used to analyze the effect of potentially explanatory risk factors on malaria prevalence. The explanatory factors such as gender, age, possession of LLINs, survey seasons, utilization of LLINs, IRS spray status, keeping cattle and other property at night and travel history were used to explain outcome variable (Prevalence of malaria). Crude associations (univariate analysis) between explanatory factors and outcome variable were estimated by regressing a single factor against malaria prevalence. To control for confounding factors, multivariate logistic regression analysis was performed. All variables that showed significant associations with malaria

prevalence in univariate analysis were selected and entered for multivariate logistic regression analysis to identify the most important predicators of malaria risk factors.

Analysis of variance (ANOVA), correlation coefficient and multiple linear regressions were used to assess the impact environmental variables and IRS spray status on mean larval density of *Anopheles* mosquito. Number of larvae per 100 dips was considered as dependent variables. Considering six month residual efficacy for Bendiocarp (sprayed in Sept, 2013) and Propoxur (sprayed in Sept, 2014 and 2015) in mud wall (FMOH, 2012), the surveyed months were categorized as sprayed and non-sprayed for the IRS sprayed localities. ANOVA was also used to compare variations of mean larval density among months and study localities. The Turkey Honestly Significant (HSD) test was carried out to distinguish the months and study localities and season with maximum larval density. Similarly ANOVA was used to test variations among localites and season, indoor host seeking density, and indoor and outdoor resting densities of *An. arabiensis* during data collection period. The HSD test was also carried out to distinguish the months and study localities, with higest feed mosquitoes, indoor host seeking density and indoor and outdoor resting densities. In order to observe association between host seeking and indoor resting densities with some household factors (Utilization of LLINs and IRS spray status) and environmental factors, mean comparison and ANOVA were carried out for the primary and secondary vectors. Percentage of mortality and knockdown was calculated according to WHO protocol for insecticide susceptibility test (WHO, 2013b). Probit analysis was used to calculate KDT50 (the time taken to knock down 50% of mosquitoes) and KDT90 (the time taken to knock down 90 % of mosquitoes) for DDT and Deltamethrin.

3.9. Ethical issue

Permission for the conduct of the study was obtained from Addis Ababa University, Collage of Natural Science ethical committee. Permission was also obtained from North Gondar and Dembia District Health offices. Dembia District Health Office informed the local administeration about the purpose of the study and written consent was obtained from the study participants. For children less than 12 year olds, assent was obtained from household heads or guardians. Blood smear was obtained with finger prick using disposal blood lancet and cotton immersed in 75% alcohol. Using a national treatment guideline (FMOH, 2012), treatment for confirmed cases were given in collaboration with health post workers of respective health posts.

4. Results

4.1. Trends of malaria incidence in Dembia District, 2001-2015

During the last 15 years (2001-2015) 338,297 febrile cases were examined microscopically or using RDT for malaria infection at the health centers and health posts of Dembia District. Out of these, 41.2% were microscopically/ RDT confirmed. The annual incidence of malaria ranged from 8.1 per 1000 population in 2008 to 57.6 per 1000 population in 2013. After an elevated trend of malaria incidence (>30/1000) between 2001 and 2005, a decrease (<20-10/1000) was observed from 2006 to 2008 and in 2014. A significant (P<0.001) increase in malaria incidence was observed during other years. In general, there was a statistically significant (P <0.001) inter-annual variation of malaria incidences in Dembia District during the period 2001-2005 (Figure 5).

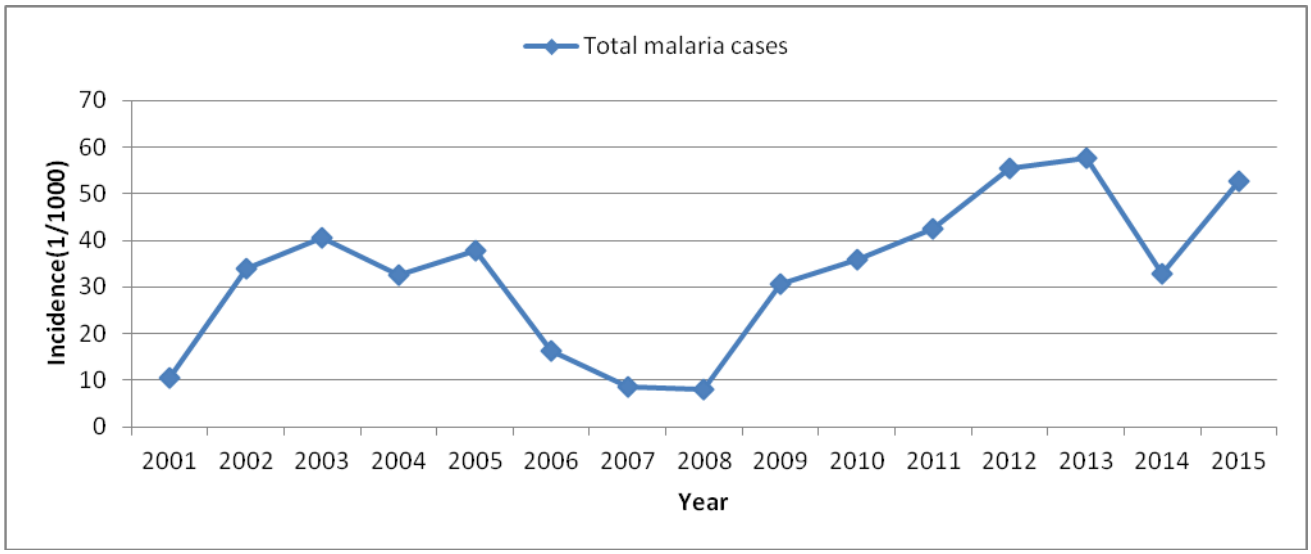


Figure 5. Retrospective data on annual incidence of malaria in Dembia District, 2001-2015.

P. falciparum was the dominant species accounting for 70.1% of malaria infection in Dembia District during the last 15 years. Twenty-seven point six and 2.3% of malaria infection were due to *P. vivax* and mixed infection, respectively. Similar to total malaria cases, incidence of *P. falciparum* and *P. vivax* increased year to year after a notable reduction of malaria in the study area between 2006 and 2008. Like total malaria incidence, inter-annual variation for *P. falciparum* and *P. vivax* was also statistically significant (P<0.001) (Figure 6).

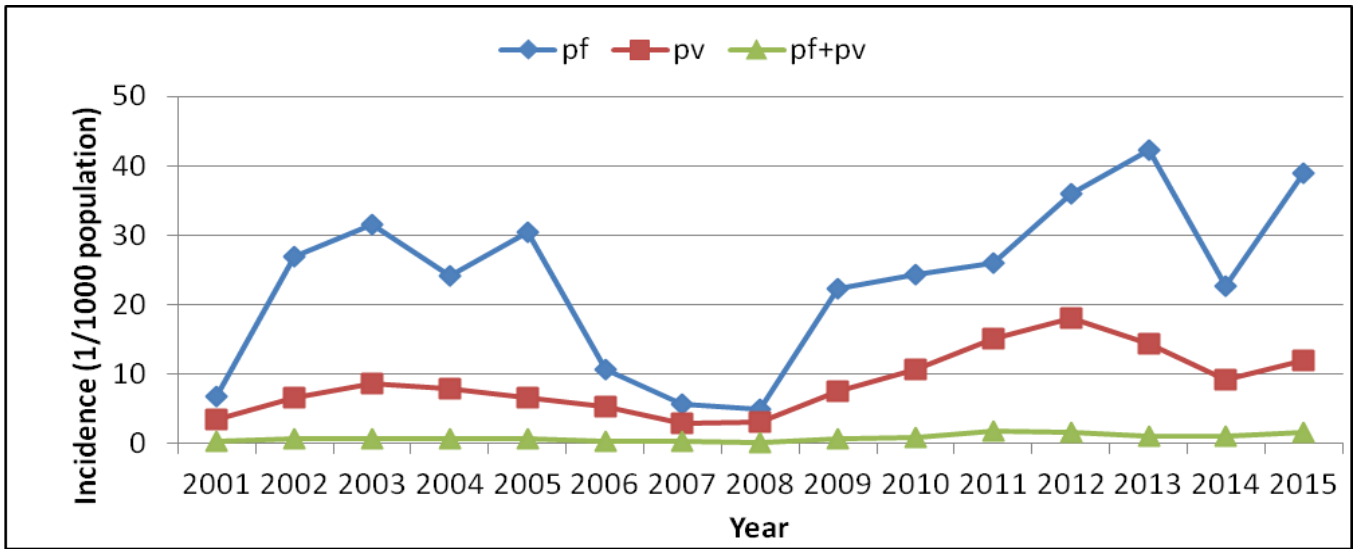


Figure 6. Retrospective data on annual incidences of *P. falciparum*, *P. vivax* and mixed infection in Dembia District, 2001-2015.

Age categories of incidence of total malaria (1/1000 population), *P. falciparum* and *P. vivax* during the last 15 year is presented in Figure 7. The incidence of malaria infection, *P. falciparum* and *P. vivax* were higher in children less than 5 year olds up to the year 2010. However, after 2010 higher incidence of malaria was observed in children 5-14 year olds except in 2014 where the incidence of malaria was higher in adult greater than 15 years old. Similarly, the incidence of

malaria was higher in adult greater than 15 years old than children less than 5 years old in 2014 and 2015. Statistically significant ($P<0.05$) variation of total malaria cases and each species were observed among different age categories (Figure 7).

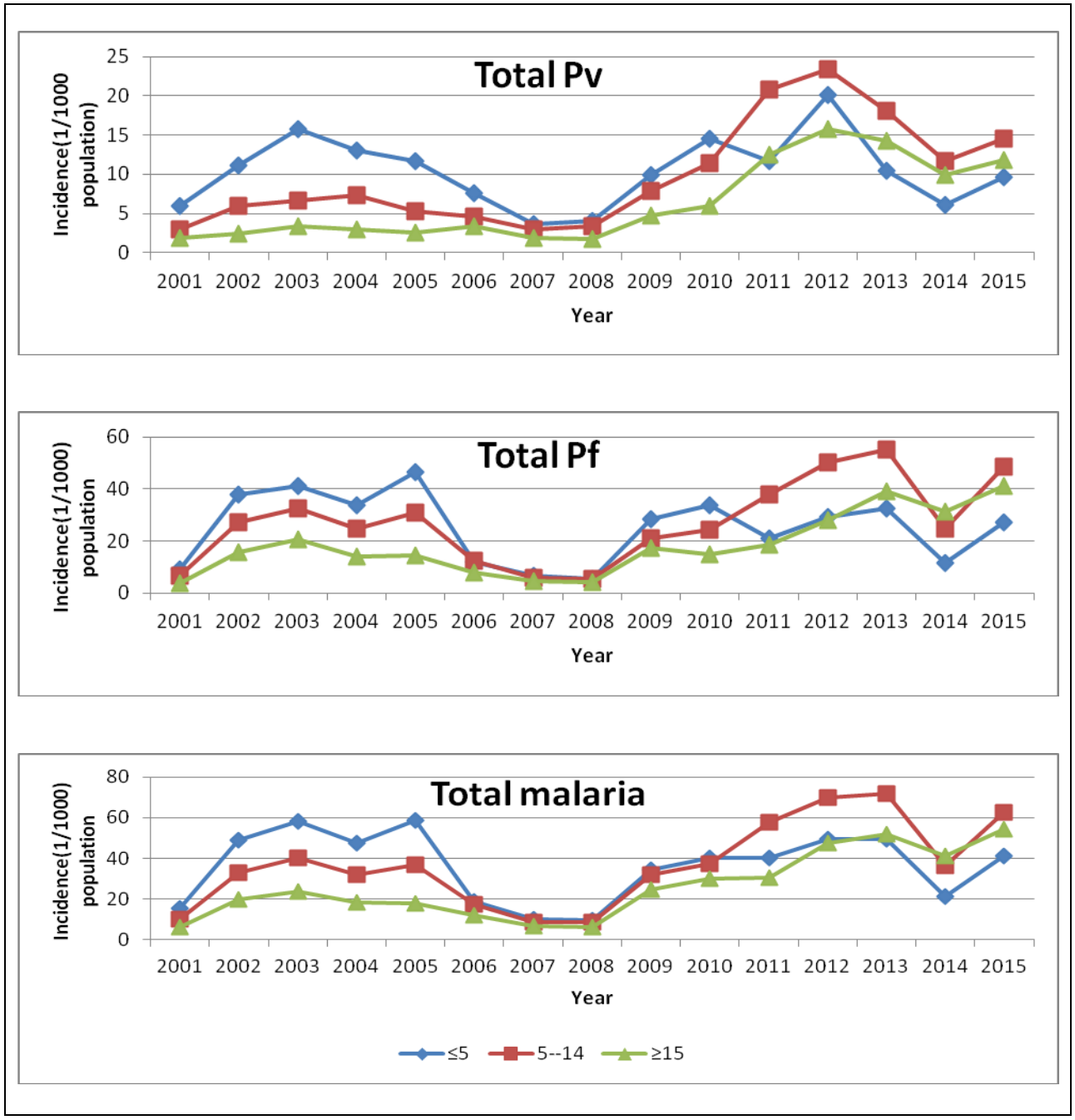


Figure 7. Retrospective data on annual incidence of total malaria, *P. falciparum* and *P. vivax* (1/1000 population) in different age categories recorded in health centers and posts of Dembia District between 2001- 2015.

During the study period malaria was observed in almost every month of the year although there was significant (P<0.01) variation in the incidence of malaria with respect month in the District. The lowest malaria incidence with 0.03 cases per 1000 population and the highest incidence with 13.5 cases per 1000 population were reported in Feb, 2008 and Jun, 2012, respectively. The highest incidences of malaria were reported in Sep-Nov and May-August in most years during the last 15 years. The lowest incidence of malaria was detected in January and February almost in all years (Figure 8).

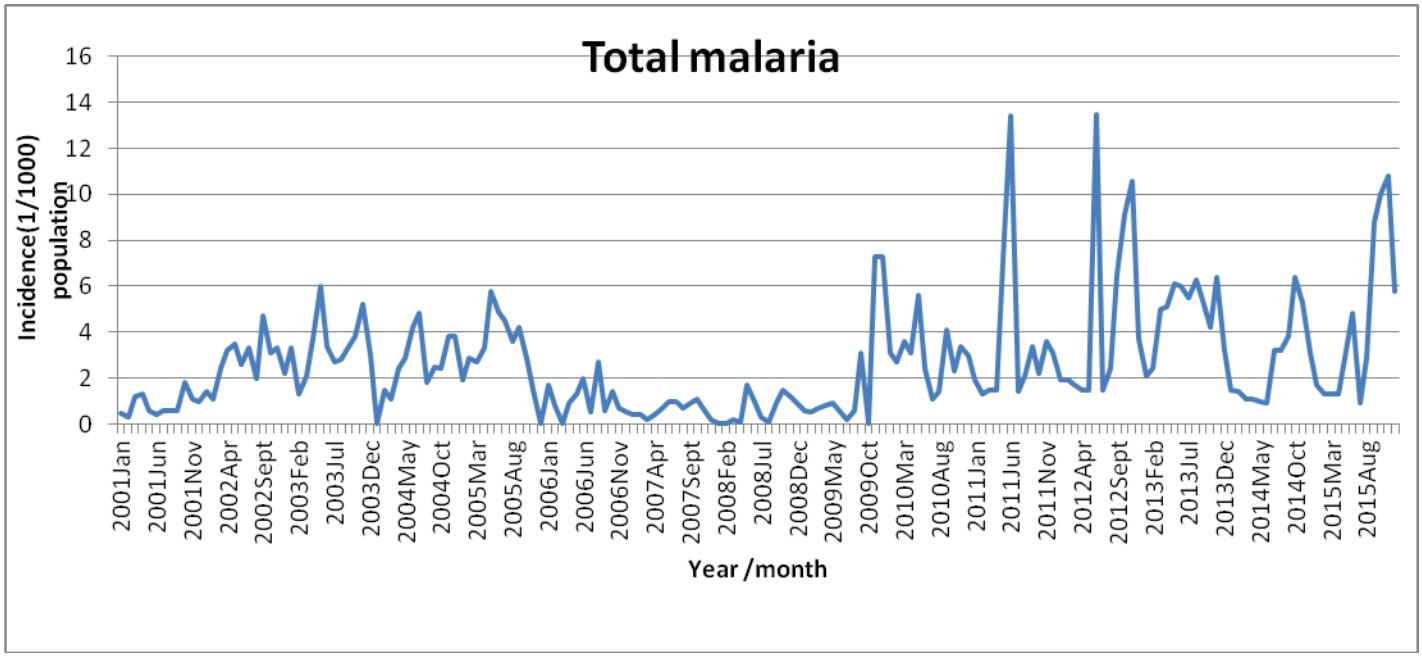


Figure 8. Retrospective data on monthly incidence of malaria in Dembia District, 2001-2015.

4.2. Malaria incidence and insecticidal residual spraying in Dembia District, 2001-2015

Although all the rural (40) and urban (5) localities in Dembia District are malaria endemic, IRS was sprayed only in some selected localities situated in relatively low altitude. DDT was sprayed from the year 2001 to 2009 while deltametrin was used in 2009 and 2010. However, after 2010, propoxur and bendiocarp were sprayed in the District (Dembia District Health Office report, 2015). Proportion of population and localities at risk protected and malaria incidence in Dembia District, during the last 15 years is presented in Figure 9. Almost in all years, IRS operations were carried out at the beginning of September. Although, the proportion of the population at risk protected by IRS rose from 0% (2001 and 2003) to 48.6%, in 2011, more than half of the populations at risk of malaria infection were not protected by IRS. The incidences of malaria increased in most of high coverage years, except in 2003, 2005, 2012 and 2013 where the incidence was increased when the proportion of people and localities protected decreased. Statistically insignificant positive correlation were observed between incidence of malaria and proportion of population (correlation coefficient=0.480; P=0.070) and localities (correlation coefficient=0.361; P=1.86) at risk is protected during the last 15 years. However, statistically significant positive correlation was observed between incidences of monthly malaria and proportion of population (correlation coefficient=0.178; P=0.017) and localities (correlation coefficient=0.169; P=0.029) at risk is protected by IRS during data collection period (Figure 9).

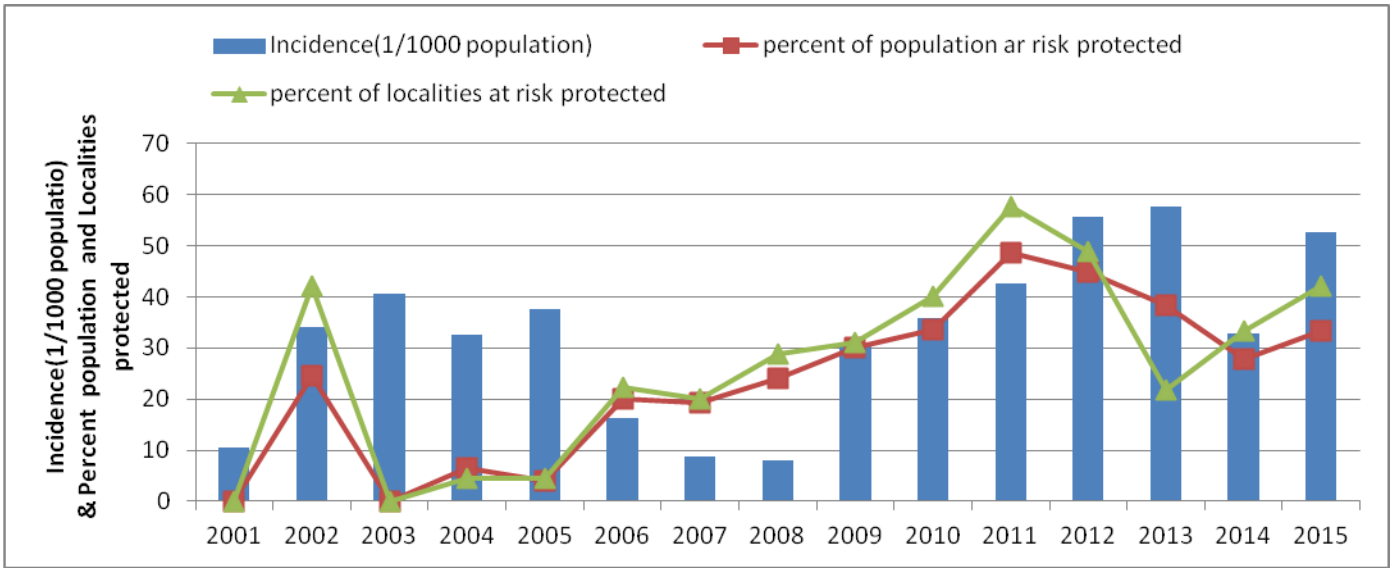


Figure 9. Reterospective data on incidence of malaria, proportion of localities and population at risk protected by IRS in Dembia District, 2001-2015.

4.3. The association of meteorological variables and malaria incidence

Results from correlation analyses of monthly incidence of total malaria cases, *P. falciparum* and *P. vivax* at zero to four months lags of meteorological parameters is summarized in Table 1. To observe the relationship between monthly malaria cases and meteorological measures (*i.e.* maximum temperature, minimum temperature, mean temperature and total rainfall) at zero to four months lag spearman's correlation and linear regression analyses were conducted. At zero months lag, only minimum temperature (P=0.016) and mean temperature (P=0.039) were positively and significantly correlated with total monthly *P. vivax* cases. Statistically insignificant negative linear association was observed at zero months lag monthly minimum temperature with monthly incidence of total malaria and *P. falciparum* during the last 15 years. However, statistically significant positive correlation was observed between total monthly

malaria (P= 0.031) and *P. falciparum* (P= 0.038) cases with average temperature at one month lagged effect. Similarly positive and statistically significant association was also detected between monthly *P. vivax* incidence with maximum temperature (P=0.033) and average temperature (P=0.039) at one month lagged effect. Statistically significant positive associations were also observed between monthly total malaria and *P. falciparum* cases and total monthly rainfall for one to four months lag during the study period (Table 1).

Table 1. Retrospective data on monthly incidence (1/1000 population) of total malaria, P. falciparum and P. vivax, with meteorological variables at zero to four months lag effect in Dembia District, 2001-2015.

Meteorological variables and monthly malaria incidence	**Correlation coefficient at zero month lag**	**Correlation coefficient at one month lag**	**Correlation coefficient At two months lag**	**Correlation coefficient at three months lag**	**Correlation coefficient at four months lag**
Total malaria					
Rainfall	0.048	0.178*	0.190*	0.256**	0.209**
Max Temp	0.062	0.123	0.084	0.040	0.092
Min Temp	-0.011	0.082	0.090	0.051	-0.041
Averg Temp	0.128	0.169*	0.099	0.047	0.027
P.falciparum					
Rainfall	0.032	0.161*	0.221**	0.300**	0.231**
Max Temp	0.044	0.098	0.069	0.003	0.077
Min Temp	-0.057	0.046	0.065	0.034	-0.062
Averg Temp	0.99	0.151*	0.092	0.001	0.013
P.vivax					
Rainfall	-0.055	-0.051	-0.045	-0.048	-0.051
Max Temp	0.071	0.159*	0.113	0.045	0.003
Min Temp	0.227**	0.081	0.015	-0.087	-0.119
Averg Temp	0.154*	0.153*	0.087	-0.015	-0.044

*Correlation is significant at the 0.05 level ** Correlation is significant at the 0.01

To assess the interaction effects of monthly cumulative rainfall and minimum, maximum and average temperature on monthly total malaria, *P. falciparum* and *P. vivax* cases, multiple linear regression analysis was conducted. When all meteorological parameters were entered into a multiple linear regression model, the only significant variable with a monthly malaria incidence at zero months lag effect was average temperature (Beta=0.282; P=0.041; 95% CI: 0.111, 0.553). Monthly cumulative rainfall was the only significant variable with a monthly malaria incidence when entered into a multiple linear regression model together with minimum, maximum and average temperature at one month lagged effect (Beta= 0.007; P=0.000; 95% CI: 0.003, 0.010) and two months lagged effect (Beta=0.008; P=0.000; 95% CI: 0.004, 0.011). Similarly, the monthly average temperature (Beta=0.220; P=0.038; 95% CI:0.012,0.428) and monthly cumulative precipitation (Beta=0.004; P=0.004; 95% CI: 0.001, 0.007) were associated significantly with *P. falciparum* at zero months and one month lagged period respectively, when entered into a multiple linear regression model together with maximum, minimum and average temperatures. Statstically significant association was also observed between *P. vivax* and minimum temperature at zero month lag in multiple linear regression analysis (Table 2).

Table 2. The interaction effect of all meteorological variables on monthly malaria incidence (1/1000 population) of total malaria, *P. falciparum* and *P. vivax,* at zero and one month lag effect in Dembia District, 2001-2015

Monthly malaria incidence	**Meteorological variables**	**Beta (95% CI) zero month lag**	**Beta (95% CI) One month lag**
Total malaria	Rainfall	0.03 (-0.001,0.007)	0.07(0.003,0.010)*
	Max Temp	-0.032(0.228,0.168)	0.087(-0.094,0.268)
	Min Temp	-0.143 (0.376,0.090)	0.028(-0.168,0.224)
	Average Temp	0.282 (0.11,0.553)*	0.218(-0.045,0.481)
P. falciparum	Rainfall	0.002(-0.001,0.005)	0.004(0.001,0.007)*
	Max Temp	-0.046(-0.197,0.105)	0.053(-0.088,0.194)
	Min Temp	-0.166(-0.345,0.0012)	-0.005(-0.157,0.147)
	Average Temp	0.220(0.012,0.428)*	0.150(-0.054,0.355)
P. vivax	Rainfall	-0.04(-0.025,0.017)	0.007(-0.013,0.027)
	Max Temp	-0.145(-1.175,0.886)	0.759(-0.228,1.747)
	Min Temp	1.411(0.190,2.663)*	0.545(-0.523,1.613)
	Average Temp	0.459(0.962,1.880)	0.061(-1.373,1.495)

* Significant at the 0.05 level

4.4. Malaria prevalence and associated risk factors in Dembia District

4.4.1. Characteristics of study participants

The age and sex distribution of the study participants are presented in Table 3. A total of 770 study participants were enrolled in the first survey conducted during December, 2013. Most of the study participants were above 15 years old (56.1%) with a mean (±SD) age of 23.1 (±16.7),

and the range was between one year and 83 years. Above half (52.2%) of the participants were females (Table 3).

Table 3. Age and sex distribution of the study participants in four selected *Kebeles* of Dembia District during the first survey, Dec, 2013.

Age group	**Study localities**	**Total examined n(%)**		**Total n (%)**
		Male	**Female**	
<5	Girargii	3(0.8)	7(1.7)	10(1.3)
	Atkelit-Telifit	18(4.9)	8(2.0)	26(3.4)
	Jangua	10(2.7)	7(1.7)	17(2.2)
	Guramba-Mickael	7(1.9)	8(2.0)	15(1.9)
5-9	Girargii	7(1.9)	10(2.5)	17(2.2)
	Atkelit-Telifit	19(5.2)	17(4.2)	36(4.7)
	Jangua	15(4.1)	9(2.2)	24(3.1)
	Guramba-Mickael	8(2.2)	10(2.5)	18(2.3)
9-14	Girargii	16(4.3)	19(4.7)	35(4.5)
	Atkelit-Telifit	30(8.2)	25(6.2)	55(7.1)
	Jangua	18(4.9)	27(6.7)	45(5.8)
	Guramba-Mickael	24(6.5)	16(4.0)	40(5.2)
≥15	Girargii	46(12.5)	49(12.2)	95(12.3)
	Atkelit-Telifit	43(11.7)	75(18.7)	118(15.3)
	Jangua	53(14.4)	63(15.7)	116(15.1)
	Guramba-Mickael	51(13.9)	52(12.9)	103(13.4)
Total		368(47.8)	402(52.2)	770(100)

n: number of people ; % percentage

The same individuals were followed for the next five visits, these were 756 (May, 2014), 758 (Nov, 2014), 758 (Feb, 2015), 759 (July, 2015), and 767 (October, 2015). The entry and exit of some individuals due to various reasons were the causes for inconsistency of sample size in the different survey periods.

4.4.2. Malaria prevalence in four selected *Kebeles* of Dembia District

From six month surveys (2013-2015), 4568 blood films were examined by two independent laboratory technologists and their diagnostic outcome had a statistically significant level of agreement (Kappa= 0.926; P= 0.000). Out of the total 4568 blood films examined, 339 (7.4%) were infected. Malaria was observed in all of the six surveys conducted in two non-IRS sprayed *Kebeles* (Girargii and Atkelit-Telifit) and two IRS sprayed *Kebeles* (Jangua and Guramba-Mickael). Two hundred fifty two of 339 (74.3%) and 69(20.4%) were due to *P. falciparum* and *P. vivax*, respectively. Three *P.ovale* (0.9%*)* and 15 mixed infections (4.4%) were also detected during the data collection period (Table 4).

Table 4. Prevalence of malaria in four selected *Kebeles* of Dembia District during the six month surveys, 2013-2015.

Study *Kebeles*	**Averg Altit masl***	**Total Examined (%)**	***P. falciparum* (%)**	***P. vivax* (%)**	***P. ovale* (%)**	***P. falciparum* + *P. vivax* (%)**	**Total positive (%)**	**X^2(P.value)**
Girargii	1960	894(19.5	32(3.6)	10(1.1)	0	1(0.1)	43 (4.8)	
Atkelit-Telifit	1872	1410(30.9)	77(5.5)	21(1.5)	2(0.1)	6(0.4)	106(7.5)	23.9(0.021)
Jangua	1810	1209(26.5)	64(5.3)	19(1.6)	0	2(0.2)	85(7.1)	
Guramba-Mickael	1802	1055(23.1)	79(7.5)	19(1.8)	1(0.1)	6(0.2)	105(10.0)	
Total		4568(100)	252(5.5)	69(1.5)	3(0.1)	15(0.3)	339	

*Average altitude of sampled households

In the first survey, which was conducted on Dec, 2013, 49 (6.4%) of 770 individuals examined had malaria. In this survey, *P. falciparum* was the dominant species, accounting for 81.6% (40/49) of the infection. In Dec, 2013 statistically significant variations with higher malaria prevalence, 11.4% was observed in Guramba-Mickael where 57.9 % of study participant's houses were sprayed with Bendiocarp in Sept, 2013. The *Kebele* with the second most malaria prevalence in December, 2013 survey was Atkelit-Telifit, 6.4 % where the IRS was not-sprayed in September 2013. The prevalence of malaria was 3.5 % and 4.5% in low-lying sprayed *Kebele* (Jangua) and at a relatively higher altitude and non-sprayed *Kebele* (Girargii), respectively in

Dec, 2013 survey (Table 5). All age groups were affected by malaria, although the infections were higher in adults age 15 and above (6.9%) and in children age 9 to 14 years old (6.8%) in Dec, 2013 survey. Malaria prevalence was higher in males, (8.2%) than females, (4.7%), although the difference was not statistically significant (Table 5).

Table 5. Prevalence of *Plasmodium* species among *Kebeles*, age groups and sex in Dembia District, December, 2013.

Variables	**Average altitude Masl***	**Total examined**	***P. falciparum* (%)**	***P. vivax* (%)**	**Total (%)**	**X^2 (P.value)**
Kebeles						
Girargii	1960	157	6(3.8)	1(0.6)	7(4.5)	
Atkelit-Telifit	1872	235	13(5.5)	2(0.9)	15(6.4)	12.587(0.05)
Jangua	1810	202	6(3.0)	1(0.5)	7(3.5)	
Guramba-Mickael	1802	176	15(8.5)	5(2.8)	20(11.4)	
Total		770	40(5.2)	9(1.2)	49(6.4)	
Age groups						
<5	-	68	3(4.4)	0	3(4.4)	
5-9	-	95	2(2.1)	2(2.1)	4(4.2)	4.657(0.589)
9-14	-	175	9(5.1)	3(1.7)	12(6.8)	
≥15	-	432	26(6.0)	4(0.9)	30(6.9)	
Total	-	770	40(5.2)	9(1.2)	49(6.4)	
Sex						
Male	-	368	25(6.8)	5(1.4)	30(8.2)	6.0 (0.140)
Female	-	402	15(3.7)	4(1.0)	19(4.7)	
		770	40(5.2)	9(1.2)	49(6.4)	

*Average altitude of sampled households

In the second survey, which was conducted in May, 2014, lowest cases were detected in Gerargii, 3.4% in a relatively higher altitude and non-sprayed *Kebele*. Malaria incidence was 5.1 % in a non-sprayed and mid-level altitude *Kebele* (Atkelit-Telifit). On the other hand, the incidence of malaria was higher in lower altitude *Kebeles*, Guramba-Mickael (8.0%) and Jangua (7.0%) during May, 2014 survey, although the difference was statistically insignificant. Unlike, a December 2013 survey, there was a small difference in the number of malaria cases in Guramba-Mickael and Jangua (Table 6). Malaria was observed in all of the age groups, although statistically insignificant higher malaria cases were detected in children age 9-14 years old (7.6%) and children age 5-9 years old (7.4%). Like in the first survey, *P. falciparum* was the dominant species 73.3 %(33/45). *P. vivax* and mixed infection accounted for 20.0 % (9/45) and 6.7 % (3/45) of the infection during May, 2014 survey. Statistically significant higher malaria cases were observed in males (8.1%) than females (4.1%) (Table 6).

Table 6. Incidence of *Plasmodium* species among *Kebeles*, age groups and sex in Dembia District, May, 2014.

Variables	**Average altitude masl***	**Total examined**	***P. falciparum* (%)**	***P. vivax* (%)**	***Pf+Pv* (%)**	**Total positives (%)**	**X^2(P. value)**
Kebeles							
Girargii	1960	146	4(2.7)	1(0.7)	0	5(3.4)	
Atkelit-Telifit	1872	235	9(3.8)	2(0.9)	1(0.4)	12(5.1)	6.996(0.638)
Jangua	1810	199	11(5.5)	3(1.5)	0	14(7.0)	
Guramba-Mickael	1802	176	9(5.1)	3(1.7)	2(1.1)	14(8.0)	
Total		756	33(4.4)	9(1.2)	3(0.4)	45(6.0)	
Age groups							
<5	-	65	0	1(1.5)	0	1(1.5)	
5-9	-	94	5(5.3)	2(2.1)	0	7(7.4)	5.905(0.749)
9-14	-	172	10(5.8)	2(1.2)	1(0.6)	13(7.6)	
≥15	-	425	18(4.2)	4(0.9)	2(0.5)	24(5.6)	
Total	-	756	33(4.4)	9(1.2)	3(0.4)	45(6.0)	
Sex							
Male	-	361	23(6.4)	4(1.1)	2(0.6)	29(8.1)	7.158(0.05)
Female	-	395	10(2.5)	5(1.3)	1(0.3)	16(4.1)	
Total	-	756	33(4.4)	9(1.2)	3(0.4)	45(6.0)	

*Average altitude of sampled households

In the survey conducted in November 2014, 71 (9.4%) individuals examined were positives for malaria parasites. Like the two surveys, *P. falciparum* was the commonest species, accounting for 67.6% (48/71) of the infection while 19.7% (14/71), 1.4% (1/71) and 11.3% (8/71) were due to *P. vivax*, *P.ovale* and mixed infections, respectively. Like the previous two surveys, malaria was more prevalent in the lower altitude *Kebele*, Guramba-Mickael, 15.9 % which was not sprayed in Sept, 2014. The second highest cases, 9.8 % were detected in non-sprayed mid-altitude *Kebele,* Atkelit-Telifit. Similar to the previous survey, the lowest malaria cases, 5.5 %

were observed in Girargii, a relatively high altitude and non-sprayed *Kebele*. The incidence of malaria in Jangua was 5.9% (12/202) in Nov, 2014 survey, which was lower than Guramba-Mickael and Atkelit-Telifit and slightly higher than Girargii, a relatively high altitude non-sprayed *Kebele.* Jangua was the only sprayed *Kebele* in Sept, 2014. In general, statistically significant difference was observed between localities in Nov, 2014 survey. The incidence of malaria was more in adults age greater than 15 year olds (10.4%), although the difference was not statistically significant. Statistically significant higher malaria incidence was observed in males (11%) than females (7.8%) (Table 7).

Table 7. Incidence of *Plasmodium* species among *Kebeles*, age groups and sex in Dembia District, Nov, 2014

Variables	Averg altit Masl*	Total examined	*P. falciparum* (%)	P. vivax (%)	P. ovale (%)	Pf+Pv (%)	Total positivs (%)	X^2 (P.value)
Kebele								
Girargii	1960	145	5(3.4)	2(1.4)	0	1(0.7)	8(5.5)	
Atkelit-Telifit	1872	235	17(7.2)	4(1.7)	0	2(0.9)	23(9.8)	25.5(0.09)
Jangua	1810	202	4(2.0)	6(3.0)	0	2(1.0)	12(6.0)	
Guramba-Mickael	1802	176	22(12.5)	2(1.1)	1(0.6)	3(1.7)	28(15.9)	
Total		758	48(6.3)	14(1.8)	1(0.1)	8(1.1)	71(9.4)	
Age group								
<5	-	65	2(3.1)	2(3.1)	0	0	4(6.2)	
5-9	-	94	4(4.3)	1(1.1)	0	0	5(5.4)	9.6(0.649)
9-14	-	173	10(5.8)	5(2.9)	0	1(0.6)	16(9.3)	
≥15	-	426	32(7.5)	6(1.4)	1(0.2)	7(1.6)	46(10.7)	
Total	-	758	48(6.3)	14(1.8)	1(0.1)	8(1.1)	71(9.4)	
Sex								
Male	-	364	29(8.0)	4(1.1)	1(0.3)	6(1.6)	40(11.0)	
Female	-	394	19(4.8)	10(2.5)	0	2(0.5)	31(7.8)	8.7(0.05)
Total		758	48(6.3)	14(1.8)	1(0.1)	8(1.1)	71(9.4)	

* Average altitude of sampled households

In the dry season survey, February, 2015, slightly higher malaria incidence was observed in Guramba-Mickael, 5.1% and Jangua, 5.0 %. The incidence of malaria was 3.0 % and 2.1 % in Atkelit-Telifit and in Girargii, respectively (Table 8). Similar to the other surveys malaria was observed in all groups but statistically insignificant higher malaria incidence was observed in children age 5-9 year olds (4.3 %). Statistically insignificant higher malaria incidence was observed in males (4.7 %) than females (3.8 %) (Table 8).

Table 8. Incidence of *Plasmodium* species among *Kebeles*, age groups and sex in Dembia District, Feb, 2015.

Variables	**Average altitude masl***	**Total examined**	***P. falciparum* (%)**	**P. vivax (%)**	**Total positives (%)**	**X^2(P.value)**
Kebeles						
Girargii	1960	146	1(0.7)	2(1.4)	3(2.1)	
Atkelit-Telifit	1872	235	4(1.7)	3(1.3)	7(3.0)	9.099(0.168)
Jangua	1810	202	8(4.0)	2(1.0)	10(5.0)	
Guramba-Mickael	1802	175	3(1.7)	6(3.4)	9(5.1)	
Total		758	16(2.1)	13(1.7)	29(3.8)	
Age groups						
<5	-	65	2(3.1)	0	2(3.1)	
5-9	-	94	3(3.2)	1(1.1)	4(4.3)	
9-14	-	174	3(1.7)	3(1.7)	6(3.4)	2.794(0.834)
≥15	-	425	8(1.9)	9(2.1)	17(4.0)	
Total	-	758	16(2.1)	13(1.7)	29(3.8)	
Sex						
Male	-	364	9(2.5)	8(2.2)	17(4.7)	1.438(0.487)
Female	-	394	7(1.8)	5(1.3)	12(3.1)	
Total		758	16(2.1)	13(1.7)	29(3.8)	

* Average altitude of sampled households

The overall incidence of malaria was 7.4% in the survey conducted in July, 2015 and the highest cases were detected in Jangua, 10.9%. Like the previous four surveys, *P. falciparum* was the dominant species, accounting for 82.1% (46/56) of the infections. The second malaria prevalent *Kebele* in July, 2015 survey was Guramba-Mickael, 8.6 %. Like the previous three surveys, the lowest cases were detected in Gerargii, 4.1%, which had relatively high altitude and non-sprayed. The incidence of malaria in other non-sprayed mid-level altitude *Kebele* (Atkelit-Telifit) was 5.6%. Statistically significant variations of malaria incidence were observed in the four

Kebeles during the summer survey. Statistically insignificant higher malaria incidence was observed in adult age more than 15 year olds. Similar to the other surveys statistically insignificant higher malaria incidence was observed in male study participants (Table 9).

Table 9. Incidence of *Plasmodium* species among *Kebeles*, age groups and sex in Dembia District, July, 2015.

Variables	**Average altitude masl***	**Total examined**	***P. falciparum***	***P.vivax***	***P. falciparum +P. vivax***	**Total positives (%)**	**X^2(P. value)**
Kebeles							
Girargii	1960	146	6(4.1)	0	0	6(4.1)	
Atkelit-Telifit	1872	235	7(3.0)	4(1.7)	2(0.9)	13(5.6)	17.606(0.040)
Jangua	1810	202	19(9.4)	3(1.5)	0	22(10.9)	
Guramba-Mickael	1802	176	14(8.0)	1(0.6)	0	15(8.6)	
Total		759	46(6.1)	8(1.1)	2(0.3)	56(7.4)	
Age groups							
<5	-	65	2(3.1)	0	0	2(3.1)	
5-9	-	94	4(4.3)	1(1.1)	0	5(5.4)	13.676(0.134)
9-14	-	174	9(5.2)	0	2(1.1)	11(6.3)	
≥15	-	426	31(7.3)	7(1.6)	0	38(8.9)	
Total	-	759	46(6.1)	8(1.1)	2(0.3)	56(7.4)	
Sex							
Male	-	364	23(6.3)	5(1.4)	1(0.3)	29(8.0)	0.784(0.853)
Female	-	359	23(5.8)	3(0.8)	1(0.3)	27(6.9)	
Total	-	759	46(6.1)	8(1.1)	2(0.3)	56(7.4)	

* Average altitude of sampled households

The highest malaria incidence, 11.6% was observed in the survey conducted in October, 2015 compared to the other five previous surveys. Malaria was more prevalent in Atkelit-Telifit, 15.3% compared to the other two sprayed *Kebeles,* Guramba-Mickael, 10.8 % and Jangua, 9.9 %. Both of the low altitudes Kebeles (Guramba-Mickael and Jangua) were fully coverd in September, 2015. The incidence of malaria in Gerargii, the other non-sprayed *Kebele* was 9.1 % during this survey. However, the differences were not statistically significant between non-sprayed (Atkelit-Telifit and Gerargii) and sprayed *Kebeles* (Jangua and Guramba-Mickael). In October, 2015 survey, statistically significant higher malaria incidence was observed in adults age 15 years and above (15.4%) and males study participants (15.7%). Similar to the previous surveys *P. falciparum* was the dominant species, accounting for 77.5 % (69/89) of the infections (Table 10).

Table 10. Incidence of *Plasmodium* species among *Kebeles*, age groups and sex in Dembia District, Oct, 2015.

Variables	**Average altitude masl***	**Total examined**	***P. falciparum* (%)**	***P. vivax* (%)**	***P. ovale* (%)**	***P. falciparum* +*P. vivax* (%)**	**Total positives (%)**	**X^2 (P. value)**
Kebeles								
Girargii	1960	154	10(6.5)	4(2.6)	0	0	14(9.1)	
Atkelit-Telifit	1872	235	27(11.5)	6(2.6)	2(0.9)	1(0.4)	36(15.4)	11.1(0.52)
Jangua	1810	202	16(7.9)	4(2.0)	0	0	20(9.9)	
Guramba-Mickael	1802	176	16(9.1)	2(1.1)	0	1(1.1)	19(11.3)	
Total		767	69(9.0)	16(2.1)	2(0.3)	2(0.3)	89(11.6)	
Age groups								
<5	-	68	3(4.4)	1(1.5)	0	0	4(5.9)	
5-9	-	95	4(4.2)	1(1.1)	0	0	5(5.3)	16.1(0.05)
9-14	-	173	13(7.5)	1(0.6)	0	0	14(8.1)	
≥15	-	431	49(11.4)	13(3.0)	2(0.5)	2(0.5)	66(15.4)	
Total	-	767	69(9.0)	16(2.1)	2(0.3)	2(0.3)	89(11.6)	
Sex								
Male	-	367	46(12.5)	8(2.2)	2(0.5)	2(0.5)	58(15.7)	15.6(0.04)
Female	-	400	23(5.8)	8(2.0)	0	0	31(7.8)	
Total	-	767	69(9.0)	16(2.1)	2(0.3)	2(0.3)	89(11.6)	

* Average altitude of sampled households

4.4.3. Malaria risk factors in Dembia District

Univariate and multivariate logistic regression analysis of individual and household risk factors and malaria prevalence obtained from generalized estimating equations model is presented in Table 11. The univariate and multivariate logistic analysis of individual and household risk factors with prevalence of malaria showed non-utilization of LLINs, keeping cattle and other

property outdoors at night and travel history were associated with statistically significant increased risk of malaria infections. However, statistically significant reduced risk of malaria infection was observed in Dec, 2013, May, 2014, Feb, 2015 and July, 2015 surveys compared to October, 2015 survey (Table 11).

On the other hand, univariate logistic regression showed statistically significant increased risk of malaria infection in male study subjects and in the households that did not posses LLINs. Similarly, statistically significant reduced risk of malaria infection was observed in IRS sprayed households by multivariate analysis (Table 11).

Table 11. Individual and household risk factors of malaria in four selected *Kebeles* in Dembia District, Dec, 2013 to Oct, 2015.

Risk factors	**Crude OR (95% CI)**	**P. value**	**Adjusted OR (95% CI)**	**P.value**
Sex				
Male	1.692(1.350,2.120)	0.000	1.208(0.657,2.220)	0.543
Female	1		1	
Age of study subjects				
>5	0.475(0.287,0.788)	0.004	0.729(0.423,1.254)	0.253
5-9	0.625(0.363,1.0771)	0.090	0.729(0.423,1.254)	0.271
9-14	0.777(0.424,1.426)	0.416	0.777(0.419, 1.441)	0.423
≥15	1			
Survey seasons				
Dec, 2013	0.538(0. 373,0.776)	0.001	0.458(0.312,0.672)	0.000
May, 2014	0.870(0.584,1.296)	0.001	0.379(0.250,0.576)	0.000
Nov, 2014	0.843(0.606,1.171)	0.843	0.827(0.597,1.180)	0.295
Feb, 2015	0.315(0.204,0.485)	0.000	0.219(0.139,0.344)	0.000
July. 2015	0.619(0.434,0.882)	0.008	0.586(0.396.0.868)	0.08
Oct, 2015	1		1	
Possession of LLINs				
No LLINs	1.631(1.122,2.369)	0.010	1.047(0.705,1.5557)	0.820
One LLINs	1.411(1.010,1.971)	0.043	0.874(0.604,1.265)	0.475
Two or more	1		1	
Utilization of LLINs				
Not hanged	3.527(1.834,6.785)	0.000	3.343(1.731,6.458)	0.000
Hanged one	2.315(1.870,3.067)	0.000	2.569(1.967,3.357)	0.000
Hanged two	1		1	
IRS spray status				
Yes	0.773(0.577,1.034)	0.083	0.721(0.527,0.968)	0.040
No	1		1	
Keeping cattle or other property at night				
Yes	3.297(2.297,2.0929)	0.000	2.996(2.267,3.960)	0.000
No	1		1	
Travel history				
Yes	4.737(2.593,8.654)	0.000	3.387(1.658,6.921)	0.000
No	1		1	

4.4.4. Possession and utilization of LLINs in different seasons during data collection period, 2013-2015

Longitudinal study on possession and utilization of LLINs was carried out during the six months follow up study conducted between December, 2013 and October 2015 concurrently with parasitological surveys. In the first survey which was conducted in December 2013,196 households were participated in KAPs study. Most of the households were followed for the next five visits, there were 193 (May, 2014), 193 (Nov, 2014), 193(Feb, 2015), 191 (June, 2015), and 196 (October, 2015). In first survey which was conducted in December 2013, 77.5 % (152/196) of the household owned at least one LLIN. In this survey, 40.8% of them possess only one LLIN while 36.7% of them possess two or more LLINs. In the second survey, which was conducted in May, 2014 household owned at least one LLIN was 67.4% (130/193). The number of households' posses two or more LLINs was greatly reduced in May, 2014 (6.7%) survey. Most of the LLINs observed in December, 2013 and May, 2014 surveys were distributed freely for the study population in June, 2010. In June, 2014, free LLINs were distributed in the study population free of charges like the first distribution. In the third survey, which was conducted in Nov, 2014, 96.4% of the study populations possess at least one LLIN. More number of households possesses two or more LLINs in Nov, 2014 (65.3%) survey compared to the previous two surveys. Similar to Nov, 2014 survey, possession of at least one LLIN was 96.4% (186/193) during February, 2015 survey. However, households possess two or more LLINs were lower in February, 2015 survey compared to November, 2014 survey but higher than the others two surveys conducted previously. In the fifth survey, which was conducted in July, 2015 ownership of at least one LLIN was 89.0 % (170/191). Possessions of two or more LLINs were lower in

July, 2015 (27.8%) compared to November, 2014 and February, 2015 surveys. In the last survey, which was conducted in October, 2015, the ownership of at least one LLIN was 89.8%. In general, statistically significant variations in ownership of LLINs were observed in six survey period (X^2=182.958; P=0.000) (Fig, 11). There was statistically significance variation (X^2 =180.686; P=0.000) in utilization of LLINs in different seasons of the survey period. The highest (74.7%) and the lowest (38.2%) LLINs utilization was observed during July, 2015 survey in the rainy season and February, 2015 survey during dry season respectively (Fig 10).

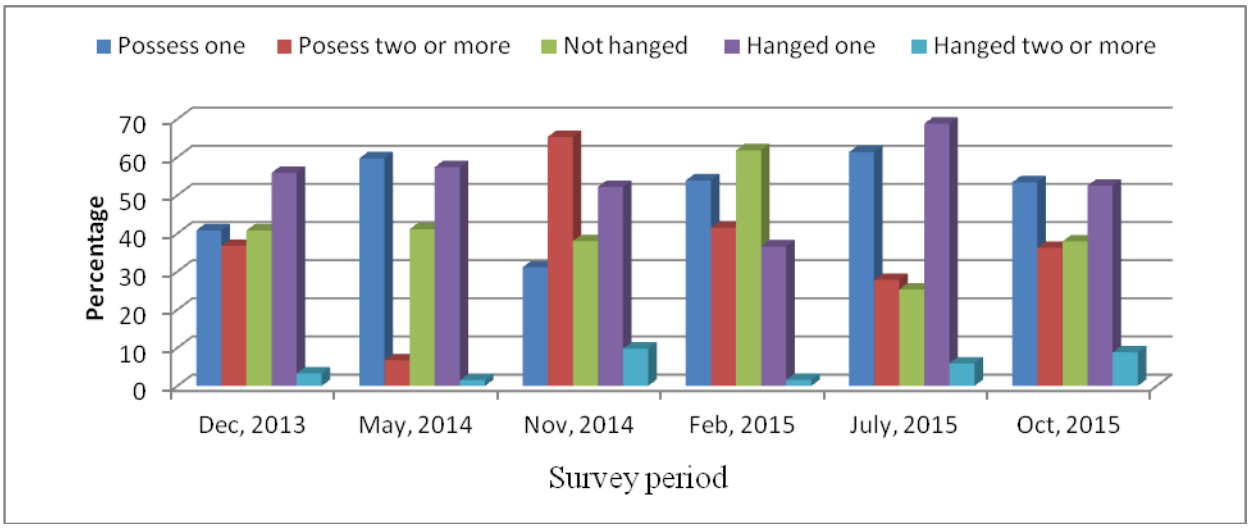


Figure 10. Possession and utilization of LLINs in four selected *kebeles* of Dembia District during data collection period, 2013-2015

4.4.5. Possession and utilization of LLINs in four K*ebeles* of Dembia District during data collection period, 2013-2015

Of the surveyed households, self reported average ownership for at least one LLIN during data collection period was 92.6% where 22.5%, 52.8% and 24.7% self reported possession of one, two and three or more LLINs respectively. The average LLINs ownership among self reported respondent was 2.1 LLINs *per* household. The difference in self reported ownership of LLINs in four *Kebeles* of the District was not statistically significant (X^2 =2.758;0.431). On the other hand, the observed ownership of LLINs in four *Kebeles* of the District was 85.2% during data collection period. Unlike self reported ownership, the majority of LLINs owned households had one (56.2%) and two (38.6%), LLIN irrespective of their household size. The observed mean LLINs ownership was 1.4 per household during data collection period. Statistically significant variations between *Kebeles* were not observed in ownership of LLINs during data collection period (X^2=4.49; 0.212). Of the households that owned LLINs, 59% showed at least one properly hanged LLINs during data collection period. From those properly hanged LLINs, 90.7 % and 9.3 % households hanged one and two LLINs, respectively. There was statistically significant variations in utilization (X^2 =27.885; P=0.001) of LLINs in four selected *Kebeles* of Dembia District during data collection period. Highest utilization (64.7%) and lowest utilization (48.4%) was observed in Atkelit-Telifit and Girargii, respectively. Respondents were interviewed about the family member slept under hanged LLINs the night prior to the survey day. Majority of respondents (55.1%) replied younger children and mother slept under the hanged LLINs the previous night of the survey day. Household head and spouse (23.5%) was the second most frequently mentioned household member that slept in hanged LLINs during data

collection period. Most households that hanged two LLINs gave priority for older children for the second hanged LLINs. The main reasons respondents cited for non- utilization of LLINs during the survey periods were: The LLINs are not available in their house (30.6%), absence of mosquitoes bite (16.3%), the LLINs are worn out (10.2%), absence of malaria in the area (9.2%), presence of biting bugs in the LLINs (8.2%) (Table 12).

Table 12. Average ownership, utilization and reason for non-utilizing LLINs in four selected *Kebeles* of Dembia District during data collection period, 2013-2015.

Variables	Atkelit-Telifit Frequency (%) n=59	Girargii Frequency (%) n=39	Jangua Frequency (%) n=55	Guramba-Mickael Frequency (%) n= 43	Total Frequency (%) n=196	X^2;P-value
Ownership of LLINs (self reported)						
Yes	55 (93.2)	36 (92.3)	52 (94.5)	39 (90.7)	181(92.3)	
No	4 (6.8)	3 (7.7)	3 (5.5)	4 (9.3)	15 (7.7)	2.758;0.431
Ownership of LLINs (confirmed by observation)						
Yes	51 (86.4)	31 (79.5)	49 (89.1)	35 (81.4)	167 (85.2)	4.49;0.212
No	8 (13.6)	8 (20.5)	6 (10.9)	8 (18.6)	29 (13.9)	
Number of LLINs in the household (self reported)	n=55	n=36	n=52	n=35	n=182	
One	13 (23.9)	10 (27.8)	10 (19.2)	8 (20.5)	41(22.5)	94.829;0.000
Two	20 (35.8)	18 (50.0)	32 (61.6)	26 (66.7)	96(52.8)	
Three or more	22 (40.3)	8 (22.2)	10 (19.2)	5 (12.8)	45(24.7)	
Number of LLINs in the household (observed)	n= 51	n= 31	n= 49	n= 35	n= 166	
One	29 (56.7)	20 (64.5)	24 (49.0)	22 (62.9)	95 (57.2)	42.141;0.000
Two	19 (37.1)	10 (32.3)	22 (44.9)	13 (37.1)	64(38.6)	
Three or more	3 (6.2)	1 (3.2)	3 (6.1)	0	7 (4.2)	
Households showing properly hanged LLINs	n=51	n=31	n=49	n=35	n=166	
Not hanged	18 (35.3)	16 (51.6)	21 (42.8)	13 (37.2)	68(41.0)	26.089;0.02
Hanged one	31 (60.8)	14 (45.2)	24 (49.0)	20 (57.1)	89(53.6)	
Hanged two	2 (3.9)	1 (3.2)	4 (8.2)	2 (5.7)	9 (5.4)	
Which family member slept under hanged LLINs last night	n=33	n=15	n=28	n=22	n=98	
Older children (8-14 years old)	3 (9.1)	2 (13.3)	3 (10.7)	2 (9.1)	10 (10.2)	34.321;0.003
Household head and spouse	8 (24.2)	3 (20.0)	7 (25.0)	5 (22.7)	23 (23.5)	
Younger children and mothers (0-7years old)	19 (57.6)	9(60.0)	15 (53.6)	11 (50)	54 (55.1)	
Adult household member(>15 years old)	2 (6.1)	1 (6.7)	2 (7.1)	2 (9.1)	7 (7.1)	
Outdoor household head or adult	1 (3.1)	0	1 (3.6)	2 (9.1)	4 (4.1)	
Reason for non utilizing LLINs	n=25	n=24	n= 27	n=22	n=98	
No malaria	2 (8)	2(8.3)	3 (11.1)	2 (9.1)	9 (9.2)	
No mosquito	4 (16)	3 (12.5)	5 (18.5)	4 (18.2)	16 (16.3)	62.361;0.000
No bed	2 (8)	2 (8.3)	2 (7.4)	1 (4.5)	7 (7.1)	
Suffocation/too hot	2 (8)	2 (8.3)	1 (3.7)	1 (4.5)	6 (6.1)	
Worn out	3 (12)	3 (12.5)	2 (7.4)	2 (9.1)	10 (10.2)	
Presence of biting bugs in LLINs	2 (8)	3 (12.5)	2 (7.4)	1 (4.5)	8 (8.2)	
IRS was sprayed	0	0	3 (11.1)	1 (4.5)	4 (4.1)	
Used in matters at night	2 (8)	1 (4.2)	3 (11.1)	2 (9.1)	8 (8.2)	
No LLINs	8 (32)	8 (33.3)	6 (22.2)	8 (36.4)	30 (30.6)	

4.5. Entomological studies

4.5.1. Larval mosquito surveys

A total of 16,094 3rd and 4th instar anopheline larvae were collected in different larval habitat in four *Kebeles* of Dembia District during 14 months survey which was conducted between Dec, 2013 and Dec, 2015 and examined microscopically for species identification. Eight species of anopheline larvae were identified during the study period among which *An. arabiensis* (39.4%*)* and *An. christyi* (34.2%) were the major species. *An. pharoensis* is the third abundant species which accounted for 9.1% of the collection. The other five species *An. garnhami* (4.5%), *An. pretoriensis* (3.7%), *An. funestus s.l.* (4.2%), *An. theileri* (3.2%) and *An. coustani* (1.7%) comprised only 17.3% of the collection. More number of anopheline larvae was collected in Atekelit-Telifit than relatively low lying sprayed *Kebeles*, Guramba-Mickael and Jangua during data collection period. Mosquito larvae were less abundant in Gerargii, a relatively high altitude and non-sprayed *Kebele* (Table 13).

Table 13. Total numbers of late instar (III and IV) anopheline mosquito larvae collected in different larval habitats in four *Kebeles* of Dembia District between Dec, 2013 and Dec, 2015.

Locality	***An. christyi***	***An. garnhami***	***An. arabiensis***	***An. pretoriensis***	***An. funestus s.l.***	***An. pharoensis***	***An. theileri***	***An. coustani***	**Total**
Girargii	1049	139	1315	286	125	284	185	0	3383(21)
Atkelit-Telifit	1785	241	1831	312	168	357	265	0	4959(30.8)
Jangua	1750	234	1615	0	193	359	58	149	4358(27.1)
Guramba-Mickael	927	109	1580	0	168	472	9	131	3396(21.1)
Total	5511(34.2)	723(4.5)	6341(39.4)	598(3.7)	654(4.2)	1472(9.1)	517(3.2)	280(1.7)	16,096(100)

Fourteen months surveys of the mean larvae density of the primary (*An. arabiensis*) and the secondary (*An. pharoensis* and *An. funestus s.l.*) vectors of malaria in the four *Kebeles* and monthly rainfall is presented in Figure 11. Mean larvae density of *An. arabiensis* start to rise in all surveyed localities when the amount of monthly rainfall increases. The difference was statistically significant between the dry season and the wet season in the low altitude IRS spray *Kebeles* and non-sprayed mid and high altitude *Kebeles* (ANOVA; F=11.938, df =13; P=0.000). Mean larval density of *An. arabiensis* was higher in low-lying sprayed *Kebeles* (Jangua and Guramba-Mickael) than non-sprayed high (Girargii) and mid-level (Atkelit-Telifit) altitude

Kebeles in the surveys conducted between July and August, although the difference was not statistically significant (Turkey Honestly Significant Test >0.05).

The lowest larval density in the two IRS sprayed *Kebeles*, Guramba-Mickael and Jangua were observed between September, 2015 and December, 2015 surveys, immediately after IRS sprayed in the two *Kebeles* in Sept, 2015. Statistically significant positive correlation was observed between mean larval densities of *An. arabiensis* and monthly rainfall in all *Kebeles* (correlation coefficient=0.738; P=0.000). *An. pharoensis* and *An. funestus s.1*. were collected more in dry season than wet season. *An. pharoensis* mean densities were higher in Guramba-Mickael in most surveyed months than the other three surveyed localities during the study period. Statistically significant negative association was observed between monthly mean larval densities of *An. pharoensis* (correlation coefficient=-0.312; P=0.019) and *An. funestus s.1*. (Correlation coefficient=-0.334; P=0.012) with monthly rainfall during the study period.

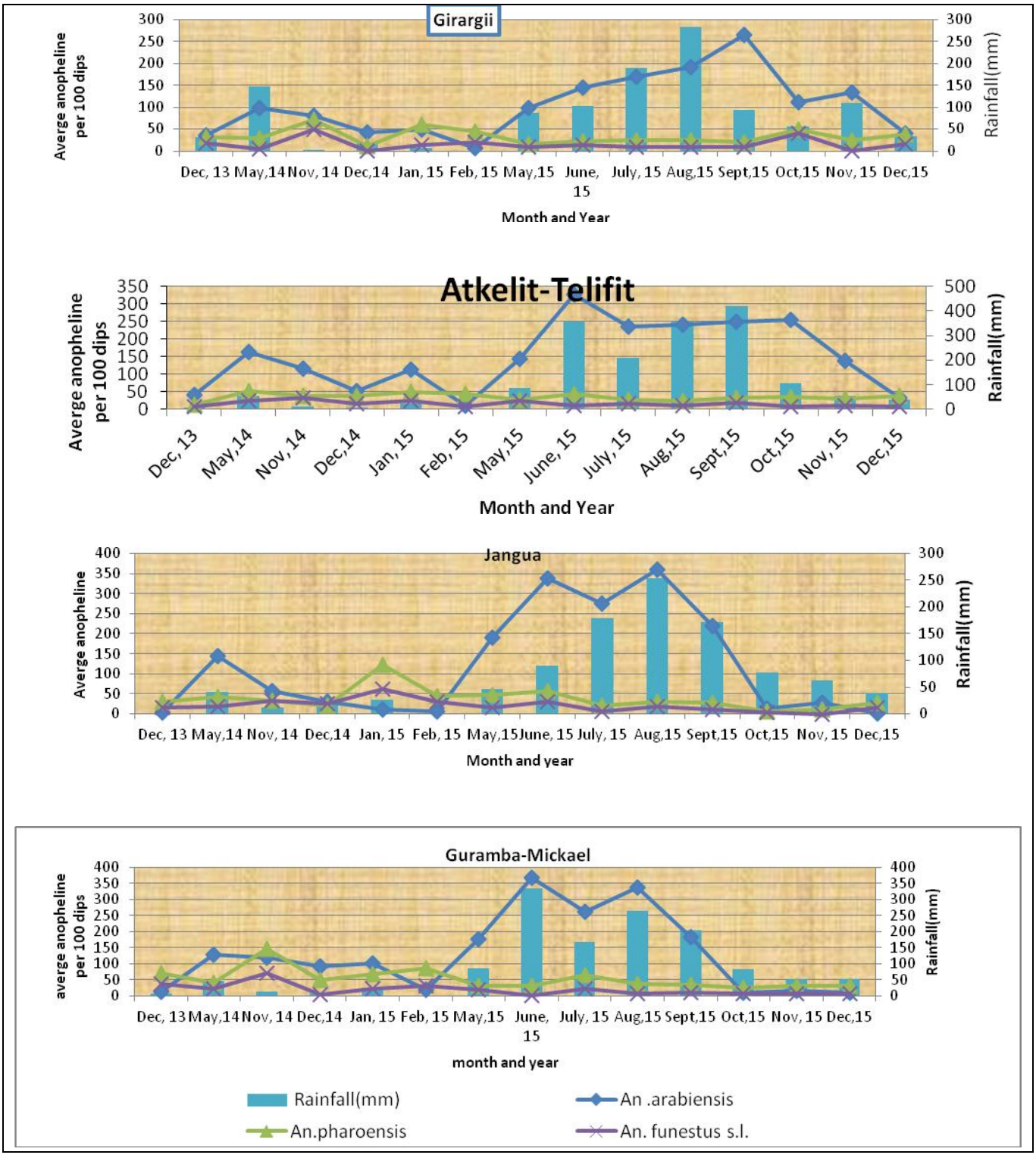
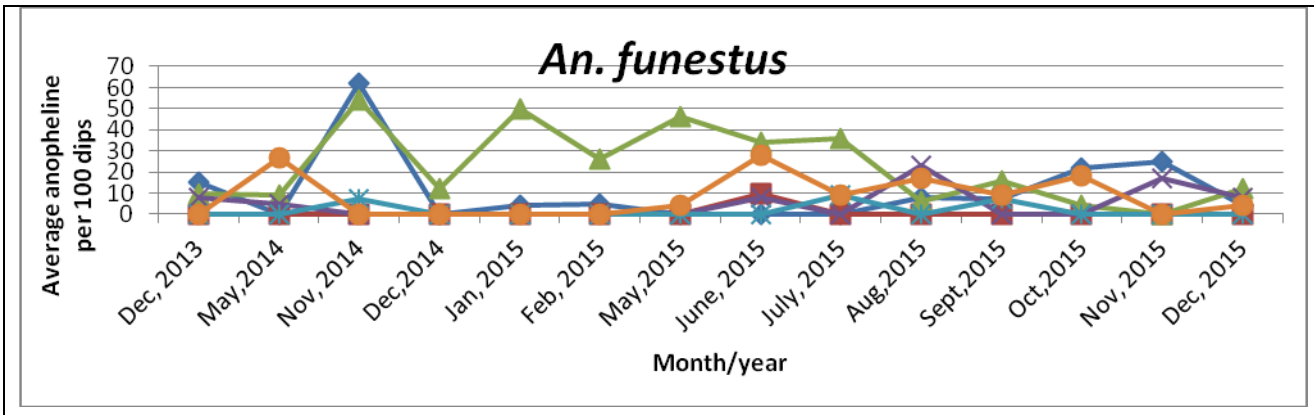
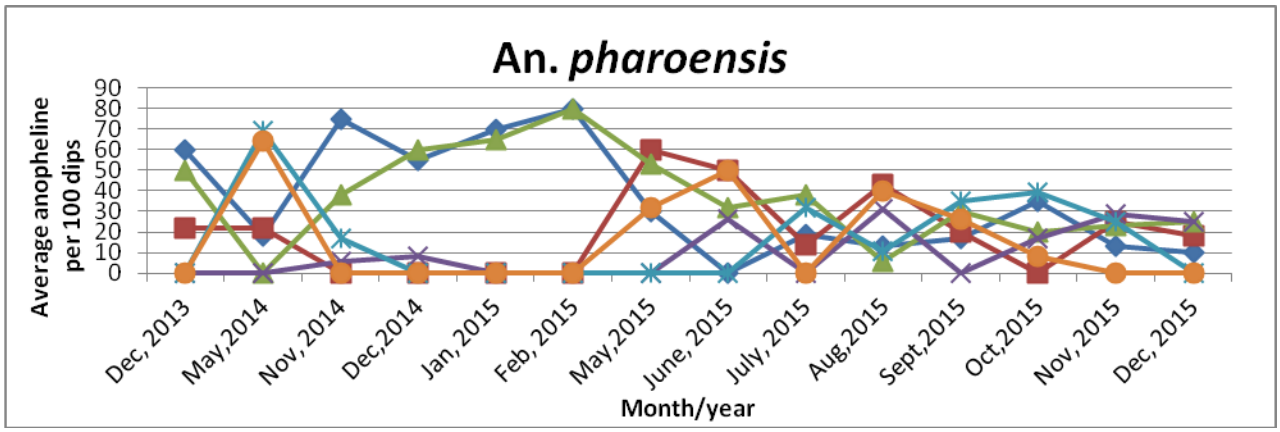
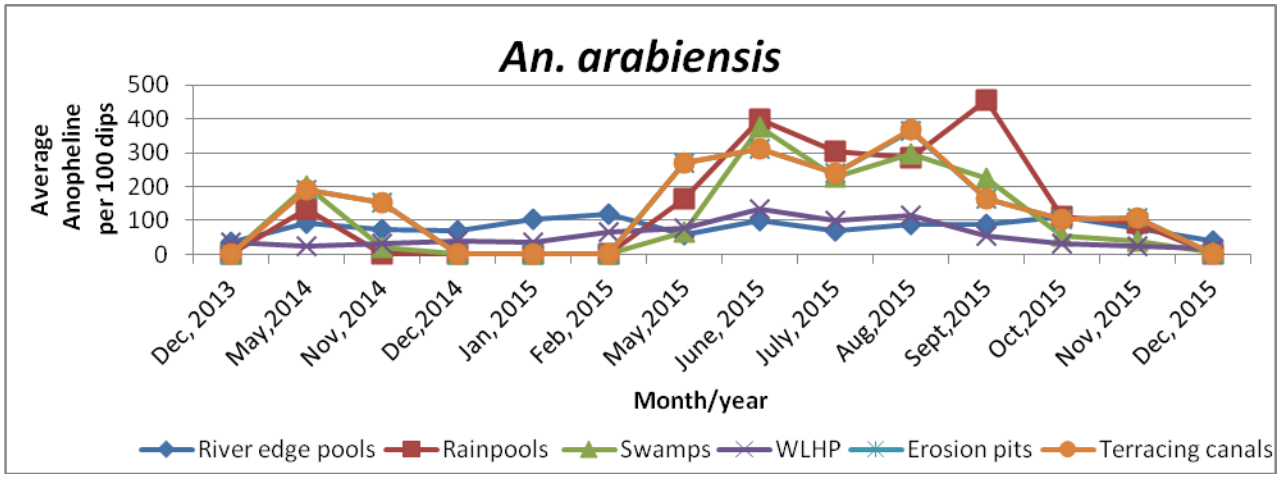


Figure 11. Monthly larval density of the primary and the secondary vectors of malaria and rainfall in four *Kebeles* of Dembia District during data collection period, 2013-2015.

Figure 12 shows the major breeding habitats of the primary and secondary vectors of `malaria in four selected *Kebeles* of Dembia District. *An. arabiensis* larvae were collected most abundantly from rain pools. However, *An. arabiensis* larvae were also collected from permanent breeding habitats such as swamps and stream/river edge pools during the study period. During the dry season, stream / river edge pools and water leaked from hand pipes were positive for *An. arabiensis* larvae. The stream / river edge pools surveyed during the dry season was created due to blockage of the stream / river at various points because of irrigation activities. Water leaked from hand pipes also support small number of *An. arabiensis* during the dry season. Substantial numbers of *An. arabiensis* larvae were collected in terracing canals during the rainy season which was made for erosion protection during the rainy season. Although found in all habitat, *An. pharoensis* and *An. funestus s.1*. were collected more in swamps and river edge pools during the dry season (Figure 12).







WHLP; water leaked from handpipe

Figure 12. Major breeding habitats of the primary and the secondary vector of malaria in Dembia District during study period, 2013-2015.

4.5.2. Adult mosquito surveys

A total of 1947 adult female *Anopheles* mosquitoes corresponding to seven species were collected during the 12 month sampling period in four selected *Kebeles* of Dembia District. The non-sprayed relatively higher altitude (Gerargii) and mid-altitude *(*Atkelit-Telifit) *Kebeles* were the localities showing highest anopheline diversity with seven species each while in the sprayed and lower altitude *Kebeles,* Jangua and Guramba-Mickael, five species were detected (Table 14). Since all of the 62 *An. gambiae s.l.* were found to be *An. arabiensis,* by species-specific PCR, the *An. gambiae* s.l. collected in these study were considered as *An. arabiensis*. *An. arabiensis* was the most abundant species (48.7%) followed by *An. christyi* (18.6%) and *An. pharoensis* (13.5%), *An. funestus s.l.* (7.6%), *An. garnhami* (5.3%), *An. pretoriensis* (3.4%) and *An. theileri* (2.8%).

Similar to the larval survey, more numbers of adult anophelines were collected in mid-level altitude and non-sprayed *Kebele,* Atkelit-Telifit (29.2%) than low-lying sprayed *Kebeles* (Jangua and Guramba-Mickael). The lowest numbers of adult anophelines were collected in a relatively higher altitude and non-sprayed *Kebele* (Girargii), during the study period. Although there were differences in the number of anophelines collected in four surveyed *Kebeles,* the difference was not statistically significant (ANOVA; F=0.564, df=3; P=0.641) when the number of female anopheline collected by all methods were pooled during the study period. *Anopheles arabiensis* was collected more indoors (85.7%) than outdoors (14.3%). However, *An. pharoensis* were collected only indoors during data collection period. *An. pharoensis* was not collected outdoors in artificial pit sheleter. Similar to the other species most *An. pharoensis* and *An. funestus s.l.*

collected indoors from animal sheleters. Compared to *An. arabiensis* and *An. pharoensis* outdoor pit sheler collection of *An. funestus* (28.4%) were higher (Table 14).

Table 14. Number of adult female *Anopheles* collected indoors and outdoors during the study period in four *Kebeles* of Dembia District, 2013-2015.

***Anopheles* species**	**Girargii (%)**		**Atkelit-Telifit (%)**		**Jangua (%)**		**Guramba-Mickael (%)**		**Total**
	Indoors	**Outdoors**	**Indoors**	**Outdoors**	**Indoors**	**Outdoors**	**Indoors**	**Outdoors**	
An. arabiensis	178(18.8)	22(2.3)	228(24.1)	35(3.7)	203(21.4)	42(4.4)	203(21.4)	37(3.9)	948(48.7)
An. pharoensis	48(18.3)	0	70(26.6)	0	65(24.7)	0	80(30.4)	0	263(13.5)
An. funestus s.l.	22(14.9)	8(5.4)	27(18.2)	10(6.8)	28(18.9)	11(7.4)	29(19.6)	13(8.8)	148(7.6)
An. christyi	68(18.8)	26(7.2)	76(21)	27(7.5)	49(13.5)	24(6.6)	66(18.2)	26(7.2)	362(18.6)
An. garnhami	15(14.4)	5(4.8)	20(19.2)	9(8.7)	18(17.3)	10(9.6)	18(17.3)	9(8.7)	104(5.3)
An. pretoriensis	27(40.3)	4(6.0)	29(43.3)	7(10.4)	0	0	0	0	67(3.4)
An. theileri	20(36.4)	4(7.3)	26(47.3)	5(9.1)	0	0	0	0	55(2.8)
Total	378(19.4)	69(3.5)	476(24.4)	93(4.8)	363(18.6)	87(4.5)	396(20.3)	85(4.4)	1947(100)

The monthly host seeking density of *An. arabiensis* is shown in Figure 13. Seasonal and spatial variations in the number of host seeking *An. arabiensis* were observed in IRS sprayed *Kebeles* (Jangua and Guramba-Mickael) and non-sprayed *Kebeles (*Atkelit-Telifit and Girargii). Host seeking density starts to increase in May, 2015 and the highest densities with 4.1 /CDC-LT/night were observed in non-sprayed mid-altitude *Kebele* (Atkelit-Telifit), in Sept, 2015. The host seeking densities were higher in sprayed *Kebeles* (Jangua and Guramba-Mickael) than non-sprayed *Kebeles* (Atkelit-Telifit and Girargii) during May to August surveys. However, the host seeking density was higher in non-sprayed *Kebele* (Atkelit-Telifit and Girargii) than sprayed *Kebeles* (Jangua and Guramba-Mickael) between September 2015 and Nov, 2015 immediately after IRS was sprayed in September, 2015 (Figure 13). The lowest host seeking density with 0.1/CDC-LT/night/house was collected in all surveyed *Kebeles* except Guramba-Mickael in Nov, 2014 survey. CDC light trap catch showed, the presence of *An. arabiensis* in all surveyed months with highest during the wet season and lowest during the dry season (Figure 13). Statistically significant variations were observed between the dry and wet seasons in all study *Kebeles* (ANOVA; F=12.605, df=11; P=0.000).

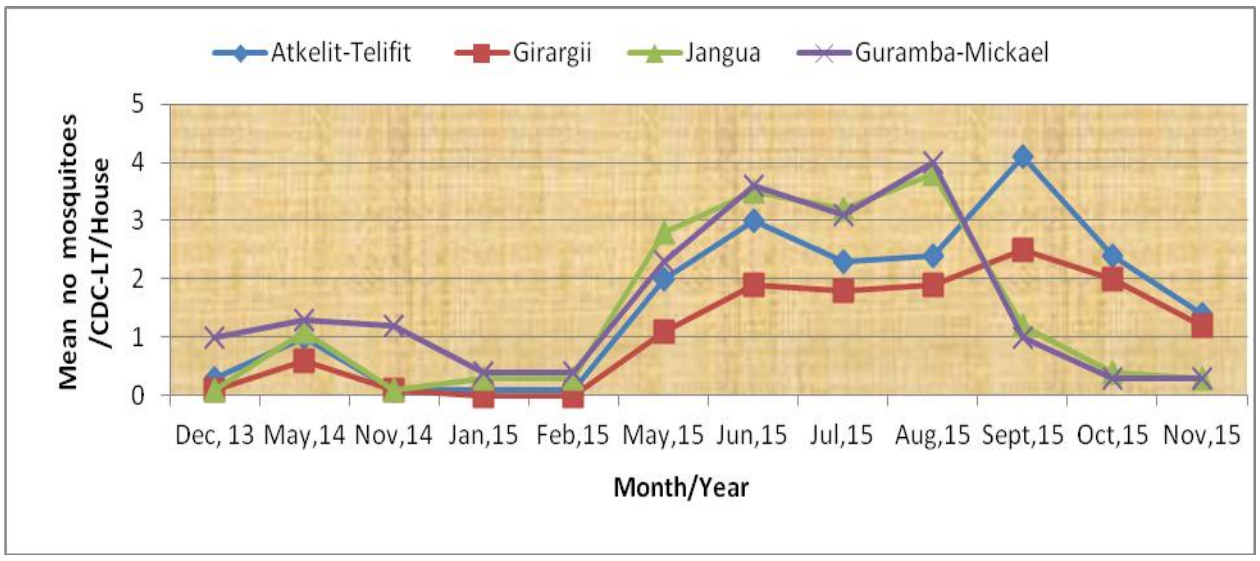


Figure 13. Monthly host seeking density of *An. arabiensis* in four *Kebeles* of Dembia District, North west Ethiopia, 2013-2015.

Monthly indoor resting density of *An. arabiensis* was also varied significantly (ANOVA; F=5.307, df=11; P=0.000) with a maximum of 2.7/PSC/day/house observed in Atkelit-Telifit, non-sprayed mid altitude *Kebele*, during September, 2015 survey. Like the host seeking density, indoor resting density of *An. arabiensis* was higher in relatively low-lying and sprayed *Kebeles* (Jangua and Guramba-Mickael) than non-sprayed higher altitude (Girargii) and mid-level altitude (Atkelit-Telifit) *Kebeles* during the wet season. However, after IRS was done in September, highest indoor resting density was observed in non-sprayed mid-level (Atkelit-Telifit) and high (Girargii) altitude *Kebeles*, although the difference was not statistically significant (Figure 14).

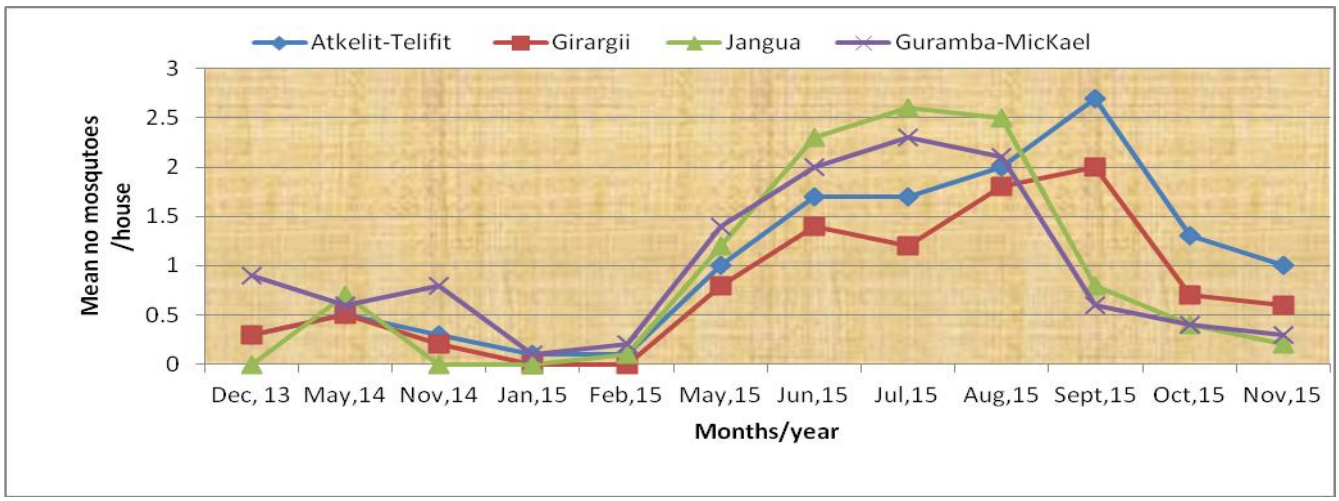


Figure 14. Monthly mean indoor resting densities of *An. arabiensis* in four *Kebeles* of Dembia District, North west Ethiopia, 2013-2015.

The higest malaria prevalence in Guramba-Mickael, in Dec, 2013 (11.6%) and Nov, 2014 (15.9%) corresponding with the highest indoor densities of *An. arabiensis* in Dec, 2013 and Nov, 2014 of that *Kebele* when 57.3% and none of sampled houses were sprayed in September of respective years. Similarly the incidence of malaria in Jangua (10.9%) was highest in July, 2015 reflecting higher indoor densities of *An.arabiensis* in June and July, 2015 in that *Kebele*. The second highest incidence was decteced in Guramba-Mickael (8.5%) similar to the second highest indoor densities of *An. arabiensis* (Figure 15). However, highest incidence of malaria (15.3%) was dectected in non-sprayed mid-level altitude *Kebele* (Atkelit-Telifit) in October, 2015 when higher indoor densities of *An. arabiensis* were observed in that *Kebele* in Sept, 2015. Although indoor densities were decresed after spraying of IRS in low altitude sprayed *Kebeles* (Guramba-Mickael and Jangua), compared to high-altitude *Kebele (*Girargii), malaria incidence was slightly

lower in relatively high altitude *Kebele*, Girargii (9.1%) than low altitude *Kebeles*, Guramba-Mickael (10.8%) and Jangua (9.9%) in October, 2015 (Figure 15).

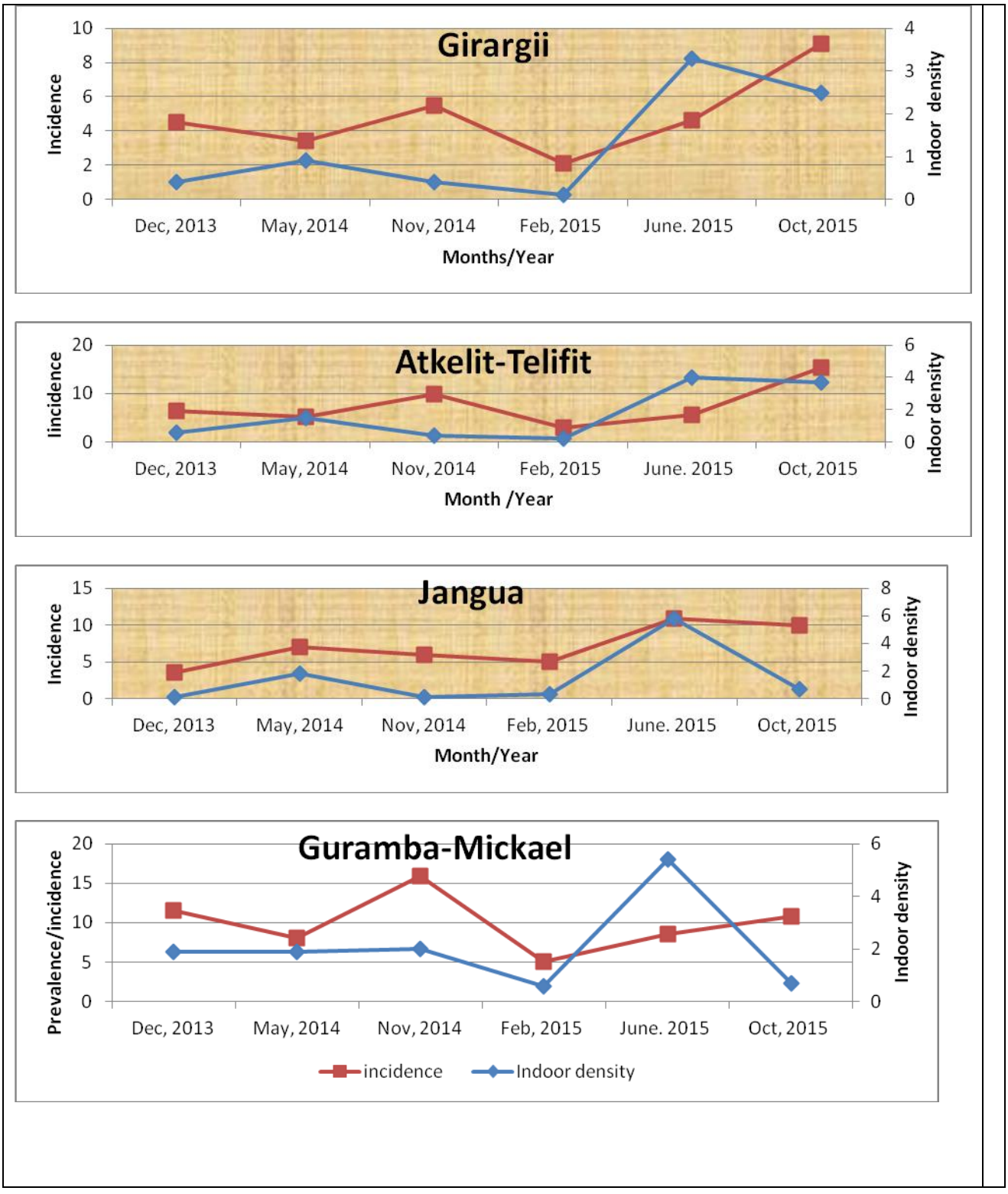


Figure 15. Monthly indoor densities of *An. arabiensis* and incidence of malaria in four *Kebeles* of Dembia District, 2013-2015.

Monthly variations were also observed in outdoor resting densities of *An. arabiensis* **(**ANOVA; F=5.307, df=11; P=0.000) with a maximum of 2/pit shelter/day in Jangua and Guramba-Mickael and a minimum of 0/pit shelter/day in Girargii during August and January 2015 survey, respectively. Unlike host seeking and indoor resting densities, outdoor resting densities in low lying sprayed *Kebeles* were slightly higher in most months except in mid-level altitude *Kebele*, Atkelit-Telifit where the outdoor density was higher between September and December, 2015 survey after the IRS was sprayed in Guramba-Mickael and Jangua in September, 2015 (Figure 16).

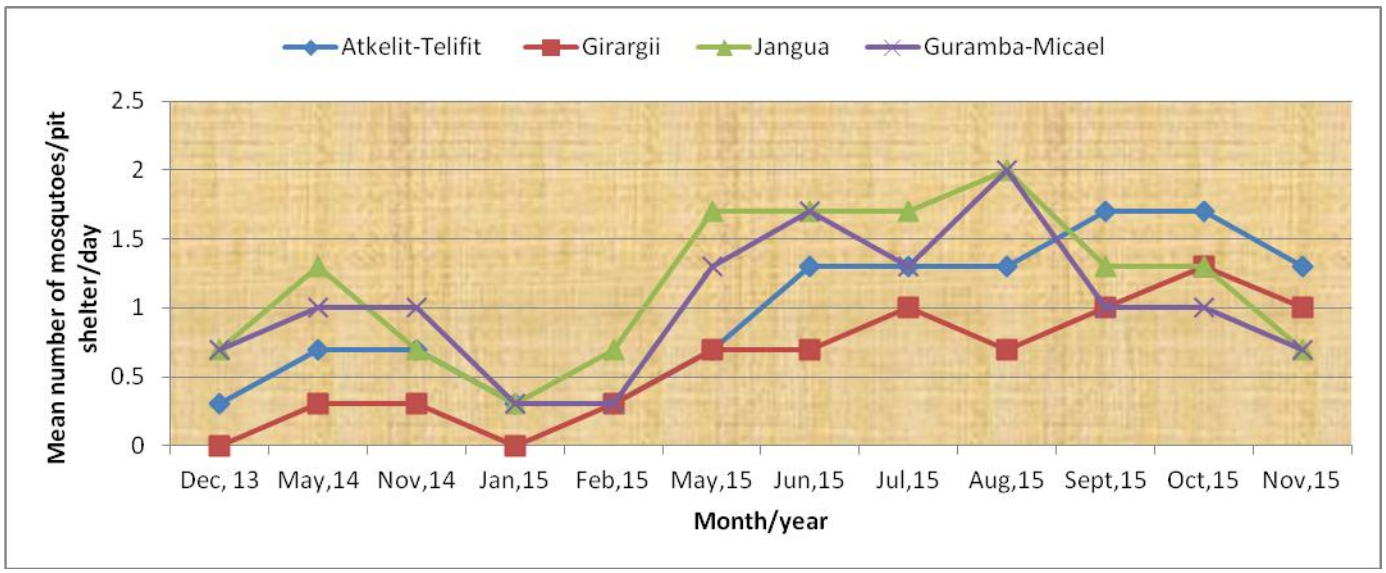


Figure 16.Monthly outdoor densities of *An. arabiensis* in four *Kebeles* of Dembia District, North west Ethiopia, 2013-2015.

Similar to highest outdoor density highest malaria incidence was observed in Guramba-Mickael in Dec, 2013 and Nov, 2014. Similarly highest outdoor resting densities and highest malaria

incidence was observed in Atkelit-Telifit in October, 2015 (Figure 18). The outdoor density of Guramba-Mickael and Jangua was slightly higher than Gerargii in September, 2015. Similarly malaria incidence was slightly higher in these *Kebeles* during Oct, 2015 survey (Figure 17).

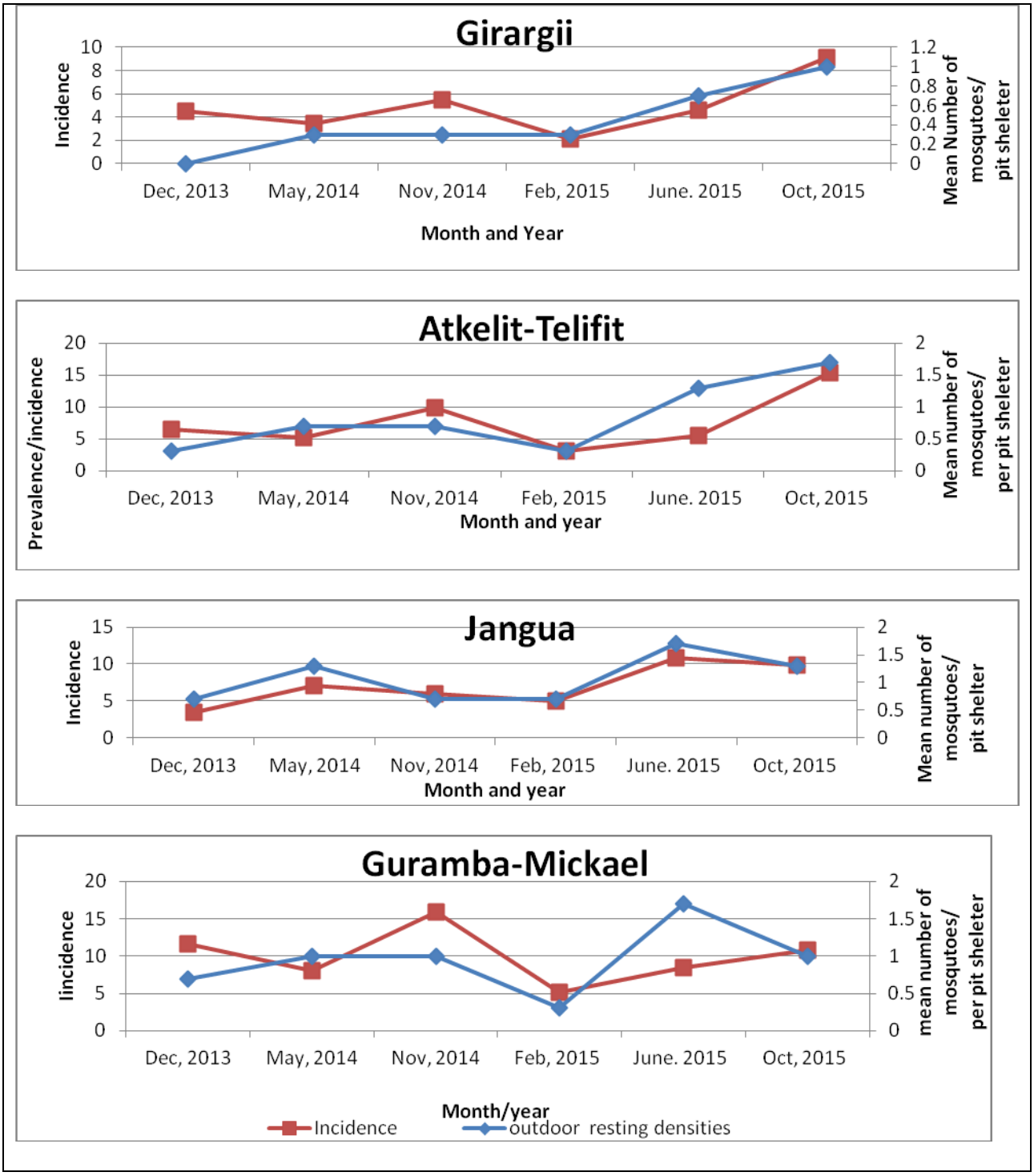


Figure 17. Monthly outdoor densities of *An. arabiensis* determined by pit shelter sampling and incidence of malaria in four *Kebeles* of Dembia District, 2013-2015.

Indoor densities of the primary and secondary vectors were lower in those households that utilize two LLIN than households that lack LLIN and utilize one LLIN always although the difference was not statistically significant. Stastically significant lower indoor densities were observed in IRS sprayed houses than non-sprayed houses for *An. arabiensis* and *An. funestus s.l.* (Table 15).

Table 15. The relationship between LLINs utilization and IRS spray status and indoor host seeking and resting density of primary and secondary vectors in Dembia District, 2013-2015.

Factors	Variables and collection methods	*An. arabiensis*			*An. funestus s.l.*			*An. pharoenis*		
		Mean±S.E	F	P	Mean±S.F	F	P	Mean±S.E	F	P
Surveyed locality	**Indoor host seeking density**									
	Atkelit-Telifit	1.48±0.11	1.68	0.17	0.16± 0.037	0.558	0.0643	0.42±0.060	3.686	0.01
	Girargii	1.07±0.09			0.14 ± 0.063			0.30±0.047		
	Jangua	1.26±0.15			0.20 ± 0.041			0.35±0.053		
	Guramba-Mickael	1.29±0.14			0.19 ±0.040			0.55±0.063		
	Indoor resting density									
	Atkelit-telifit									
	Girargii	0.89±0.085	0.17	0.92	0.11±0.033	0.026	0.994	0.29±19	1.052	0.37
	Jangua	0.79±0.086			0.11±0.033			0.20±12		
	Guramba-Mickael	0.83±0.106			0.10±0.031			0.30±20		
		0.83±0.096			0.11±0.033			0.28±19		
LLINs utilization	**Indoor host seeking density**									
	No LLINS	1.33±0.101	0.412	0.66	0.20± 0.048	2.359	0.096	0.46±047	1.763	0.17
	Hanged one LLINs	1.26 ±0.98			0.16± 0.029			0.40±0.069		
	Hanged two LLINs	1.15±0.163			0.08 ±0.037			0.35±0.039		
	Indoor resting density									
	No LLINS	0.89±0.070	0.58	0.55	0.14±0.027	2.335	0.098	30±0.036	0.778	0. 46
	Hanged one LLINs	0.80±0.068			0.14±0.024			26±0.036		
	Hanged two LLINs	0.77±0.144			0.04±0.027			21±0.057		
IRS spray status	**Indoor host seeking density**									
	Yes	0.21±0.047	83.83	0.000	0.08±0.031	5.772	0.017	0.31±0.059	2.794	0.095
	No	1.54±0.072			0.19±0.023			0.43±0.032		
	Indoor resting density									
	Yes	0.23±0.049	47.02	0.000	0.03±0.035	7.26	0.007	0.19±0.045	2.494	0.115
	no	0.939±0.054			0.13±0.029			0.29±0.028		

Table 16 shows the blood meal orgins of the primary and secondary vectors of malaria collected indoors and outdoors from four rural *Kebeles* of Dembia District. The blood meal analysis of *An. arabiensis* showed an overall preference of bovine blood (64.5%) than human blood meal (41.7%) when mixed blood was included both in HBI and BBI. The HBI was higher for *An. arabiensis* collected indoors (44%) than for those collected outdoors (23.9%). The BBI (74.1%) of *An. pharoensis* was higher than HBI (31.8%) indicating the species prefers bovine blood than human blood (Table 16). Similar to *An. arabiensis* and *An. pharoensis, An. funestus s.l.* has shown a preference for bovine blood meal than human blood meal with bovine blood meal alone rates of 67.9% . Unlike, the other species, human blood meal alone was not dectected in samples of *An. funstus s.l.* A small proportion of *An. arabiensis* had blood meals of unknown origin (8.9%) (Table 16).

Table 16. Source of blood meals of the primary and secondary vectors collected indoors and outdoors from four selected *Kebeles* of Dembia District, 2013-2015.

Anopheles species	**Collection site**	**No. analyzed (HBI, %)**	**Blood meal source**			
			Human N (%)	Bovine N (%)	Mixed N(%)	Unknown N(%)
An.arabiensis	Indoors	350(44)	98(28)	167(47.7)	56(16)	29(8.3)
	Outdoors	56(34)	10(17.9)	30(53.6)	9(16.1)	7(12.5)
	Total	406(41.9)	108(25.9)	197(48.5)	65(16)	36(8.9)
An. pharoensis	Indoors	85(31.8)	14(16.5)	50(58.8)	13(15.3)	8(9.4)
	outdoors	0	0	0	0	0
	Total	85(31.8)	14(16.5)	50(58.8)	13(15.3	8(9.4)
An. funstus s.l.	Indoors	19(0)	0	14(71.4)	2(10.5)	3(15.8)
	Outdoors	26(11.6)	0	18(69.2)	3(11.6)	5(19.2)
	Total	45(11.1)	0	32(71.1)	5(11.1)	8(17.8)
Overall *Anopheles*		536(38.3)	122(22.8)	279(52.1)	83(15.5)	52(9.7)

When computing for human blood index (*HBI*) mixed blood meals were added to the number of human blood. Mixed blood meals = human + bovine, unknown blood meals are negative for both human and bovine antibodies.

A total of 913 indoor and outdoor caught *Anopheles* mosquitoes comprising *An. arabiensis* (n=622), *An. christyi* (n=98), *An. pharoensis* (n=80), *An. funestus s.l.* (n=49), *An. garnahami* (n=35), *An. pretoriensis* (n=17) and *An. theileri* (n=12) were tested for the presence of *Plasmodium* sporozoites by ELISA and four *An. arabiensis* were positives for *P. falciparum* sprozoites. None of the tested *An. arabiensis* was positives for *P. vivax* sporozoites and none of the other anophelines were positive for sporozoites.

The overall *P. falciparum* sporozoite rates of *An. arabiensis* collected by the three methods were 0.6% in the study area. Three of sporozoite positives *An. arabiensis* were collected by CDC-LT in June, August and September, 2015 in Jangua, Guramba-Mickael and Atkelit-Telifit, respectively, whereas the remaining one sporozoite positive *An. arabiensis* was collected from outdoor by artificial pit sheleter in Sept, 2015 in Jangua. The sporozoite rates of *An. arabiensis* collected from indoors and outdoors by the three collection methods were 1.2 in low-lying sparyed *Kebele* (Jangua), while the sprozoite rates were 0.7% in the other low-lying and sprayed *Kebele* (Guramba-Mickael). *P. falciparum* sporozoite rate was 0.6 % in non-sprayed mid- level altitude *Kebele* by all collections methods (Atkelit-Telifit) while *P. falciparum* infected *An. arabiensis* was not detected in relatively higher altitude and non-sprayed *Kebele (*Girargii). All of *P. falciparum* infected *An. arabiensis* were collected during the wet season (June, August and September 2015) (Table 17).

Table 17. Monthly sporozoite rates of *An. arabienis* collected indoors by light traps and space spary catchs and outdoors by artificial pit sheleter from four selected *Kebeles* of Dembia District, 2013-2015.

Month and year	**Study localities**									
	Atkelt-Telifit		Girargii		Jangua		Guramba-Mickael		Total	
	No tested	Pf positive (%)	No tested	Pf positive (%)	No tested	Pf positive (%)	No tested	Pf positive (%)	No tested	Pf positives %
Dec,2013	4	0	2	0	3	0	12	0	21	0
May, 2014	10	0	9	0	14	0	10	0	43	0
Nov, 2014	6	0	5	0	6	0	5	0	22	0
Jan, 2015	6	0	4	0	3	0	5	0	18	0
Feb, 2015	4	0	2	0	2	0	5	0	13	0
May, 2015	14	0	16	0	22	0	17	0	69	0
Jun, 2015	19	0	17	0	30	1(3.3)	25	0	91	1(1.1)
Jul, 2015	21	0	18	0	25	0	21	0	85	0
Aug, 2015	23	0	17	0	25	0	25	1(4)	90	1(1.1)
Sept, 2015	27	1(3.7)	17	0	18	1(5.6)	14	0	76	2(2.6)
Oct, 2015	22	0	14	0	8	0	7	0	51	0
Nov, 2015	16	0	15	0	6	0	6	0	43	0
Total	172	1(0.6)	136	0	162	2(1.2)	152	0.7	622	4(0.6)

Monthly entomological inoculation rate of *An. arabiensis* from the three localities are shown in Table 18. The EIR estimated from CDC light trap catches were zero in most months. However, 18.2 *P. falciparum* infectious bites for *An. arabiensis* in August, 2015, in low-lying sprayed *Kebele* (Guramba-Mickael) were detected. The estimated annual *P. falciparum* EIR of *An. arabiensis* from CDC light trap was 9.0 infectious bites per year in the study area. Estimates of the EIR of *An. arabiensis* were also made individually for the three study *Kebeles* and for sprayed and non- sprayed *Kebeles* (Table 18). The Annual *P. falciparum* EIR was 3.0, 2.7 and

3.3 infectious bites per person per year in Atkelit-Telifit, Jangua and Guramba-Mickael, respectively. However, when EIR estimated from CDC light trap were categorized as sprayed (Jangua and Guramba-Mickael), and non-sprayed *Kebeles* (Girargii and Atkelit-Telifit), the annual EIR in sprayed localities (6.1 ib/p/y) was 2.1 times higher than non-sprayed localities (2.9ib/p/y).

Table 18. Monthly entomological inoculation rates of *An. arabiensis* from CDC light trap catches in three *Kebeles* of Dembia District, 2013-2015.

Month and year	**Study localities and monthly EIR**			
	Atkelit-Telifit PfEIR*	Janga PfEIR	Guramba-Mickael PfEIR	Total
Dec,2013	0	0	0	0
May, 2014	0	0	0	0
Nov, 2014	0	0	0	0
Jan, 2015	0	0	0	0
Feb, 2015	0	0	0	0
May, 2015	0	0	0	0
Jun, 2015	0	11.2	0	3.7
Jul, 2015	0	0	0	0
Aug, 2015	0	0	18.2	3.7
Sept, 2015	12.3	0	0	2.8
Oct, 2015	0	0	0	0
Nov, 2015	0	0	0	0
Total EIR	3	2.9	3.3	9

* PfEIR: *P.falciparum* entomological inoculation rate

4.5.3. Status of insecticide susceptibility of *An. arabiensis* in Dembia District

Table 19 shows the knockdown time and mortalities of the six insecticides tested for *An. arabiensis* collected in four selected *Kebeles* of Dembia District. Deltamethrin resulted in highest knockdown with 97.2% but DDT resulted in lowest knockdown with 14.8%. Deltamethrin had the lowest KDT50 (25.2 minutes) and KDT90 (54.7 minutes) values, whereas DDT resulted in only 14.8% knockdown within 60 minutes of exposure time. The mortality rate of *An. arabiensis* after the 24-hour recovery period was 13.3% for DDT, 54.2% for Deltamethrin and 51.9% for Malathion which was lower than the susceptibility boundary set by WHO (80%) (WHO, 2013). *An. arabiensis* was susceptible for Fenitrothion, Propoxur and Bendiocarp because the mortality rate was 100%, 98% and 98.9%, respectively (Table 19). The mortality rate calculated for the experimental tests was not corrected because mortality in the controls was always less than 5%.

Table 19. Inseticide susceptibility of *An. arabiensis* collected in four *Kebeles* of Dembia District, June-Agust, 2015

Insecticides tested	**Number exposed**	**% of knockdown**	**KDT50 (95%CI)**	**KDT90 (95CI)**	**% of mortality**	**Status (WHO, 2013)**
DDT	113	14.8	*	**	13.3	Resistant
Deltamethrin	107	97.2	25.2(20.3-29.0)	54.7(49.8-61.0)	54.2	Resistant
Malathion	104	36	*	**	51.9	Resistant
Fenitrothion	92	38	*	**	100	Susceptible `
Propoxur	99	97	41.0(36.5-44.6)	56.3(51.3-65.8)	98	Susceptible
Bendiocarp	98	97.9	38.9(34.9-42.3)	58.2(53.3-66.1)	98.9	Susceptible

*50% was not Knockdown ** 90% was not Knockdown, CI confidence interval

5. Discussion

Retrospective data compiled from Health centers and posts of the District showed an increment of malaria incidence in most years, except in the years 2006-2008 and 2014. The decrement of malaria cases between 2006 and 2008 was also observed in retrospective studies conducted in different regions of the country. These included Fogera District (Kassa and Beyene, 2014), Koladeba health center (Alemu *et al.,* 2012) and Jimma town (Alemu *et al.*, 2011a). The reduction of malaria incidence in Dembia District and different parts of Ethiopia between 2006 and 2008 might be explained by the introduction of Artemether/lumefantrine (coartem) as first line treatment for uncomplicated *P. falciparum* malaria at the national level (FMOH, 2012). Such post-coartem introduction effect on malaria incidence has been reported by John *et al.* (2009) from Kenyan highlands where treatment of children with coartem combined with IRS spray significantly reduced gametocyte carriage and density compared to the period prior to intervention. Reduced malaria prevalence during intial deployment of coartem in 2005 in Tigray region of Ethiopia was also reported (Barnes *et al.,* 2009). Free ITN distribution was the other possible reason for the reduction of malaria in that period (Dembia District health office report, 2006). High amount of average monthly rainfall (> 200mm) during the major rainy season (May-Sept), in these years (2006-2008) (National meteorology Sevice Agency), might be an additional reason for the fall in malaria incidence, since continuous and heavy rainfall disturbs mosquito breeding habitats.

The overall prevalence of malaria during the six month prospective surveys was higher (7.4%) than those reported from lowland and highland fringes of Butajira (0.93%) (Woyessa *et al.,* 2012) southcentral Ethiopia; Jimma town (5.2%) (Alemu *et al*., 2011b), Western Ethiopia, the overall average (4.1%) reported from three regions (Amhara; Oromia and SNNP) of Ethiopia (Graves *et al*., 2009). Differences in environmental and meteorological conditions together with differences in the malaria control interventions might explain the variations in malaria prevalence reported from different parts of Ethiopia. The higher prevalence compared to other parts of Ethiopia shows Dembia District to be a highly favorable environment for malaria transmsion.

The higher proportion of *P. falciparum* (70.3%) in the reterospective data and from the prospective longutidinal study (74.3%) in Dembia District is in agreement with malaria parasite distribution in Ethiopia that *P. falciparum* is the dominant species in altitudes less than 2000 masl (FMOH, 2014).

The retrospective data collected from health centers and posts of Dembia District showed all age groups to be affected by malaria showing poor immune protection in the population, which is the characteristic of mesoendemicity of malaria in the District. The incidence of malaria was expectedly higher in children less than five years old up to the year 2010; however, shifing to children aged 5 to 14 years old then after. The possible explanation of the higher malaria incidence in the older children (5-14) after 2010 might be due to the introduction of LLIN to protect younger children, which would increase malaria risk to the unprotected people by

diverting mosquitoes away from users and concentrating their host seeking efforts on non-users (Pullen *et al.* 2010). Higher incidence of malaria in older children age 5-14 due to the lack of coverage of LLIN was reported in Ziway (Gari *et al*., 2016) and Chano Mille (Loha and Lindtjørn, 2012) in southern Ethiopia. Furthermore, the prospective study showed failure to use LLIN to be associated with statistically significant increased risk of malara infections. Therefore, low LLIN coverge and its improper utilization, when available, may have been impeding effective malaria control in the District as it is the case in other parts of the country (Alemu *et al.,* 2011b: Loha and Lindtjørn, 2012).

The lack of adequate LLIN coverage and the increased outdoor activities of adult males might explain the higher incidence of malaria in male adults above 15 years old as the findings from the prospective study (2013-2015) indicated. The finding that nocturnal outdoor activities in all six surveys were associated with statistically significant increased risk of malaria infection is an indication of outdoor malaria transmsion. Similar to the present study, outdoor occupation and activity at night was associated with increased risk of malaria infection in Kenya and Ghana (Homan *et al.,* 2016; Monroe *et al*., 2015). Furthermore, statistically significantly increased risk of malaria infection with recent travel history in adult males in the prospective study can be explained by the movement of people from Dembia into malarious areas with large scale commercial farming such as in Metema, Quara, Humera, Sanja and Abrah Jira during months of peak mosquito biting (Adino *et al.,* 2015). In line with the present study, malaria surveys conducted in highland fringes of Northwest Ethiopia (Alemu *et al.,* 2014) and the malarious lowlands of Southern Ethiopia (Yukich *et al.,* 2013) had shown recent travel history to be associated with increased risk of malaria infection. In addition, the detection of *P. ovale,* a

parasite not common in the District, in residents who went to commercial farm areas is good evidence that population movements in the region does introduce malaria to different localities. Therefore, increasing outdoor activities and population movement must have been contributing to the perpetuation of malaria and hence contributing toward the sustained malaria transmsion in the District.

In addition, the observed lack of adequate supply of coartem in health posts and the emergence of deltamethrin resistance, in *An. arabiensis* (Balkew *et al.*, 2010; Abate and Hadis, 2011) with which the distributed LLINs were treated would explain the rise of malaria in the District. The lack of statistically significant difference in indoor biting *An. arabiensis* in households that utilized LLIN and households that lacked LLIN in the prospective entomological study strengthens the lack of the chemical barrier of the LLINs due to deltamethrin resistance or the loss of residual efficacy of the net as a result of long age of the LLIN. Thus, since insecticide susceptibility test of deltamethrin showed the moratality rate to be only 54.2 % in the study area; the LLIN is ineffective for those household members (adult and older age children) that were not physically protected. This is similar to a study conducted in Dielmo, Senegal where in that study ITNs quickly selected resistant mosquitoes with long lifespan and unchanged feeding behavior (Ndiath *et al.*, 2014).

Assesssment of the year round monthly malaria prevalence has revealed an addtional feature perhaps unique to Dembia District, to the overall epidemiological picture of malaria in Ethiopia where malaria incidence increases after the major rainy season (FMOH, 2012). This is the June

to August transmsion that was revealed in the present study both by retrospective and prospective malaria prevalence data, which was also supported by the major vector mosquito (*An. arabiensis*) population dynamics. The reason for highest malaria incidence during the rainy season might be the moderate rainfall distribution (50-100mm) recorded during those periods (NMSA). Moderate rainfall increases the creation of small rainpolls which become favorable mosquito breeding habitats for *An. arabiensis* (WHO, 1975). The lower malaria incidence observed in frequently sprayed low-altitude *Kebele* (Jangua) compared with a similar low altitude *Kebele* (Guramba-Mickael) appears to be due to the inconsistent IRS operation practices, whereby the operation was only partial.

In Dembia District, IRS spraying was carried out at the beginning of September during the last 15 years and June-August transmsion in the lowland areas and the mid-altitude malarious areas were not taken into account. Thus, lack of judicious planning for effective targeting the transmsion period though IRS contributed to malaria persistence in the District. In addition to this, re-plastering of residential house walls following application of IRS has also undermined the control effort. This impedent is compounded by the lack of trained persons on IRS operation and spraying inadequate doses of IRS on the walls of the households as detected through the longutidinal prospective study. Furthermore, the short-life of insecticides used in IRS, such as Bendiocarp and Propoxur (WHO,2011) has been shown to be ineffective beyond four months due to waning of residual effect as observed in a study conducted in northern Uganda (Tukei *et al.*, 2017). Therefore, the prospective longutidinal study provide evidence that irregularity in IRS operation practices in low altitude *Kebeles* and not including the mid-level *Kebeles* in IRS spraying operation is the reason for the persitance of malaria in the District.

Not considering control of outdoor malaria transmission such as by using mosquito coils, topical repellents, vapour phase insecticides and mass administration of gametocytocidal drugs to reduce human infectiousness prior to the transmission season, is additional reason for the the failure to control malaria in Dembia District since outdoor activities to keep cattle and other properties are common in the study area.

The considerable numbers of malaria cases during the dry season are due to the presence of permanent breeding habitats in the District as shown by the presence of *An. arabiensis* larvae in river edge pools and water leaked from hand pipes. It is also possible that the secondary vectors, *An. pharoensis* and *An. funestus s.l.,* which were abundant in the swamps and river edge pools and adults, collected indoors and outdoors may have role in the dry season transmsion. Furthermore, the possibilities of relapse in *P. vivax* and recrudescence of *P. falciparum* during the dry season will contribute to the ease of burden (Cattamanchi *et al.,* 2003; Golassa and White, 2017).

The significant association between monthly *P. vivax* and minimum temperature at zero month lag indicated that *P. vivax* tolerates minimum temperature more than *P. falciparum* (Pats and Olson, 2006). Similar to the present study, statistically significant association was observed between monthly *P. vivax* incidence with monthly minimum and average temperature in Motuo county, Tibet (Haung *et al.*, 2011).

Stastically significant positive association by multiple linear regression between monthly total malaria and *P. falciparum* and average temperature at zero month lag showed the interaction effect of temperature and rainfall for the transmsion of *P. falciparum* in the District. In addition, the District with the monthly average temperature of 20^0C-25^0C, the ideal temperature required for the development of the parasite in the vector and for the longevity of *Anopheles* mosquito (Cohen *et al.,* 2008) has a favorable temperature to sustain falciparum malaria transmsion.

The significant positive correlation observed between monthly incidence of total malaria and *P. falciparum* with monthly rainfall at one to four months lag during the study period was similar to the report from Burundi (Nkurunziza *et al.,* 2010) and Botswana (Chirebvu *et al.,* 2016). The long lag effect might be that heavy rains destroy existing breeding habitats, interrupt the development of mosquito eggs or larvae, or flush the eggs, larvae or pupae out of breeding habitats (Tian *et al.,* 2008). The other possible explanation for the long lag effect of rainfall might be that evaporation of pools keeps relative humidity at high level prolonging the longevity of vector mosquitoes (WHO, 1975). The vertisol and flavisol soil types of Dembia District (Berhanu, 1985) are suitable for the formation of fast creating pools, since the soil had high water holding capacity. Therefore, favorable rainfall distribution with special type of soil create many *Anopheline* breeding sites during the rainy reasons that it has not been possible to effectively control malaria in Dembia District.

The detection of high larval and indoor density of *An. arabiensis* during the wet season (May-August) in sprayed low altitude *Kebeles*, before IRS operation was carried out, could be the

possible explanation for the high malaria incidence observed in wet season (May-Aug) in the retrospective study and the highest malaria incidence detected in lower altitude *Kebeles* during May, 2014 and July, 2015 prospective study.

River edge pools created due to blockage of large Rivers (Megech and Dirma) and many other small streams for the purpose of irrigation activities were responsible for the high density of the secondary vectors (*An. pharoensis* and *An. funestus s.l.*) during the dry season. In addition, the small number of *An. arabiensis* collected during the dry season was mainly from the river edge pool localities. Terracing canals constructed by mobilization of peoples on January for the purpose of preventing soil erosion became suitable habitats for *An. arabiensis* and other anophelines, including *An. pharoensis* and *An. funestus s.l.* in the study area, during the rainy season Therefore, change in water pool creation due to irrigation activities, construction of terracing canals and unproper use of hand pipes might have contributed for transmsion of malaria in Dembia District .

The presence of considerable number of cases in IRS sprayed *Kebeles* in Oct, 2015 in the prospective study despite lower indoor densities of *An. arabiensis* might be attributed to outdoor biting and resting behavior of *An. arabiensis.* This behaviour presents obstacles for interventions with ITNs and IRS which is therefor one of the reason for the persistence of malaria in the District despite malaria control intervetions. The higher indoor density of *An. arabiensis* compared to outdoor resting density in the present study is due to the collection of most of *An. arabiensis* during the wet season when the low altitude *Kebeles* were not sprayed.

Similar to studies conducted in different parts of Ethiopia (Habtewold *et al.,* 2004; Massebo *et al.,* 2013a: Yewhalaw *et al.,* 2014), *An. arabiensis* in this study preferred to feed on bovine blood than human blood. Different studies found that, proximity or location of livestock relative to humans influenced malaria risk. For instance, Ghebreyesus *et al.* (2000), Seyoum *et al.* (2002) and Iwashita *et al.* (2014) reported increased malaria risk when animals were housed inside at night, or in close proximity to sleeping rooms. In contrast, when livestock were housed in separate shelters some distance away, malaria risk decreased (Palsson *et al.,* 2004: Tirados *et al.,* 2006). Because in this study humans and cattles live in the same house, the presence of cattle might have contributed to the abundance of *An. arabiensis* which in turn will be responsible for the persistence of malaria in the District.

Although members of *An. funestus* complex were not identified to species level by molecular identification, the zoophilic tendency indicated, the species might be either *An. rivulorum or An. parensis* or both. However, since earlier molecular identification of *An. funestus* s.s., collected from Ethiopia had shown *An. parensis* to be the only member of the complex (Weeto *et al.,* 2004), the species may be presumptly considerd to be *An. parensis.* Historically extensive use of IRS was successful in controlling *An. funestus* in areas where it was the primary vector, by taking advantage of its highly anthrophilic and endophilic behaviors (Smith, 1966). Due to a long history of IRS spray activities in the present study area (Cox *et al.,* 1999), the highly anthropophilic species, *An. funestus s.s.* might have been replaced by the zoophilic *An. parensis*. This makes *An. arabiensis* the major vector of in Dembia making the malaria control effort complicated because of the difficulty of controling *An. arabiensis* by the major malaria control intervetion methods (IRS and ITN) due to its oppurtunstic behavior.

The sporozoite infection rate and EIR of the present study reflect malaria incidence of the reterospective and prospective study. For instance, the highest malaria incidences in June, 2015 in Jangua and October, 2015 in Atkelit-Telifit *Kebeles* coincided with the highest EIR in June, 2015 and September, 2015 in the two respective *Kebeles*. In addition, the highest EIR observed in June and August, 2015 in the low altitude *Kebeles* (Jangua and Guramba-Mickael), strengethen the highest incidence of malaria during the rainy season in the retrospective study appears to be due to inappropriate IRS spray operation timing. The presence of higher EIR in the mid-altitude *Kebele* during September, 2015 after spraying of the low altitude *Kebeles* strengethens the concern that exclusion of mid-altitude *Kebeles* from IRS spray operations would increase EIR in non-sprayed mid-altitude *Kebeles*. Similar to the present study, more *P. falciparum* CSPs infected *An. arabiensis* were collected during the wet season when the localities were not sprayed (Shililu *et al.*, 2003). Additionally, the detection of *P. falciparum* CSP infected *An. arabiensis* collected from artificial pit shelter immediately after IRS spraying was a strong evidence that outdoor transmsion is taking place in the area, making malaria control ineffective.

The very high level of resistance of *An. arabiensis* to DDT in the four study *Kebeles* could be expected since DDT spraying is carried out starting from 1957 WHO pilot program in the District (Cox *et al.*, 1999). The percent mortality rate of *An. arabiensis* (13.3%) for DDT is comparable to earlier study in Gorgora, near the present study sites (11.3%) for DDT (Abate and Hadis, 2011). This suggest that vector resistance to DDT, the main insecticide used, had set in long ago and hence malaria persist in the District.

Although, *An. arabiensis* was susceptible to propoxur and bendiocarp, (the IRS sprayed during the last five years), these two insecticides are more expensive and are not efficient insecticides with respect to residual efficacy and repellency compared to DDT. For instance, DDT has 6–12 months of residual efficacy depending on dosage and nature of the substrate (WHO, 2011). For instance, a study in Ethiopia showed bendiocarp to have only 2 to 4 months residual efficacy on mud and dung surface whereas the residual efficacy of propoxur was 4 to 5 months in the mud and dung surfaces, the most used material in the area (Ye-ebiyo *et al.,* 2016). In addition, DDT has a spatial repellency and an irritant effect on malaria vectors (WHO, 2011) that strongly limit human-vector contact. Therefore, the high malaria prevalence in sprayed *Kebeles* after five months of IRS application can be considered due to low residual efficacy of propoxur (Yeebiyo *et al.,* 2016) in addition to re-plastering of walls and the low quality of IRS operation in the study area.

6. Limitations of the study

Not including serological and molecular study to better understand malaria transmission dynamics and not incorpriating higher altitude (>2000masl) locality in sampling is the limition of this study.

7. Conclusions and recommendations

7.1. Conclusions

The study findings point to:

6.1.1. The lack of effective malaria control activities is responsible for the persistence of malaria in Dembia District.

6.1.2. The year to year favorable local meteorological conditions for malaria transmsion have contributed to high malaria burden in Dembia District.

6.1.3. The population movement from Dembia District attracted by the large scale commercial crop farming in lowland areas, where malaria control program are difficult (due to night working and sleeping of laborer in the farm un protected by control measure) seem to have have been maintaining malaria circulation in the District.

6.1.4. The tradition of staying outdoors at night to keep livestock and other properties supports outdoor transmsion, making LLIN and IRS targeting of the mosquto vectors inefficient.

6.1.5. The poor utilization and accessibility of LLIN together with plastering of the sprayed walls are additional factors that impede the effectiveness of malaria control efforts.

6.1.6. The creation of suitable larval habitats due to increasing irrigation activites and construction of terracing canals, for soil conservation and rain water harvesting, can enhance vector breeding, which can increase mosquito density leading to sustained malaria prevalence in the District.

7.2. Recommendations

6.2.1. The use of IRS application for prevention and control of malaria should include the mid-level altitude (1850-2000 masl) *Kebeles.*

6.2.2. The time the IRS operation is carried out should be in the beginning of June instead of limiting spraying only to September.

6.2.3. The use of retrospective data should be strengthened for decision making for IRS operation and priorities of localities should be guided by the incidence data.

6.2.4. Study on the extent of population movement and its effect on transmission of malaria in Dembia District should be conducted in order to devise effective malaria control strategy considering migrant laborers.

6.2.5. Control methods targeting outdoor transmsion such as mosquito coils, topical repellents, vapour phase insecticides for protecting open space, treating clothes with contact insecticides, mass administration of gametocytocidal drugs to reduce the human infection prior to the transmission season and larval source mangment should be implemented.

6.2.6. Larval surveys should be considered to assess the effectiveness of IRS applications by comparing the density of *An. arabiensis* before and after the IRS operation.

6.2.7. Sero-epidemological study should be conducted in order to understand the immune status of the population and malaria transmission dynamics in Dembia District.

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9. Appendices

9.1. Household questionnaire (English version)

Addis Ababa University, Collage of Natural Sciences, Department of Microbial, Cellular and Molecular Biology

Questionnaire to collect data on malaria prevention and control practices

General Information		
GI1	Household code	_____ GPS data Longitude _____Latitude_____
GI2	Location	Region _____ Woreda_____ Kebele _____
GI3	Personnel (name and signature)	a) Interviewer_____ b) Field Supervisor_____
GI4	Date of visit	[_____|_____| _____| dd | mm| yyyy

Introduction and Consent

My name is_____ and I'm working for Gondar University. We are conducting a survey about malaria in collaboration with the Woreda Health Office. We would very much appreciate your participation in this survey. This information will help the region in particular and the country in general. This interview could take less than 20 minutes to complete. Whatever information you provide will be kept strictly secret and will not be shown to other persons. Participation in this survey is voluntary and you can choose not to answer any individual questions or all of the questions. However, we hope that you will participate fully in this survey since your views are important.

Do you have any questions about the survey? May I begin the interview now?

A) Socio-demographic and economic characteristics

No	**Question**	**Response**
A1	List household members and Guests of the household who stayed here last night, starting with the head of the household.	**Name** **Age** **Sex** **Occupation** **1.**_____ _____ _____ _____ **2.**_____ _____ _____ _____ **3**_____ _____ _____ _____

		4. _____ _____ _____ _____ **5. _____ _____ _____ _____** **6. _____ _____ _____ _____** **7. _____ _____ _____ _____** **8. _____ _____ _____ _____** **9_____ _____ _____ _____** **10 _____ _____ _____ _____** **11. _____ _____ _____ _____** **12_____ _____ _____ _____** **Sex** **Education status** **Occupation** **1= Male** **1= Illiterate** **1=Employed** **2= Female** **2= Read and write only** **2= House wife** **3= Farmer** **4= Day laborer** **5= Fishery** **6= Trader** **7= Student** **8=No job/dependent** **9=Housemaid** **10= other, specify**
A2	What is the main source of drinking water for members of your household?	A2.1. Piped into dwelling A2.2 Piped into yard/plot A2.3. Public tap/standpipe A2.4. Tube well or borehole A2.5. Protected well A2.6. Unprotected well A.2.7. Unprotected spring water A.2.8. Protected spring water A.2.9. River A.2.10. Other, specify

A3	What kind of toilet facility does your household use?	A3.1. Flush to piped sewer system A3.2. Flush to septic tank A3.3. Flush to pit latrine A3.4. Ventilated improved pit latrine (VIP) A3.5. Pit latrine with slab A3.6. Composting toilet A3.7. Hanging toilet/hanging latrine A3.8. No facility/bush/field A3.9. Other (specify)
A4	Does your household have: Electricity? A radio? A television? A telephone? A refrigerator	yes no Electricity 1 2 Radio 1 2 Television 1 2 Telephone 1 2 refrigerator 1 2
A5	What type of fuel does your household mainly use for cooking?	A5.1. Electricity A5.2. lpg/natural gas A5. 3. Biogas A5. 4. Kerosene A5.5. Charcoal A5.6.firewood A5.7. dung A5.8. Other (specify
A6	Main material of the floor. Record observation.	A6.1. Earth/sand A6.2. Dung A6.3. Floor wood A6.4.Planks palm/bamboo A6.5. Parquet or polished wood A6.6. Vinyl or asphalt strips A6.7. Ceramic tiles

		A6.8. Cement A6. 9. Other (specify
A7	Main material of the wall. Record observation	A7.1 bamboo/wood with mud A7.2. Bamboo/wood without mud A7.3. Stone with mud A.7.4. Cement A7. 5. Stone with lime/cement A7. 6. Cement blocks A7.7. Other (specify
A8	Main material of the roof. Record observation.	A8.1. Thatch/leaf A8.2. Sticks and mud A8.4. Reed/bamboo A.8.5. Wood planks A.8.6. Corrugated iron A.8.7. other (specify)
A9	Windows record observation	A9.1. Yes A9.2. No
A10	If yes, how many	Total number of windows ------
A11	If yes, what type of window	A11.1. window without cover A11.2 windows with glass A11.3. windows with screens A11.4 windows with curtains or shutter A11. 5 other, specify
A12	How many separate rooms are in this household? Include all rooms, including kitchen, toilet, sleeping rooms, salon, etc.	Number of rooms --------
A13	How many separate sleeping spaces are there in your	Number of sleeping rooms

	household? Include all sleeping spaces, including if there is more than one sleeping space in each room used for sleeping	
A14	How many of the following animals /birds does your household own? Cattle? Goats? Sheep? Pigs? Chickens? Dogs? Cats?	Cattle? Yes no Goats? Yes no Sheep? Yes no Pigs? Yes no Chickens? Yes no Dogs? Yes no Cats**?** Yes no Other specify
A15	Number of animals in the household	Number _____
A16	Do household have separate rooms/space for animals/birds Record observation.	A16.1. Yes A16.2. No
A17	If yes, where he/she keeps the livestock	A17.1 Keeps livestock animals around home A17.2 keeps livestock animals within 10 m from home
A18	Does any member of this household own: A bicycle? A motorcycle? An animal-drawn cart? A car or truck?	Yes No Bicycle……..……...…….1 2 Motorcycle……...…….1 2 Animal-drawn cart……...…….1 2 Car/truck……...……...…….1 2

A19	Does any member of this household own any land that can be used for agriculture?	Yes----------1 No……2
A20	If yes for QA19 How much of Agricultural land does members of this household own?	_____ local units
A21	How far is the nearest irrigation ditch from your house?	Minutes [____|____] Hours [____|____] Very close to the house
A22	Is there outdoor work at night in your family	A21. Yes A22. No
A23	If yes, what type work	A23.1. Livestock keeping A23.2. Keeping crops A23.3. Business work A23.4 Other (specify)
A24	If yes for B16, which member of the household most frequently do the outdoor work	A.24.1.. Male household head A.24.2 Female household head A.24.3. Children A.24.4. Other, specify

B. Knowledge and practice of the respondent

No	Questions	Response
B1	At any time in the past 6 months, has anyone sprayed the interior walls of your dwelling against mosquitoes?	B1.1. Yes B1.2. No

B2	How many months ago was the house sprayed against mosquitoes?	B2.1. Less one month B2.2. Month ago (specify**)**
B3	Who sprayed the house against mosquitoes?	B3.1.Government worker/program B3.2. Household member B3.3. Other (specify)
B4	At any time in the past 12 months, have the walls in your dwelling been plastered or painted?	B4.1. Yes B4.2. No
B5	How many months ago were the walls plastered or painted?	B5.1. Less than one month B5.2. Month ago (Specify)
B6	Does your household have any mosquito nets	B6.1. Yes B6.2. No
B7	How many mosquito nets does your household have?	B7.1. one B7.2. two B7.3. three B7.4 four B7.5. five B7.6. more than five
B8	Ask respondent to show you the net(s) in the household.	Net 1 B81.1. observed B81.2. not observed Net 2 B81.1. observed B8.2. not observed Net 3 B8.1. observed B8.2. not observed Net 4. B8.1. observed B8.2. not observed Net 5. B8.1. observed B8.2. not observed

B9	How long ago did your household obtain the mosquito net?	Year Month Net 1---------- ---------- --------- Net 2---------- ---------- ---------- Net 3---------- ---------- ---------- Net 4---------- ---------- ---------- Net 5---------- ---------- ----------
B10	Where did you obtain the net	Net.1 B10.1. Government B10.2. |Clinic/hospital B10.3 Neighborhood B10.4. Health committee B10.5. Health extension worker B10.6 Retail shop /pharmacy B10.7. Workplace B10.8 Other (specify)_____
B11	Please record or ask the general condition of the net	Net 1 B.11.1. good (no holes) B.11.2 fair (no holes that fit a torch battery) B.11.3. poor (1-4 holes that fit a torch battery) B.11.4. Unsafe (>5 holes that fit a torch battery B.11.5. unused (still in package) B.11.6. unknown
B12	Did anyone sleep under this mosquito net last night?	Net 1 B12.1 Yes B12.2 No
B13	Why did no-one sleep under this mosquito net last night?	Net 1 B13.1. No malaria

		B13.2. No nuisance/insects B13.3. No space for net B.13.4. Irritation B13.5 suffocation /too hot B13.6 difficult hanging net shape B13.7 absence from home B13.8 other (specify)
B14	How do you the use the net at night	B14.1.Hanging over the bed to the floor B14.2Hanging over the bed tucked in B14.3 Hanging over sleeping mat/matters B14.4 Other/ specify
B15	If there are not enough nets for everyone in a household, who should be given priority when deciding who can sleep under a net? Do not provide answers multiple responses possible probe once (anything else)	B15.1. Elderly people B15.2. Head of household B15.3. Young children B15.4. Pregnant women B15. 5. People who contribute the most money to the household B15.6. Person who obtained /bought the net B.15 . Other (specify)_____
B16	Travels history	B16.1 Recent travel malarias rural area B16.2 Recent travel malarias urban area B16.3 regular travel to malarias rural area B16.4 Regular travel to malarias urban area

9.2. Household questionnaire (Amharic version)

አዲስ አበባ ዩኒቨርስቲ የተፈጥሮ ሳይንስ ኮሌጅ የማይክሮብያል ሴሉላር እና ሞሊኪውላር ባዮሎጂ ት/ክፍል

የወባ መከላከያ ዘዴዎች፣ ትናንሽ የመስኖ ልማቶች ፣እርከኖች እና ያካባቢው የአየር ፀባይ በደንብያ ወረዳ የወባ በሽታ ስርጭት ላይ ያላቸው ተፅዕኖ ለማጥናት የተዘጋጀ የቤተሰብ መጠይቅ

ማሳሰቢያ

1. **ሁሉም መረጃ ሰብሳቢወች መረጃ በሚሰበሰብበት ወቅት መረጃ ለመሰብሰብ የተፈቀደላቸውን ደብዳቤ መያዝ ይጠበቅባቸዋል**
2. **መረጃ ሰብሳቢዎች መረጃውን የሚሰበስቡት አላማ እና ፈቃድነታቸውን መረጃውን ከመሰብሰባቸው በፊት ለቤተሰብ ሀላፊ ማስታወቅ ይጠበቅባቸዋል**

ተ ቁ	ጥያቄ	መልስ	ኮድ
ሀ 1	የተጠያቂው ፆታ	ሀ 1.1 ወንድ ሀ 1.2 ሴት	
ሀ.2	የቤተሰብ አባላት ከቤተሰብ ሀላፊ ጀምሮ እና በትላንት ምሽት በእንግድነት እቤት ውስጥ ያደሩ ሰዎች ስም ዝርዝር ፃፍ	1. _____ 2. _____ 3. _____ 4. _____ 5. _____ 6. _____ 7. _____ 8. _____ 9. _____ 10._____	
ሀ.3	የተጠያቂው ዕድሜ		
ሀ.4	የተጠያቂው የት/ደረጃ	ሀ.4.1 ማንበብና መፃፍ የማይችል ሀ.4.2 ማንበብና መፃፍ የሚችል ሀ.4.3 የመጀመሪያ ደረጃ ትምህርት(1-6) ሀ.4.4 ሁለተኛ ደረጃ(7-12) ሀ.4.5 ከ12ኛ ክፍል በላይ ሀ.4.6 ሌላ ካለ	
ሀ.5	የተጠያቂው እምነት	ሀ.5.1 ኦርቶዶክስ ተዋህዶ ሀ.5.2 ካቶሊክ ሀ.5.3 ፕሮቴስታንት ሀ.5.4 እስልምና ሀ.5.5 ሌላ ካለ ይገለፅ	

ሀ.6	የተጠያቂው የገቢ ምንጭ	ሀ.6.1 ግብርና ሀ.6.2 ንግድ ሀ.6.3 ደመወዝ ሀ.6.4 የቤት እመቤት ሀ.6.5 ሌላ ካለ ይገለፅ	
ሀ.7	የቤተሰብ እማወራ እርግዝና ሁኔታ	ሀ.7.1 አዎ ሀ.7.2 በቅርብ የተጋባን ሀ.7.3 ፈት ነኝ ሀ.7.4 የለም ሀ.7.5 ሌላ ካለ ይገለፅ	
	ለ፡ የቤተሰብ ሶሾ ኢኮኖሚ መረጃ		
ተ.ቁ	ጥያቄ	መልስ	ኮድ
ለ.1	ቤተሰብ የመጠጥ ውሃ ከየት ነው የሚያገኘው	ለ.1.1 ከቤት ውስጥ ያለ የባንባ ውሃ ለ.1.2 ህብረተሰቡ በጋራ የሚጠቀምበት የቧንባ ውሃ ለ.1.3 የጉድጉድ ውሃ (የተጠበቀ መዝጊያ ያለው) ለ.1.4 ክፍት የጉድጉድ ውሃ ለ.1.5 በአጥር ያልተከለለ የምንጭ ውሃ ለ.1.6 በአጥር የተከለለ የምንጭ ውሃ ለ.1.7 የወንዝ ውሃ ለ.1.8 ሌላ ካለ ይገለፅ	
ለ.2	ቤተሰቡ ከዚህ በታች የተዘረዘሩት አገልግሎቶች አለት ወይ? 1.ኤሌክትሪክ 2. የሚሰራ ራዲዮ 3. የሚሰራ ቴሌቪዥን 4. ስልክ(ተንቀሳቃሽ ወይም ኬብል) 5. ማቀዝቀዣ	<u>አዎ</u> <u>የለም</u> 1.ኤሌክትሪክ ------- ------- 2. የሚሰራ ራዲዮ------- ------- - 3. የሚሰራ ቴሌቪዥን------- ------- 4. ስልክ(ተንቀሳቃ ወይም ኬብል) --------- ------ 5. ማቀዝቀዣ --------- -------	
ለ.3	የቤቱ ወለል የተሰራበት ምንድን ነው ወደ ወለሉ በመመልከት መመዝገብ	ለ.3.1. የአፈር ወለል ለ.3.2 በኩበት የተመረገ ወለል ለ.3.3 የእንጨት ወለል ለ.3.4 የቀርቅሃ ወለል ለ.3.5 የሲሚንቶ ወለል ለ.3.6 የሴራሚክ ወለል ለ.3.7 ሌላ ካለ ይገለፅ	
ለ.4	የቤት ግድግዳ በምን አይነት እቃ ነው የተገነባው(የተሰራው)	ለ.4.1 የእንጨት ግድግዳ በጭቃ የተመረገ	

	ምልክትህን መዝግብ	ለ.4.2 የእንጨት ግድግዳ በሞቃ ያልተመረገ ለ.4.3 ቀርቅሃ ለ.4.4 በሞቃ የተያያዘ ድንጋይ ለ.4.5 በስሚንቶ የተለሰነ ግድግዳ ለ.4.6 የቡልኬት ግድግዳ ለ.4.7 ሌላ ካለ ይገለፅ	
ለ.5	የቤቱ ጣሪያ የተሰራበት ዋና እቃ ወደ ጣሪያው በመመልከት መመዝገብ	ለ.5.1 የሳር ክዳን ለ.5.2 የእንጨት ጣሪያ በሞቃ የተያዘ ለ.5.3 የቀርቅሃ ጣሪያ ለ.5.4 የእንጨት ጣሪያ ለ.5.5 የቆርቆሮ ክዳን ጣሪያ ለ.5.6 ሌላ ካለ ይገለፅ	
ለ.6	የቤቱ ግድግዳ ክፍተት የለውም ወይ? ምልክትህን መዝግብ	ለ.6.1 አዎ ለ.6.2 የለም	
ለ.7	የተጠያቂው ቤት መስኮት አለ ውሃ	ለ.7.1 አዎ ለ.7.2 የለም	
ለ.8	መስኮት ካለ ስንት መስኮት ነው ያለው?	ብዛት----------	
ለ.9	የቤቱ መስኮት ምን አይነት ነው	ለ.9.1 ሽፋን የሌለው የብረት መስኮት ለ.9.2 የእንጨት መስኮት ለ.9.3 የመስታውት መስኮት ለ.9.4 የሸቦ ወይም ሌላ ሽፋን ያለው መስኮት ለ.9.5 ሌላ ካለ ይገለፅ	
ለ.10	ቤቱ ስንት ክፍሎች አሉት	የክፍሎች ቁጥር----------	
ለ.11	ቤተሰቡ ምን ያህል የተለየ መኝታ ክፍል ወይም የመኝታ ቦታ አለው	ቁጥር----------	
ለ.12	ከዚህ በታች የተዘረዘሩት የቤት እንስሳት አለ ወይ? ካለ ቁጥሩን ፃፍ ከብቶች፣ ፍየሎች፣ በጉች ፣አሳማዎች ደሮዎች ፣ውሻ/ዎች እና ድመት/ዎች	**<u>አዎ</u>** **<u>የለም</u>** **<u>ቁጥር</u>** 1.ከብቶች 2.ፍየሎች 3.በጉች 4.አሳማዎች 5.ደሮዎች 6.ውሻ/ዎች 7.ድመት/ዎች	

ለ.13	በአጠቃላይ ከቤት ውስጥ የሚኖሩ የቤት እንስሳት ብዛት	----------	
ለ.14	ለእንስሳት የተለየ ቤት ወይም ቦታ አለ ወይ?	ለ.14.1 አዎ ለ.14.2 የለም	
ለ.15	የተለየ ቦታ ካለ ከዋናው ቤት በምን ያህል ርቀት ይርቃል?	ለ.15.1 ከቤት ውስጥ ለብቻው በተከለለ ቦታ ለ.15.2 ከቤት ውጭ በበረት	
ለ.16	ከቤተሰብ አባላት ከቤት ውጭ ማታ ማታ የሚሰራ ስራ አለ?	ለ.16.1 አዎ ለ.16.2 የለም	
ለ.17	ከቤት ውጭ የሚሰራ ካለ ምን አይነት ስራ ነው?	ለ.17.1 የቤት እንስሳት መጠበቅ ለ.17.2 አዝመራን መጠበቅ ለ.17.3 ንግድ ለ17.4 ሌላ ካለ ይገለፅ	
ለ.18	ከቤተሰቡ አባላት የትኞቹ ናቸው በብዛት በውጭው ስራ የሚሳተፉት?	ለ.18.1 የቤተሰብ አባወራ ለ.18.2 የቤተሰብ እማወራ ለ.18.3 ልጆች ለ.18.4 ሌላ ካላ ይገለፅ	
ለ.19	የቤተሰቡ አባወራ ወይም ሌሎች የቤተሰብ አባላት ለግብርና የሚውል መሬት አላቸው ወይ	1.አዎ 2.የለም	
ለ20	ለግብርና የሚውል መሬት ካለ ስንት ቃዳ መሬት ነው		
ለ21	ቤተሰቡ ከግብርና በተገኘ ሀብት ከተማ ውስጥ ቤት ሰርተዋል ወይ	1. አዎ 2. የለም	
ለ22	የመስኖ እንቅስቃሴ የሚካሄደው ከቤቱ በምን ያህል ርቀት ነው	ደቂቃ---------- ስአት---------- በጣም ቅርብ ቦታ የመስኖ እንቅስቃሴ አይካሄድም	

	ሐ. የተጠያቂው በወባ በሽታ ያለው ግንዛቤ እና በሽታውን ለመከላከል የሚያደርጉቸው ጥረቶች የሚመለከት መጠይቅ	
ሐ.1	ቤተሰብዎ የአልጋ አጎበር አለው ወይ ?	ሐ.11.1 አዎ ሐ.1.2 የለም
ሐ.2	ስንት የአልጋ አጎበር በቤትዎ ውስጥ አለ?	ሐ.2.1 አንድ ሐ.2.2 ሁለት ሐ.2.3 ሶስት ሐ.2.4 አራት ሐ.3.5 አምስት ሐ.2.6 ከአምስት በላይ
ሐ.3	ተጠያቂውን /ዋን የአልጋ አጎበር	የአለጋ አጎበር ቁጥር አንድ 1. አለ

	እነዲያሳዩት ይጠይቁት	2.የለም የአልጋ አጎበር ቁጥር ሁለት 1. አለ 2.የለም የአልጋ አጎበር ቁጥር ሶስት 1.አለ 2.የለም የአልጋ አጎበረ ቁጠረ አራት 1.አለ 2.የለም	
ሐ.4	መቼ ነው የአልጋ አጉበሩን/ዎችን ያገኙት	የአልጋ አጉበር1---------- 2---------- 3---------- 4---------- 5----------	
ሐ.5	የአልጋ አጉበሩን ከየት ነው ያገኙት	የአልጋ አጉበር ቁጥር 1 ሐ.5.1 የአካባቢው ጤና ጣቢያ ሐ.5.2. ከመንግስት ጤና ተቆማት ተሰጥቶን ሐ.5.3. ከከጎረቤት ሐ.5.4. ከጤና ኤክስቴንሽን ሐ.5.5. በግል ገዝተን ሐ.5.6. ከምንሰራበት ቦታ ሐ.5.7. ሌላ ካለ ይገለፅ	
ሐ.6	ባለፈው ማታ በዚህ አጉበር ውስጥ የተኛ ሰው አለ	ሐ.6.1 አዎ ሐ.6.2 የለም	
ሐ.7	የአልጋ አጉበሩን ካለፈው ማታ ካልተጠቀሙበት ያልተጠቀሙበት ምክኛት ምንድን ነው	ሐ.7.1 በአካባቢው በሽታ ስሌለለ ሐ.7.2 የወባ ትንኝ ስሌለለ ሐ.7.3 የአልጋ አጉበሩን ለመጠቀም በቂ የመኝታ ቦታ ስሌለለ ሐ.7.4 ስለሚያፍን(ሙቀት ስለሚፈጥር) ሐ.7.5 የአጉበሩ ቅርፅ ለመጠቀም አስቸጋሪ ስለሆነ ሐ.7.6 የአልጋ አጉበሩ በአሁኑ ግዜ ቤት ስሌለለ ሐ.7.7 ሌላ ካለ ይገለፅ	

ሐ.8	የአልጋ አጉበሩን እንዲት ነው ማታ ማታ የሚጠቀመበት (የሚዘረጉት)	ሐ.8.1 የአልጋ አጉበሩን ከጣሪያ በአልጋው በትዩዩ ወደ መኝታ መዘርጋት ሐ.8.2 አልጋውን በአልጋ አጉበር በመሸፈን ወዳ መሬት በመዘርጋት ሐ.8.3 ከፍራሹ በላይ የአልጋ አጉበሩን በመዘርጋት ሐ.8.4 ፍራሹን በአልጋ አጉበር በመሸፈን ውስጥ ገብቶ መተኛት ሐ.8.5 ሌላ ካለ ይገለፅ	
ሐ.9	የአልጋ አጉበሩን አጥብወት ያውቃሉ	ሐ.9.1 አዎ ሐ.9.2 የለም	
ሐ.10	የአልጋ አጉበሩን የሚያጥቡት ከሆነ በምንያህ ግዜ ነው የሚያጥቡት	ሐ.10.1 የአልጋ አጉበሩ በቆሸሸ ግዜ ሐ.10.2 በአመት አንድ ግዜ ሐ.10.3 በአመት ሁልት ግዜ ሐ.10.4 በአመት ሶስት ግዜ- ሐ.10.5 በአመት አራት ግዜ ሐ.10.6 ሌላ ካለ ይገለፅ	
ሐ.11	የአልጋ አጉበሩን ከአጠቡት በሆላ እንደሌላው ግዜ ትንኞችን ይገላል	ሐ.11.1 አዎ ሐ.11.2 የለም	
ሐ.12	በቤትወ ውስጥ የአልጋ አጉበር በበቂ ሁኔታ ከሌለ የአልጋ አጉበሩን እንዲጠቀሙ ቅድሚያ ለማን ትሰጣላቹሁ	ሐ.12.1 በእድሜ ትልልቅ ለሆኑ ቤተሰብ አባላት ሐ.12.2 ለቤተሰብ አባወራ (እማወራ) ሐ.12.3 ለህፃናቶች ሐ.12.4 ለእርጉዝ እናት ሐ.12.5 ለቤተሰብ ብዙ ገቢ ለሚያስገባ ሐ.12.6 የአልጋ አጉበሩን ላገኘው ወይም ላመጣው ሰው ሐ.12.7 ሌላ ካለ ይገለፅ	
ሐ.13	ከቤተሰብ አባል በዚህ ሶስት ሳምንት ወደ ወባማ ቦታ የሄደ ወይም ከወባማ ቦታ መቶ በእንግድነት ያረፈ ሰው አለ ?	ሐ.13.1 አዎ ሐ.13.2 የለም	

9.3. Consent Form (English version)

A study will be conducted under the objective of determining malaria transmission dynamics and investigate the relationship between mosquito populations and associated risk factors in order to assess the impact of malaria control intervention in Dembia district. A trained health professional will take a blood sample from selected and volunteer study participants 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 project. In addition to blood sample the researcher collect adult mosquitoes by different collection methods the whole night for two days monthly. Study participants have a right to withdraw from a project at any time if they feel discomfort from the purpose of study or any personal reasons. If a study participants or any responsible body have any questions about the project please contact with principal investigator Ato Solomon Tesfaye (Phone number 0913413375) or Addis Ababa university Microbial, cellular and molecular biology department (Phone number-0118959216).

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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9.4. Laboratory activities

9.4.1. Insecticide susceptibility test

9.5.2 Sporozoite Dectection by ELISA

9.4.3. Blood film diagnosis and *Anopheles* mosquto species identification by microscopy

Declaration

I, the undersigned, here honestly declare that this PhD dissertation is my own original work and has not submitted by me or any other body elsewhere at another university or other accadamic institution to fulfill a similar pupose. All materials used for this thesis has been dully acknowledged.

PHD candidate Signiture _____ Date_____