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Decline of Malaria using Combined Long Lasting Insecticide Treated Nets and DDT House Spraying Strategies in Adami Tulu Jido Kombolcha District, Central Ethiopia: A Longitudinal Study from Parasitological and Entomological Indices Data

Damtew Bekele[1]*, Beyene Petros[2], Wakgari Deressa[3] and Yeshambel Belyhun[4]

[1]*Biology Department, Faculty of Natural and Computational Sciences, Biomedical Sciences, Debre Markos University, Debre Markos, Ethiopia*
[2]*Microbial, Cellular and Molecular Biology Department, Life Science Faculty, Biomedical Sciences, Addis Ababa University, Ethiopia*
[3]*School of Public Health, Epidemiology, and Biostatics Unit, Addis Ababa University, Ethiopia*
[4]*Department of Microbiology, Immunology, and Parasitology, College of Medicine and Health Sciences, Gondar University, Ethiopia*

Abstract

Background: Dichlorodiphenyltrichloroethane (DDT) house spraying and long lasting insecticide treated nets (LLITNs), as integrated strategies, are key components in malaria prevention and control in Adami Tulu Jido Kombolcha District. The aim of this study was to determine the impact of this combined malaria control strategies on malaria incidence/prevalence, and Anopheles density in Aneno Shisho kebele (the lowest administrative unit of Ethiopia).

Methods: A longitudinal parasitological and entomological study was conducted in Aneno Shisho (received LLITNs and DDT), Kamo Garbi (received LLITNs), and Jela Aluto (did not receive either LLITNs or DDT). Parasitological surveys were conducted in the three kebeles after the main rain season (October/November, 2006), and during the small rains (April, 2007), in the three kebeles of Adami Tulu Jido Kombolcha District. Monthly collection of adult mosquitoes was undertaken from October-December 2006 and April-May 2007. Data on net coverage and knowledge of residents with LLITNs use for malaria control was also collected.

Results: The total prevalence of malaria was 8.6% in Jela Aluto, 4.4% in Kamo Garbi, and 1.3% in Aneno Shisho in the two season surveys, and this prevalence was significantly different among the three kebeles ($P<0.05$). There was a protective incidence rate ratio (RR) of 0.17 (95% CI: 0.039, 0.77) in Aneno Shisho compared with Kamo Garbi and Jela Aluto, this estimates reached statistical significance at $P<0.05$. Anopheles gambiae s.l. and Anopheles pharoensis densities also remained very low in Aneno Shisho compared with the two kebeles.

Conclusion: Use of LLITNs and DDT house spraying by the community of Aneno Shisho kebele in Adami Tulu Jido Kombolcha District resulted in a decline in human-mosquito contact, a decrease in the number of mosquitoes, and a reduction in malaria morbidity. Therefore, LLITNs should be distributed to the whole communities in Ethiopia. In addition, use of DDT house spraying judiciously and health education on malaria in a combined approach, should be implemented in the country to interrupt malaria transmission.

Keywords: Malaria control; *Anopheles*; *Plasmodium*; Dichlorodiphenyltrichloroethane; Long lasting insecticide treated nets; Adami Tulu Jido Kombolcha

Abbreviations: DDT: Dichlorodiphenyltrichloroethane; LLITNs: Long Lasting Insecticide Treated Nets; ITNs: Insecticide Treated Nets; IRS: Indoor Residual Spraying; WHO: World Health Organization; RBM: Roll Back Malaria; WDP: Water-dispersible powder; RR: Rate Ratio; IVM: Integrated Vector Management; a.s.l: above sea level; KAP: Knowledge, Attitudes and Practices; FMOH: Federal Ministry of Health

Background

Among the most important public health problems, the World Health Organization (WHO) reports that 300-500 million cases per year, with at least one million of these cases resulting in death [1]. According to Roll Back Malaria (RBM), malaria is one of the major public health challenges facing the poorest countries in the world, not only causing sickness and death, but also hinder economic development; as a result of lost working hours and the high costs of treating those affected, and of vector control [2]. In Ethiopia, it is estimated that almost 75% of the land is malarious, and nearly 68% of the population is living in this area [3]. The disease is unstable mainly due to the country's topographical and climatic features; hence the population at risk is exposed to frequent waves of epidemics. The country has also experienced the worst malaria epidemics in 1958, with three million malaria cases and 150,000 deaths [4].

Malaria is caused by protozoans of the genus *Plasmodium*, and is transmitted to humans by female *Anopheles* mosquitoes. All four species of *Plasmodium*: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* are known to occur in Ethiopia [5]. However, the two epidemiologically important species are

***Corresponding author:** Damtew Bekele, Biology Department, Faculty of Natural and Computational Sciences, Biomedical Sciences, Debre Markos University, Debre Markos, Ethiopia, E-mail: damtish99@yahoo.com

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Plasmodium vivax and *Plasmodium falciparum*, and the most deadly type of malaria is caused by the latter species [5]. The magnitude of malarial transmission can vary greatly from one region of a country to another, where variables such as altitude, terrain, and temperature influence the density of the vector mosquito population [6]. In Ethiopia, malaria transmission is seasonal ranging in transmission from September through November, as the major transmission season following the cessation of the big rains, and minor transmission occurs in April and May, following the little rains of January and February.

Thirty-four species and two subspecies of *Anopheles* have been found in Ethiopia [7,8]. Malaria in Ethiopia is transmitted by anopheline mosquitoes. Of which, *Anopheles arabiensis* is the small rain pool species, and is the major malaria vector, whereas *Anopheles pharoensis* is the permanent shore water species, and *Anopheles funestus* is the running water species. *Anopheles arabiensis* and the two latter species are considered as primary and secondary vectors, respectively [7].

The initiative which focuses more on Sub-Saharan Africa proposes as the key control features, with the use of insecticide treated nets (ITNs) and early and effective case management, and indoor residual spraying (IRS) programs in countries with unstable malaria [9]. Long lasting ITNs are recently promoted as a way to prevent malaria through the distributions of millions of this commodity in Africa, and as a tool to gain the attention of the public, and raise new funds [10]. Also, malaria can be prevented, or its effects can be greatly reduced by sleeping underneath a bed net treated with insecticide, taking anti-malaria medicines, destroying adult mosquitoes and their young by clearing ponds, pits, old cans, or broken pots that collect water, and raising mosquito-eating fish in ponds or lakes [11]. On the other hand, chemical insecticides are used for malaria control, and are usually classified on the basis of their chemical categories as follows: organochlorines (DDT, dieldrin, and lindane); organophosphates (malathion, fenitrothion, primiphosmethyl, and temephos); carbamates (propoxur and bendiocarp); pyrethroids (permethrin, deltamethrin, and lmbdacyhalothrin). The insecticides in the same group are usually similar in their chemical structure and mode of action against the mosquito vector [12].

Dichlorodiphenyltrichloroethane house spraying

Dichlorodiphenyltrichloroethane has come back into use in different ways than before. It is now recommended only for limited use indoors, in a method of indoor residual spraying (IRS) [13]. This is spraying small amounts of DDT on the inside walls of a house to kill mosquitoes that land there. One study in India found that controlled use of DDT led to a significant reduction in the number of malaria cases, with an even more remarkable drop in cases among infants [14]. Preparedness of insecticide for residual house spraying in Ethiopia include: DDT 75% of 535 gms in eight liters of water for one time use; DDT 100% of 400 gms in eight litres of kerosene for one time use [15]. It has been applied mainly in the form of 75% water-dispersible powder (WDP), at a target dosage of 2 gm active material per square meter by hand carried compression sprayers [16]. The same source described that 75% DDT at 2 g/m2 gives good control of vectors for six months on mud and thatch surfaces, but on impervious surfaces a 100% DDT is used as a solution in kerosene.

Residual insecticide house spraying is among the most important malaria control methods, which has been used in Ethiopia for nearly five decades [16]. Most spraying operations are carried out in June or July, just before the major transmission season, and in January and February to prevent transmission following the little rains. Residual effect (persistence of chemical on a given surface) of DDT remain high for at least 6 months, thereby cover the malaria transmission season, and to prevent the need for multiple applications.

Until recently, the control of malaria in Ethiopia relied on case treatment and vector control through application of insecticide spraying. However, the development of drug resistance of the parasite to the commonly used anti-malarial drugs calls for search for alternative control methods. Therefore, use of LLITNs is an addition to the range of malaria control strategies available.

Long lasting insecticide treated nets

The rectangular LLITNs are commonly preferred and used in Ethiopia; this is because of the nets are pooled and fixed on four sides, to prevent contact with different parts of the human body, and it is easy to fix and use by those who prefer to sleep out door [15]. These long lasting insecticide treated nets (PermaNets™), are nets that are treated with a higher dose of wash-resistant deltamethrin at the factory level, by a process that binds or incorporates insecticide into the fibers. They are designed to maintain their biological efficacy in the field against mosquito vectors for at least 3 years, under recommended conditions of use, and can resist at least 20 washes [3].

Insecticide treated nets protect from mosquitoes through repellent and knocking down effect of the insecticide used to impregnate the nets [17]. Treated nets are more effective when the target vectors are anthropophilic (primarily human biting), endophagic (feeding indoors), and when peak feeding times occur when people are under the protection of the ITNs. A net must be lowered and tucked under a mattress or sleeping mat to prevent entry by mosquitoes. Regarding the use of ITNs, it has to be considered that to prevent malaria, everyone should sleep under ITNs, every night, and all year round [18].

In a study conducted in northern Ghana, insecticide impregnated bed nets were found to substantially reduce occurrence of malaria in the population, especially in the wet season [19]. Although use of ITNs has been shown to have beneficial impact in the fight against malaria, in addition to increased ownership of ITNs, regular treatment and choice of insecticide remain a major challenge. Recently, the introduction of long lasting insecticide treated nets (LLITNs); ideally lasting the entire lifetime of a mosquito net, is considered a possible solution to this problem.

Combined strategies

Distinguishing characteristics of integrated vector management (IVM) are described as advocacy, social mobilization and legislation, collaboration within the health sector and with other sectors, integrated approaches, evidence-based decision-making, and capacity-building [20]. In 2004, the global strategic framework for IVM was prepared establishing new, broad principles and approaches to vector control, that are applicable to all vector-borne diseases [20]. Moreover, as part of the global plan to combat neglected tropical diseases for 2008-2015, the WHO has called for the strengthening of IVM and capacity building as one of the strategic areas for action [21].

Integrated disease control comprises various interventions that could be considered in combination, and/or single, depending on suitability and feasibility of their implementation [16]. This available interventions can be divided into two major categories: simple and require individual or household efforts, and provide individual protection, the second category includes large scale interventions requiring more concerted community efforts and aimed at reducing

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mosquito population, thus providing community protection [16]. The same source described that personal protective measures include those giving individual protection to reduce mosquito bites, such as use of repellents, protective clothing and use of mosquito nets, and those giving protection to a whole household, such as insect proofing of house use of insecticide sprays, coils or local herbs, and prevention of breeding in and around houses. These measures are simple and easy to apply, require no particular expertise, and can be applied by individuals or household for their own protection.

Currently to reduce mortality and morbidity due to malaria, in addition to DDT spraying, wide scale use of LLITNs is underway in the country. In view of this wide scale usage, the present study aims to investigate the impact of this combined control strategies on malaria transmission in Adami Tulu Jido Kombolcha District. Therefore, it becomes imperative to evaluate the impact of the malaria prevalence/incidence, and the mosquito abundance of this control strategy.

Methods

Study area and population

The study was conducted in malarious area in Adami Tulu Jido Kombolcha District, which is part of the East Showa Zone of the Oromia Regional State in between October 2006 and May 2007. Geographically, the area is located between 38°20' and 38.5°5' and 7°35'and 8°05'. The District covers an area of 1403.3 km^2 (unpublished data from Adami Tulu Jido Kombolcha District agricultural development office in 2006/07). Ecologically, Adami Tulu Jido Kombolcha is found in the Central Rift Valley of Ethiopia, and it is located at 160 km away from Addis Ababa.

The distance between Aneno Shisho and Kamo Garbi was 14 km, and between Aneno Shisho and Jela Aluto, 12 km (Figure 1). These kebeles were mainly selected due to the fact that there was a major malaria epidemic in the area, since in 1992 (the area, with altitudes ranging from 1,600-1,700 metres, is epidemic prone) [22]. The other reason is about four decades before the time of this study, detailed entomological studies had been conducted in the area, and both *Anopheles gambiae* s.l. and *Anopheles pharoensis* are known to exist in the area, hence these could be used as baseline information for undergoing this study.

People in the area live in circular tukuls with thatched conical roofs and mud or thatch walls. There are a few rectangular houses with roofs of corrugated metal sheets. Major occupation was agriculture and livestock herding. Aneno Shisho has an average altitude of 1,660 m above sea level (a.s.l), Kamo Garbi and Jela Aluto have average altitude of 1,680 m a.s.l. and 1,675 m a.s.l., respectively. Among the three kebeles; Aneno Shisho was received DDT and LLITNs (population, 4179), Kamo Garbi was received LLITNs only (population, 4100), and Jela Aluto did not receive either DDT or LLITNs (population, 4286). The three kebeles had comparable population size, and their total

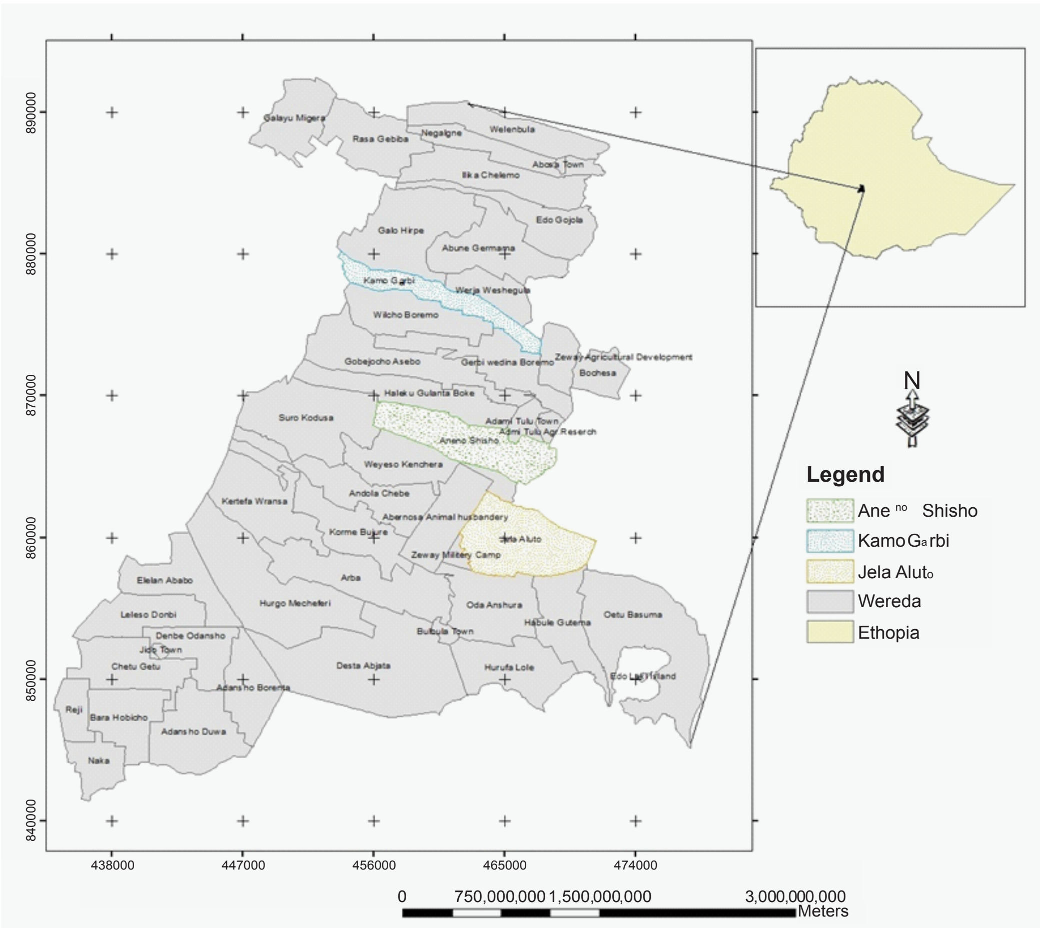

Figure 1: Sketch map of Ethiopia showing the study District (Adami Tulu Jido Kombolcha).

population was 12,565, according to a census taken in 1994 (Ethiopian Central Statistical Authority). In Aneno Shisho and Kamo Garbi, an average of two LLITNs (PermaNets) per household were first supplied free of cost on January 2005 (Haile Gebre-head of malaria control center of Adami Tulu Jido Kombolcha District, Personal Communication).

According to the data of malaria control activities of Adami Tulu Jido Kombolcha District (unpublished report), 17,199.4 kg of DDT was sprayed during the period 2001-2006, and in 2005-2007, 27,912 free LLITNs were distributed to the population in these areas. As health service based malaria morbidity data in Adami Tulu Jido Kombolcha District (unpublished report) during the period 2005/2006 showed *Plasmodium falciparum* was the dominant species in the area. The prevalence of *Plasmodium falciparum* was highest in 1998/1999, and peaking again in 2003/2004. On contrary, *Plasmodium vivax* infection rate fluctuated less from year to year. However, in 2003/2004, the infection rate of *Plasmodium vivax* tripled compared to 2002/2003. Furthermore, the *Plasmodium vivax* infection rate in 2004/2005 was higher than that of *Plasmodium falciparum*. In 2001 to 2003, reports showed that the use of DDT spraying was high (8767 kg) in the District. In these corresponding years, the malaria infection was low. On contrary, in 2003/2004, the use of DDT was very low (only 2684 kg), as a result of which there was peak malaria. In 2004/2005, the DDT spraying coverage was very high (4693 kg), and the malaria infection was low. On contrary to this, in 2005/2006, the DDT spraying coverage was very low (1053 kg), and the malaria infection was high. Therefore, the rate of the annual increase in DDT spraying was correlated with population protection from malaria. Spraying of DDT has been carried out in Aneno Shisho, beginning from July 2005, and the second round spraying has been carried out in January 2006. Also, DDT has been used in July 2006 and January 2007, as its residual life span is for six months.

Study design

The study design was composed of a longitudinal survey. This study was conducted to determine the prevalence and incidence of malaria, together with *Anopheles* density in Aneno Shisho, Kamo Garbi and Jela Aluto kebeles in Adami Tulu Jido Kombolcha District. This study was divided into two phases, the first (major transmission season) starting from the October/November, 2006, and the second (minor transmission season) during the April, 2007 in the study area. Eight year data (1998-2005) of average malaria prevalence (15.8%) was obtained from Zeway health office (unpublished report), and average malaria prevalence rate (6.8%), during six months study period (July 1 to December 30 in 1994), from the same District reported was taken for the determination of sample size [22]. Using this prevalence rate, the sample size (n) was found to be 240, by using the formula for estimating a single proportion and the total sample size was 720 after adding a 3% as contingency for non-response. The list of households of the three kebeles was used as the sampling frame, and a total of 218 household heads were selected from a random start. Then, to sample 240 people per kebele systematic random selection was used at every 5th households, from which all present were sampled.

Parasitological evaluation

A blood survey was conducted in October/November 2006, and in April 2007 in Jela Aluto, Kamo Garbi and Aneno Shisho kebeles. In conducting this study, two blood slide collectors: research worker and assistant were together involved as a slide-taker and registering population. The parasite rate in the representative sample population was determined; whereby finger prick blood specimens were collected on coded slides from a sampled population, regardless of the presence of symptoms of malaria, and malaria parasite positivity were determined from thick smear Giemsa stained films. Species identification was carried out from thin smear slide preparations. Parasite rate refers to the proportion of the sample population with species specific positive blood films, for either *Plasmodium falciparum* or *Plasmodium vivax* infections. One or more parasites per field were considered positive, and at least 100 high power fields per slide was observed before concluding the specimen as malaria negative. The point prevalence of malaria infection was expressed in terms of slide positivity rate. Examination for parasites was made in the Zeway Health Center, and confirmatory examination was carried out in the biomedical laboratory of Biology Department, Addis Ababa University.

Entomological evaluation

Densities of malaria vector were collected from human dwellings and associated structures, such as cowsheds and stables, as per the WHO techniques [23]. In collecting them from indoors and outdoors, a suction tube (aspirator), torch-light, CDC light traps, cotton and paper cups were used. Two dry cell battery operated CDC light traps were set indoors in the randomly selected houses form the three kebeles, and operated from 18:00 to 06:00 hours to collect adult mosquitoes. Indoor resting mosquito collections from human dwellings were conducted monthly in the three kebeles for five consecutive morning hours (6:00-8:30), between October/December 2006 and April/May 2007. Mosquitoes collected were transferred to a labeled paper cup, until identification. For mosquito identification: killing tube, chloroform, forceps, pill boxes, tissue paper, and 10X pocket lens were used. The anopheline mosquitoes collected were counted and identified to species, following the morphological descriptions [8]. To assess the association between temperature and *Anopheles* density, the seasonal/monthly minimum and maximum temperature in °C were used (Source: National Meteorological Services Agency of Ethiopia, unpublished data).

Knowledge, Attitude and Practice (KAP) study

Semi-structured questionnaires were prepared beforehand and administered in the local language (Afan Oromo), which was spoken by all members of the community. This survey was done by a house-to-house visit with a view to collecting blood samples, *Anopheles* mosquito collection and gathering LLITNs coverage, and use information from the members of the households selected after informed consent was obtained.

Data analysis

Data collected on parasitological, entomological and KAP surveys was captured on Excel spreadsheets, and were managed and analyzed using a statistical computer program SPSS version 13.0. The comparisons between kebeles in malaria prevalence, malaria incidence, mosquito density and LLITNs coverage were carried out, using simple frequency counts and the Pearson's chi-square test. To assess the difference in malaria incidence rate ratio among the three kebeles OpenEpi, version 2 was used [24]. A P- value<0.05 was considered to be statistically significant.

Ethical clearance

At the beginning of the study, the purpose of the investigation was explained to the kebele leaders and household heads in their vernacular language (Afan Oromo), and verbal informed consent was

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obtained from all participating households. During the study period, antimalarial drugs were given to all febrile cases.

Results

Malaria prevalence

The malaria point prevalence in October/November 2006 in Jela Aluto which did not receive LLITNs or DDT, was significantly higher (10.4%) than Kamo Garbi which received LLITNs (5.4%) (Chi square (X^2)=4.115, P<0.05), and in Aneno Shisho, which received both LLITNs/DDT as integrated malaria control (1.7%), only (X^2=16.185, P<0.05). Furthermore, the difference in malaria prevalence between Kamo Garbi and Aneno Shisho kebeles was statistically significant (X^2=4.940, P<0.05).

During the April 2007 survey, malaria point prevalence in Jela Aluto was 6.7%; 3.4% in Kamo Garbi, and 0.8% in Aneno Shisho (Table 1). The parasite prevalence in Jela Aluto was significantly higher than Aneno Shisho (P<0.01); also the prevalence in Jela Aluto was significantly higher than Kamo Garbi (P<0.05). The total parasite prevalence was significantly different among the three kebeles (P<0.05), as presented in table 1.

Malaria incidence

New cases of malaria were detected in the three kebeles during April 2007 survey (Table 1). The incidence of malaria in Aneno Shisho was only 0.8% cases per six months, as compared with Kamo Garbi and Jela Aluto, which were nearly 2% and 5% cases, respectively. This represents a protective incidence rate ratio of 0.17, with efficacy of 84.6% against *Plasmodium falciparum* and *Plasmodium vivax* (95% CI: 0.039, 0.77) in Aneno Shisho compared with Jela Aluto, and this estimates reached statistical significance at P<0.05.

Anopheles density

The densities of *Anopheles gambiae* s.l. and *Anopheles pharoensis* were highest in Jela Aluto, where it reached 73% and 64%, respectively, as compared with Aneno Shisho, where the densities of *Anopheles gambiae* s.l. (7%) and *Anopheles pharoensis* (9%) was observed (Figure 2). In the association between meteorological data of the average minimum and maximum seasonal temperature with *Anopheles* density and malaria prevalence, indicated the anopheline density and parasite rate matched (Figure 3). It just indicated it is a vector borne disease, or malaria transmission.

Knowledge, attitude and practices (KAP) survey

Characteristics of the study population: Among a total of 145 individuals took part in the KAP study in Aneno Shisho and Kamo Garbi kebeles, the mean age was 40.8 years, ranging from 18-75 years. Most respondents either had no schooling (67.9%), or only attended

Kebeles	Months		
	October/November, 2006	April, 2007	
	%malaria prevalence	%malaria prevalence	(%)malaria incidence/6month
Jela Aluto	25/240 (10.4%)	16/238 (6.7%)	11/222 (4.9%)
Kamo Garbi	13/240 (5.4%)	8/238 (3.4%)	5/230 (2.1%)
Aneno Shisho	4/240 (1.7%)	236 (0.8%)	2/234 (0.8%)

Table 1: Incidence of malaria among study participant in Jela Aluto, Kamo Garbi and Aneno Shisho kebeles in Adami Tulu Jido Kombolcha District, between October/November 2006 and April 2007.

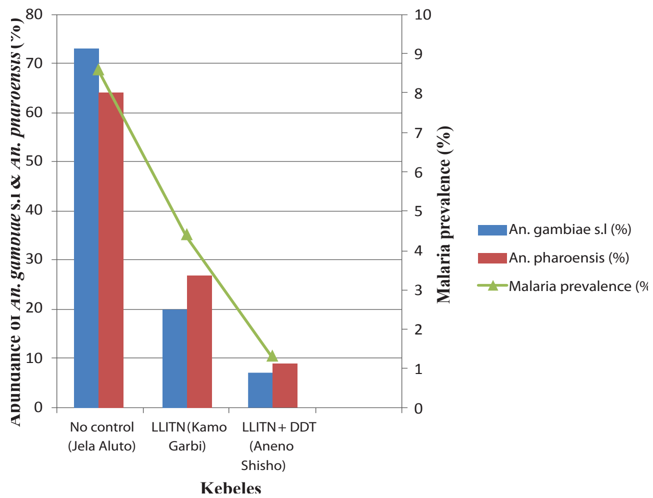

Figure 2: The relative density of *Anopheles gambiae* s.l. and *Anopheles pharoensis* with malaria prevalence in the three kebeles of Adami Tulu Jido Kombolcha District in October/November 2006 and April 2007.

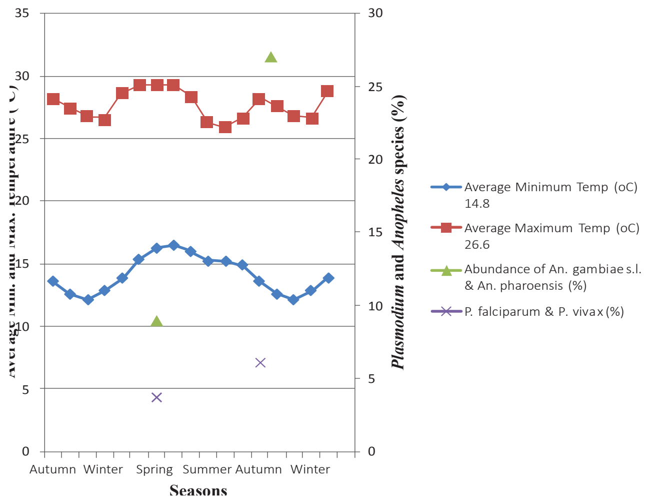

Figure 3: The seasonal average minimum and maximum temperature (2000-2007) in Adami Tulu Jido Kombolcha District and *Anopheles* density and malaria prevalence in the study area in 2006/07.

primary school (32.1%). Farmer was the most commonly reported occupation (82.6%), followed by daily labor and merchant (13.3% and 4.1%), respectively. The majority of respondents (80%) claimed to be of the Muslim faith, 17.4% to be of Protestants beliefs, and 2.5% to have Orthodox.

Long lasting insecticide treated nets: In Kamo Garbi and Aneno Shisho kebeles respondents (n=73 and n=72), respectively, said they had nets in the home (Table 2). A total of 287 nets were reported by respondents, resulting in an average two nets per household. Slightly more than two-thirds of the nets (67%) were used by children, either on their own or with their mothers, and a third (33%) covered adults. Concerning when they would use the nets, more than two-thirds of the respondents (69.7%) said all year; and nearly a third (30.3%) said only during the rains (because this is when mosquitoes are abundant). Of the 145 respondents that have nets, 67.6% said that someone slept under it the previous night, whereas 32.4% said not using the net in the

Sleeping under the LLITNs (%)	Kebeles		
	Kamo Garbi (n=73)	Aneno Shisho (n=72)	LLITNs coverage
Husband and wife	8 (11.0)	6 (8.3)	10%
Children alone	28 (38.4)	33 (45.8)	42%
Mother and child	19 (26.0)	18 (25.1)	25%
All family members	18 (24.7)	15 (20.8)	23%

Table 2: Long lasting ITNs use pattern within the family in Aneno Shisho and Kamo Garbi kebeles in Adami Tulu Jido Kombolcha District, 2006/2007.

previous night. Reasons given for not using the net was that there were no mosquitoes at that time.

Discussion

The point prevalence of malaria in October/November 2006 peak was 10.4% in Jela Aluto (no malaria control), 5.4% in Kamo Garbi (malaria control by LLITNs), and 1.7% in Aneno Shisho (Table 1). In the second survey (April 2007) peak, the point prevalence was 6.7% in Jela Aluto, 3.4% in Kamo Garbi and 0.8% in Aneno Shsisho (Table 1). It was observed that a major peak malaria month (October/November) was two times higher than the minor April peak in Aneno Shisho, which is a difference of around 33% in the annual prevalence rate, or 50% decrease from October/November (Table 1). Therefore, up to 25% of the April 2007 peak, malaria might be due to relapse from the old reservoir (October/November peak). That makes 75% of the point prevalence with the repeated visits of relapse cases. This indicates out of the 67% case rate, 58% *Plasmodium vivax* and 8% *Plasmodium falciparum*. On the contrary, a study carried out over the last 14 years in the same District showed a shift in malaria species ratio by a 100% rise in *Plasmodium falciparum*, and the dominance rate was reaching 5 *Plasmodium falciparum* to 1 *Plasmodium vivax* during the peak malaria months of October/November, in the autumn of 1991 [25].

In the present study area (in Aneno Shisho kebele), DDT spraying was applied in the inner walls of human dwellings at six-month intervals to kill and repel mosquitoes that landing on these walls, before or after taking a blood meal. Another study in India was showed where the mosquito bit humans primarily indoors, DDT spraying was found to remarkably reduce malaria incidence rates, when used in controlled amounts [26]. Also, residual house spraying has the advantage that adult mosquitoes are at risk every time that they enter a sprayed house, and given reasonably good spray coverage, little chance that any individual will survive long enough for sporozoites to mature in it [27]. The same author also pointed out the disadvantages that include: many people perceive it as dangerous (without scientific justification); households often refuse return visits by spray men; many *Anopheles* populations are resistant to cheaper insecticides; and trained spray teams and complex logistics (timely transportation) are required.

In our study, the protective effect of LLITNs and DDT on *Plasmodium falciparum* and *Plasmodium vivax* incidence among the population of Aneno Shisho compared with Jela Aluto) were 84.6% (95% CI=0.039-0.77). This showing the risk of development of malaria infection is 84.6% lesser, or a greater reduction in *Plasmodium* species incidence among users of LLITNs and DDT, compared with the non-users in Adami Tulu Jido Kombolcha District. Similarly, ITNs/IRS use can reduce malaria parasite transmission by 90% or more, and can correspondingly reduce malaria incidence, malaria prevalence, high parasite density, and clinical malaria [28-30]. Another malaria control strategy was investigated in Madagascar, whereby yearly residual spraying was used in conjunction with antimalarial drugs in rural populations [31]. The same author proved that the strategy was effective in keeping vector population at a minimum, and thereby reducing infection rates.

The findings of the Solomon Islands study also found that malaria infection rates went down by two-thirds using DDT house spraying, together with impregnated nets [32]. Accordingly, the use of multiple mosquito control methods simultaneously has been shown to be almost always more effective. In Eritrea, the attainment and even the overcoming of the targets fixed by RBM in just the first 5 years of program is mainly due to the use of IRS and ITNs [28]. In the present study, the use of LLITNs should be integrated with case treatment for the reduction of source of infection, and also by combining IRS with health education, better malaria control results can be obtained (Haile Gebre-head of malaria control center of Adami Tulu Jido Kombolcha District, personal communication). In connection with this, it was reported that IVM can complement other existing malaria control strategies (ITN use, access to effective treatment), by avoiding reliance on any single intervention to reduce the burden of malaria [33,34].

From the total density of mosquitoes collected-*Anopheles gambiae* s.l. and *Anopheles pharoensis* from Jela Aluto, Kamo Garbi and Aneno Shisho kebeles were in the ratio of 8.5: 3: 1, respectively. Long lasting treated mosquito nets were shown to tremendously reduce mosquito population, because mosquitoes landing on the net would be killed before having taken a blood meal, or they would be repelled by the insecticides. So, both *Anopheles gambiae* s.l. and *Anopheles pharoensis* are almost equally sensitive to integrated control measures, and the efficacy of LLITNs and DDT triples in Aneno shisho compared with Jela Aluto. Regarding malaria cases, in the April 2007 in Aneno Shisho, the number of new malaria cases was 2 as compared to 11 in Jela Aluto, which means 81.9% drop in malaria incidence in Aneno Shisho. This indicates the reduction in *Anopheles* density resulted in a major reduction in malaria cases, among the users of LLITNs and DDT. In line with this study, positive feedback has come from a study province in South Africa, where the reintroduction of DDT-based IRS has allowed the eradication of one vector species, *Anopheles funestus*, that was reappeared in the province after the withdrawal of DDT in 1996 [35]. In another study, among the total adult female anophelines collected, 53.1% were *Anopheles pharoensis*, 36.6% *Anopheles gambiae* s.l, and 9.0% *Anopheles* coustani [22].

In this study, DDT spraying in human dwelling can still reduce longevity of mosquitoes, thereby reducing transmission of malaria in Aneno Shisho kebele. In the same study area, the sporozoite detection test was carried out on dried and desiccated mosquitoes collected for the presence of *Plasmodium falciparum* and *Plasmodium vivax* sporozoite antigens by the Enzyme-linked Immunosorbent Assay method, and none were found infected [36]. This finding is in agreement with the study carried out in the same District that from *Anopheles arabiensis* and *Anopheles pharoensis* tested for the sporozoite antigens; none were found infected [22]. However, mosquitoes have and continue to develop resistance to insecticides, thereby reducing the efficacy of vector control and personal protection measures, and the options for effective malaria prevention. In connection with this, 20%-30% of the *Anopheles arabiensis* was resistant to DDT [22]. And the mortality rate of *Anopheles arabiensis* and *Anopheles pharoenisis* after one hour exposure to 4% DDT-impregnated papers were 69.7% and 17.5%, respectively. This indicated *Anopheles pharoensis* is more highly resistant to DDT. Another study in Gambella showed *Anopheles pharoensis* are efficient vectors of *Plasmodium vivax* [37].

Long lasting insecticide treated nets have been widely accepted by the population in the study areas (Aneno Shisho and Kamo Garbi kebeles), since they are considered to increase the quality of life and reduce the mosquito bites, nuisance insects, head lice, fleas, bedbugs, and cockroaches (Berhanu Terafa, personal communication). Insecticide treated nets offer individual protection, thereby reducing morbidity and mortality, especially in infants, but carryover to the community by reducing transmission has also been demonstrated [38].

On the other hand, the thermo climatic variation and the anopheline density curve indicate there was no anopheline swarm in the warm spring, as the study area was with temporary water collections. Consequently, the spring (April and May months) anopheline abundance was nearly half the autumn (October and November months) peak. With this sporogonic adjustment, the spring/Autumn ratio was nearly 33%: 66%, which was comparable with *Plasmodium* species rate. The increase in temperature should exacerbate the risk of malaria spreading around the world because malaria is largely a tropical disease [39]. The same author pointed out that as the world temperature increases, the range of malaria would become larger because of the mosquito season gets longer. Climate changes contributed to the increase in malaria [15]. The same author stated that the temperature affects the malaria transmission as follows: high temperature speeds up the development of the life cycle of a mosquito; high temperature accelerates the length of the development of the life cycle of malaria parasite within the mosquito host, thereby the sporogonic cycle shortened. Accordingly, mosquitoes are able to thrive best under optimum conditions of temperature ranging from 25-27°C [23]. The other factor that contributes to proliferation of *Anopheles* species in Ethiopia is since people in the rural areas use soil to make mud bricks, which they use in constructing their homes. This activity leaves pits in the soil that can fill with water, in which the *Anopheles* species breeds [39]. Mud bricks are sometimes the only material available for home construction in rural areas of Ethiopia. If other materials are available for home construction, most of the people could not afford them.

In protected kebeles (Aneno Shisho and Kamo Garbi), nearly two-thirds of the children were using LLITNs alone, or with their mother (Table 2). The LLITNs protection level was 70% in <5 children in relation to adults, as observed from the KAP study. More than two-thirds of the net-owners said that they intended to sleep under the net all year. Similarly, two-thirds said that someone had slept under the net the previous night, as the October/November falls within the peak malaria season. These results can illustrate the presence of understanding of the role of the ITN in malaria prevention. In this study area LLITNs as personal protection against mosquitoes were at a lower coverage (two LLITNs per household); this was based on WHO guidelines focused primarily on providing nets for children under five and pregnant women. However, in a study from Kenya has shown that expanding use of the nets to all people in targeted areas increases coverage and enhances protection of vulnerable groups, while protecting all community members [40].

In conclusion, based on the findings of this study, malaria control by using DDT house spraying and LLITNs were the most successful method for reducing the incidences of malaria and density of the target vector population. However, due to the lack of whole coverage of LLITNs and DDT house spraying strategies, interruption of malaria transmission could not be reached in Ethiopia. Therefore, combining control strategies including LLITNs distribution, insecticide house spraying, and health education on malaria should be implemented to control malaria in Ethiopia.

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