Variation in Malaria Transmission in Southern Ethiopia

The impact of prevention strategies and a need for targeted intervention

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To
Serawit,
Nahum and Hildana

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Summary

In Ethiopia, 60 per cent of the population is at risk of malaria. The transmission of the disease is unstable, and hence, the possibility of epidemics demanded continuous vigilance and preparedness of the health system. Meanwhile, the complexity of the transmission of the disease has become an impediment to retain the effectiveness of prevention and control strategies. Understanding factors that play role in disease transmission at different locations, the pattern of disease transmission, the impact of prevention and control strategies and challenges in control efforts were deemed crucial for the way forward.

This thesis analysed the local variations in the link between potential determinants of transmission – meteorological factors and malaria incidence. For this, we used datasets from 35 locations found in the Southern Nations and Nationalities People's Region and registered within the period 1998 to 2007. The findings implied that the variability in the models to be principally attributed to regional differences, and a single model that fits all locations was not found. Although there is a biological link between meteorological factors and malaria transmission, the link is affected by local conditions and non-meteorological factors.

With the understanding of a need to incorporate non-meteorological factors, in an attempt to predict disease incidence, a detailed investigation was carried out in Chano Mille Kebele – one of the malarious Kebeles of Arba Minch Zuria district, Gamo Gofa zone, south Ethiopia. A prospective cohort study was conducted for two years with a weekly visit to each of 1,388 households. The findings showed that rainfall increased and indoor residual spraying with Deltamethrin reduced falciparum malaria incidence. Higher disease incidence was observed among males, children 5–14 years old, insecticide-treated net non-users, the poor, and people who lived closer to vector breeding site. Meanwhile, we identified spatio-temporal clusters of high disease rates within a 2.4 sq.km area of the Kebele.

Mass distribution of insecticide-treated nets neither showed community-wide benefit nor influenced the spatio-temporal clustering of malaria, though proved to be protective at the individual level. Further analysis on insecticide-treated nets showed that the proportion of insecticide-treated net use reached a maximum of 69 per cent despite a near universal coverage (98.4 per cent) was achieved. Sleeping under the insecticide-treated nets was influenced by gender, age and proximity to the vector breeding site. Factor compromising the

usable life of insecticide-treated nets and a lack of convenient space to hang more than one net were reported.

The local variations in meteorology-malaria link, the heterogeneous risk carried by different population segments and the observed effect of prevention strategies may help to revisit the approaches towards malaria – for which I forwarded specific recommendations.

List of original papers

This thesis is based on the following papers, which will be referred to in the text by the respective Roman numerals:

Paper I   *Eskindir Loha and Bernt Lindtjørn*. Model variations in predicting incidence of *Plasmodium falciparum* malaria using 1998-2007 morbidity and meteorological data from south Ethiopia. *Malar J.* 2010; 9:166.

Paper II   *Eskindir Loha and Bernt Lindtjørn*. Predictors of *Plasmodium falciparum* malaria incidence in Chano Mille, south Ethiopia: a longitudinal study. *Am. J. Trop. Med. Hyg.* 2012; 87(3): 450–459.

Paper III   *Eskindir Loha*, *Torleif Markussen Lunde and Bernt Lindtjørn*. Effect of bed nets and indoor residual spraying on spatio-temporal clustering of malaria in a village in south Ethiopia: a longitudinal study. *PLoS ONE.* 2012; 7(10): e47354.

Paper IV   *Eskindir Loha, Kebede Tefera and Bernt Lindtjørn*. Freely distributed bed nets use among Chano Mille residents, south Ethiopia: a longitudinal study. *Malar J.* 2013; 12:23

Abbreviations

ACT	Artemisinin-based combination therapy
ARIMA	Autoregressive integrated moving average
CI	Confidence interval
DDT	Dichlorodiphenyltrichloroethane
GEE	Generalized estimating equation
IPTi	Intermittent preventive treatment in infants
IPTp	Intermittent preventive treatment in pregnancy
IRR	Incidence rate ratio
IRS	Indoor residual spraying
ITN	Insecticide-treated net
LLIN	Long-lasting insecticidal net
malERA	Malaria Eradication Research Agenda
MEWS	Malaria Early Warning System
PCA	Principal component analysis
RBM	Roll Back Malaria
RDT	Rapid diagnostic test
SMC	Seasonal malaria chemoprevention
SNNPR	Southern Nations and Nationalities Peoples' Region
TF	Transfer function
WHO	World Health Organization

Contents

Chapter I: Introduction

1.1. General overview

Malaria is an ancient disease caused by parasites of the genus *Plasmodium* and transmitted by several species of female anopheline mosquitoes. The term 'malaria' originates from *mal'aria* (Italian) – signifying 'bad air' or miasmas arising from marshes. Cognizant of the burden of the disease in antiquity, several efforts have been made to understand the disease – notably, the detection of the *Plasmodium* parasite in the blood of infected humans in 1880,[1] as well as proof of the complete life cycle of malaria parasites in mosquitoes in 1897.[2-3] Among 200 *Plasmodium* species identified,[1] *P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi* are known to be responsible for human malaria,[4-6] while mortality due to malaria is mostly attributed to infections with *P. falciparum.*[4-5, 7]

1.2. Burden of malaria

1.2.1. Global

Malaria transmission exits in 99 countries throughout world,[5] and the greater burden of the disease is carried by African countries.[5, 8] According to the World Health Organization (WHO), the estimated cases of and deaths due to malaria in 2010 were 219 million and 660,000, respectively,[5] with malaria deaths steadily decreasing since 1980 in countries outside of Africa. However, inside Africa, malaria deaths in 2004 exceeded those reported in1980, and only a 30% (from 2004) reduction was observed in 2010, which was believed to be associated with the international donor-dependent massive intervention programmes launched after 2004.[9] Despite the decline in the burden of malaria with the scaling-up of interventions,[10] the fact that the estimated (uncertainty exists) number of malaria deaths in 2010 exceeded that of 1980[9] calls for more efforts in the prevention and control of the disease in Africa.[11]

1.2.2. Ethiopia

According to the World Malaria Report of 2012, more than 60% of the Ethiopian population was at risk of malaria, and approximately 62% of all malaria cases were due to *P. falciparum.*[5] Malaria prevalence has exhibited a decline since 2005, with the decline

attributed to the scale-up of malaria-related interventions.[12-13] Nonetheless, according to the two consecutive national malaria indicator surveys, an 'increase' in malaria prevalence was observed in 2011 (1.3%) compared to 2007 (0.9%) in areas <2,000 metres above sea level.[14]

1.3. The malaria vector

Anopheles arabiensis, An. funestus, An. gambiae s.s., An. melas, An. merus, An. moucheti and *An. nili* have been reported as the dominant vector species in Africa. Among these, *An. gambiae s.s.* and *An. arabiensis* are the most efficient vectors in malaria transmission.[15-16] Small-, temporary-, clear-, sunlit- and shallow fresh water pools are necessary for the breeding of *An. arabiensis* – the dominant vector in Ethiopia.[17-18] Moreover, temperature and moisture (measured as precipitation or humidity) are the key environmental determinants for the vector life cycle.[19]

1.4. Life cycle of malaria parasite

An infected female *Anopheles* mosquito inoculates sporozoites into a human host. The uptake of sporozoites subsequently initiates asexual reproduction, which gives rise to the formation of gametocytes to be ingested by the mosquito. The sexual reproduction taking place in the mosquito produces sporozoites ready for further inoculation into a human host – perpetuating the cycle unless interrupted [Figure 1].
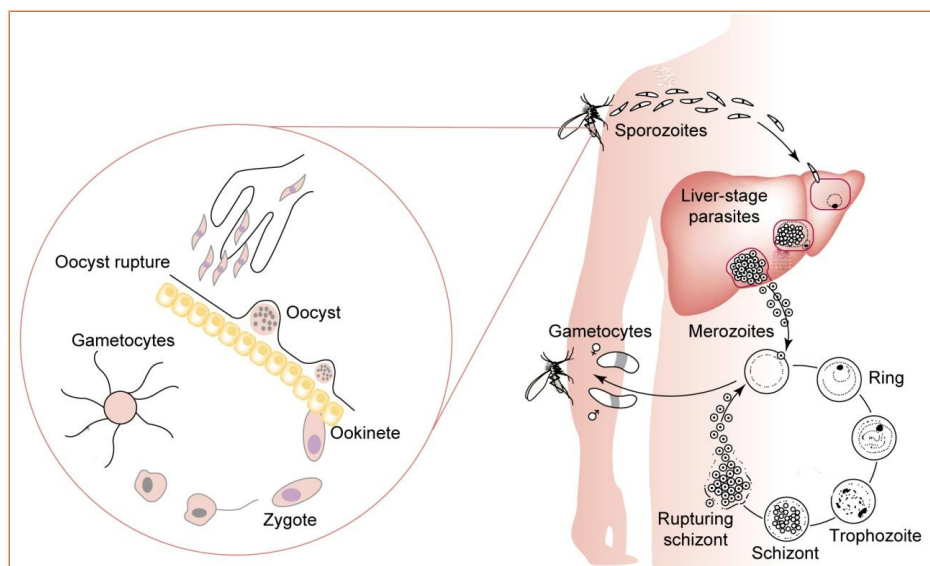
Figure 1: Life cycle of malaria parasite (adapted from Targett GA[20])

1.5. Factors favouring malaria transmission

1.5.1. Climate/meteorology

Minimum[21-23] and maximum[24-27] temperature and rainfall[28-30] influence malaria transmission, whereas several studies have indicated that the effect of such meteorological conditions on malaria epidemiology to be subject-to local variations.[27, 31-39] The use of climate to predict the burden of infectious diseases, including malaria, was of interest;[40] however, future changes in temperature and precipitation may not necessarily result in an increase in malaria endemicity[32, 41] – thereby indicating a need to consider other potential determinants.

1.5.2. Environmental change

Among others, environmental changes affecting the incidence of malaria include water control projects (reservoirs, irrigation canals and micro/macro dams), road construction, flooding, deforestation and the initiation of crop agriculture. Such either man-made or natural environmental alterations can result in favourable conditions for vector breeding and hence an increase in the risk of malaria.[42-45] On the contrary, evidence showed that an increase in urbanization was coincident with a reduction in the global malaria burden.[46]

1.5.3. Demographic and socio-economic factors; population movement

Studies have indicated that factors including age, sex and socio-economic conditions all play a role in malaria epidemiology.[47-54] Additionally, population movement to and from malaria-endemic areas also affects the distribution of the disease.[55-56]

1.6. Economic and social impacts of malaria

The attempts to measure the direct and indirect costs of malaria have revealed a major economic burden on households,[57] as malaria epidemics usually coincide with planting and harvesting seasons, thus reducing labour productivity and in effect jeopardizing the household economy,[58] with the effect being worse for those who are socially vulnerable.[54] Overall, evidence has shown that the burden of malaria is inversely related to a country's economic growth.[52, 59-60]

1.7. Malaria prevention and control: Historical perspectives

The WHO's global malaria eradication campaign (launched in 1955) formulated a plan to eradicate malaria in 10–15 years with the indoor residual spaying (IRS) of

dichlorodiphenyltrichloroethane (DDT).[61] The prevention of breeding of vectors and measures against malaria parasites were also considered in the eradication package,[62] which had taken lessons from earlier control efforts and lasted until 1969. Though it was not highly successful in African countries – due to inability of the health services to manage control programmes,[63] consequently leading to technical difficulties to pursue eradication,[64] it resulted in eliminating malaria from most of Europe and North America.[65] The understanding that the eradication plan was impossible with a sole or similar strategy across the globe[66] demanded the development of new insights and tools to combat the disease starting in 1969.[64] In 1992, the recognition of malaria as a global priority was revitalized, which led to designing a global strategy[67] for malaria control that employed four basic technical elements:

- Providing early diagnosis and prompt treatment;
- Planning and implementing selective and sustainable preventive measures, including vector control;
- Early detection, containment and prevention of epidemics;
- Strengthening local capacities in basic and applied research.

The vector control measures included the use of insecticides, biological agents and environmental management, out of which more of an emphasis was given to indoor residual spraying. However, the selection of vector control measures should rely on expert judgment.[67]

Subsequently, a global plan of action for the years from 1993 to 2000 was developed to guide the implementation of the global malaria control strategy, emphasizing the need to improve the involvement of both the public and private sectors, communities and individuals at risk of malaria.[68]

The use of bed nets impregnated with long-acting insecticides (such as synthetic pyrethroids), known as insecticide-treated nets (ITNs), as a personal protection was considered a promising tool to combat malaria.[68] Nevertheless, considering the observed low re-treatment practices of ITNs, the WHO prompted the use of long-lasting insecticidal nets (LLINs), which have been regarded as a major breakthrough in malaria prevention.[69]

Since malaria continues to be a major public health problem, and to help provide a coordinated global response to malaria – envisioning 'a world free from the burden of malaria', the roll back malaria (RBM) partnership was established in 1998.[70] This was followed by the Abuja Declaration in 2000, which was made by the 44 malaria-affected African countries, who agreed to halve malaria mortality by the year 2010 and commit themselves to implementing the RBM strategies.[71] In the meantime, the discovery of Artemisinin-based combination therapy (ACT) – "anti-resistance malaria medicine", while resistant parasites to other anti-malarial drugs became rampant, reinforced the RBM's fight against malaria.[72]

The impact of unexpected malaria epidemics is huge in terms of morbidity and mortality in areas where malaria transmission is unstable and the larger segment of the population lacks immunity. This called for designing a mechanism to inform about the possibility of epidemics in advance, both in time and space. As a result, the Malaria Early Warning System (MEWS) was formulated in 2001 in order to salvage the lives of 110 million people in 23 countries.[73] Such a need for the early detection, containment and prevention of epidemics was also indicated in the global strategy for malaria control in 1993.[67] The MEWS made use of the following three indicators:[73]

- Vulnerability indicators, which include low immunity, malnutrition and population movement;
- Transmission risk indicators, which include unusual increases in rainfall;
- Early detection indicators such as malaria morbidity data, which were obtained from a health facility.

Following a call by Bill and Melinda Gates,[74] a Global Malaria Action Plan was endorsed in 2008 with an "ambitious but achievable" goal, i.e. a near zero death from malaria by the year 2015 and eradication in the long-term through progressive elimination wherever feasible. Taking into account the regional differences in malaria epidemiology, the Global Malaria Action Plan made distinct strategies for Africa, the Americas, Asia-Pacific, the Middle East and Eurasia.[75]

1.8. Existing strategies to combat malaria and the challenges
The WHO recommendations[5] for malaria prevention and control include:

- IRS with insecticides
- ITNs
- Larval control
- Preventive chemotherapy
- Diagnosis and treatment

1.8.1. Indoor residual spraying with insecticides

The effectiveness of IRS with insecticides (DDT) for malaria prevention and control in the pre-eradication era[63] paved the way for IRS to be the prominent control measure during the 'eradication' era,[62] and in the process also becoming one of the most important tools in recent times.[5, 75-76] However, rapidly developing resistant vectors to available insecticides jeopardize this strategy, thereby implying the need for the continuous monitoring of insecticide resistance to sustain the benefit of IRS.[77-79]

1.8.2. Insecticide-treated nets/Long-lasting insecticidal nets

As a physical barrier from the mosquito nuisance, the use of bed nets has existed for many years.[80] The impregnation of a bed net with insecticides made it more effective as a result of the added actions of repelling and/or killing mosquitoes.[81-83] The efficacy of this tool in preventing malaria is documented,[84] and it is one of the three primary interventions for effective malaria control. Consequently, the scaling-up of this intervention was believed to have made a substantial contribution in achieving the United Nations Millennium Development Goals.[85] However, recent evidence is mounting regarding the reduced effectiveness of this tool due to the development of insecticide resistance[86-88] and factors related to its utilization.[87, 89-95]

1.8.3. Combination of IRS and LLINs

The WHO questions the financial sustainability ("while potentially being very effective") of the broad deployment of IRS and LLINs in combination,[5] although evidence is lacking regarding the added value of using both tools compared to the application of each strategy separately. A mathematical model showed the interaction of the effects of both methods, which may result in a reduced efficacy of the tools when compared to being used alone.[96] Recent data has shown contradictory evidence, i.e. the absence[97] and presence[98-100] of the benefit of combining the two strategies, although these studies utilized different

methodologies. The combined effect of IRS and LLINs remains a subject for further scrutiny.[101-102]

1.8.4. Larval source management
Larval habitat manipulation (temporary)/modification (long-lasting) and chemical and biological larviciding have all been used as larval source management strategies in certain countries.[5] Unlike with IRS and LLINs, and despite the effectiveness of this strategy[103-104] to the extent of it providing malaria extinction in some regions of the world,[105] it was not taken as a core strategy to prevent and control malaria since its application was considered circumstantial, i.e. the larval habitat should be well-defined and relatively few.[5, 106-107]

1.8.5. Preventive chemotherapy
Preventive chemotherapy is recommended for pregnant women and infants in countries with a moderate-to-high/stable transmission of malaria. In addition, seasonal malaria chemoprevention for children 3–59 months old is recommended in areas with highly seasonal malaria transmission.[5, 108-110] The preventive chemotherapy recommendations are:

- Intermittent preventive treatment in pregnancy (IPTp) – providing Sulfadoxine-Pyrimethamine at each scheduled ante-natal care visit (each dose to be given at least one month apart) starting early in second trimester.[108]
- Intermittent preventive treatment in infants (IPTi) – providing three doses of Sulfadoxine-Pyrimethamine to infants along with the second and third Diphtheria-Pertussis-Tetanus and Measles vaccines.[109]
- Seasonal malaria chemoprevention (SMC) – providing Amodiaquine plus Sulfadoxine-Pyrimethamine, to a maximum of four doses, for children 3–59 months in areas where there is highly seasonal malaria transmission.[110]

Preventive chemotherapy is not included in the package of malaria prevention in Ethiopia because of the country's unstable malaria transmission, as well as the high level of resistance of *P. falciparum* to Sulfadoxine-Pyrimethamine.[111]

1.8.6. Diagnosis and treatment
Prompt diagnosis and treatment is required to reduce complications and death due to malaria.

The diagnosis of malaria entails:[112]

- Clinical diagnosis: Considered to be less specific and results in over-treatment since it primarily considers fever or a history of fever.

- Parasitological diagnosis: Performed with the help of light microscopy (gold standard) and rapid diagnostic tests (RDTs). Misclassification may arise in both methods, thus implying the need for strong quality assurance.[113]

The WHO strongly recommended the use of Artemisinin-based combination therapy (ACT) for uncomplicated falciparum malaria, which includes Artemether plus Lumefantrine, Artesunate plus Amodiaquine, Artesunate plus Mefloquine and Artesunate plus Sulfadoxine-Pyrimethamine, while for severe *P. falciparum* malaria intravenous Artesunate is the drug of choice. Chloroquine is effective against malaria infections caused by *P. vivax*, *P. ovale* and *P. malariae* species, and in areas where Chloroquine resistant *P. vivax* exists, ACTs (except Artesunate plus Sulfadoxine-Pyrimethamine) are recommended.[112] Ethiopia adopted ACT (Artemether plus Lumefantrine) starting in 2004.[5]

In light of the parasite resistance to monotherapies in most countries of the world, the change of regimen to ACTs was inevitable regardless of the countries' inability to afford the new drugs.[114-116] Despite the notion that malaria parasites are less likely to develop a resistance to ACTs[117] and its proved efficacy,[118-119] the recent development of resistance in some parts of the world calls for a concerted effort in the containment of resistance[120] and new drug development.[121-122] Unfortunately, the rampant circulation of counterfeit drugs in southeast Asia and sub-Saharan Africa complicates the problem, thereby highlighting the need for an urgent solution.[121, 123-124]

1.9. Future aspects of malaria epidemiology: "Shrinking the malaria map"

The reduction of the burden of malaria in high-transmission settings and the possible elimination in areas experiencing low transmission were believed to be realistic goals with the rapid scale-up of existing tools against malaria – given that these tools continued to be effective.[125] For achieving the goals, however, a continuous programme reorientation in accordance with the disease burden, including a commitment at all levels, a health system strengthening and the development of new intervention tools, is essential. The programme reorientation extends from 'control' to 'consolidation' (a high and stable malaria transmission

set-up), 'consolidation' to 'pre-elimination', 'pre-elimination' to 'elimination' and 'elimination' to the 'prevention of reintroduction'.[125-126]

Of 99 malaria-endemic countries, 67 are controlling- and 32 are eliminating malaria.[127] Malaria control refers to "reducing the disease burden to a level at which it is no longer a public health problem", whereas elimination was defined as "interrupting local mosquito-borne malaria transmission in a defined geographical area, that is, zero incidence of locally contracted cases, although imported cases will continue to occur. Continued intervention measures are required".[125]

Aiming at a regional elimination plus long-term global eradication, the Malaria Eradication Research Agenda (malERA) initiative was established in 2008 to come up with key research and development issues to support the global malaria action plan, which resulted in key research agendas including the need for new vector control approaches and vaccine development aiming at the interruption of transmission. A sustained commitment of all stakeholders and advancing the capacity of researchers from malaria-endemic countries were indicated as the way forward to change the dream to reality.[128]

Ethiopia is in the control phase of malaria,[5] and according to the Ethiopian National Malaria Strategic Plan (2010-2015), it is expected to achieve malaria elimination within specific geographical areas with historically low malaria transmission and a near zero malaria transmission in the remaining malarious areas of the country by 2015. Consequently, there is a plan to embark on malaria elimination in 2020 with an integrated community health approach.[129]

1.10. Rationale for this study

Predicting malaria epidemics, if possible, allows time for a preparation to employ preventive measures, in effect lessening the impact of the deadly disease.[130-132] Several attempts have been made to predict malaria, mainly relying on the biological relationship between malaria transmission and meteorological/climatic conditions.[21-26, 28-29, 133] Despite several studies documenting the existence of the link between malaria transmission and meteorological factors, inconsistencies exist – which could be attributable to the influence of local conditions.[31-39] This implies that a prediction using meteorological/climatic factors may

provide false alarms or be unreliable. Therefore, understanding the local variations helps in the search for other potential determinants of malaria transmission to develop better predictive models. Hence, the use of empirical data to show the link between local meteorological factors and the incidence of malaria - to elucidate the local variations - was essential [Paper I]. Meanwhile, studying both the climatic and non-climatic determinants of malaria transmission addresses the limitation of models employing only meteorological factors for malaria prediction [Paper II].[21-22, 34, 36, 41, 133-134]

A variability in the risk of malaria infection was observed within a micro-environment, thus making malaria a very local disease. This knowledge allows the interventions to hit the target – improving efficiency and effectiveness.[135-141] In Ethiopia, malaria transmission is mostly seasonal and unstable,[129] with the seasonal nature of the disease leading to epidemic situations unless prevention and control efforts are in place. However, the responsiveness of both the seasonal nature and variability in risk among the population of the sub-groups to the previously available prevention and control tools should be known. This information helps to increase the awareness of the effectiveness of the interventions and guide policy making, with Papers II and III addressing these issues.

One of the key strategies to prevent malaria transmission is the use of ITN,[85] although enquiries have been posed against the effectiveness of this strategy.[87, 142] The reported reasons that may contribute to the 'failure' of this intervention include problems related to consistent and proper use of ITNs due to several factors,[89-95] in addition to the insecticide resistance issue. To advance the benefits of this strategy, investigating ITN utilization among people at risk of malaria is imperative [Paper IV].

Chapter II: Objectives

2.1. General objective

The overall aim of the thesis is to assess the variation in malaria transmission, practice and impact of malaria prevention tools in southern Ethiopia.

2.2. Specific objectives

1. To find out whether variations in rainfall and temperature can consistently predict falciparum malaria incidence at different locations [Paper I];

2. To assess the effect of local meteorological and environmental conditions, indoor residual spraying with insecticides and insecticide-treated nets use at the individual- and community levels, as well as socio-economic and other individual-level factors on the incidence of falciparum malaria [Paper II];

3. To assess the effect of mass insecticide-treated nets distribution and indoor residual spraying with insecticides on the spatio-temporal clustering of malaria [Paper III];

4. To characterize the pattern of- and assess the factors related to insecticide-treated net use [Paper IV].

Chapter III: Methods

3.1. Study locations

The study locations are found in the Southern Nations and Nationalities Peoples' Region (SNNPR), which is one of the administrative regions of Ethiopia, with the total population of the region estimated to reach 16.5 million in 2010.[143] Over 45 ethnic groups (56% of the more than 80 ethnic groups of Ethiopia) are indigenous to this region, which has 14 administrative zones, 131 districts and 22 city administrations. There are 3,602 rural and 324 urban Kebeles (a Kebele is the lowest administrative structure). Of the 110,931.9 sq.km area of the region, 57.4%, 34% and 8.6% are regarded as hot and semi-arid-, tropical sub-humid- and tropical humid agro-ecologic zones, respectively.

Data from 33 health centres and two hospitals with 35 nearby meteorological stations (altitude ranging from 1,182 to 2,582 metres above sea level) were used in Paper I. In the meantime, the details of malaria epidemiology were also studied in the Chano Mille Kebele, the Arba Minch Zuria District and the Gamo Gofa Zone [Papers II, III and IV]. The Arba Minch Zuria District is one of the 54 malaria hot-spot districts in the region. Chano Mille Kebele is one of the 11 malarious Kebeles in the district, and is located 492km to the southwest of Addis Ababa at an altitude of 1,206 metres above sea level. The southeast boundary of the Kebele is Lake Abaya, which is surrounded by swampy areas. Moreover, many hoof prints from cattle and hippopotami in the swampy areas produce small, sunlit and shallow water bodies favourable for the malaria vector life cycle. The main source of income of the residents is agriculture (primarily maize, banana and mango), which is supported by an irrigation scheme (made from concrete) running from the west. Our first census in April 2009 enumerated 7,038 residents (1,212 households) in a 2.4 sq.km area. The Kebele has one health post (run by a health extension worker) providing preventive services, in addition to diagnostic (with RDT kits) and curative (with Co-Artem) services for malaria. The health post is located at $6^{°}6.666'$ N and $37^{°}35.775'$ E, with Figure 2 showing the geographic coordinates of the study sites:
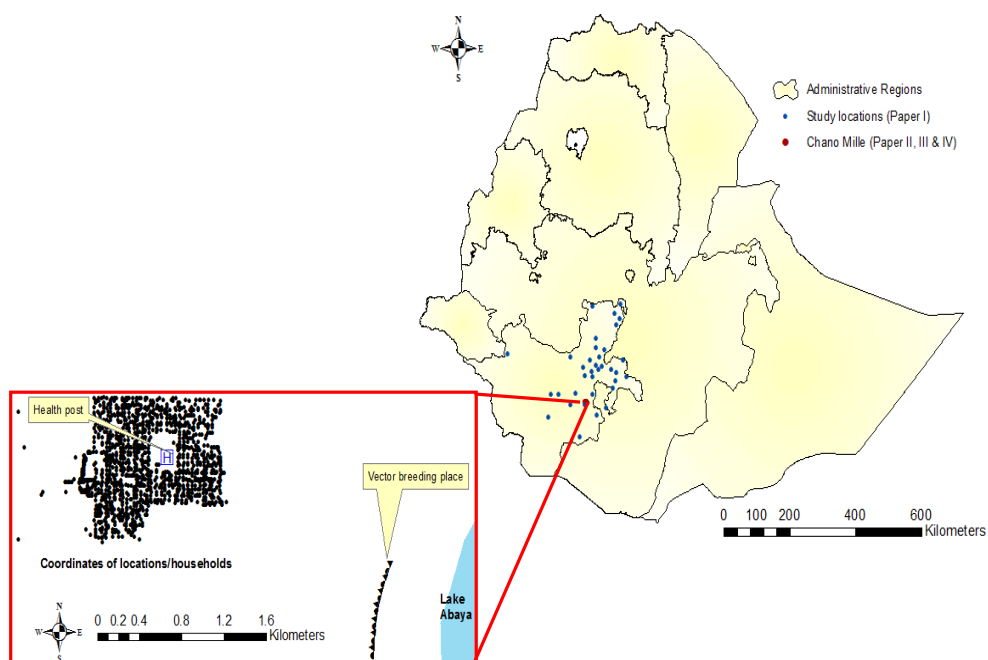
Figure 2: Map of Ethiopia showing the geographic coordinates of the study locations

3.2. Study design and data

A retrospective study [Paper I] was conducted using historical data of the incidence of falciparum malaria and local meteorological variables from 35 locations, and data were collated from 42 locations. The minimum serial length required for a time series analysis is 50,[144] and this excluded datasets from five locations, while datasets from two locations were dropped due to missing data exceeding 15% of the total observations. The inclusion of meteorological variables was determined by the availability of records. Three meteorological stations recorded temperature, rainfall and relative humidity; 14 stations recorded temperature and rainfall, and the remaining 18 stations recorded only rainfall. Meteorological data were obtained from the southern branch office of the Ethiopian Meteorological Agency, Hawassa, and falciparum incidence data were obtained from the SNNP Regional Health Bureau, with both datasets spanning from 1998 to 2007. A total of 210,659 microscopically confirmed falciparum malaria cases were reported from the 33 health centres and two hospitals during the 10-year study period.

For Papers II, III and IV, a prospective open cohort study design was employed. Chano Mille Kebele was purposely because it was of interest to see malaria epidemiology in a more focused manner in the presence of an irrigation scheme and a nearby lake. All residents of the Kebele were included in the study and were followed weekly for two years (101 weeks) from April 2009 to April 2011. Each household was given an identification card with a number corresponding to a unique number printed on a metal plate, and posted on the main entrance of each house. Subsequently, the geographical coordinates of each house were recorded with a handheld GPS apparatus with an accuracy of ±5m.

A census was conducted three times – at the beginning, in the middle (Week 50) and at the end of the study, and the total number of the study participants was 8,121 in 1,388 households. We used both active and passive surveillance schemes, and each week, a data collector visited each household to collect data on ailments (fever, cough and diarrhoea) from the last seven days preceding the date of interview and wrote down the names of household members who slept under the ITN the night before the interview. The data collector also asked for any febrile case in the household at the time of the visit and when present, measured the axillary temperature – if it was ≥ 37.5 degree Celsius, the case was referred to the health post to be diagnosed and treated (active surveillance). A mechanism was also designed to cross-check whether the referred case went to the health post on the same day. In the meantime, residents were consistently advised to self-report (with an identification card) to the health post whenever they developed a fever between the weekly visits (passive surveillance). Blood samples were taken at the health post using the appropriate techniques. For the sake of treating the patient, RDT kits were used, and the laboratory technician (specifically hired for this research) prepared thick and thin blood films using WHO guidelines.[145] Two senior laboratory technologists made microscopic examinations of 2,573 blood slides collected from febrile cases, and when a discordant reading was found, a confirmation by a third reader was sought. All readers were unaware of each other's readings.

During the study period, the government interventions were recorded. IRS with DDT was carried out in Week 7 (June 2009), the free distribution of LLINs was done during Week 48 (March 2010) and IRS with Deltamethrin was done in Week 63 (July 2010). Post-intervention surveys (during weeks 23, 50 and 65) were done to measure coverage and the

practice of re-plastering the sprayed surfaces and to count the number of freely distributed LLINs to each household [Figure 3].
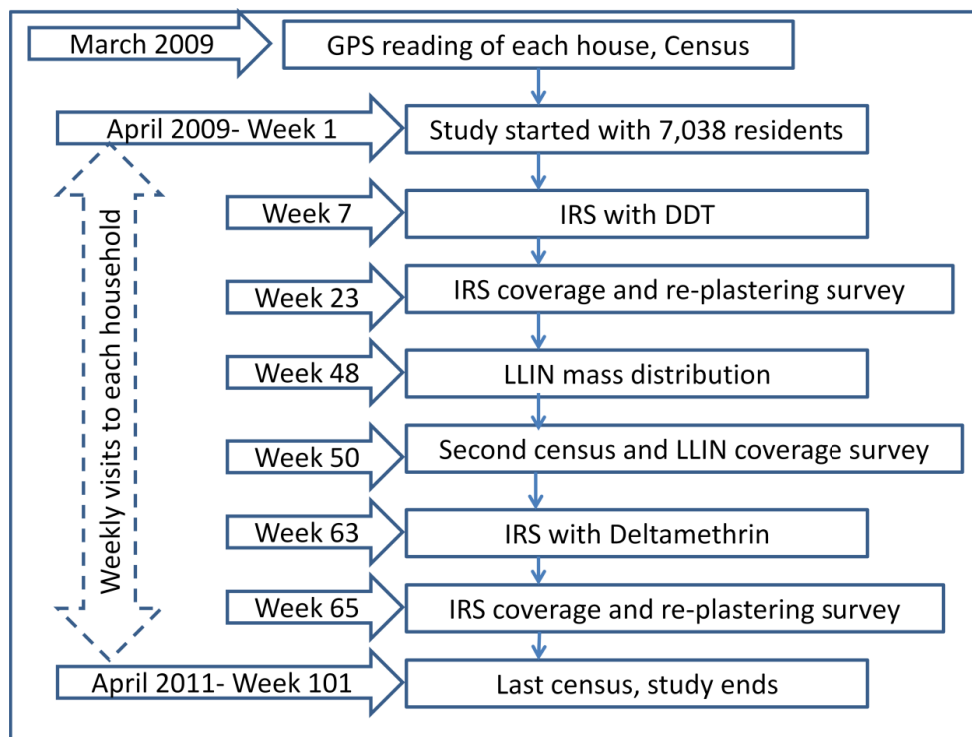
Figure 3: Timeline of major events of the Chano Mille study [Papers II, III and IV]

3.3. Statistical analysis

A time series analysis [Papers I and II] was carried out using an autoregressive integrated moving average (ARIMA) model.[146] The non-seasonal (*p,d,q*) and seasonal (*P,D,Q*) ARIMA orders were interpreted. To make room for the predictor series, in addition to the univariate ARIMA, transfer function (TF) models[144] were constructed. Likewise, with the ARIMA orders, there are both non-seasonal and seasonal TF orders: *numerator*, *denominator* and *difference*. We used an R squared coefficient of determination to assess the goodness of fit of the models. For a differenced data series, a stationary R squared was used. A Ljung-Box Q statistic was employed as a model diagnostic tool – models were accepted provided that the Ljung-Box Q statistic had a P value >0.05. The Expert modeller method was applied in Paper I, and user-specified/custom ARIMA and TF models were employed in Paper II.

A principal component analysis (PCA)[147-148] was used to construct a wealth index using 15 socio-economic variables, including the main material of the floor, wall and roof [Paper II]. A generalized Poisson log-linear model was used to predict malaria episodes [Papers II and III], and to deal with over dispersion, a negative binomial probability distribution model was used to predict ITN use [Paper IV]. To take into account the repeated measurements (weekly data), a generalized estimating equation (GEE) was used with a logit link function to predict falciparum malaria [Paper II]. Additionally, pair-wise comparisons were also carried out for the different age categories [Papers II and IV].

SPSS 17 [Paper I] and PASW 18 [Papers II, III and IV] (Chicago, IL, USA) were used for data analysis. Statistical significance was set at a P value <0.05, and an incidence rate ratio (IRR) with a 95% confidence interval (CI) was reported. Next, a distance from each household to the identified malaria vector breeding place was calculated using the proximity analysis tool of ESRI ®ArcMap[TM] 9.3 (Redlands, CA, USA) [Papers II, III and IV]. To incorporate the number of households between a household and malaria vector breeding site (household count) into a generalized Poisson log-linear model, we used R[149] to make the count at different search angles. A search angle of 1° did not show multicollinearity with a variable "distance from the vector breeding site", and was used in the multivariate model [Paper III].

For a spatial and space-time statistical analysis [Paper III], we used SatScan v9.1.1 (http://www.satscan.org/). A discrete Poisson based model was applied, and we employed 9,999 Monte Carlo replications. A combination of standard Monte Carlo, sequential Monte Carlo and Gumbel approximations yielded P values,[150] and a circular window with various spatial cluster size restrictions (50%, 35%, 25% and 15%) was used – searching for areas of high rates.

Table 1 presents a summary of major statistical methodologies employed in this thesis:

Table 1: Major statistical methods used for analysis

Statistical Method Used	Paper
Autoregressive Integrated Moving Average; Transfer Function	• Paper I (Serial length: 51–118) • Paper II (Serial length: 95)
Generalized Estimation Equation	• Paper II (N= 582,846)
Generalized Poisson Log-linear	• Paper II (N=8,121) • Paper III (N=8,121)
Space-time: Discrete Poisson	• Paper III (9,999 Monte Carlo replications)
Negative Binominal Probability Distribution	• Paper IV (N=8,121)
Principal Component Analysis	• Paper II
Descriptive statistics	• Paper I • Paper II • Paper III • Paper IV

3.4. Ethical considerations

The Regional Health Research Ethics Review Committee of the SNNP Regional
Health Bureau approved this research. Permission was sought from local
administrators, while informed verbal consent was obtained from all participants. For
minors, consent was obtained from caregivers or legal guardians. Using a national
treatment guideline,[151] malaria cases were treated immediately at the health post based
on the RDT result. During the study period, Co-Artem was supplied by the
government for the treatment of falciparum malaria, and Chloroquine was supplied by
this research project for the treatment of vivax malaria.

Chapter IV: Results

Paper I: Model variation in predicting *P. falciparum* malaria incidence

Thirty-five datasets qualified for the analysis, and time series modelling was carried out using TF models and univariate ARIMA when there was no significant predictor meteorological variable. Of the 35 models, five were discarded because of the significant value of Ljung-Box Q statistics. Past *P. falciparum* malaria incidence alone (17 locations) or when coupled with meteorological variables (four locations), was able to predict *P. falciparum* malaria incidence within statistical significance. All seasonal ARIMA orders were from locations at altitudes above 1,742 m. Monthly rainfall and minimum and maximum temperature was able to predict incidence at four, five and two locations, respectively. In contrast, relative humidity was not able to predict *P. falciparum* malaria incidence. The R squared values for the models ranged from 16% to 97%, with the exception of one model, which had a negative value. Furthermore, models with seasonal ARIMA orders were found to perform better.

Meanwhile, the average rainfall data of 23 locations resulted in monthly rainfall being a significant predictor at a lag of four months coupled with an autoregressive order of 1 (monthly rainfall was a significant predictor only in four locations when the datasets were analysed separately). The stationary R squared of this model was 67%. This model structure was applied to each of the 23 locations, but did not produce any significant results.

The models for predicting *P. falciparum* malaria incidence varied from location to location, as well as among lagged effects, data transformation forms, ARIMA and TF orders. Variability in the models was principally attributed to regional differences, and a single model was not found that fit all locations. Lastly, past *P. falciparum* malaria incidence appeared to be a better predictor than meteorology.

Paper II: Predictors of *P. falciparum* malaria incidence

The potential effects of local meteorological and environmental conditions, IRS with insecticides, ITN use at individual and community levels and individual factors on *P. falciparum* malaria incidence were assessed.

There were 317 microscopically confirmed falciparum malaria episodes over a period of two years, of which 29.3% occurred among temporary residents. The incidence density was

3.6/10,000 person-weeks of observation, and we observed a higher malaria incidence among males, children 5–14 years of age, ITNs non-users, the poor and people who lived closer to vector breeding sites. Rainfall increased and IRS with Deltamethrin reduced the falciparum incidence. Although ITNs prevented falciparum malaria for the users, we did not find that free mass ITNs distribution reduced falciparum malaria on a village level.

Paper III: Effect of prevention tools on spatio-temporal clustering of malaria

The total number of both types of malaria episodes analysed was 622, yielding 45.1 episodes per 1,000 persons per year; among these, episodes of *P. falciparum* and *vivax* infection numbered 316 (22.9 per 1,000 per year) and 306 (22.2 per 1,000 per year), respectively. IRS with DDT, and later with Deltamethrin and free mass distribution of ITNs, were carried out during the study period. There was space-time clustering of malaria episodes at a household level. The spatio-temporal clustering of malaria was not influenced by the free mass distribution of ITNs; however, the time span of the spatio-temporal clustering of malaria cases ended after IRS with Deltamethrin. The presence of clusters on the southeast edge of the village was consistent with the finding of an increasing risk of acquiring malaria infection for individuals who lived closer to the identified vector breeding site.

Paper IV: Freely distributed bed net use

The total number of ITNs available at the beginning of the study was 1,631 (1.68 ITNs per household). In Week 48, 3,099 new ITNs (PermaNet2.0) were freely distributed (2.3 ITNs per household), and the number of households who received at least one new ITN was 1,309 (98.4%). The percentage of children <5 years and pregnant women not using ITNs exceeded that of other adults. The mean (range; standard deviation) ITN use fraction before and after mass distribution was 0.20 (0.15-0.27; 0.03) and 0.62 (0.47-0.69; 0.04), respectively. Before mass ITN distribution, the most frequent reason for not using ITN was having worn out the bed nets (most complained the bed nets were torn by rats), and after mass ITN distribution, there was a lack of convenient space to hang more than one ITN. Males, younger age groups (mainly 15–24 years) and those living away from the vector-breeding site were less likely to use ITNs. The ITN use fraction reached to a maximum of 69% despite a near universal coverage (98.4%) being achieved.

Interrater agreement

The interrater agreement of microscopic readings of the first two readers was checked with Kappa statistics, and a better agreement was achieved in the readings of the vivax- than in the falciparum species, 0.87 *versus* 0.80, respectively [Table 2]. All of the discordant readings were confirmed by a third reader.

Table 2: Interrater agreement for the readings of 2,573 microscopic slides

		Second reader					
		P. falciparum			*P. vivax*		
		Positive	Negative	Total	Positive	Negative	Total
First reader	Positive	255	49	304	265	41	306
	Negative	59	2,210	2,269	31	2,236	2,267
	Total	314	2,259	2,573	296	2,277	2,573
Kappa		0.80			0.87		
95% CI for Kappa		0.76–0.84			0.83–0.90		
Interpretation[§]		Good agreement			Very good agreement		

[§]Strength of agreement[152]: <0.2 = Poor, 0.21–0.40 = Fair, 0.41–0.60 = Moderate, 0.61–0.80 = Good, and 0.81–1.00 = Very Good

Chapter V: Discussion

5.1. Methodological discussion
Study design

A retrospective study [Paper I] is an efficient way of driving information. Nonetheless, a lack of full control over quality, completeness and potential confounders limits its use.[153]

With the objective of measuring the incidence of malaria, we employed a prospective cohort study design [Papers II, III and IV]. The word cohort refers to a group of people, and a cohort study is defined as tracking people forward in time from exposure to outcome. The temporal sequence between the exposure and outcome is very clear, and it is also possible to provide a risk of developing the outcome if exposed to a factor of interest. It also gives a chance to document a change in exposure variables across time, though the costly nature of the study, an inability to minimize lost-to-follow up, being unsuitable for studying rare outcomes and having less control over confounders (than randomized controlled trials) are among the limitations.[154-155] The longitudinal nature of the study design helped us to see the effects of preventive tools, and since the practice of ITN use changes overtime, it was possible to document these changes and introduce their lagged effects to the outcome of interest.

Sample size

The nature of the time series (seasonality, non-stationarity), the autoregressive and moving average orders, the presence of outliers and data transformation (differencing) determine the sample size in a time series modelling, which is referred to as the serial length. On some occasions, a sample size of less than 50 may provide adequate power, while in certain circumstances a sample size of even more than 150 may not do so. Therefore, as a general rule of thumb, a time series modelling should be applied if the serial length is greater than 50 observations,[144] which was the reason to drop five datasets since they did not qualify with this criterion. The minimum serial length we considered for modelling was 51, with the maximum being 118 (on monthly time scale). However, given the presence of retrospective data of a longer duration, we believe that we might have developed models that better captured important structures in the data such as seasonality. Meanwhile, 16 (of 35) locations exhibited a bimodal rainfall pattern that could 'double' the serial length, hence improving the capability of the models to capture seasonality [Paper I]. Besides the need to have a longer serial length, modelling malaria may also be affected by the time scale employed, which is

primarily due to the frequent fluctuation of the exogenous series. Studies recommended using a shorter time scale, e.g. a week, to improve the sensitivity of the models to show the association between falciparum malaria and meteorology.[22, 24, 26] This was addressed by the Chano Mille study, in which we measured both the exogenous and endogenous series on a weekly time scale [Paper II].

We did not calculate a sample size in this thesis – we included all residents of Chano Mille as study participants, and a total of 8,121 individuals were followed for 101 weeks, thus yielding 627,172 person weeks of observation. We believe that this sample size was adequate enough, as it produced statistically significant results for most of the expected predictors [Papers II, III and IV] – leaving no reason to do post-hoc power calculations.[156-157]

In a SatScan, Monte Carlo replications are said to be the computer-simulated random replications of the data set under the null hypothesis assumption; and to ensure an adequate power of the test, the recommended number of Monte Carlo replications is 999.[150] Though it was time consuming, we used 9,999 Monte Carlo replications to achieve more power in identifying areas of high malaria risk within the perimeter of the study location [Paper III].

Internal validity

Internal validity refers to conclusions about the studied participant being true, and can be evaluated with regard to the role of selection bias, information bias and confounding.[154] Moreover, studies without adequate power may also result in a statistically non-significant output even if there exists a real effect – thereby compromising the internal validity.

Selection bias

Selection bias arises in the process of selecting the study subjects. We studied all residents in the Kebele [Paper II, III and IV], thus there was no selection bias. All of the residents voluntarily participated in the study for the entire period. However, there was no mechanism to include newcomers to the study area the moment that they joined the cohort; as a result, these newcomers were not followed for some of the time they spent in the study area. Considering the observed unexpected population movement, we did a census in the middle (Week 50) and at the end to update our denominator. In addition, those newcomers enumerated at Week 50 were included in the study, which helped to incorporate potential risk factors of malaria transmission among the in-migrants.

Information bias

Information bias refers to whether the information is gathered from the study subjects in the same way regardless of their exposure status,[158] the presence of measurement error[154] or both. In this thesis, a measurement error could arise while diagnosing, documenting and reporting falciparum malaria cases, documenting and reporting meteorological variables [Paper I], interviewing the study participants during census and weekly visits [Papers II, III and IV] and preparing and reading microscopic slides [Papers II and III].

For Paper I, we relied on secondary data sources; hence, we admit that the quality of data obtained through the routine recording and reporting system in developing countries is of poor quality, mostly due to underreporting. We used only those cases with microscopically confirmed falciparum malaria, although observer error may lead to misdiagnosis. Nevertheless, we considered that the presence of basic elements in the data such as trend, seasonality and monthly variations would suffice for the modelling exercise. In addition, we strictly used the Ljung-Box Q statistics as a model diagnostics to accept or drop the model – that led to ignoring five models. This statistic provides an indication of whether the model was correctly specified with regard to the presence of structure in the observed series, which was not accounted for by the model.

Publication bias arises when researchers tend to report only positive findings.[153] In our case, publication bias is unlikely since we reported all types of models [Paper I], including models with a positive or negative result with regard to the relationship between the incidence of falciparum malaria and meteorological factors. Additionally, the reported models had an R squared coefficient of determination ranging from a negative value (worse than the baseline model) to as high as 0.97.

Original data were collected for Papers II, III and IV, and we used a pre-tested (in the neighbouring Kebeles) data collection format for the census, as well as well-trained data collectors and supervisors. The weekly ITN use (the night before the interview) data was based on self-reporting. In the first four weeks of follow-up, we asked about ITN use with a 'Yes/No' type of question; however, realizing that this approach was more predisposed to bias starting from week 5, we wrote down the names of the household members who slept under the bed net the night before the interview. This approach (asking whether an individual has slept under the bed net to measure ITN use), though used by others as well,[90] could not be

free from bias. Even so, the fact that the maximum ITN use fraction (69%) not approaching the ITN coverage (98.4%) shows that social-desirability bias was less worrisome. Observing household members while sleeping under the bed net in the middle of the night without prior notice may provide unbiased information, but is not ethically justified.

We employed standard procedures to prepare microscopic slides,[145] and two experienced laboratory technologists read the slides independently [see Table 2 under results], while microscopic slides with discordant readings were checked by a third reader. In Papers II and III, a malaria case is defined as an individual with microscopically confirmed malaria parasites by at least two experienced laboratory technologists.

An edge effect results in a biased risk estimate, which has been a concern in spatio-temporal analyses given that the clusters are observed at the perimeter of the study location and the absence of data on the adjacent area.[159-161] In Paper III, we reported that malaria cases were clustered at the edge of the village. However, the adjacent area was also part of the Kebele with no residential houses – an agricultural land extending to the shore of the Lake Abaya, which could rule out the existence of an edge effect.[161-162]

Confounding

Confounding is a blurring or mixing of effects, as the researcher measures the effect of a third hidden factor - a confounder - while attempting to establish an association between the exposure and the outcome. Therefore, it is required to control for potential confounders using the available statistical methodologies,[154, 158] and we used multivariate techniques to address this issue. For instance, the effect of meteorological factors was controlled for preventive measures and lagged falciparum incidence [Paper II]. In addition, the effect of preventive measures was controlled for socio-demographic and economic characteristics, proximity to the vector breeding site [Paper II and III] and the number of households between a household and the vector breeding site [Paper III]. Moreover, the effect of gender was controlled for age and distance from vector breeding site in the study of factors associated with ITN use [Paper IV].

Chance

Statistical significance tests are designed to rule out the role of chance. A P value cut-off point <0.05 and a 95% CI that does not cross a null hypothesis value are used to make

statistical decisions – rejecting the null hypothesis. The 95% CI is said to be more informative since it holds the effect size,[163-164] and that the width of the interval also signifies the power of the test.[157] Our data consistently revealed statistical significance while employing different statistical models, e.g. a Poisson log-linear and generalized estimating equation with a logit link function. The existence of space-time malaria clusters (with high risk) on the side of the vector breeding site was confirmed by an increasing risk (with different analyses approaches) in malaria incidence for the households near the vector breeding site. We did separate analyses for *P. falciparum* [Papers I, II and III], *P. vivax* and for both species [Paper III], and we also did pair-wise comparisons for different age categories [Papers II and IV].

External validity
External validity refers to the generalizability of the research findings to the people outside the study area.[154] We considered only those locations with available datasets (long enough to exercise time series modelling) of malaria incidence and meteorological factors, which did limit us to select locations randomly, hence compromising generalizability. However, we included qualified datasets of varying altitude ranging between 1,182 and 2,582 metres above sea level with the intention of showing how models could differ across locations, thereby questioning the use of general predictive models [Paper I].

For the sake of detail scrutiny on malaria epidemiology in the presence of irrigation schemes, a nearby lake and routine prevention practices, we carried out a study in Chano Mille Kebele for two years [Papers II, III and IV]. Chano Mille is a resettlement area where residential houses are built close to each other; however, the way of living is typical of rural areas in southern Ethiopia. The data from this Kebele could reflect the malaria transmission dynamics in similar setups, and the findings on the use and impact of prevention strategies could help shed light on the existing challenges in malaria prevention and control efforts.

5.2. Discussion of main findings
Forecasting or the early detection of epidemics lessens the impact of malaria epidemics, primarily in regions where there is a low or unstable transmission, including the country where this study was conducted: Ethiopia. And such a justification led to the establishment in 2001 of MEWS, which uses three main groups of indicators (vulnerability, transmission risk

and early detection), and was expected to greatly support the public health system to better prevent (and control) deadly epidemics.[73] However, the effectiveness of MEWS is governed by the availability of quality data, robust modelling strategies and functional health system. Transmission risk indicators, including the use of unusually high rainfall to predict malaria epidemics, were not always successful – thus exhibiting local and temporal variations.[27, 31-39] This questioned the use of general or regionally based predictive models, and implied a need for a continuous effort to add a piece of information to the existing knowledge base.[32]

We attempted to show the local variations in the link between falciparum malaria incidence and meteorological factors using historical data from different locations of varying altitude, and also their averaged effect. The models we reported showed the presence of a link between malaria incidence and meteorological factors, though not in the majority of the locations, thus imposing a shift of emphasis to other potential risk factors. It is worth noting that the biologically driven link of meteorological factors and malaria incidence[165-166] may not always be direct or predictable.[167] Although they were limited by providing a shorter lead time,[73] our models favoured the role of early detection (rather than meteorology) since 21 of 30 models revealed the significance of the lagged effect of falciparum malaria incidence.

Using only meteorological factors, the prediction of malaria incidence exhibited inconsistent results. This implied the need to consider other determinants, including prevention and control measures, as well as socio-demographic and economic factors.[34, 36, 41, 134] The effect of local meteorological factors was evaluated by controlling for malaria prevention and control interventions and also past disease incidence, while we also investigated the net effects of environmental factor (the proximity of each household to the malaria vector breeding site), socio-demographic and economic factors (age, sex, education of the household head and wealth index) and the practice of sleeping under ITN using different statistical modelling strategies.

The vector breeding site was identified in the perimeter of the study site at the shore of Lake Ababa. This extensive swampy area – while serving as a grazing field, also nurtures the deadliest mosquitoes. The impact increases during the rainy season due to overflow (and later contraction) of the lake, hence resulting in an extended effect of rainfall on malaria epidemiology. The proximity of the household to the identified vector breeding site was the strongest risk factor for malaria in the study area, and in all approaches of analysis the effect

was consistent. This implies the need to consider larval source management as equally (even more) as the other routinely practiced prevention tools - ITN and IRS - in such locations.

Studies reported that housing structure is an important determining factor for malaria transmission that needs to be considered in malaria prevention and control efforts.[168-171] We did not analyse the effect of housing structures separately; however, we incorporated the housing structure while constructing a composite wealth index – three of the 15 variables used to construct the wealth index were of the housing structure. Our findings showed an inverse relationship between wealth index and malaria incidence, thus implying that those who lived in the impoverished houses had more of a risk of malaria infection controlling for other factors.

Studies have shown that the risk of malaria infection varied according to gender. Some studies have reported more of a risk among males than females,[50, 172] but a review of earlier studies reported the risk being equal for both genders.[51] Our study revealed that females had a lower risk of malaria infection with the falciparum species (with no significant difference for the vivax species). Furthermore, ITN use among females consistently exceeded that of males for the entire study period with statistical significance. Even so, the fact that we observed less a risk of malaria among females being controlled for other factors, including ITN use, implied the need to look for further explanation.

In regions with stable malaria transmission, children less than five years of age (and pregnant women) suffer more from malaria infection than the other population segments,[51] which may be due to an acquired immunity to *Plasmodium* parasites.[173-176] It was difficult to categorize the study area under "stable malaria transmission" since we observed seasonality, though we observed a higher incidence of malaria among the younger age group. The seasonal nature of malaria transmission in the study area may not allow for a continuous exposure to the parasite, yet still might have offered some immunity to the older population,[175] but we did not measure this. The disparity in ITN utilization among different age categories may play a role in the different incidence rate ratios for different age categories, e.g. children aged 5–14 years were the least of the ITN users [Paper IV] and suffered more from malaria [Papers II and III]. In addition, the observed age shift with regard to the risk of malaria infection after mass ITN distribution and before IRS with Deltamethrin (a greater risk was observed in the lesser ITN

using age group between 15–24 years old than the risk observed before the distribution) substantiated this finding [Paper II].

The population movement both to and from the study site was unexpectedly high, and the inability to characterize temporary in-migrants was unfortunate. Nonetheless, a higher incidence of malaria was observed among these temporary residents, which implied a need to consider the role of population movement on malaria epidemiology in such studies. This is because the in-migrants may have a role in malaria transmission given that their original domicile is less malarious and that their practice of prevention strategies is low.[55]

A disparity was observed between ITN coverage and use, and different determinants of ITN use were also identified. The residents of Chano Mille living closer to the identified vector breeding site reported a higher use of ITN, and this may imply that the presence of nuisance mosquitoes, the risk perception or both were motivating factors for sleeping under ITNs. The older residents used ITNs more frequently than the younger ones despite the high malaria risk carried by the latter group – which may be due to a less frequent use of ITN, a lack of acquired immunity or both. Such disproportions in ITN use and a higher malaria risk among the younger population may bring the advocacy of "ITN use by the vulnerable" back to the table for such localities. Meanwhile, the inability to hang more than one ITN due to the absence of a convenient space in the house reflected that calculations of ITN coverage - the number distributed over the total population - were unrealistic. It also implies a waste of resources, the need to do a prior assessment of the housing structure and post-distribution 'hang-up' campaigns.[177-178] As most residents of the study site reported, the presence of rodents may compromise the usable of life of LLINs – three years in field conditions,[85] which requires attention.

Our results showed that sleeping under an ITN was protective at the individual level; nevertheless, it was incapable of protecting the community at large, and the simple logic of claiming that a considerable proportion of the community receives protection through ITNs (e.g. 35%–65% ITN coverage), thus protecting the community at large,[179] could not work. This may be explained by the inherent nature of the ITNs – a balance between the excito-repellency *versus* the insecticidal properties. If excito-repellency wins out, then ITNs will act as a risk factor for the non-users.[142, 180] The different statistical models we used consistently showed that following the mass distribution of ITNs, there was no significant impact on

malaria epidemiology in the study area. Therefore, we suggest that the expected community-wide benefits of ITNs can be achieved if and only if the insecticidal effect of ITNs surpassed the excito-repellency, with a fairly high rate of not only ITN coverage, but also of consistent use.

The study period was long enough to evaluate the effectiveness of different strategies employed to prevent and control malaria, and it was evident that IRS was more effective than ITN mass distribution in terms of providing a community-wide benefit. However, the effectiveness of IRS was a function of the insecticide resistance level since IRS with DDT was not as effective as IRS with Deltamethrin. This finding was consistent with the report from the same study site (and period), which demonstrated a greater efficacy of Deltamethrin compared to DDT, 47% *versus* 10%, respectively.[18] Even so, the fact that the IRS with Deltamethrin (the same insecticide was used to coat the ITNs – PermaNet2.0[181]) reduced the transmission of malaria indicated the effectiveness of the strategy, rather than the efficacy of the insecticide per se.

We observed malaria to be a very local disease, such that significant risk differences within a small area characterize its transmission. A segment of a 2.4 sq.km area of the study site carried a significantly greater risk, and it was found near the identified vector breeding site. Such an area was described as a "hot-spot"[182] – a proof of heterogeneity in the risk of malaria transmission within a micro-environment. Identifying such a place may be an advantage so as to organize meagre resources for a focused, efficient and effective utilization.[135, 140, 183-185] In the meantime, we observed that the increased risk in the identified "hot-spot" was aborted by IRS with Deltamethrin, but not by the mass distribution of ITNs.

The burden of malaria in Chano Mille was almost equally shared by the two *Plasmodium* species – vivax and falciparum. However, we had expected falciparum malaria to dominate, whereas others reported a greater proportion of vivax malaria in some locations in the country.[186] This implies the need to duly consider both species while deploying rapid diagnostic tests and antimalarial drugs in similar locations. An emphasis was sought to avert the neglect of vivax malaria[187] since there may not be significant gains to be achieved if strategies target only the falciparum species in areas where a significant contribution of the vivax malaria is documented. We had to provide Chloroquine for vivax malaria during the entire study period, but recently Chloroquine has been available at the health post level.

5.3. Implications for policy

Malaria epidemics threaten the health system by imposing immense financial and logistic constraints, and deaths due to malaria are common during epidemics. While a near zero death rate due to malaria is envisaged by the year 2015, the importance of a malaria early warning system that informs about the occurrence of epidemics in advance is unquestionable. Still, the application of meteorological factors in an early warning system should take local variations into consideration, while using past disease incidence as an early detection indicator may be helpful.

The choice of malaria prevention strategies should be tailored to the pattern of malaria transmission in the specific setups. There is also a need to evaluate the cost-effectiveness of different strategies in different scenarios, as the move towards 'blanket coverage' by interventions such as LLINs may not guarantee future success. In our case, the mass distribution of LLINs did not prove significant gains. In addition, there is a need to opt for other strategies such as larval source management. Such a focused move may address the very local nature of the disease, and targeting the 'hot-spots' may become an essential part of prevention and control efforts than a 'one-size-fits-all' approach. A particular locality may be labelled as a 'hot-spot' or 'malaria risk' area, but may have a 'hot-spot' or what is sometimes called a 'micro-cluster' within itself. Hence, the interchangeable use of the terms 'malaria risk', 'hot-spot', 'cluster' and 'micro-cluster' should be revisited. In this thesis, we considered 'hot-spots' to refer to groups of households that have an increased risk of malaria infection within a malaria risk area.

The observed disparity in LLINs' coverage (98.4%) and use (maximum 69%) implies that the health system may not need to rely on the number of LLINs distributed, while there is also a need to establish a mechanism by which the continuous monitoring of the use of LLINs by the beneficiaries is practiced. Differences in the risk of malaria infection were attributable to different levels of ITN use among the study participants (among other factors), as adults 24 years and above were favoured more than the younger population and pregnant women. Equity in use may need to be addressed to enhance the effectiveness of this strategy. Additionally, factors compromising the expected usable life of the LLINs under field conditions should be addressed; otherwise, the functionality of the LLINs may not go with the distribution schedule.

Despite the lower level of efficacy of Deltamethrin,[18] IRS with Deltamethrin significantly reduced the malaria burden in the study area. The fact that IRS (unlike the ITNs) provides protection to all members of a household compels us to opt for this strategy if the continuous and consistent use of ITN by all members of the household cannot be assured.

Chapter VI: Conclusions and Recommendations

6.1. Conclusions

- Linked with meteorological data, the models of *P. falciparum* malaria incidence revealed local variations and that a single model was not found that fit all locations. Past *P. falciparum* malaria incidence was a superior predictor than meteorology, and there is also a need for the inclusion of non-meteorological factors in malaria modelling.

- The malaria incidence rate was higher among males, children 5–14 years of age, ITNs non-users, the poor and people who lived closer to vector breeding sites. Rainfall increased, and IRS with Deltamethrin reduced falciparum incidence. ITNs provided personal protection, but free mass ITNs distribution did not reduce the malaria burden.

- There was a spatio-temporal clustering of malaria of both species, consequently showing that the risk of getting a malaria infection varied significantly within one village. The free mass distribution of ITNs did not influence the spatio-temporal clustering of malaria, although IRS might have eliminated malaria clustering.

- The coverage of ITN was 98.4%, but the ITN use fraction did not go beyond 69%. Gender, age differences and distance from vector breeding site were associated with ITN use. A lack of convenient space to hang more than one ITN (for those receiving more than one) and factor compromising the usable life of ITNs were reported.

6.2. Recommendations

Operational

- The mass distribution of ITNs should be accompanied with 'hang-up' campaigns, regular follow-up on utilization and measures to prolong the usable life of ITNs.
- Education campaigns should emphasize an equity of ITN use within the household and, prioritize the vulnerable (younger and pregnant women) whenever it is not possible to hang more than one ITN in the house, in which one ITN could not accommodate all family members.

- The use of historical data to learn future trends of malaria transmission should be strengthened.
- The identification of 'hot-spots' of malaria transmission should receive more attention, and the need of using alternative strategies such as larval source management should be assessed.

For policy

- The use of IRS for the prevention and control of malaria should be strengthened.
- Mechanisms to narrow the gap between ITN coverage and utilization should be an integral part of mass distribution campaigns.
- The choice of prevention and control strategies may benefit from prior and detail knowledge of local conditions.
- The burden of vivax malaria should be duly considered.

For research

- The role of population movement in malaria transmission and its implications for prevention and control efforts should be studied.
- Studies should assess the effectiveness of ITNs at a community level and the very reasons for not sleeping under the net while having one.
- Data are required to inform the cost-effectiveness of targeted intervention on malaria 'hot-spot' areas compared to the current universal coverage (of all malaria risk areas) approach.
- Alternative ways of identifying malaria clustering activity (as an early detection) should be tried, e.g. a prospective analysis of 'alive' clusters with available software packages using the incoming weekly data from the passive surveillance scheme.
- Studies on the socio-economic impact of vivax malaria may enhance concerns about this *Plasmodium* species.
- The economic and epidemiological advantage of a combination of strategies, e.g. ITN with IRS, should be studied.

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Original articles

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MALARIA
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RESEARCH

Model variations in predicting incidence of *Plasmodium falciparum* malaria using 1998-2007 morbidity and meteorological data from south Ethiopia

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Abstract

Background: Malaria transmission is complex and is believed to be associated with local climate changes. However, simple attempts to extrapolate malaria incidence rates from averaged regional meteorological conditions have proven unsuccessful. Therefore, the objective of this study was to determine if variations in specific meteorological factors are able to consistently predict *P. falciparum* malaria incidence at different locations in south Ethiopia.

Methods: Retrospective data from 42 locations were collected including *P. falciparum* malaria incidence for the period of 1998-2007 and meteorological variables such as monthly rainfall (all locations), temperature (17 locations), and relative humidity (three locations). Thirty-five data sets qualified for the analysis. Ljung-Box Q statistics was used for model diagnosis, and R squared or stationary R squared was taken as goodness of fit measure. Time series modelling was carried out using Transfer Function (TF) models and univariate auto-regressive integrated moving average (ARIMA) when there was no significant predictor meteorological variable.

Results: Of 35 models, five were discarded because of the significant value of Ljung-Box Q statistics. Past *P. falciparum* malaria incidence alone (17 locations) or when coupled with meteorological variables (four locations) was able to predict *P. falciparum* malaria incidence within statistical significance. All seasonal AIRMA orders were from locations at altitudes above 1742 m. Monthly rainfall, minimum and maximum temperature was able to predict incidence at four, five and two locations, respectively. In contrast, relative humidity was not able to predict *P. falciparum* malaria incidence. The R squared values for the models ranged from 16% to 97%, with the exception of one model which had a negative value. Models with seasonal ARIMA orders were found to perform better. However, the models for predicting *P. falciparum* malaria incidence varied from location to location, and among lagged effects, data transformation forms, ARIMA and TF orders.

Conclusions: This study describes *P. falciparum* malaria incidence models linked with meteorological data. Variability in the models was principally attributed to regional differences, and a single model was not found that fits all locations. Past *P. falciparum* malaria incidence appeared to be a superior predictor than meteorology. Future efforts in malaria modelling may benefit from inclusion of non-meteorological factors.

Background

Over 100 million people worldwide are affected by malaria and *P. falciparum* malaria is responsible for approximately one million deaths annually, with many of those deaths occurring in children under the age of five

years. Unfortunately, 90% of the global malarial burden is carried by sub-Saharan Africa [1,2]. Malaria transmission is complex and not yet fully understood; the recent focus of developed nations on global warming has spawned the suspicion of a climate-malaria link.

The possible association of changes in temperatures to variations in malaria epidemiology is merited by the well-defined biological effects on life-cycle stages of the

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Anopheles insect transmission vector and the *Plasmodium* malaria parasite [3]. For example, increasing of the temperature to 31°C results in a shortened sporogonic period of the *Plasmodium* parasite, an effect which differs among the *P. falciparum* and *P. vivax* species. Higher mean daily temperatures are not favourable for vector survival since increased temperatures speed up development of the aquatic stages of the vector's life cycle.

Many researchers, therefore, have proposed developing improved tools to forecast malaria epidemics by using variations in regional temperatures. These efforts have resulted in the medical literature using vastly inconsistent terminology to describe malaria risks, and to distinguish between long-term forecasts, early warning and early detection of epidemics.

Long-term epidemic forecasting is usually based on climate forecasting, and relies on such datasets as the El Niño Southern Oscillation indices to predict epidemic risk months in advance over large geographical areas. Such a forecast allows time for the population to prepare for a possible epidemic in the upcoming malaria season.

Malaria epidemic early warning is based on surveying transmission risks to predict timing of an increase based on abnormal rainfall or temperatures. Often, such risks are influenced by population vulnerability, such as history of low rates of malaria transmission. Such predictions of malaria epidemics can provide lead times of weeks to months.

The long-term and early warning approaches should, however, be distinguished from epidemic early detection, which involves noting the beginning of an unusual epidemic. As such, this surveillance approach is limited in that is offers little lead time (days to weeks) for preparation and implementation of preventive measures. When used in an effective manner, it is able to prevent sickness and death.

The aim of this study was to examine if the spatio-temporal distribution of surface temperature and rainfall are useful factors to predict changes in malaria incidence, as a malaria epidemic early warning strategy. This evaluation was based on an assumption that the link between climate and occurrence of malaria is constant and similar for different regional settings.

Incorporating prediction and forecasting approaches, however, calls for sound understanding of the complex factors involved in malaria transmission. It has been suggested that the major driving force of malaria transmission is climate [4-8]. However, the data has been largely inconsistent as to exactly how climate influences malaria transmission. In some geographic regions, the minimum temperature has been shown as an important contributory factor for malaria transmission [6,7,9], while in others the maximum temperature has been implicated [5,10-12]. Onset of malaria epidemics often coincide with the rainy season or significant rainfall [13,14], but this is not always the case [9]. Inconsistent findings also exist in studies focusing on the number of climate lags, both for rainfall and temperature, associated with malaria epidemics [5-7]. Prediction strategies based on climate information have been most accurate when considering colder locations [6]; still, not all studies have been able to confirm the utility of meteorological variables at varying altitudes [7]. The effect of rainfall on transmission rates has also been found to vary between urban and rural areas [6], suggesting the presence of an additional cofounding factor in one of both of these communities. To date, many different models have been developed based on the simple assumption that a defined set of climatic variables influence malaria incidence; however, the models have different statistical or mathematical forms, incorporate different variables and lag combinations, and demanded different forms of data transformation and analysis [5-8]. This might reflect the complexity between climatic variables and malaria transmission [15] while striving to address biological plausibility. Unfortunately, such biological approaches are able to describe malaria transmission but are not powerful enough to yield reliable predictions of incidence [16]. This is also a limitation that affects implementation of climate-based malaria early warning and forecasting [17]. Attempts have been made to improve the models using historical morbidity and climatic variables [6-8,12].

Nonetheless, the impact of climate on malaria transmission has yet to be firmly established. Thus, there exists a need to consider local variations in climates in order to fully understand the relationship between climate and malaria transmission [11,15,16,18-23]. Taking the average of conditions across large geographic areas, or even making similar assumptions stating the effects of climatic variables to be constant across different locations [7], might cause an underestimation of local variations and disable a models accurate ability to predict malaria incidence [16,17].

In addition to the incorporation of climatic causes, some researchers have suggested building models that consider non-climatic factors such as land use, population movement, immunity, topography, parasite genotypes, vector composition, drug resistance, vector control measures and availability of healthcare services [6-8,18,20,24]. The study presented herein did not include non-meteorological data because of limited data availability; however, local variations were considered in the link between malaria incidence and meteorological factors [10,18,20]. In areas such as the Ethiopian highlands endemic malaria occurs at altitudes above 2100 m [25]. It has been suggested that global warming will drive malaria transmission at higher altitudes, mainly because of corresponding changes in the distribution of the *Anopheles*

vector [26]. However, the study period covered less than 10 years and, thus, was too brief to evaluate the potential effects of global warming on vector distribution and on malaria incidence. Therefore, the principal objective of this study was to explore if variations of meteorological factors are able to consistently predict *P. falciparum* malaria incidence at different locations in south Ethiopia.

Methods

Data inputs and inclusion criteria

A total of 42 locations in the southern region of Ethiopia were examined for data on varying serial length of *P. falciparum* malaria incidence during 1998-2007; available data from local meteorology stations were also collected for the analysis. The minimum serial length was set at 50 [27], resulting in five locations being excluded from further analysis. To ensure against imputation effects on model structure, the threshold of allowable missing data was set at 15% of the total series; this criterion led to the exclusion of two locations. The final number of locations in this study was, therefore, 35.

Microscopically-confirmed *P. falciparum* malaria cases only were considered in this study. The total number of cases was 210 659 and covered a period of 6.7 years from the 35 locations. The mean serial length was 80 months (range: 51-118 months). The available meteorological data included: monthly total rainfall in millimetres (all locations), monthly average maximum, minimum and average temperature in Celsius (17 locations) and monthly average relative humidity as percentage (three locations). While taking averaged rainfall data for each month in the series, 19 locations exhibited a unimodal rainfall pattern (which peaked from June to September) and the remaining 16 locations exhibited a bimodal pattern (peaks in February to April and August to October). The bimodal rainfall pattern could 'double' the serial length and, hence, improve the chance of observing any link between *P. falciparum* malaria incidence and rainfall pattern. The altitude of the meteorology stations ranged from 1182-2582 m, and 14 locations were below 1750 m. Figure 1 presents the coordinates of each location.

Data source

A health centre provides basic curative and preventive health services for a population of about 25,000 people. Each health centre is staffed by nurses and health officers, and by trained laboratory technicians. The institutions routinely performed thick and thin blood film examinations for malaria parasites. Rapid diagnostic tests for malaria were not used. Each month, all health institutions reported suspected malaria cases and confirmed *P. falciparum* and *P. vivax* cases to the regional health authorities.
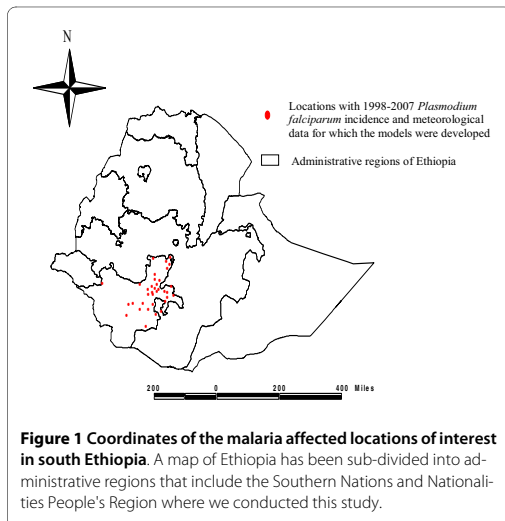
Figure 1 Coordinates of the malaria affected locations of interest in south Ethiopia. A map of Ethiopia has been sub-divided into administrative regions that include the Southern Nations and Nationalities People's Region where we conducted this study.

Microscopically-confirmed *P. falciparum* malaria cases ($n = 210,659$) were obtained from the reports made to the Southern Nations and Nationalities Regional Health Bureau from 33 health centres and from two district hospitals.

The meteorological data used for analyses were obtained from the Southern Branch office of the National Meteorological Agency of Ethiopia. This agency operates over 200 meteorological stations, with records spanning 15 to over 50 years. From the year 1970 onward, the proportion of missing data is low [28]. In this study, meteorological data from 35 locations was used (Figure 1).

Missing data handling

The Box-Jenkins method [29] was used, hereafter referred to as the Autoregressive integrated moving average (ARIMA). As this model requires discrete time series data with no missing values, missing data replacement for both dependent and independent series was carried out. Missing values were replaced with the mean or the median of the period in which the observation was missing. The mean was used for data of *P. falciparum* malaria incidence, temperature and relative humidity. Since rainfall data was heavily skewed, the median was used to replace the missing values of rainfall.

Assumptions

1. The underlying data of malaria transmission was assumed to be stochastic, whereby local variations and other unmeasured causes play important roles. Others have reported local variations in the association between climate and malaria incidence

[15,16,18-22]. Therefore, building models for each location would remove crudeness of models and enable reliable forecasts. In addition, this might contribute to our understanding of the impact of meteorology with respect to varying altitudes.

2. The quality of data obtained through routine reporting in developing countries may be questionable, mainly because of under-reporting. However, the data sets were assumed to hold the basic elements of malaria transmission like trend, seasonality or monthly variations, which could suffice for modelling exercise [12]. The need to use available data at the regional level, check whether these data could be used to model *P. falciparum* malaria incidence and assist malaria epidemic early warning was considered.

3. The meteorology station correctly captures climate data within a 10 km radius [Southern Branch office of National Meteorological Agency of Ethiopia, personal communication], and this matches to the service area coverage of the corresponding health centre. This assumption does not apply for the two district hospitals since the service area coverage of a district hospital is beyond the 10 km radius [30].

4. In Ethiopia, malaria transmission is largely unstable [31] and, hence, the population has insignificant immunity, putting all age groups at equal risk of contracting the disease. Therefore, demographic changes were assumed to have had minimal impact in malaria transmission during the study period. Meanwhile, there is lack of proper denominator for health facility-based data in this country. As a result, the number of malaria cases was used instead of its fraction out of the total population.

Scope

This paper sought to unveil the local variations in the predictive power of lagged effects of the number of past *P. falciparum* malaria cases and climatic variables on incidence of *P. falciparum* malaria. As model structures of each location were presented, detail of each model, forecasting and validation were beyond the scope of this research effort.

Data processing and analysis

SPSS version 17.0 Expert Modeler (Chicago, IL, USA) was used to automatically determine the best-fitting model. Malaria incidence was the dependent variable, and all available climatic variables were fed into the model as predictors. The Expert Modeler keeps the predictor series in the model only if it is significant. The resultant model was checked for consistency by inserting the model criteria set and significant predictor identified by the Expert Modeler. To do this, custom ARIMA models were used and several logical combinations of criteria

to look for better models were considered. The best-fitting model built by the Expert Modeler was subsequently used. For the locations of Cheleklektu and Buee, a constant value of 1 was added to the dependent series to enable log transformation. Automatic detection of outliers was made and the outliers were modelled accordingly, thus trimming was not performed. The same procedure was followed for all data sets.

Goodness of fit

The R-squared measurement was used as an indicator of goodness of fit for the models if there was no differencing. The R-squared coefficient of determination suggests the proportion of variance of the dependent variable explained by the model. The stationary R-squared was used instead whenever the Expert Modeler considered differencing. The stationary R-squared was used to capture trend or seasonality, which is the basis for differencing. The stationary R-squared and the ordinary R-squared values were the same when there was no data transformation to any form. It is noted that if the series was log transformed without differencing, stationary R-squared would overestimate the ordinary R-squared and underestimate for the square root transformation.

Diagnostic statistics

The Ljung-Box Q statistic, also known as the modified Box-Pierce statistic, was used to provide an indication of whether the model was correctly specified. A significant value less than 0.05 was considered to acknowledge the presence of structure in the observed series which was not accounted for by the model; therefore, we ignored the model if it had significant value.

The residual autocorrelation function was expected to agree with the white noise assumption. White noise, the most common model of noise in time series analysis, is a stationary time series or a stationary random process with zero autocorrelation. In other words, in white noise $N(t)$ any pair of values $N(t_1)$ and $N(t_2)$ taken at different moments t_1 and t_2 of time are not correlated; that is, the correlation coefficient $r(N(t_1), N(t_2))$ is equal to null. The SPSS 17.0 forecasting menu provides autocorrelations that provides p values for each lagged noise residual series using the Ljung-Box statistics. It was possible to see which lagged noise residual was significantly autocorrelated. For each data set, autocorrelation of noise residuals was carried out, and the results were consistent with that of the model statistics table of Ljung-Box Q statistics.

The model

Since meteorological variables were used as predictors, addition of the Transfer Function (TF) model to the basic univariate ARIMA model was considered. Whenever the

Expert Modeler dropped the predictor series, the model was found to take on the univariate ARIMA form.

ARIMA orders

In ARIMA (p, d, q) (P, D, Q), the first parenthesis held the non-seasonal autoregressive (p), differencing (d) and moving average (q) orders. A non-seasonal autoregressive order of 1 specified the value of the series in one time period from the past to be used to predict the current value. A first order differencing implied a linear trend in the series. Meanwhile, moving average order of 2 specified the deviations from the mean value of the series from each of the last two time periods to be considered when predicting the current value. The second parenthesis held their seasonal counterparts. For monthly data, the seasonal order of 1 implied that the current value was affected by the series value 12 periods prior to the current one.

Transfer function orders

Numerator specified which previous values from the predictor series were to be used to predict the current value of the dependent series.

Denominator specified how deviations from the series mean, for previous values of the predictor series, were to be used to predict the current value of the dependent series.

Difference specified the order of differencing applied to the predictor series before estimating the model occurred.

The seasonal orders were built using the same strategy as that for the ARIMA orders.

Delay

Setting a delay is known to cause the predictor's influence to be delayed by the number of intervals specified. For instance, a delay of 4 implies the value of the predictor at time t does not affect forecasts until four periods have elapsed ($t + 4$). A delay of 4 essentially equals lag of four time periods.

See Additional file 1 for details of the model.

Data transformation

The ARIMA model is an analysis in the temporal domain applied to stationary data series. Thus, the presence of outliers, random walk, drift, trend, or changing variance in the series might have resulted in nonstationarity. And the stationarity of the series could be achieved when both the mean and the variance remained constant over time. For this, variance stabilizing transformations, like natural log (LN) and square root (SQR), and detrending using differencing were used when necessary. In addition, the Expert Modeler was set to detect outliers (if any) and model them automatically.

Results

Model inclusion and exclusion

Data from 35 locations were analysed using Time Series modelling. Models of five locations were ignored because of the significant results of the diagnostic statistics, the Ljung-Box Q, including models built for the two hospital locations.

Data description

We analysed 210 659 microscopically-confirmed *P. falciparum* malaria cases from 35 localities (Figure 1). During the same period, these institutions also reported 112 354 microscopically-confirmed *P. vivax* malaria cases. The ratio of *P. falciparum* to *P. vivax* malaria cases was 1.87 to 1.00.

The pattern of meteorological variables and *P. falciparum* malaria monthly cases was not uniform across the locations, indicating local variations. Sequence charts were generated for each of the 35 locations and for the mean meteorological conditions of 23 (rainfall) and 14 locations (rainfall and temperature). The lagged effect of rainfall on *P. falciparum* malaria incidence was more visible for the mean meteorological conditions (Additional file 2).

Past *Plasmodium falciparum* malaria incidence

Of 30 models, 21 were based on lagged effect of incidence data alone (17 locations) or coupled with meteorological predictors (4 locations). Among those 21 models, 16 had a non-seasonal AR order of 1 (13 locations) or 2 (3 locations). Three locations had both seasonal and non-seasonal AR orders of 1. Two locations had only a seasonal AR order of 1. Non-seasonal and seasonal first order differencing was used for five and three locations, respectively. Five locations had a non-seasonal MA order of range 1-6, and there was no seasonal MA order. Seasonal ARIMA orders were specified for six locations of altitude 1742 m or higher, constituting one-third of the locations above this altitude (Additional file 3, Tables S1-S4).

Meteorological data

Rainfall data were available from all locations, however, it was found to be a significant predictor for only four of the locations (altitude: 1182, 1431, 1618 and 2054 m). A delay of 2 months was significant for 2 of these. A delay of 2 months with numerator TF order of 0 refers to a 2 months lagged effect (Additional file 3, Table S1). Besides the delay value of 2, including the numerator TF orders of 2, 1 and 0 was interpreted as the rainfall data corresponding to the previous four, three and two months to predict the current incidence. The denominator TF orders of 2 and 1 indicated that the model used the deviations of rainfall data of the 4th and 3rd lagged months (delay 2) from the series mean. One model specified numerator TF

order of 2, 1 and 0 without setting a delay; that is, rainfall data of the last two consecutive months coupled with the current one were used to predict incidence (Additional file 3, Table S2). A single location had 3 months lagged effect of rainfall (Additional file 3, Table S4).

Minimum and maximum temperatures were available for 17 of the locations. Minimum temperature was found to be a significant predictor in five locations. Delays of 2, 4 and 5 months with numerator TF order 0 predicted incidence in three locations (altitudes: 2582, 1220 and 2331 m). Of those, first order non-seasonal differencing was required for the location with the lowest altitude (1220 m). Incidence (two locations) and maximum temperature (one location) were included in the models (Additional file 3, Tables S1 and S4). For the remaining two locations, numerator TF order of 0 without a delay was specified; that is, the current value of the predictor was used to determine the current incidence with no contribution for forecasting. For one of the models, however, it impacted incidence to achieve higher goodness of fit statistics. The other model had the worst goodness of fit statistic (negative value) of all models (Additional file 3, Table S2).

Maximum temperature at a lag of 4 months coupled with the deviations of a lag of 5 and 6 months from the series mean predicted incidence at an altitude of 1221 m (Additional file 3, Table S1). Meanwhile, coupled with minimum temperature, the value at lag of 4 when added to the lag of 2 months predicted incidence (Additional file 3, Table S4).

Only three locations had data available on relative humidity, but none proved significant.

Goodness of fit of models

Except for one model which produced a negative value, the range of the R-squared was 16-97%. Of 30 models, 20 had values greater than 50% and seven had values exceeding 85%. The range for models with any of the seasonal ARIMA orders was 60-97%. The models were reasonably good for explaining the total variations of the data sets. According to the Spearman's rho correlation coefficient, there was no significant correlation between the R-squared values and the serial length (r = 0.29) or the average incidence per month (r = -0.01).

Model similarities and variations

The model predicted incidence fairly well by its lagged values in most locations. Models of seven locations were similar with ARIMA (1, 0, 0) (0, 0, 0) with no transformation. Nevertheless, the other incidence models applied different forms of transformation (LN, SQR or differencing) or incorporated different meteorological variables. Some models did not contain incidence at any AR or MA orders. Meanwhile, meteorological variables were signifi-

cant predictors for only seven of the locations without any apparent reiteration in line with the altitude. For two of the data sets, the Expert Modeler revealed the absence of a significant predictor with reasonable goodness of fit statistics. And for five of the data sets, the model did not comply with the criterion of diagnostic statistics. In summary, the variations outweighed the similarities of the models made for different locations for the given incidence and meteorological data.

Mean meteorological conditions

It was not possible to engage all (thirty) data sets to evaluate the utility of taking mean meteorological conditions for prediction because aggregates of *P. falciparum* malaria incidence of all data sets produced a serial length of 29 (below 50). Therefore, the mean condition of 23 locations with a serial length of 62 was used. This resulted in monthly rainfall being a significant predictor at a lag of 4 months coupled with AR order of 1 (monthly rainfall was a significant predictor only in four locations when the data sets were analysed separately). This model was applied to each of the 23 locations but did not produce any significant results. The mean condition of temperature (for 14 locations) with or without rainfall was also checked, but the diagnostic statistics disqualified the model (Additional file 3, Table S4).

Discussion

Statistical modelling is used for understanding and prediction of multifactorial based events; as such, reproducibility, biological plausibility and robustness govern the applicability and effectiveness of each resultant model. Malaria transmission is one such complex event as many underlying causes have been associated with its frequency and duration, including regional factors. The Malaria Early Warning System (MEWS) has been established to enable reliable predictions of *P. falciparum* malaria epidemics using transmission risk indicators like unusual increase in rainfall [32]. Nevertheless, producing accurate predictions using climate data remains a challenge [15]. This study set forth to evaluate large populations affected by malaria and residing in widely variable geographical areas; specifically, we considered local variations to determine their contribution and ability to limit effective prediction of *P. falciparum* malaria incidence by using statistical modelling methods. This strategy is expected to also provide evidence to support the meteorology-malaria link and to determine the validity of using mean meteorological conditions to create a general predictive model.

The Ljung-Box Q provided the diagnostic statistics to check the presence of structure in the observed series which was not accounted for by the model. Five models were ignored with significant values according to this

diagnostic statistic, but the underlying reasons were not immediately clear. It was likely that the only two data sets that came from hospitals might not have properly coincided with the station-specific climate data since the catchment area of those hospitals was wider than that of the meteorological stations. Thus, it remains to be seen whether linking hospital data with wider catchment to station-specific meteorological data would benefit evaluations of the proposed meteorology-malaria link.

Malaria transmission is known to be associated with gametocyte prevalence in a population [33]. The finding that past *P. falciparum* malaria incidence often predicts the current incidence may support this relationship. Likewise, the AR term in this study was found to be much more important than the meteorological variables for prediction of *P. falciparum* malaria. Other studies have also shown that models using the previous month's incidence for malaria prediction outweigh the impact of climate variables [6-8,12]. In this study, the MA order was also used. Seasonal ARIMA orders were specified at higher altitudes only, starting at 1742 m. This might reflect the seasonal nature of malaria transmission in these locations. However, two-thirds of the locations above this altitude did not have seasonal ARIMA orders. So, it was not possible to conclude that malaria was seasonal at areas above the altitude of 1742 m, or that erratic rainfall patterns or other unmeasured potentially confounding factors have eluded the seasonality.

As has been shown by others [34,35], this study confirms the biologically driven link of temperature and rainfall with malaria transmission. However, the link is complex and sensitive to the effects of other factors, and it remains to be seen whether direct and predictable relationships really exist [36]. This study suggests that temperature and rainfall are significant predictors for only a few of the locations examined. The absence of temperature data for 13 of the locations might have limited our findings. A serial length above the minimum requirement for the ARIMA models was used, but it might not have had enough statistical power to yield significant values or be long enough in duration to capture seasonality. Nevertheless, the rainfall pattern was bimodal in 16 out of 35 locations, offering a better chance to see the effect of rainfall on *P. falciparum* incidence. Some researchers have suggested the use of weekly data to model malaria incidence [5,7,12]. Thus, the monthly morbidity and meteorology data used in this study might not be sensitive enough to reveal the association between meteorology and *P. falciparum* malaria incidence.

Considering mean conditions or aggregated data might disguise real effects [16,17]. This urges approaches of modelling to specific locations at the expense 'all-fit-one' or simple model. This study also examined the effect of mean meteorological conditions and compared it with the area-specific results. Meteorological variables, particularly rainfall, were able to predict *P. falciparum* malaria incidence on a wider geographic scale. However, the local variations seen in the link between *P. falciparum* malaria incidence and meteorological factors have not allowed such approaches of prediction. More importantly, if malaria transmission cannot be explained by the meteorology-malaria link, it is necessary to include non-climatic factors like vector composition, vector control measures and healthcare services in statistical modelling, as is advocated by others [6-8,18,20,24,37].

Conclusions

This study shows that models of climate-malaria link varied from place to place, and one model could not fit all locations. In several locations, it was found that past *P. falciparum* malaria incidence was a more robust predictor than any of the meteorological variables. It is possible that more accurate malaria modelling may require the inclusion of non-climatic causes as well. Nonetheless, statistical time-series modelling to analyse meteorology-malaria link appears to be a promising approach to predicting malaria incidence and merits further investigation.

Additional material

Additional file 1 Details of the model. Formulae and main features of Transfer Function and univariate ARIMA models.

Additional file 2 Sequence charts. Sequence charts for each of the 35 locations examined, mean meteorological conditions of 23 and 14 locations. The separate sheets in the Excel file are labeled by the name of the locations corresponding to the data. Data displayed include altitude, available meteorological variable(s) and *P. falciparum* incidence.

Additional file 3 Tables S1 to S4: Time series models to predict *Plasmodium falciparum* malaria incidence at different locations in south Ethiopia. The 35 locations were divided among four tables for ease of presentation. All tables included data on location, altitude, available data used, model structure, goodness of fit, significant variables and model description, serial length and average incidence per month.

Authors' contributions
EL conceived the study, collated, analysed and interpreted the data, and prepared the draft manuscript. BL conceived the study, guided the analysis, interpreted the data and helped to draft the manuscript. Both authors have read and approved the submitted version of the manuscript.

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Additional file 1: Details of the model

Notations used :

$a_t (t = 1,2,...,n)$ White noise series normally distributed with mean zero and variance σ^2

p Order of the non - seasonal autoregressive part of the model

q Order of the non - seasonal moving average part of the model

d Order of the non - seasonal differencing

P Order of the seasonal autoregressive part of the model

Q Order of the seasonal moving average part of the model

D Order of the seasonal differencing

s Seanality or period of the model

$\phi_p(B)$ AR polynomial of B of order $p, \phi_p(B) = 1 - \varphi_1 B - \varphi_2 B^2 - ... - \varphi_p B^p$

$\theta_q(B)$ MA polynomial of B of order $q, \theta_q(B) = 1 - \vartheta_1 B - \vartheta_2 B^2 - ... - \vartheta_q B^q$

$\Phi_P(B^s)$ Seasonal AR polynomial of BS of order $P, \Phi_P(B^s) = 1 - \Phi_1 B^s - \Phi_2 B^{s2} - ... - \Phi_p B^{sP}$

$\Theta_Q(B^s)$ Seasonal MA polynomial of BS of order $Q, \Theta_Q(B^s) = 1 - \Theta_1 B^s - \Theta_2 B^{s2} - ... - \Theta_Q B^{sQ}$

Δ Differencing operator $\Delta = (1 - B)^d (1 - B^s)^D$

B Backward shift operator with $BY_t = Y_{t-1}$ and $Ba_t = a_{t-1}$

$Z\sigma_t^2$ Prediction variance of Z_t

$N\sigma_t^2$ Prediction variance of noise forcasts

A Transfer Function model describing the relationship between the dependent and predictor series has the following form:

$$Z_t = f(Y_t)$$

$$\Delta Z_t = \mu + \sum_{i=1}^{k} \frac{Num_i}{Den_i} \Delta_i f_i(X_{it}) + \frac{MA}{AR} a_t \tag{1}$$

Droping the predictor series yields univariate ARIMA:

$$\Delta Z_t = \mu + \frac{MA}{AR} a_t \tag{2}$$

Main features of the model :

- Initial data transformations of the dependent and predictor series
- A constant term μ
- The unobserved i.i.d., zero mean, Gaussian error process a_t with variance σ^2
- The moving average lag polynomial $MA = \theta_q(B)\Theta_Q(B^s)$ and the auto - regressive lag polynomial $AR = \phi_p(B)\Phi_P(B^s)$
- The difference/lag operators Δ and Δ_t
- Predictors are assumed given. Their numerator and denominator lag polynomials are of the form :

$Num_i = (\omega_{i0} - \omega_{i1} B - ... - \omega_{iu} B^u)(1 - \Omega_{i1} B^s - ... - \Omega_{iv} B^{vs})B^b$ and

$Den_i = (1 - \delta_{i1} B - ... - \delta_{ir} B^r)(1 - \Delta_{i1} B^s - ...)$

- The "noise" series

$N_t = \Delta Z_t - \mu - \sum_{i=1}^{k} \frac{Num_i}{Den_i} \Delta_i X_{it}$ is assumed to be a mean zero, stationary ARMA process.

Table S1: Time series models to predict _Plasmodium falciparum_ malaria incidence at different locations in south Ethiopia

Location (Health centre, unless specified)	Altitude	Available data used (Temperature- Max and Min)	Model Structure (ARIMA)	Goodness of fit (R^2, if *: stationary R^2)	Significant Variables and model description	Serial Length	Average Incidence per month
Daramalo Wacha	1182	Incidence Rainfall	(1,0,0)(0,0,0)	0.56	**Incidence** (SQR) at lag 1 **Rainfall** at delay 2 and numerator TF order of 0.	68 (Mar 2002-Oct 2007)	45
Tepi	1205	Incidence Temperature Rainfall	(1,0,0)(0,0,0)	0.30	**Incidence** (LN) at AR lag 1	117 (Jan 1998-Sep 2007)	57
Arba Minch	1220	Incidence Temperature Rainfall Relative Humidity	(0,1,6)(0,0,0)	**0.53***	**Incidence** (LN) at MA lag 1 and 6 and first order non-seasonal differencing **Min temperature**(LN) at delay 4, numerator TF order of 0 and first order non-seasonal differencing	118 (Jan 1998-Oct 2007)	83
Mirab Abaya	1221	Incidence Temperature Rainfall Relative Humidity	(0,0,0)(0,0,0)	0.30	**Max temperature**(LN) at delay 4, numerator TF order of 0, denominator TF orders of 1 and 2	105 (Feb 1999-Oct 2007)	54
Bele	1240	Incidence Rainfall	(1,0,0)(0,0,0)	0.64	**Incidence** at AR lag 1	64 (Jul 2002-Oct 2007)	133
Tercha _Hospital_	1335	Incidence Temperature Rainfall	Model ignored (Ljung-Box Q P value <0.05)			63 (Aug 2002-Oct 2007)	53
Jinka _Hospital_	1373	Incidence Temperature Rainfall	Model ignored (Ljung-Box Q P value <0.05)			62 (Jul 2002-Aug 2007)	126
Sawla	1380	Incidence Temperature Rainfall	(2,0,0)(0,0,0)	0.69	**Incidence** at AR lags 1 and 2	81 (Dec 2000-Aug 2007)	91

Table S2: Time series models to predict *Plasmodium falciparum* malaria incidence at different locations in south Ethiopia

Location (Health centre, unless specified)	Altitude	Available data used (Temperature- Max and Min)	Model Structure (ARIMA)	Goodness of fit (R², if *: stationary R²)	Significant Variables and model description	Serial Length	Average Incidence per month
Konso Karat	1431	Incidence Temperature Rainfall	(1,0,0)(0,0,0)	0.94	**Incidence** at AR lag 1 **Rainfall** at delay 2, numerator TF orders of 0, 1 and 2, and denominator TF orders of 1 and 2.	118 (Jan 1998–Oct 2007)	64
Gesuba	1552	Incidence Temperature Rainfall	(0,0,0)(0,0,0)	-0.03	**Min temperature**(LN) at numerator TF order of 0	64 (Jul 2002–Oct 2007)	62
Bedesa	1609	Incidence Rainfall	(1,0,0)(0,0,0)	0.65	**Incidence** at AR lag 1	53 (Jun 2003–Oct 2007)	65
Humbo Tebela	1618	Incidence Rainfall	(0,0,0)(0,0,0)	0.19	**Rainfall** at numerator TF orders of 0, 1 and 2	64 (Jul 2002–Oct 2007)	98
Amaro Kele	1659	Incidence Temperature Rainfall	(1,0,0)(0,0,0)	0.77	**Incidence** at AR lag 1	97 (Sep 1999–Sep 2007)	24
Cheleklektu	1701	Incidence Rainfall	(1,0,0)(0,0,0)	0.21	**Incidence** (LN) at AR lag 1	75 (Sep 1999–Nov 2005)	24
Wondo Genet	1742	Incidence Rainfall	(0,0,2)(1,0,0)	0.97	**Incidence** at non-seasonal MA lag 2 and Seasonal AR lag 1	118 (Jan 1998–Oct 2007)	90
Areka	1752	Incidence Rainfall	(0,1,0)(0,0,0)	**0.55***	No significant predictor	118 (Jan 1998–Oct 2007)	353
Alaba	1772	Incidence Temperature Rainfall	(0,1,1)(0,0,0)	0.46	**Incidence** (LN) at first order of non-seasonal differencing and MA lag 1 **Min temperature**(LN) at numerator TF order of 0, first order of non-seasonal and seasonal differencing	64 (Jul 2002–Oct 2007)	302
Yirg Alem	1786	Incidence Temperature Rainfall	Model ignored (Ljung-Box Q P value <0.05)			64 (Jul 2002–Oct 2007)	45

Table S3: Time series models to predict *Plasmodium falciparum* malaria incidence at different locations in south Ethiopia

Location (Health centre, unless specified)	Altitude	Available data used (Temperature- Max and Min)	Model Structure (ARIMA)	Goodness of fit (R², if *: stationary R²)	Significant Variables and model description	Serial Length	Average Incidence per month
Kebado	1807	Incidence Rainfall	Model ignored (Ljung-Box Q P value <0.05)			64 (Jul 2002-Oct 2007)	23
Wolayta Soddo	1854	Incidence Temperature Rainfall Relative Humidity	(1,0,0)(0,0,0)	0.36	**Incidence** (LN) at AR lag 1	65 (Jul 2002-Nov 2007)	42
Koshe	1876	Incidence Rainfall	(1,0,0)(1,1,0)	**0.91***	**Incidence** at AR lag 1 (both non-seasonal and seasonal) and first order of seasonal differencing	89 (Sep 1998-Jan 2006)	63
Wolkite	1884	Incidence Rainfall	(0,1,0)(1,1,0)	**0.86***	**Incidence** at AR lag 1 (seasonal), non-seasonal and seasonal first order of differencing	118 (Jan 1998-Oct 2007)	151
Kemba	1895	Incidence Rainfall	(1,0,0)(0,0,0)	0.62	**Incidence** at AR lag 1	52 (Jul 2003-Oct 2007)	51
Laska	1910	Incidence Rainfall	(1,0,0)(0,0,0)	0.67	**Incidence** at AR lag 1	56 (Feb 2003-Sep 2007)	30
Aleta Wondo	1947	Incidence Rainfall	(0,0,1)(0,1,0)	**0.88***	**Incidence** at MA lag 1 and first order seasonal differencing	86 (Sep 2000-Oct 2007)	43
Derara	1950	Incidence Rainfall	(1,0,0)(0,0,0)	0.89	**Incidence** at AR lag 1	94 (Jan 2000-Oct 2007)	73
Shone	1959	Incidence Rainfall	Model ignored (Ljung-Box Q P value <0.05)			64 (Jul 2002-Oct 2007)	98
Tora	1987	Incidence Rainfall	(0,0,0)(0,0,0)	0.30	No significant predictor	60 (Nov 2002-Oct 2007)	69
Yirba	2023	Incidence Rainfall	(1,0,0)(1,0,0)	0.68	**Incidence** at AR lag 1 (both non-seasonal and seasonal)	63 (Jul 2002-Sep 2007)	92

Table S4: Time series models to predict *Plasmodium falciparum* malaria incidence at different locations in south Ethiopia

Location (Health centre, unless specified)	Altitude	Available data used (Temperature- Max and Min)	Model Structure (ARIMA)	Goodness of fit (R^2, if *: stationary R^2)	Significant Variables and model description	Serial Length	Average Incidence per month
Boditi	2043	Incidence Temperature Rainfall	(2,0,0)(0,0,0)	0.16	**Incidence** (LN) at AR lag 1 and 2	89 (May 2000-Sep 2007)	98
Buee	2054	Incidence Rainfall	(1,0,0)(1,0,0)	0.60	**Incidence** (LN) at AR lag 1 (both non-seasonal and seasonal) **Rainfall** at delay 3 and numerator TF order of 0.	86 (Sep 2000-Oct 2007)	30
Butajira	2074	Incidence Temperature Rainfall	(1,0,0)(0,0,0)	0.94	**Incidence** at AR lag 1	118 (Jan 1998-Oct 2007)	50
Hossana	2306	Incidence Temperature Rainfall	(2,1,1)(0,0,0)	**0.66***	**Incidence** at AR lag 1 and 2, first order of differencing and MA lag 1	64 (Jul 2002-Oct 2007)	56
Angacha	2331	Incidence Temperature Rainfall	(1,0,0)(0,0,0)	0.39	**Incidence** (LN) at AR lag 1 **Min temperature**(LN) at delay 5 and numerator TF orders of 0	64 (Jul 2002-Oct 2007)	14
Arbegona	2582	Incidence Temperature Rainfall	(0,0,0)(0,0,0)	0.62	**Max temperature** at delay 2 and numerator TF orders of 0 and 2. **Min temperature** at delay 2 and numerator TF orders of 0	51 (Mar 2003-May 2007)	30
Mean condition of 23 locations		Incidence Rainfall	(1,0,0)(0,1,0)	**0.67***	**Incidence** at AR lag 1 and first order seasonal differencing **Rainfall** at delay 4 and numerator TF order of 0 and first order seasonal differencing	62 (Jul 2002-Aug 2007)	2579
Mean condition of 14 locations		Incidence Temperature ±Rainfall	Model ignored (Ljung-Box Q *P* value <0.05)			51 (Mar 2003-May 2007)	1146

II

Predictors of *Plasmodium falciparum* Malaria Incidence in Chano Mille, South Ethiopia: A Longitudinal Study

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Abstract. We assessed potential effects of local meteorological and environmental conditions, indoor residual spraying with insecticides, insecticide-treated nets (ITNs) use at individual and community levels, and individual factors on *Plasmodium falciparum* malaria incidence in a village in south Ethiopia. A cohort of 8,121 people was followed for 101 weeks with active and passive surveillance. Among 317 microscopically confirmed *P. falciparum* malaria episodes, 29.3% occurred among temporary residents. The incidence density was 3.6/10,000 person-weeks of observation. We observed higher malaria incidence among males, children 5–14 years of age, ITNs non-users, the poor, and people who lived closer to vector breeding places. Rainfall increased and indoor residual spraying with Deltamethrin reduced falciparum incidence. Although ITNs prevented falciparum malaria for the users, we did not find that free mass ITNs distribution reduced falciparum malaria on a village level.

INTRODUCTION

Malaria is a major public health problem in Ethiopia, and in 2011 about two-thirds of the population was at risk of malaria.[1] Results of the 2007 national malaria indicator survey showed prevalence of 0.7% and 0.3% of *Plasmodium falciparum* and *Plasmodium vivax* malaria, respectively[2]; the dominant vector is *Anopheles arabiensis*. Indoor residual spraying (IRS) with insecticides has been used since 1960, and distribution of insecticide-treated nets (ITNs) to all age groups and provision of free artemisinin-based combination therapy were in use since 2004.[1]

In the southern part of the country, malaria remains the leading cause of outpatient morbidity, admission, and death comprising 27.6%, 28.7%, and 46.4%, respectively, in the year 2009/2010.[3] In 2010/2011, 1,360,101 individuals received antimalarial drugs, and 490,729 were laboratory-confirmed cases. In the same year, IRS with Deltamethrin was carried out in 1,129,158 houses covering a total of 3,850,808 residents, and 525,177 ITNs were distributed to 262,589 households. The estimated coverage of ITNs was 95%,[4] and this was greater than the national average of 72%.[5] Despite these interventions, a more than expected number of malaria cases was reported from some areas, but no mention was made on the possible reasons for the increase.[4]

Understanding the multifaceted determinants of malaria transmission is important. Many factors play a role in malaria transmission including local meteorological conditions,[6,7] population movement,[8,9] age, sex, socio-economic factors,[10–15] and prevention and control measures.[16–18]

Some studies showed that IRS and ITNs prevent and control malaria transmission using a varying scale of analyses for model, time, and space.[16–19] The simultaneous use of IRS and ITNs might be beneficial.[20,21] However, rising insecticide resistance of vectors could hamper the effectiveness of such insecticide-based interventions.[22] In addition, a high percentage of ITNs ownership may not be a guarantee for a sustained lower incidence of the disease caused by factors possibly linked to lack of efficacy or lack of proper use.[23–26]

Although a comprehensive analytical approach to understanding malaria epidemiology may not be possible, investigating the roles of as many factors as possible might shed light on prevention and control strategies. Therefore, our study aimed to assess the effect of local meteorological and environmental conditions, IRS, ITNs use at individual and community levels, and socio-economic and other individual-level factors on falciparum malaria incidence in a village in south Ethiopia.

METHODS

Study setting. This study was carried out in rural Chano Mille Kebele of Arba Minch Zuria district that is 492 km south of Addis Ababa. The Kebele was selected purposely to study malaria epidemiology and vector biology in detail. Kebele is the smallest administrative region and each Kebele had at least one government health post. A health post provides basic health services including malaria diagnosis using rapid diagnostic test (RDT) kits and treatment with Co-Artem (for falciparum malaria only), which is free. The research project provided Chloroquine tablets (for vivax malaria). The Kebele area was 2.4 sq km, and the health post was located at 6°6.666′ N and 37°35.775′ E. The altitude is 1,206 meters above sea level. Almost all households had mango plantation within their compound. Mango, banana, and maize were the major cash crops. Geographical location and description of the study area is shown in Figure 1.

Study design. This cohort study was carried out in collaboration with the village health post. The study covered 101 weeks, from April 2009 to April 2011. All residents in the Kebele were taken as study subjects. Every household was visited weekly looking for febrile cases. A febrile case was a case whose axillary temperature was ≥ 37.5°C. The active surveillance team used to refer a febrile case to the health post for diagnosis and treatment. At the end of each day there was a cross-check whether that case had visited the health post. All households were given a unique identification card with a number corresponding to the unique number posted on a metal plate on the main entrance of each house. Residents were advised to come to the health post with the identification card if they got febrile in the days between the weekly visits (passive surveillance).

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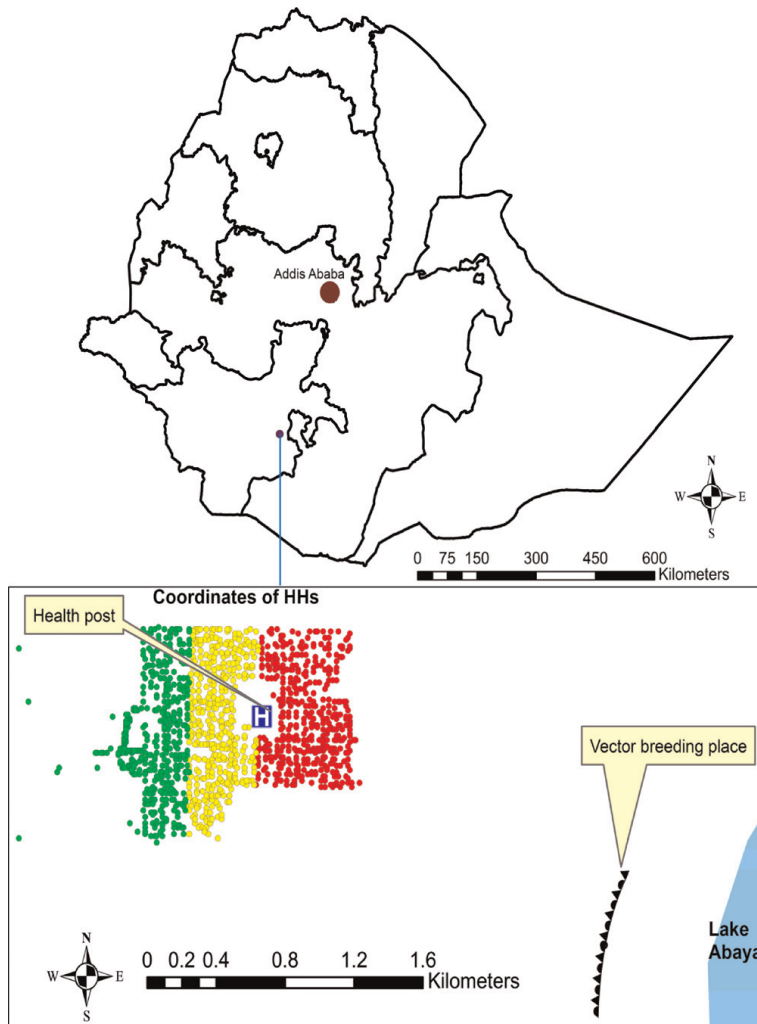
FIGURE 1. Map of Ethiopia, location of Chano Mille Kebele and coordinates (dots) of households subdivided into sub-Kebeles 1 (green), 2 (yellow), and 3 (red), 2009–2011.

Data. The geographic coordinates of all houses and vector breeding places were recorded by using a handheld global positioning system receiver with an accuracy of ± 5 m. Census was done at the beginning, on Week 50, and at the end. In the first census, 7,038 (1,212 households) individuals were registered, and the second census added 1,083 subjects—making the total number of studied subjects 8,121 in 1,388 households. The average number of persons per household was 5.9. Figure 2 shows the study profile. The month in which the study subjects came into or left the study area was recorded. The month in which the study subjects left or moved to the study area was considered to contribute 2 weeks to the total number of weeks observed.

Blood samples were collected from all febrile cases using World Health Organization (WHO) standard procedures[27]; a single finger prick was used to take a blood sample for the RDT and to prepare thick and thin blood films for microscopic evaluation. The result of RDT was used to treat the febrile case on the spot. The confirmations of at least two of the three experienced readers were sought to label a study subject as a malaria case. This work dealt with microscopically confirmed (by at least two readers) malaria cases only.

Three major malaria-related interventions (IRS with DDT and later with Deltamethrin and mass ITNs distribution) were carried out by the government within the study period. The brand of the ITNs was PermaNet2.0 (Vestergaard, Frandsen,
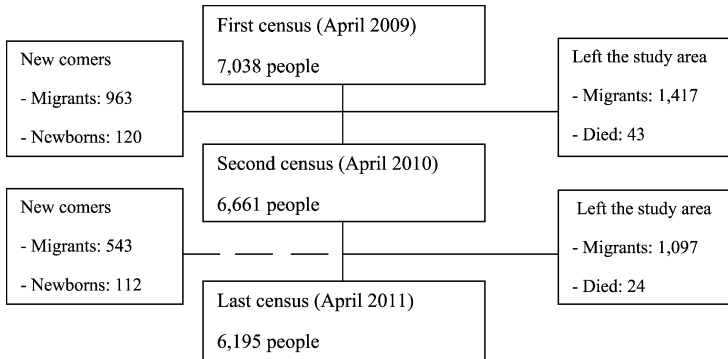
FIGURE 2.   Study profile: broken line indicates that follow-up on insecticide-treated nets (ITNs) use was not done, Chano Mille Kebele, south Ethiopia, 2009–2011.

Switzerland). We did post-intervention surveys to record ITNs and IRS coverage, and document replastering of the insecticide sprayed surfaces in the houses. The efficacies of DDT and Deltamethrin were assumed to be 10% and 50% (taken from Massebo and colleagues, unpublished data of the same study area) on the sprayed week and to decay over time at a constant rate at each time step—becoming nil after 24 weeks and 18 weeks,[21,28] respectively. The initial efficacy was multiplied by the coverage minus the proportion of households that practiced replastering of the sprayed surface.

Starting from Week 5, we recorded each household member who slept under the ITNs the night before the interview.

The main vector breeding place was identified by the research team. We explored potential places within and surrounding the Kebele and the place where we found larvae of *Anopheles* species was the swampy area near the Lake Abaya. There were several small water bodies created mainly by hoofprints of cattle and hippopotamus. Being on the shore of the lake, these tend to dry slowly after the rainy season—producing extended effect of rainfall. Overflow of the lake during the rainy season followed by shrinkage resulted in more favorable condition for vector breeding.

The meteorological data were obtained from the nearest local meteorological station at Arba Minch University, which was 6 km away from the study area.

Statistical analysis. Time-series modeling (auto-regressive integrated moving average [ARIMA] with transfer function [TF]) was carried out considering rainfall, temperature (minimum and maximum), relative humidity, ITNs use fraction, and IRS as potential predictors. The timescale was a week. The ITNs use fraction and IRS was lagged by 2 weeks considering the incubation period of falciparum malaria. The ITNs use data were available from Week 5; therefore, the analysis involved malaria incidence data starting from Week 7. This made the serial length 95 weeks. The number of individuals who slept under the net the night before the interview was divided by the total population of the week as a denominator to get the ITNs use fraction. The total population varied from week to week based on the number of weeks each individual has been observed. Cross-correlations were used to get information about lag number of meteorological variables. The maximum number of lags was set to be 16. Significant peaks (peaks with a bar height beyond the upper or lower confidence

limit) in the cross-correlation function plot determined the lag numbers for further analysis. Ljung-Box Q statistics was used as model diagnostics (the *P* value should not be < 0.05 to accept the model). The methodological details of ARIMA and TF models were reported elsewhere.[29]

To construct a wealth index, principal component analysis (PCA) was carried out using 15 socio-economic variables (see Supplemental file for the details). Lessons to construct the wealth index were taken from Vyas and Kumaranayake, and Howe and colleagues.[30,31] The total variance explained by the first principal component and the corresponding Eigen value was 20.35% and 3.05%, respectively, and this was comparable with a previous study.[12] A factor score derived from the first principal component was used in further analysis as a wealth index. Wealth index categorized into tertiles was used for descriptive purposes.

A generalized Poisson log-linear model was built considering age, sex, wealth index, proximity to the identified vector breeding place, total ITNs use, and education of the head of the household as potential predictors of falciparum malaria episodes for each study subject. The mean and the variance of falciparum episodes were 0.03 and 0.032, respectively. The number of weeks an individual had been observed was considered as a scale weight variable. Pearson χ^2 was used as scale parameter method and robust estimator for the covariance matrix. The parameter estimation method was hybrid with a maximum Fisher scoring iteration of 1. Kernel was specified for the log-likelihood function. Bivariate and multivariate analyses were carried out. The term used to build the model was the main effects. The omnibus test was used to check performance of each fitted model against the intercept-only model.

Because there was weekly active surveillance for cases, the generalized estimating equation (GEE) was carried out to allow for repeated measurements. The probability distribution specified was binomial with logit link function with independent working correlation matrix. Pearson χ^2 was used as the scale parameter method and robust estimator for the covariance matrix. The parameter estimation method was hybrid with a maximum Fisher scoring iteration of 1. Kernel was specified for the log quasi-likelihood function. The term used to build the reported model was the main effects. Corrected quasi-likelihood under independence model criterion was compared for main and interaction effects but no model
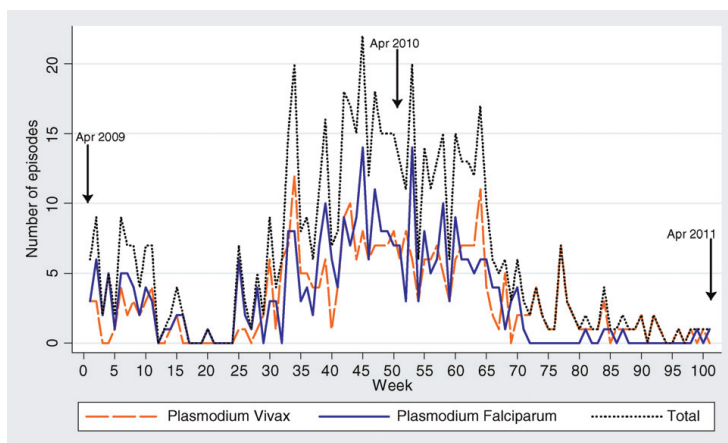
FIGURE 3. Sequence chart of *Plasmodium falciparum*, *Plasmodium vivax*, and total episodes over 101 weeks of observation, Chano Mille Kebele, south Ethiopia, 2009–2011.

improvement was achieved. The hypothesis test was based on Wald χ^2. Wealth index, age, sex, proximity to the identified vector breeding place, ITNs use, and education of the head of the household were considered as predictors. Because data on ITNs use at the individual level was collected from Week 5 and net use lagged by 2 weeks; data on ITNs use and falciparum malaria incidence ranged from Weeks 5 to 99 and Weeks 7 to 101, respectively, making the total number of weeks observed 95. The minimum and maximum number of observations per individual was 2 and 95.

Pairwise comparisons of estimated marginal means for age categories were done based on the original scale of dependent variable. The adjustment method for multiple comparisons was Sequential Sidak, which is a sequentially step-down rejective Sidak procedure that is much less conservative in terms of rejecting individual hypotheses but maintains the same overall significance level.

PASW Statistics 18 (Chicago, IL) was used for the analyses. Distance (in meters) from the vector breeding place was calculated using the proximity analysis tool of ESRI ArcMap 9.3 (Redlands, CA). To calculate the incidence rate ratio (IRR) with test-based confidence intervals (CI), we used Stata/IC 11.0 (College Station, TX).

Ethical approval. The Regional Health Research Ethics Review Committee of the Southern Nations, Nationalities and People's Regional Health Bureau approved this research project. Informed verbal consent was obtained from all study participants. For minors, consent was obtained from their caregivers or legal guardians. Patients were treated according to National guidelines[32] with antimalarial drugs immediately based on their RDT results.

RESULTS

During the 101 weeks of follow-up, there were 2,573 microscopically screened febrile episodes. Of these, 624 (24.3%) were microscopically confirmed malaria episodes; falciparum and vivax malaria accounted for 317 (50.8%) and 307 (49.2%) episodes, respectively. The pattern of malaria occurrence over the whole study period is shown in Figure 3. Descriptive statistics of meteorological variables and malaria episodes are presented in Table 1.

Of 317 falciparum malaria episodes, 224 (70.7%) were among registered residents and the rest 93 (29.3%) were temporary residents or visitors. Among 93 temporary visitor cases, 67 were males; their mean (SD) age was 14.8 (6.9) years. Only 12 of 93 cases were later registered in the last two censuses. Qualitative inquiry showed that most of the temporary residents or visitors were labor migrants such as house servants and cattle shepherds from neighboring districts and zones. Characteristics of registered residents by number of falciparum malaria episodes are presented in Table 2.

The incidence of falciparum malaria among registered subjects was 3.57/10,000 person-weeks of observation. As compared with females, males had a 64% higher rate of acquiring falciparum malaria. Likewise, the rate for the poor was greater than the rich by 64%. Among all age categories, children 5–14 years of age had higher IRR. The IRR was less for sub-Kebeles 1 and 2, and for the weeks in which the total rainfall was < 1.5 mm. Higher IRR was also documented in the first year of the study and after free mass ITNs distribution but before IRS with Deltamethrin. There was no significant difference in IRRs among different levels of education of the household head (Table 3).

TABLE 1

Descriptive statistics of meteorological variables and malaria episodes on weekly timescale, Chano Mille Kebele, south Ethiopia, 2009–2011

Variables ($N = 101$)	Minimum	Maximum	Mean (SD)
Minimum temperature (°C)	14.2	20.7	18.1 (1.2)
Maximum temperature (°C)	25.1	35.6	30.9 (2.1)
Total rainfall (mm)	0.0	147.5	15.7 (24.0)*
Relative humidity (%)	30.7	75.6	56.1 (11.0)
Plasmodium falciparum episodes	0	14	3.1 (3.4)*
Plasmodium vivax episodes	0	12	3.0 (2.9)*

*Median: 6.2 (total rainfall), 2 (*Plasmodium falciparum* episodes), and 2 (*Plasmodium vivax* episodes).

TABLE 2
Characteristics of registered residents by number of *Plasmodium falciparum* episodes, Chano Mille Kebele, south Ethiopia, 2009–2011

		Plasmodium falciparum episodes							
		0 (N = 7,916)		1 (N = 188)		2 (N = 15)		3 (N = 2)	
Variables (N = 8,121)		No.	%	No.	%	No.	%	No.	%
Sex	Male	4,100	51.8	115	61.2	10	66.7	2	100.0
	Female	3,816	48.2	73	38.8	5	33.3	0	0
Age in years*	< 5	1,033	13.0	30	16.0	3	20.0	1	50.0
	5–14	2,075	26.2	91	48.4	8	53.3	1	50.0
	15–24	2,279	28.8	38	20.2	4	26.7	0	0
	> 24	2,529	31.9	29	15.4	0	0	0	0
Wealth index: mean (SD)		0.24 (0.98)		0.06 (0.89)		−0.28 (0.85)		−0.68 (1.49)	
Distance (km) from vector breeding place: mean (SD)		2.49 (0.34)		2.22 (0.37)		2.09 (0.39)		1.95 (0.21)	
ITNs used (total number of weeks): Median		30		39		40		63	

*Mean (SD) for 0, 1, 2, and 3 episodes: 20.25 (15.41), 13.94 (11.29), 10.13 (5.57), and 4.79 (5.95) years, respectively.

We also calculated the IRRs by stratifying the ITNs use fraction into different age categories. This showed that before mass ITNs distribution, children 5–14 years had significantly higher IRR followed by under five children, but there was no significant difference between age categories 15–24 and above 24 years. After free mass ITNs distribution, the IRRs of < 5 and 5–14 years categories declined and there was a significant increase in IRR of 15–24 years category, whereas the IRR of 5–14 years category was still the highest. Meanwhile, after IRS with Deltamethrin (ITNs use fraction of 0.63), no significant differences were observed in the IRRs of all age categories (Table 4).

Regarding malaria-related interventions, IRS was carried out in June 2009 (DDT)—Week 7 and July 2010 (Deltamethrin)—Week 63; and free ITNs were distributed to all households in March 2010—Week 48. Post-intervention

surveys showed that the coverage of IRS with DDT and Deltamethrin was 91% and 97.5%, respectively. The percentages of households that practiced replastering of the sprayed surfaces was 9.2 (DDT) and 3.2 (Deltamethrin). Meanwhile, an average of 2.3 ITNs were given to each household (a household had an average of 5.9 persons).

Predictors of weekly number of falciparum malaria episodes. Time series modeling with a serial length of 95 weeks was carried out. The total number of falciparum malaria episodes from Weeks 7 to 101 was 295 including cases that did not exist in the census register. The average number of episodes per week was 3.1. The minimum, maximum, and mean ITNs use fraction after mass distribution was 0.47, 0.69, and 0.61; before mass distribution, these figures were 0.16, 0.24, and 0.2, respectively. The meteorological variable with a significant peak from the cross-correlation function plot was rainfall

TABLE 3
Characteristics of the study subjects, study year, total rainfall, and ITNs use fraction by incidence rate ratio (IRR) of *Plasmodium falciparum* malaria, Chano Mille Kebele, south Ethiopia, 2009–2011*

			Plasmodium falciparum		
Variables		Person-weeks of observation	Number of episodes	Weekly incidence per 10,000	IRR (95% CI)
Sex	Male	319,559	141	4.41	1.64 (1.25–2.14)
	Female	307,613	83	2.7	1
Age in years	< 5	88,556	39	4.4	3.23 (2.05–5.08)
	5–14	182,875	110	6.02	4.41 (3.03–6.4)
	15–24	143,306	46	3.21	2.35 (1.5–3.69)
	> 24	212,435	29	1.37	1
Sub-Kebele	1	210,396	36	1.71	0.23 (0.17–0.33)
	2	205,938	34	1.65	0.23 (0.16–0.32)
	3	210,838	154	7.30	1
Wealth status	Poor	209,155	86	4.11	1.64 (1.14–2.37)
	Medium	250,294	96	3.84	1.53 (1.07–2.2)
	Rich	167,723	42	2.50	1
Education: head of household	No education	331,838	112	3.38	2.44 (0.36–16.41)
	Primary	159,625	65	4.07	2.95 (0.45–19.35)
	Secondary	128,474	46	3.58	2.59 (0.38–17.46)
	Above secondary	7,235	1	1.38	1
Study year	1	328,656	142	4.32	1.57 (1.2–2.06)
	2	298,516	82	2.75	1
Total rainfall in mm†	< 1.5	207,491	44	2.12	0.46 (0.33–0.66)
	1.5–14.6	208,004	83	3.99	0.87 (0.65–1.17)
	> 14.6	211,677	97	4.58	1
ITNs use fraction (average)	0.20‡	269,935	106	3.93	2.76 (1.9–4.02)
	0.56§	97,011	71	7.32	5.15 (3.56–7.45)
	0.63¶	232,102	33	1.42	1
All subjects		627,172	224	3.57	NA

*ITNs = insecticide-treated nets; CI = confidence interval; NA = not applicable.
†Divided into tertiles.
‡Before free mass ITNs distribution (Weeks 5–47); data on ITNs use was not collected for Weeks 1–4.
§After free mass ITNs distribution and before indoor residual spraying (IRS) with Deltamethrin (Weeks 48–62).
¶After IRS with Deltamethrin (Weeks 63–101).

TABLE 4
ITNs use fraction and age categories by incidence rate ratio (IRR) of *Plasmodium falciparum* malaria, Chano Mille Kebele, south Ethiopia, 2009–2011*

Variables			*Plasmodium falciparum*			
ITNs use fraction (mean)	Age in years	Person-weeks of observation	Number of episodes	Weekly incidence per 10,000	IRR (95% CI)	
0.2†	< 5	34,432	21	6.10	4.3 (2.28–8.11)	
	5–14	77,904	59	7.57	5.34 (3.13–9.13)	
	15–24	65,881	13	1.97	1.39 (0.65–2.99)	
	> 24	91,718	13	1.42	1	
0.56‡	< 5	14,321	11	7.68	2.75 (1.18–6.4)	
	5–14	28,358	31	10.93	3.92 (1.97–7.79)	
	15–24	22,090	20	9.05	3.24 (1.54–6.82)	
	> 24	32,242	9	2.79	1	
0.63§	< 5	36,475	5	1.37	1.81 (0.56–5.83)	
	5–14	68,863	13	1.89	2.49 (0.98–6.35)	
	15–24	47,525	9	1.89	2.5 (0.92–6.78)	
	> 24	79,239	6	0.76	1	

*ITNs = insecticide-treated nets; CI = confidence interval.
†Before free mass ITNs distribution (Week 5–47); data on ITNs use was not collected for Weeks 1–4.
‡After free mass ITNs distribution and before IRS with Deltamethrin (Weeks 48–62).
§After IRS with Deltamethrin (Weeks 63–101).

(lag number 6). Minimum and maximum temperature and relative humidity had no significant peaks to consider. The ITNs use fraction and IRS with DDT and Deltamethrin were lagged by 2 weeks. Total rainfall and IRS with Deltamethrin significantly predicted the weekly number of falciparum malaria episodes. However, ITNs use fraction was not significant; and separate analyses using data before and after mass ITNs distribution produced a similar result. Figure 4 shows the pattern of the variables considered in the final model and the ITNs use fraction. Table 5 shows the time-series model parameter values of the significant predictors with the specified model structure and Figure 5 shows the performance of the model prediction (based on "DDT + Deltamethrin" model).

Predictors of total number of falciparum malaria episodes per individual. The total number of study subjects was 8,121, of these 4,227 (52.1%) were males. The mean (SD) age was 20.08 (15.35) years.

From Weeks 7 to 101, there were 204 episodes of falciparum malaria, of which two subjects had three episodes, 11 had two episodes, and the rest had one episode.

The constructed generalized Poisson log-linear model showed that those who were males, youngest (larger coefficient for age 5–14 years category), poorest and lived closer to the vector breeding place being more at risk. Total number of weeks in which study subjects had slept under ITNs and education of the head of the household did not predict total number of falciparum malaria episodes (Table 6). The pairwise multiple comparisons for age showed the absence of a significant difference between age category < 5 and 5–14 or 15–24 years categories. However, significant difference existed between 5–14 and 15–24 years categories.

Predictors of falciparum malaria infection at individual level: repeated measurements. The total number of subjects was 8,071 and the number of measurements per subject ranged
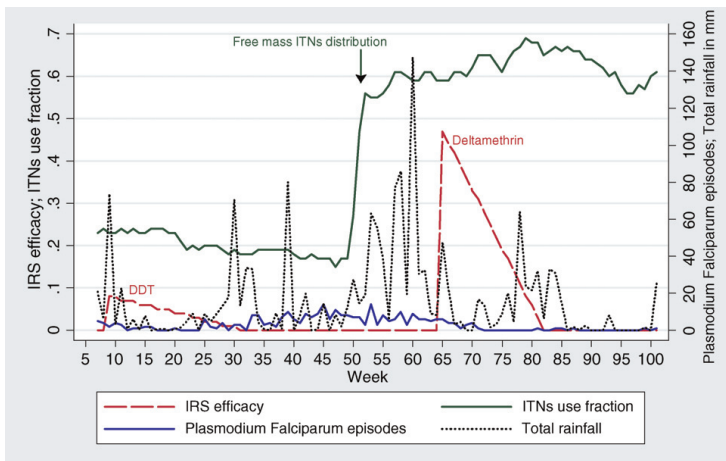
FIGURE 4. Sequence chart of *Plasmodium falciparum* episodes, total rainfall (lagged by 6 weeks), indoor residual spraying (IRS) efficacy (lagged by 2 weeks), and insecticide-treated nets (ITNs) use fraction (lagged by 2 weeks), Chano Mille Kebele, south Ethiopia, 2009–2011.

TABLE 5

Time series modeling of weekly number of *Plasmodium falciparum* episodes, Chano Mille Kebele, south Ethiopia, 2009–2011*

Model structure	Variables†			Estimate	P
ARIMA (0,1,5) (0,0,0)	Number of *Plasmodium falciparum* episodes	q	1	0.887	< 0.001
			5	−0.401	0.022
	Total rainfall (lagged by 6 weeks)‡	TF order	None	0.022	0.001
	DDT + Deltamethrin§	TF order	None	−1.867	0.001
IRS efficacy (lagged by 2 weeks)	Deltamethrin alone¶	TF order	None	−1.400	0.008
	DDT alone‖	TF order	None	−4.326	0.365

*ARIMA = auto-regressive integrated moving average; TF = transfer function; q = moving average order (both orders were significant in DDT alone and Deltamethrin alone models); IRS = indoor residual spraying.
†*Plasmodium falciparum* incidence and total rainfall had first order of non-seasonal differencing.
‡The reported estimate and P value in the table was for the model incorporated "DDT + Deltamethrin." Rainfall was significant while the model incorporated Deltamethrin alone (estimate = 0.022 and P = 0.002) and DDT alone (estimate = 0.017 and P = 0.017).
§Ljung-Box Q P = 0.747, and Goodness of fit (Stationary R-squared) = 75.2%; "DDT + Deltamethrin" represents the model that incorporated both but does not mean that both were sprayed together or their interaction term has been used.
¶Ljung-Box Q P = 0.282, and Goodness of fit (Stationary R-squared) = 71.8%.
‖Ljung-Box Q P = 0.368, and Goodness of fit (Stationary R-squared) = 70.6%.

between 2 and 95. Although taking into account the repeated measurements and the number of subjects, the total sample size became 582,846. This GEE model involved a total of 199 falciparum malaria episodes based on the availability of data on ITNs use for each individual. And to check for consistency in prediction, the variables used to build generalized Poisson log-linear model were considered.

The constructed GEE model showed those who were males, youngest (larger coefficient for 5–14 years category), poorest, and who lived closer to the vector breeding place being more at risk. The ITNs use was protective. Education of the head of the household was not significant (Table 7). The pairwise multiple comparisons for age showed that there was no significant difference among the three younger age categories except between 5–14 and 15–24 years.

DISCUSSION

The incidence of falciparum malaria was 3.57/10,000 person-weeks of observation. Of all falciparum malaria episodes, 29.1% were among temporary residents or visitors. Total rainfall, IRS with Deltamethrin, nearness to vector breeding place, sex, age,

ITNs use at individual level (not at community level), and wealth index were significant predictors of falciparum malaria.

The availability of both Co-Artem and Chloroquine motivated the residents to seek treatment at the health post, and the active search for cases facilitated prompt diagnosis and treatment. The weekly timescale provided adequate serial length for time-series modeling. The effect of local meteorological factors was evaluated controlling for malaria-related interventions. Different statistical modeling strategies allowed better scrutiny of the role of potential predictors of falciparum malaria. In addition, this study evaluated the effect of ITNs use at an individual level rather than the number of ITNs distributed. However, the data on ITNs use was based on self-report and was collected once in a week. Meanwhile, the population movement was more than expected, and there was no mechanism in place to register newcomers the moment after they arrived apart from the censuses conducted in the middle and at the end. Therefore, some study subjects could not be followed for some of the time they stayed in the study area.

The significant impact of rainfall at a lag of 6 weeks ("shorter lag") and the absence of effect of other meteorological variables were consistent with the findings of a study conducted in some districts of Ethiopia with comparable
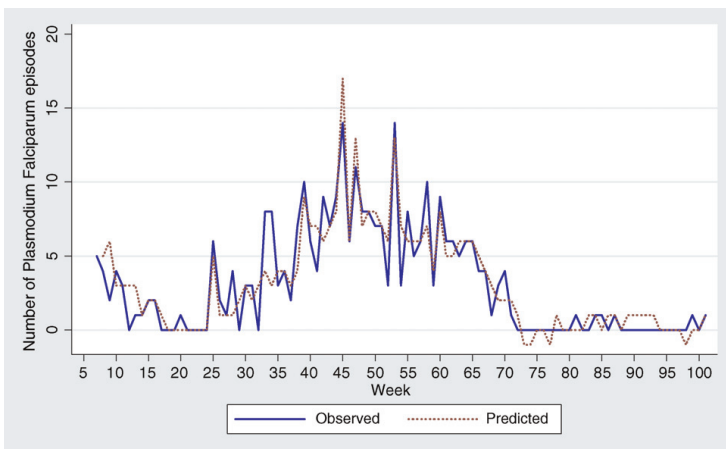

FIGURE 5. Actual and model-predicted *Plasmodium falciparum* episodes over 95 weeks of observation, Chano Mille Kebele, south Ethiopia, 2009–2011.

TABLE 6
Predictors of total number of *Plasmodium falciparum* episodes per individual: generalized Poisson log-linear model, Chano Mille Kebele, south Ethiopia, 2009–2011

Variable (N = 8,121)		Bivariate			Multivariate		
		Coefficient	Wald χ^2	P	Coefficient	Wald χ^2	P
Sex: Male		0.503	11.06	0.001	0.485	10.68	0.001
Age in years*†	< 5	1.215	21.77	< 0.001	1.117	19.47	< 0.001
	5–14	1.463	46.28	< 0.001	1.343	39.98	< 0.001
	15–24	0.742	8.72	0.003	0.747	9.09	0.003
Wealth index		−0.271	11.91	0.001	−0.186	6.49	0.011
Distance (meters) from vector breeding place		−0.002	87.12	< 0.001	−0.002	83.55	< 0.001
ITNs use: total number of weeks an individual slept under ITNs		1.913E-5‡	7.52E-5	0.993		NA	
Education: head of household‡§	No education	0.803	0.648	0.421			
	Primary	0.980	0.957	0.328		NA	
	Secondary	0.916	0.830	0.362			

*Reference category: > 24 years.
†Age as continuous variable had: bivariate (Coefficient = −0.039, Wald χ^2 = 34.67, and P < 0.001) and multivariate (Coefficient = −0.035, Wald χ^2 = 29.59, and P < 0.001).
‡Omnibus test was not significant (the model did not outperform the intercept-only model).
§Reference category: above secondary.
ITNs = insecticide-treated nets; NA = not applicable.

temperature and timescale.[6] The reason behind the analysis of local meteorological conditions in this work was to take into account the confounding effect while making conclusions about the impact of malaria prevention and control measures.

The ITNs use fraction and incidence of falciparum malaria increased in the period between mass ITNs distribution and IRS with Deltamethrin. We suggest that the episodes of falciparum malaria were dependent on other factors like rainfall rather than the ITNs use. Time series modeling showed that ITNs use fraction did not reduce incidence of falciparum malaria. This could also be explained by qualitative evaluation of Figure 4. After free mass ITNs distribution, the increase in ITNs use did not reduce falciparum malaria before IRS with Deltamethrin was introduced. Fifteen weeks is probably long enough to evaluate the impact of ITNs use fraction. Although the proportion of use by adults and children reached 35–65%, a threshold recommended,[19] the observed ITNs use might not be sufficient to reduce falciparum malaria. A previous study also showed that high ITNs ownership alone did not reduce falciparum malaria.[23]

The generalized Poisson log-linear model also showed that the study subjects were at risk of getting malaria infection regardless of the number of nights they had spent under the ITNs. This model did not consider the lag effect of ITNs use because the total number of nights spent under ITNs was obtained by cumulating the number of times that individuals

slept under ITNs and hence, could not guarantee protection against falciparum malaria unless the ITNs were used consistently. Therefore, the absence of a significant effect of total number of nights spent under ITNs on episodes of falciparum malaria might be related to inconsistent or improper use of ITNs.[33] Though both time-series and generalized Poisson log-linear modeling showed some valuable information about ITNs on the general picture of malaria epidemiology, both modeling approaches used a crude way of assessing link between ITNs and falciparum malaria infection. To compensate this, we used modeling allowing for repeated measurements and lagged effects, and it confirmed ITNs use at individual level as protective. Therefore, this study confirms that ITNs were protective at the individual level but did not show community-wide benefit with the observed ITNs use fraction. The absence of community-wide benefit suggests the excito-repellent effect of ITNs might have outweighed the insecticidal effect. This is because it is the insecticidal effect of the ITNs that results in community-wide benefit, whereas the excito-repellent effect increases the risk of infection among non-users.[34,35]

The observed data of falciparum malaria episodes and the simulated efficacy decay of DDT and Deltamethrin showed that IRS with DDT did not reduce falciparum malaria episodes, but IRS with Deltamethrin did. This suggests the community-wide benefit of the IRS with Deltamethrin

TABLE 7
Predictors of *Plasmodium falciparum* malaria episodes: GEE model, Chano Mille Kebele, south Ethiopia, 2009–2011

Variable (N = 582,846)		Bivariate			Multivariate		
		Coefficient	Wald χ^2	P	Coefficient	Wald χ^2	P
Sex: male		0.532	11.65	0.001	0.497	10.48	0.001
Age in years*†	< 5	1.184	20.09	< 0.001	1.019	15.64	< 0.001
	5–14	1.417	41.44	< 0.001	1.163	29.25	< 0.001
	15–24	0.782	9.32	0.002	0.644	6.6	0.01
Wealth index		−0.233	8.04	0.005	−0.155	4.11	0.043
Distance (meters) from vector breeding place		−0.002	85.34	< 0.001	−0.002	83.32	< 0.001
ITNs user		−0.584	13.36	< 0.001	−0.496	9.97	0.002
Education: head of household‡	No education	−0.035	0.001	0.972			
	Primary	0.112	0.015	0.903		NA	
	Secondary	−0.019	3.599E-4	0.985			

*Reference category: > 24 years.
†Age as continuous variable had: bivariate (Coefficient = −0.038, Wald χ^2 = 37.31 and P < 0.001) and multivariate (Coefficient = −0.032, Wald χ^2 = 25.31 and P < 0.001).
‡Reference category: above secondary.
GEE = generalized estimating equation; ITNs = insecticide-treated nets; NA = not applicable.

surpassed that of the ITNs (PermaNet2.0[36]) though the ITNs were coated with the same insecticide.

The greater risk among children 5–14 years of age and male subjects was comparable to other findings.[13,37] In general, younger study subjects had higher incidence rates, and this may be related to a lack of acquired immunity. The free mass ITNs distribution changed the rate of acquiring falciparum malaria among different age categories. Before free mass ITNs distribution IRR was higher in the 5–14 years than among < 5 years of age children. After free mass ITNs distribution, IRR increased among the 15–24 year age group. This may confirm the shift in rate of acquiring falciparum malaria among different age categories after such interventions, as has also been reported by others[26,38,39]; meanwhile, the absence of a significant difference in the IRRs of all age categories after IRS with Deltamethrin suggests that IRS protects all household members regardless of their age. This may also suggest the differences in IRRs among the age categories before IRS with Deltamethrin being attributable to age-related ITNs usage.

Population movement in a form of labor migration is considered important in malaria[8] and other vector-born disease[9] transmissions. This study showed that temporary residents or visitors experienced about a quarter of the number of falciparum malaria episodes. Characterizing these cases was limited because of uncertainties of their denominator. Nevertheless, their malaria risk seems to be higher because their denominator or length of stay is less or shorter than that of the permanent residents. This shows the contribution of population movement to malaria transmission, and its implication in prevention and control, and the need for further study.

In conclusion, there were 3.57 episodes of falciparum malaria per 10,000 person-weeks of observation. The data showed that rainfall at a lag of 6 weeks significantly increased, and IRS with Deltamethrin (but not with DDT) reduced falciparum malaria incidence. The ITNs use fraction did not show community-wide benefit, whereas individual ITNs prevented falciparum malaria in individuals.

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CONSTRUCTION OF WEALTH INDEX

Principal component analysis (PCA) was carried out using 15 variables. Eleven of the variables were binary and the other four variables were dichotomized into meaningful categories (some of the original categories had very few frequencies (Table SF1). Other socio-economic variables were also analyzed but did result in smaller Eigen values. The total variance explained by the first principal component and the corresponding Eigen value was 20.35% and 3.05, respectively. The inclusion of education of the household head in the PCA reduced the total variance explained by 1%, so we opted not to consider it in the wealth index construction rather to use it separately in further analysis. It is also known that the majority of the household heads depend on farming to generate income rather than being used on the merits of their educational status. Table SF2 shows frequencies, communalities, and correlations.

SUPPLEMENTARY TABLE SF1
Variables and assigned values

S. no.	Variables	Assigned values
1	Electricity	Present = 1, Absent = 0
2	Watch	Present = 1, Absent = 0
3	Radio	Present = 1, Absent = 0
4	TV	Present = 1, Absent = 0
5	Mobile	Present = 1, Absent = 0
6	Refrigerator	Present = 1, Absent = 0
7	Separate room used for kitchen	Present = 1, Absent = 0
8	Bicycle	Present = 1, Absent = 0
9	Any land used for agriculture	Present = 1, Absent = 0
10	Livestock	Present = 1, Absent = 0
11	Account in bank or credit association	Present = 1, Absent = 0
12	Main material of the floor	Cement/ceramic tiles = 1* Earth or dung = 0
13	Main material of the roof	Corrugated iron or cement/concrete = 1† Thatch or leaf = 0
14	Main material of the wall	Wood with mud/wood with mud and cement = 1‡ No wall§/only wood = 0
15	Latrine facility	Pit latrine¶ = 1, No latrine = 0

*Ceramic tile floor: 2 households.
†Cement/concrete roof: 2 households.
‡Wood with mud and cement: 3 households.
§No wall: 2 households.
¶No other specific types reported.

SUPPLEMENTARY TABLE SF2
Frequencies of the binary/dichotomized variables, communalities, and correlations with the first component

Variable (N = 1388)	Present or favorable		Communalities*	Correlations with the first component†
	Frequency	Percent		
Electricity	1,166	84.0	0.539	0.602
Watch	722	52.0	0.384	0.579
Radio	873	62.9	0.496	0.644
TV	196	14.1	0.555	0.609
Mobile	223	16.1	0.457	0.497
Refrigerator	15	1.1	0.357	0.245
Separate room used for kitchen	767	55.3	0.393	0.374
Bicycle	255	18.4	0.369	0.538
Any land used for agriculture	743	53.5	0.606	0.396
Livestock	800	57.6	0.576	0.417
Account in bank or credit association	127	9.1	0.568	0.180
Main material of the floor	67	4.8	0.322	0.353
Main material of the roof	913	65.8	0.341	0.541
Main material of the wall	1,354	97.6	0.236	0.185
Latrine facility	1,358	97.8	0.439	0.134

*Estimates of the variance in each variable accounted for by the components (4 components were extracted but we took the first component for further analysis).
†Radio was the most representative (of the first component) and followed by TV, electricity, watch, main material of the roof... and latrine facility was the least. The absence of negative values confirms that all variables were positively correlated with wealth (as expected).

Effect of Bednets and Indoor Residual Spraying on Spatio-Temporal Clustering of Malaria in a Village in South Ethiopia: A Longitudinal Study

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Abstract

Background: Understanding the spatio-temporal pattern of malaria transmission where prevention and control measures are in place will help to fine-tune strategies. The objective of this study was to assess the effect of mass distribution of bednets and indoor residual spraying (IRS) with insecticides on the spatio-temporal clustering of malaria in one malaria endemic village in south Ethiopia.

Methods: A longitudinal study was conducted from April 2009 to April 2011. The average population was 6631 in 1346 locations. We used active and passive searches for malaria cases for 101 weeks. SatScan v9.1.1 was used to identify statistically significant retrospective space–time clusters. A discrete Poisson based model was applied with the aim of identifying areas with high rates. PASW Statistics 18 was used to build generalized Poisson loglinear model.

Results: The total number of both types of malaria episodes was 622, giving 45.1 episodes per 1000 persons per year; among these, episodes of *Plasmodium falciparum* and *vivax* infection numbered 316 (22.9 per 1000 per year) and 306 (22.2 per 1000 per year), respectively. IRS with Dichlorodiphenyltrichloroethane (DDT) and later with Deltamethrin and free mass distribution of insecticide-treated nets (ITNs) were carried out during the study period. There was space–time clustering of malaria episodes at a household level. The spatio-temporal clustering of malaria was not influenced by free mass distribution of ITNs; however, the time-span of the spatio-temporal clustering of malaria cases ended after IRS with Deltamethrin. The presence of clusters on the south-east edge of the village was consistent with the finding of an increasing risk of acquiring malaria infection for individuals who lived closer to the identified vector breeding site.

Conclusion: The risk of getting malaria infection varied significantly within one village. Free mass distribution of ITNs did not influence the spatio-temporal clustering of malaria, but IRS might have eliminated malaria clustering.

Introduction

Malaria is a leading health problem in Ethiopia, where 67% of the 82 million people are estimated to be at risk. There were 1 036 316 confirmed cases of malaria in 2009. The dominant plasmodium species are *Plasmodium falciparum* and *vivax*, and the major *Anopheles* species responsible for transmission is *arabiensis* [1]. In 2005, massive expansion of malaria control programmes included the distribution of long-lasting insecticidal nets (LLINs), and the use of Artemether–Lumefantrine as a first-line treatment for *Plasmodium falciparum* malaria [1,2]. In 2007 and 2010, the percentage of households with at least one LLTN was 53.8% [2] and 72% [1], respectively. In addition, 20% of households below an altitude of 2000 metres above sea level were subjected to indoor residual spraying (IRS) [2].

Nationally, the number of cases has declined since 2005 due to an expansion in the malaria control programmes. However,

malaria admissions increased in 2009 [1], and more than the expected number of cases occurred in south Ethiopia in 2010/2011 [3]. The reasons for this apparent increase are not well documented and there have been calls for better understanding of disease transmission, and an evaluation of malaria control measures.

To implement malaria prevention and control measures, and to understand risk dynamics, application of Geographic Information System (GIS) has been emphasized, in particular to provide a precise definition of the time and location of epidemics [4]. The results of GIS can be used to explain the interactions among humans, their environment, risk factors, and changes over time and space [5,6]. Geo-referencing of malaria cases, combined with efforts to link them to potential locations of environmental exposure, increases the benefit of disease maps [7].

Spatial epidemiological studies at a finer geographic scale, such as households, help to increase understanding of the varied pattern

of malaria infection and transmission [8]. Several studies have reported significant spatial and temporal variation in malaria transmission. Mapping high-risk zones or clusters may contribute to improved prevention and control efforts by delivering limited resources to the population at higher risk [8–14]. To inform policy, it is also important to observe the spatio-temporal pattern of transmission in line with the presence or absence of prevention and control measures on the ground. Therefore, this study aimed to investigate the effect of prevention and control measures on the spatio-temporal clustering of malaria at a household level in one malaria endemic village in south Ethiopia.

Materials and Methods

Study Area

Chano Mille Kebele (Kebele is the lowest administrative unit in Ethiopia) is 492 km south of Addis Ababa (Figure 1). The altitude is 1206 m above sea level. The annual rainfall was 650 mm in 2009 and 1057 mm in 2010. The area covers 2.4 square km. There were 1388 households (1346 locations) with an average population (from the first, midway and last census) of 6631 people (the total population followed was 8121). The average household size was 5.9 (8121/1388) individuals. The number of households was greater than the number of locations because some households left the study area, replaced by others in the same locations.

Chano Mille Kebele was selected purposely for the study of malaria epidemiology. Three main irrigation ditches run from the neighbouring Kebele in the west, cross the Kebele and may also end within the Kebele. There are two adjacent Kebeles, Chano Dorga to the north-west and Chano Chalba to the south-west. The area to the east and south-east sides of the Kebele, extending to Lake Abaya, is used for agricultural purpose. Most of the households grow mango trees within their compounds.

There was one health post in the village staffed by a health extension worker. A health post provides basic health services, including malaria diagnosis using rapid diagnostic test (RDT) kits and treatment with Artemether–Lumefantrine.

Study Design

The cohort study was carried out from April 2009 to April 2011. Both active and passive surveillance schemes were used. Each household was visited every week for 101 weeks looking for cases of fever (temperature $\geq$37.5 degrees Celsius); if needed patients were referred to the health post for diagnosis and treatment of malaria (active case finding). Each day, we checked whether the referred cases had visited the health post. During the
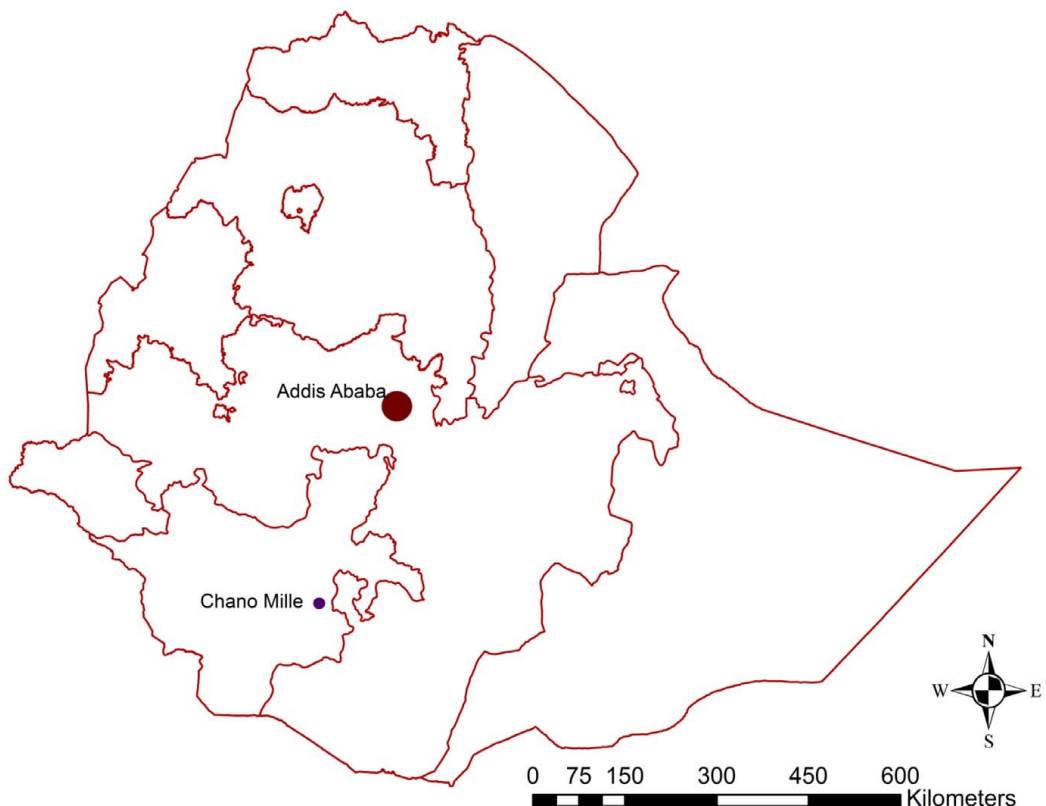
Figure 1. Map of Ethiopia and location of Chano Mille Kebele.
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days between the visits, the residents were advised to self-report to the health post if they became febrile (passive case-finding).

We gave a unique household number to each household before the first census. The geographic coordinates of all households were recorded using GPS during the first census. The GPS reading for the new households was performed during the midway census. We also recorded GPS coordinates for the main vector breeding sites. These vector breeding sites are swampy areas close to Lake Abaya, with many hoof prints of cattle and hippopotami. Such small water bodies are formed mainly after flooding of the lake during the rainy season. We did not find larvae of *Anopheles* species near the irrigation ditches or at other locations in the study area.

Blood Samples and Patient Management

The laboratory technician used a single finger prick to collect blood samples for RDT and prepared thick and thin blood films for microscopic evaluation. Based on the results of the RDT, the patients were treated with Artemether–Lumefantrine (*Plasmodium falciparum*) or Chloroquine (*Plasmodium vivax*). Chloroquine was provided by the research project. Two experienced laboratory technicians read each blood slide independently. Whenever there was a discordant reading, confirmation was obtained from a third reader. All readers were blinded to the readings of the others.

Analysis

Spatio-temporal. We used SatScan v9.1.1 (http://www.satscan.org/) software for spatial and space–time statistical analysis, to identify statistically significant retrospective space–time clusters. Two episodes of malaria (one vivax and one falciparum) were excluded from the analysis because of missing data on the location. The time precision was 1 month and a coordinate file was provided with latitude and longitude values. All cases were checked to ascertain whether they had occurred within the specified time period and geographical area. Case, population and coordinates files were prepared, considering a discrete Poisson-based model. The focus was to detect areas of high infection rate. A criterion of 'no geographical overlap' was used to report secondary clusters. The P-value was generated using a combination of standard Monte Carlo, sequential Monte Carlo and Gumbel approximation [15]. We used 9999 Monte Carlo replications. The maximum temporal cluster size was set at 50%. The spatial window shape was circular, and the maximum spatial cluster size was set at 25% of the population at risk, after evaluating the effects of switching this value to 15%, 35% and 50%. Given that we used the geographic coordinates of the households, which were close to one another, we performed the analysis by evaluating the possible changes while using different levels of maximum spatial cluster size restriction as a percentage of the population at risk. We started arbitrarily with 50%, then 35%, 25% and finally 15%. This helped to show how the SatScan software captures the clustering with varying spatial cluster size restrictions. We observed that the centres of the circles of the most likely clusters of all spatial cluster size restrictions occurred consistently very close to the south-east corner of the village.

A maximum spatial cluster size of 50% is recommended because it should capture all clustering. In our case, it captured all the smaller clusters (most likely and secondary) that we observed while running the analysis with smaller maximum spatial cluster size within one most likely cluster, and it provided no secondary cluster. The greater portion of the cluster circle had no households in it. The clusters with maximum cluster size smaller than 50% yielded smaller clusters with varying relative risks. A maximum spatial cluster size of 35% yielded a very small secondary cluster with only 34 people in it, and the greater portion of the cluster

circle did not hold households. A maximum spatial cluster size of 15%, to accommodate an additional secondary cluster, pushed the most likely cluster to the edge of the village and again the greater portion of the cluster circle contained no households. This revealed three secondary clusters, of which one contained only 21 people and the other was the same as the secondary cluster detected using the 25% maximum spatial cluster size restriction. Therefore, we decided that the 25% maximum cluster size restriction was most appropriate to show the malaria clustering activity of the study area because the portion of the most likely cluster circle with no households was relatively small and this specification yielded a secondary cluster. All the results reported here were obtained with this maximum spatial window size. The same maximum spatial cluster size was used to investigate the differences in clustering by the type of malaria and years of study. (Supplemental figures S1, S2 and S3 are provided showing space-time clusters of different maximum spatial cluster size restrictions.).

Individual level factors. The PASW Statistics 18 program (Chicago, IL, USA) was used to fit a generalized Poisson loglinear model. The dependent variables were episodes of vivax and falciparum malaria. The potential predictors considered were: distance to the vector breeding site, number of households located between each household and the vector breeding site (household count), sex, age, wealth index and total number of nights spent under insecticide-treated nets (ITNs). Every week, residents were asked whether they slept under ITNs the night before the interview and the names of the household member who had slept under ITNs were recorded. To get the total number of nights spent under ITNs, we summed-up the weekly data for each individual. The number of weeks for which each individual had been observed was used as a scale weight variable. The scale parameter method was Pearson chi-square and a robust estimator was used for the covariance matrix. The log-likelihood function was kernel. The ratio of the Pearson chi-square value to its degrees of freedom was used to rule out over-dispersion. This value was 1.34 and 1.28 for vivax and falciparum episodes, respectively. Given that these values did not deviate significantly from 1, we assumed that the Poisson distribution was a good fit for the data. Statistically significant (P-value <0.05) variables detected during bivariate analyses were considered for the multivariate model. The exponential form of the estimates was interpreted as the incidence rate ratio (IRR). We reported the IRR with 95% confidence intervals (CI).

We used ESRI ®ArcMap™ 9.3(CA, USA) to calculate the distance of each household from the vector breeding site (in km) and to produce the maps.

To get household count– the number of households between an individual household and the breeding site, we used the methodology described in supplemental file S1 (with figures S4, S5 and S6). This methodology assumes the potential possible area covered by a mosquito increases with distance travelled, and the flight pattern has a form of a circular sector. The measure is expressed in form of an angle. To highlight the sensitivity to the selection of search angle, the analysis was repeated with angles from 1 to 30 degrees (1, 5, 10, 15, 20, 25 and 30). The algorithm was implemented in R [16], and the household count for each search angle was analysed independently.

A recent paper by the authors has reported the predictors of falciparum malaria episodes, which included the effect of meteorological covariates (total rainfall, temperature and relative humidity), ITN utilization rate, efficacy of the insecticides used for IRS, and other factors. The statistical models employed were auto-regressive integrated moving average models with a transfer function model, generalized Poisson loglinear model and gener-

alized estimating equation with logit link function. Principal Component Analysis was used to construct wealth index. The variables included were presence of electricity, watch, radio, TV, mobile telephone, refrigerator, separate room used for kitchen, bicycle, any land used for agriculture, livestock, account in bank or credit association and latrine facility. In addition the main materials of the floor, the roof and the wall were included. The technical details of the construction of the wealth index were reported elsewhere [17].

Ethical Approval

The Regional Health Research Ethics Review Committee of the Southern Nations, Nationalities and People's Regional Health Bureau has approved this research project. Informed verbal consent was obtained from all study participants and recorded by the research team on the ethical consent form (with prior approval from our ethics review committee). For minors, consent was obtained from their caregivers or legal guardians. All cases of malaria were treated immediately. Given that the blood samples were collected only for the purpose of malaria diagnosis and treatment, written consent was not suggested by the Regional Health Research Ethics Review Committee.

Results

The study population (average of the three censuses) was 6631 in 1388 households (1346 locations). Within the study period, the total number of both types (*Plasmodium falciparum* and *vivax*) of malaria episode was 622, resulting in 45.1 episodes per 1000 persons per year. The number of *Plasmodium falciparum* and *vivax* episodes was 316 (in 226 locations) and 306 (in 199 locations); meanwhile, the annual rate of episodes per 1000 persons was 22.9 and 22.2, respectively. A higher number of malaria episodes occurred in males and in individuals aged 5–14 years. The mean distance of households with malaria episodes from the vector breeding site was lower (Table 1).

Space–time Analysis

Both the most likely and the secondary clusters were found on the south-east edge of the village, facing the vector breeding site (Figures 2 and 3).

The most likely cluster. The most likely space–time cluster lasted for 9 months (of the 25 months of the study) for both *Plasmodium* species. However, the *Plasmodium vivax* cluster started and ended 1 month earlier and had a smaller relative risk. The size and location of the most likely cluster of *Plasmodium falciparum* was the same as the cluster for both types of malaria. However, the relative risk for the *Plasmodium falciparum* cluster was greater. Households within the cluster were 7.49 times more at risk of contracting *Plasmodium falciparum* infection than households outside the cluster. This risk was 5.39 for *Plasmodium vivax* and 6.05 for both types of malaria (Table 2).

The secondary cluster. *Plasmodium falciparum* alone did not have a secondary cluster. The relative risk was 3.28 for both types of malaria and 5.47 for *Plasmodium vivax* alone. The time-span for both types of malaria was the same as that of the most likely cluster but was shorter by 4 months for *Plasmodium vivax* alone (Table 3).

Analysis for separate years. The space–time analysis result of both *Plasmodium* species (for separate years) was relatively similar with the analysis involving the whole study period. The location of the most likely clusters of year I and year II was almost the same. However, significant secondary cluster was found only in the first year of the study. There was also slight increase in the relative risk values (Table 4). Figure 4 shows significant space–time clusters of each study year.

Malaria Preventive Measures and Spatio-temporal Clustering

The three major preventive measures applied by the government during the study period included: IRS with Dichlorodiphenyltrichloroethane (DDT): 91% of the houses were sprayed in June 2009, free mass ITN distribution (2.3 ITNs per household) in March 2010, and IRS with Deltamethrin: 97.5% of the houses

Table 1. Malaria episodes by *Plasmodium* species, sex, age, number of locations and mean distance of locations (with and without episodes) from the vector breeding site.
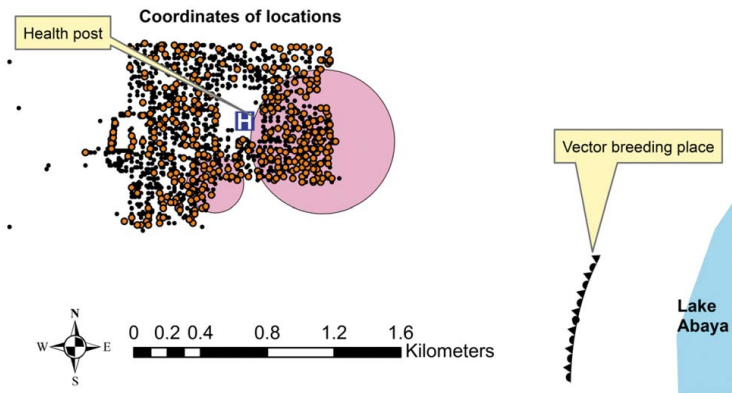
			Types of malaria			
			Plasmodium Falciparum		*Plasmodium Vivax*	
			Number	**Percent**	**Number**	**Percent**
Number of episodes	Sex	Male	208	65.8	196	64.1
		Female	108	34.2	110	35.9
	Age in years	<5	45	14.2	71	23.2
		5–14	146	46.2	130	42.5
		15–24	85	26.9	71	23.2
		>24	40	12.7	34	11.1
	Total		316		306	
Annual number of episodes per 1000			22.9		22.2	
Number of locations			226		199	
Mean (SD) distance of locations from the vector breeding site (km)	With episodes		2.28 (0.36)		2.36 (0.34)	
	Without episodes		2.53 (0.33)§		2.51 (0.34)‡	

§Number of locations: 1120.
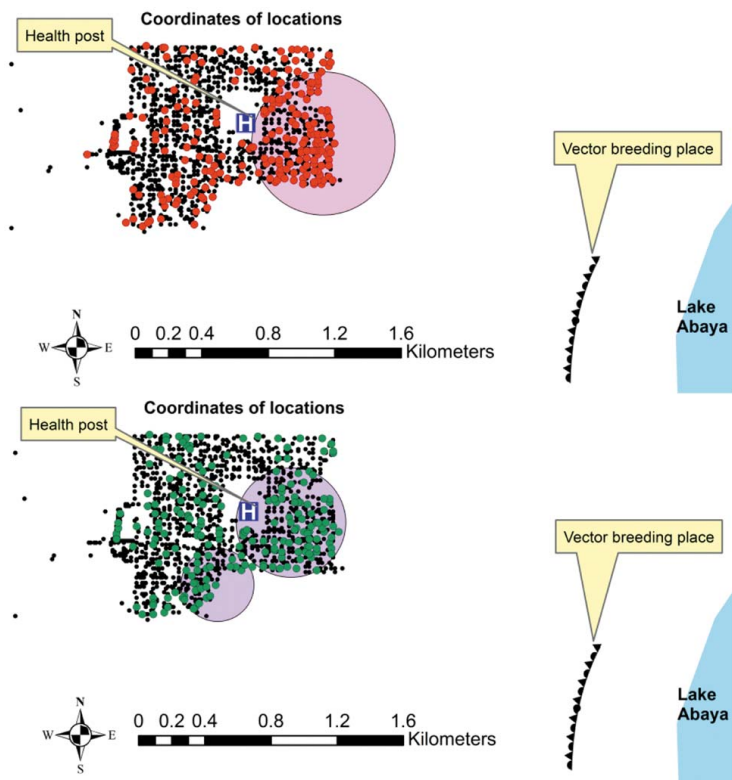‡Number of locations: 1147.
SD: standard deviation.
doi:10.1371/journal.pone.0047354.t001

Figure 2. Most likely (big) and secondary (small) clusters (shaded circles) of all malaria episodes- orange dots refer to all malaria episodes.
doi:10.1371/journal.pone.0047354.g002

Figure 3. Most likely and secondary clusters (shaded circles) of malaria episodes by *Plasmodium* **species: Red dots refer to** *Plasmodium Falciparum* **episodes that had one most likely cluster.** Meanwhile, green dots refer to *Plasmodium Vivax* episodes that had most likely (big) and secondary (small) clusters.
doi:10.1371/journal.pone.0047354.g003

Table 2. Space–time scan statistics of the most likely cluster of malaria episodes.

	Both types of malaria	Plasmodium Falciparum	Plasmodium Vivax
Number of locations included	326	326	332
Coordinates	6.1100N, 37.6005E	6.1100N, 37.6005E	6.1105N, 37.5986E
Radius (km)	0.43	0.43	0.33
Time frame	Dec. 2009 to Aug. 2010	Dec. 2009 to Aug. 2010	Nov. 2009 to Jul. 2010
Population	1626	1626	1653
Number of episodes	230	133	106
Expected episodes	54.99	27.94	27.4
Annual episodes/1000	188.6	109.0	85.8
Observed/expected	4.18	4.76	3.87
Relative risk	6.05	7.49	5.39
Log likelihood ratio	184.43	124.51	77.11
P-value	<0.001	<0.001	<0.001

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were sprayed in July 2010. The spatial coverage of IRS and ITNs are presented in Figures 5 and 6. The spatio-temporal clustering started 5–6 months after the IRS with DDT and continued for 4–5 months after free mass distribution of ITNs, but ended within 1 month after IRS with Deltamethrin. The time-span of the most likely space–time cluster generated by SatScan and the timing of the interventions in relation to the monthly incidence of malaria are presented in Figure 7.

Factors Determining Episodes of Falciparum and Vivax Malaria at Individual Level

Of the total number of falciparum and vivax episodes, 224 and 203 episodes, respectively, occurred among permanent residents for whom we had follow-up data.

Living nearer to the vector breeding site increased the risk of acquiring falciparum malaria, that is, each 1 km closer to the vector breeding site added 4.93 (95% CI: 2.59–9.35) times more risk. Household count was negatively associated with both types of malaria episodes with all vector's search angles considered during bivariate analyses. However, the household counts of the first three

lower search angles (1, 5 and 10 degrees) were found to be statistically significant in the multivariate model for falciparum episodes. As the vector's search angle decreases, the effect of household count increases. With a search angle of 1 degree, for each additional household between a household of interest and the vector breeding site, the risk of getting falciparum malaria decreases by 2%. Male participants had 1.63 (95% CI: 1.22–2.18) times more risk of acquiring falciparum malaria. When compared with adults aged >24 years, children aged 5–14 years had 3.82 (95% CI: 2.52–5.78) times more risk. Having higher wealth index was marginally failed to be protective against falciparum malaria (P-value: 0.051) in the model containing household count. Nevertheless, for search angles above 15 degrees, the wealth index regains statistical significance as the household count becomes no more statistically significant. The total number of nights spent under ITNs was not associated with the total number of falciparum episodes. Regarding vivax malaria, household count, sex, wealth index and the total number of nights spent under ITNs were not significant predictors. Meanwhile, when compared with adults aged >24 years, children <5 years old had 7.6 (95% CI: 4.2–13.74) times more risk, and living 1 km closer to the vector breeding site conferred 2.9 (95% CI: 1.2–6.99) times more risk of acquiring vivax malaria (Table 5).

Discussion

There was a space–time clustering of malaria at household level. Free mass distribution of ITNs did not affect the spatio-temporal clustering of malaria, but IRS might have. Living nearer to the vector breeding site increased the risk of acquiring malaria infection. These differences in malaria risk within a population who live in one village reflect the complexity of the disease transmission dynamics.

Our study confirms the findings of other studies that have shown spatio-temporal clustering of malaria cases at varying geographical extents [9–13], and even at household level [8,14]. The epidemiology of malaria in the study area may be unique, and this will limit the generalizability of the findings. However, the study of malaria in such a micro-environment may help to provide better knowledge on how the disease transmission is influenced by preventive and control measures.

Clustering varied by the type of malaria [8]. The similarity in size and location of the most likely clusters for both types and for

Table 3. Space–time scan statistics of the secondary clusters.

	Both types of malaria	Plasmodium Vivax
Number of locations included	81	102
Coordinates	6.1077N, 37.5947E	6.1071N, 37.5946E
Radius (km)	0.17	0.22
Time frame	Dec. 2009 to Aug. 2010	Dec. 2009 to Apr. 2010
Population	407	489
Number of episodes	43	23
Expected episodes	13.76	4.48
Annual episodes/1000	140.8	113.8
Observed/expected	3.12	5.13
Relative risk	3.28	5.47
Log likelihood ratio	20.46	19.67
P-value	<0.001	<0.001

doi:10.1371/journal.pone.0047354.t003

Figure 4. Most likely (big) and secondary (small) clusters (shaded circles) of malaria episodes (both types) according to study year-orange dots refer to all malaria episodes of the corresponding year.
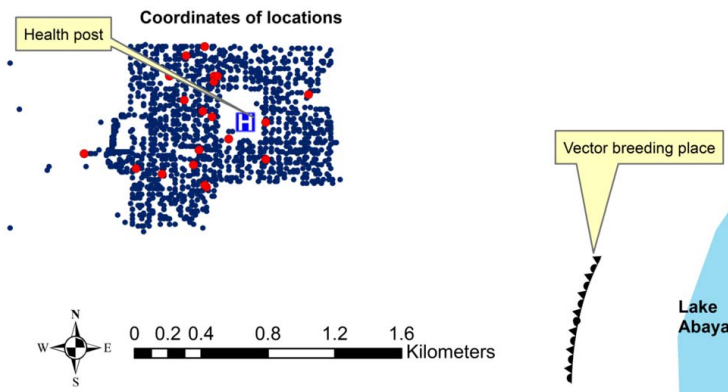doi:10.1371/journal.pone.0047354.g004

Table 4. Space–time scan statistics of the most likely and secondary clusters of malaria episodes (both types) according to year of study.

	Year I (Apr. 2009 to Mar.2010)		Year II (Apr. 2010 to Apr.2011)
	Most-likely cluster	Secondary cluster	Most-likely cluster
Number of locations included	264	79	322
Coordinates	6.1105N, 37.6009E	6.1077N, 37.5947E	6.1099N, 37.6009E
Radius (km)	0.39	0.17	0.47
Time frame	Dec. 2009 to Mar. 2010	Jan. 2010 to Mar. 2010	Apr. 2010 to Aug. 2010
Population	1335	399	1604
Number of episodes	107	22	115
Expected episodes	21.9	4.9	27.6
Annual episodes/1000	241.9	223.8	171.2
Observed/expected	4.89	4.52	4.2
Relative risk	6.77	4.77	6.21
Log likelihood ratio	97.79	16.52	93.13
P-value	<0.001	0.002	<0.001

doi:10.1371/journal.pone.0047354.t004
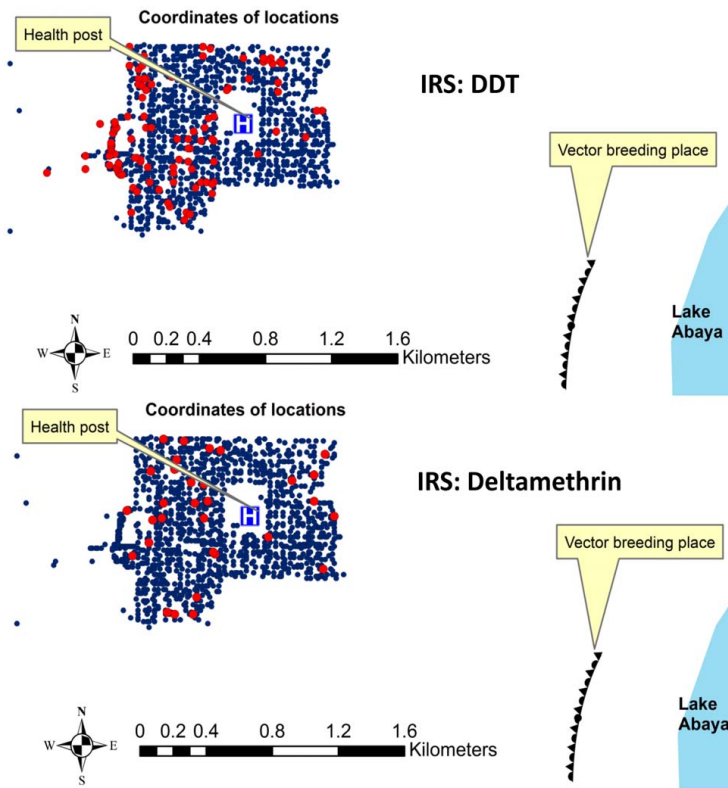
Figure 5. Spatial coverage of indoor residual spraying (IRS) with DDT and Deltamethrin: Red dots refer to houses that were not sprayed and smaller blue dots refer to sprayed houses.
doi:10.1371/journal.pone.0047354.g005

Figure 6. Spatial coverage of insecticide treated bednets (ITNs): Red dots refer to households that did not receive ITNs and smaller blue dots refer to those who received at least one ITN.
doi:10.1371/journal.pone.0047354.g006
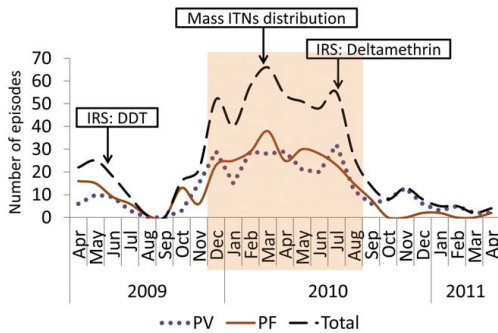
Figure 7. Monthly malaria incidence, time of interventions and time span of the most likely spatio–temporal cluster of all types of malaria. The shaded part indicates the time-span of the most likely space–time cluster.
doi:10.1371/journal.pone.0047354.g007

Plasmodium falciparum malaria may show that the clustering of *Plasmodium falciparum* dominated that of *Plasmodium vivax*. It was also shown that the relative risk of the *Plasmodium falciparum* cluster was higher than that of both types of malaria for the same cluster location and size. Similarly, the existence of a secondary cluster in the analysis of both types of malaria took into account the clustering activity of *Plasmodium vivax* (rather than *Plasmodium falciparum*) because we observed a secondary cluster only for this species (with a shorter time span and higher relative risk) when analysed separately. The most likely cluster of *Plasmodium vivax* alone was also closer to the centre of the village (farther from the vector breeding site). Descriptive statistics also showed that the mean distance of households with episodes of *Plasmodium vivax* was slightly farther from the vector breeding site than that of households with *Plasmodium falciparum* episodes. To confirm the effect of the vector breeding site on malaria clustering in

households close to it, we used Poisson regression to estimate its effect at the level of individual episodes, controlling for household count, sex, age, wealth index and the number of nights slept under ITNs. Those who lived nearer to the vector breeding site had a greater risk of acquiring infection; meanwhile, the increase in risk was greater for falciparum than for vivax malaria (4.93 versus 2.9), respectively, as a function of living 1 km closer to the vector breeding site. This may support the finding that the most likely cluster of falciparum malaria was nearer to the vector breeding site than the most likely cluster of vivax malaria, and also justifies the need for separate space–time clustering analysis for each *Plasmodium* species. There may also be a need to verify the role of new versus relapse episodes of vivax malaria on the space–time clustering.

An increase in risk of getting vivax malaria has occurred in two locations (vivax malaria had significant secondary cluster) and the relative risks within vivax malaria clusters were smaller than that of falciparum malaria cluster. This may suggest that a targeted intervention could be easier to apply for falciparum than for vivax malaria in the study area.

There is ample evidence that sleeping under ITNs protects against malaria infection [18], provided that they are used properly [19]. The absence of a significant impact of the number of nights slept under ITNs on the total number of malaria episodes an individual experienced may be related to inconsistent or improper use of ITNs. Meanwhile, in the prevention of malaria at a community level, the role of ITNs depends on the utilization rate [20]. The level of ITN utilization increased after mass distribution of ITNs (data are not shown); however it did not lower the risk for malaria clustering. Therefore, it is possible that free mass distribution of ITNs is not an effective tool with which to combat malaria without follow-up to ensure the optimal utilization of the ITNs.

The time span (December 2009 to August 2010) for the spatio-temporal clustering of both types of malaria ended when the possible effect of IRS with Deltamethrin (sprayed in July 2010) started. Although it may not be possible to reach the conclusion that IRS alone eliminated the spatio-temporal clustering of

Table 5. Factors determining malaria episodes at individual level.

Variable (n = 8121)		Plasmodium Falciparum (224 episodes)		Plasmodium Vivax (203 episodes)	
		Crude IRR (95% CI)	Adjusted IRR (95% CI)	Crude IRR (95% CI)	Adjusted IRR (95% CI)
Distance (km) from vector breeding site§		11.11(6.67–20.0)	4.93(2.59–9.35)*	4.55(2.7–7.69)	2.9(1.2–6.99)*
Household count with a search angle of 1 degree‡		0.98(0.96–0.99)	0.98(0.96–0.99)*	0.97(0.96–0.98)	0.99(0.97–1.01)
Sex : Male		1.65(1.23–2.23)	1.63(1.22–2.18)*	1.26(0.87–1.83)	NA
Age in years‡	<5	3.37(2.02–5.62)	3.07(1.87–5.03)*	7.82(4.31–14.2)	7.6(4.2–13.74)*
	5–14	4.32(2.83–6.58)	3.82(2.52–5.78)*	6.91(3.95–12.12)	6.53(3.74–11.41)*
	15–24	2.1(1.28–3.44)	2.14(1.31–3.48)*	1.84(0.91–3.74)	1.84(0.91–3.75)
Wealth index		0.76(0.65–0.89)	0.91(0.83–1.0)¥	0.95(0.78–1.16)	NA
Total number of nights spent under ITNs		1(0.99–1.01)	NA	1(0.99–1.01)	NA

‡Reference category: >24 years.
*Significant at P-value <0.05.
§The reciprocal of the IRR (95% CI) was presented to show the risk of being closer to the vector breeding site.
‡Household count refers to the number of households located between each household and the vector breeding site. For search angles of 5 and 10 degrees, the effect measures, adjusted IRR (95% CI), became 0.995 (0.991–0.999) and 0.997 (0.995–0.999), respectively.
¥P-value: 0.051.
NA: Not applicable.
doi:10.1371/journal.pone.0047354.t005

malaria without considering the effects of other factors such as rainfall and temperature, it is also not possible to state that the timing of the possible effect of IRS and the end of the clustering coincided simply by chance. Thus, we suggest that IRS with Deltamethrin has possibly suppressed the transmission to the level where little power to identify clusters remains. Meanwhile, the location where we observed the clustering activities was almost perfectly covered with IRS where only fewer than 10 households did not receive the intervention. A recent paper by the authors has discussed the reasons for the differences in the risk of falciparum malaria with regard to sex, age, wealth index, ITN use (with a 2-week lag in the effect) and other factors. We also showed that, among the meteorological covariates, rainfall (with a lag of 6 weeks) was a significant predictor of falciparum malaria. When controlled for the effect of rainfall, IRS with Deltamethrin significantly reduced the incidence of falciparum malaria; however, utilization rate of ITNs did not [17]. This finding is consistent with the negative effect of IRS with Deltamethrin and the 'null' effect of mass distribution of ITNs on the clustering of malaria episodes presented here.

All the clusters observed were on the south-east side of the community, and near to the identified vector breeding site on the shore of Lake Abaya. This implies that the greater risk of malaria infection among these households served as a 'barrier' between the breeding site and households that lived to the north-west of the cluster. This was supported by the analysis showing the significant effect of number of households located between each household and the vector breeding site while using the vector's search angle scenarios of 1, 5 and 10 degrees. Meanwhile, the clustering activity observed close to the vector breeding site in our study area could provide an example of what Bousema et al. described as "hotspots of malaria transmission in the dry and wet season". These hotspots were referred to as groups of households that have an increased risk of malaria infection within a focus of malaria transmission [21], and cognizant of the need for focused intervention, studies suggested ways of identifying malaria hotspots [22,23].

The edge effect is worth mentioning, because we observed clustering activity on the edge of the village. An edge effect may result in a biased estimate of risk at the edge of a study area provided that there are no data for the adjacent localities [24–26]. In this study, beyond the edge of the study area in which we observed the clustering activity, there were no residential houses or populations at risk – farmland extended to the vector breeding site. Therefore, we presume that the relative risk reported here is unlikely to have been influenced by an edge effect [26,27]. Qualitative evaluation of the figures also confirms that malaria episodes were more concentrated in the location where the SatScan identified the clustering. In addition, we chose a spatial window size that caused the greater portion of the cluster circle to move inwards [28].

Conclusions

The risk of malaria infection varied within one village, and there was spatio-temporal clustering of malaria episodes at household level. The vector breeding site identified may have played a role in the clustering of malaria. Mass distribution of ITNs did not influence the spatio-temporal clustering of malaria, but IRS with Deltamethrin might have eliminated the clustering activity. Local knowledge of malaria transmission and follow-up on ITN use, combined with targeted interventions, may improve the existing malaria prevention and control efforts.

Supporting Information

Figure S1 Space–time clusters of maximum spatial cluster size restriction of 15 per cent.
(TIF)

Figure S2 Space–time clusters of maximum spatial cluster size restriction of 35 per cent.
(TIF)

Figure S3 Space–time clusters of maximum spatial cluster size restriction of 35 per cent. Figures S1, S2 and S3 show how the different maximum spatial cluster size restrictions given to the SatScan affect quantitative and qualitative outcomes. The largest/larger circle in each figure represents the most likely cluster, meanwhile, the smaller circles/circle represent/s significant secondary clusters. The third secondary cluster in Figure S1 is indicated by a pink dot to the south of the health post. This cluster is the smallest with a radius of 11 meters and composed of 21 people in three households. The relative risk (14.19) of this cluster is the highest of all clusters so far presented.
(TIF)

Figure S4 Number of households (59) between the breeding site (B) and a household (H) using the simplistic (rectangular) approach.
(TIF)

Figure S5 Illustration of the parameters used to estimate the number of households between a breeding site (B), and a household (H), separated by a distance (D), and a mosquito flying with a search angle (A).
(TIF)

Figure S6 Taking the approach of a search angle rather than a constant search width alter the perception of how many houses a mosquito potentially must pass to reach a given house. This figure is showing three search angles: $10°$ (567 houses), $5°$ (284 houses), and $1°$ (55 houses).
(TIF)

File S1 Description of methodology employed to get number of households located between each household and the vector breeding site.
(DOCX)

Author Contributions

Conceived and designed the experiments: EL BL. Performed the experiments: EL. Analyzed the data: EL TML. Contributed reagents/materials/analysis tools: EL TML BL. Wrote the paper: EL BL.

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Figure S1

Maximum spatial cluster size: 15 per cent
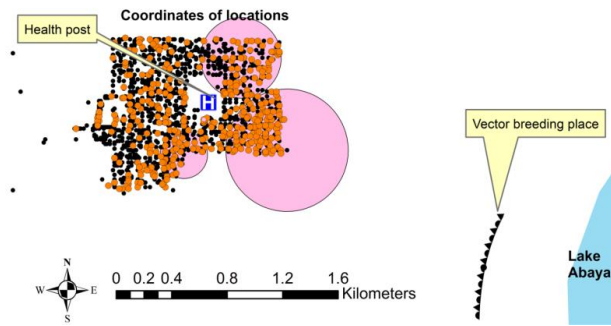
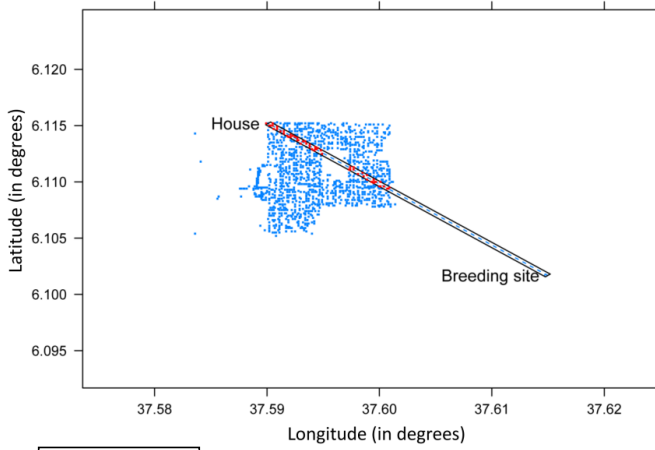**Figure S2**

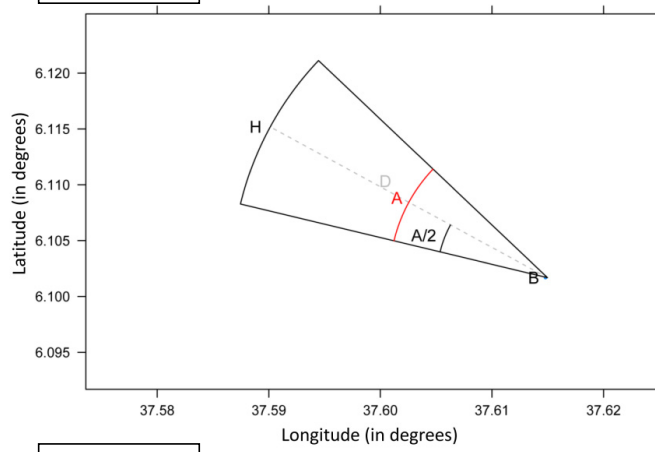**Maximum spatial cluster size: 35 per cent**
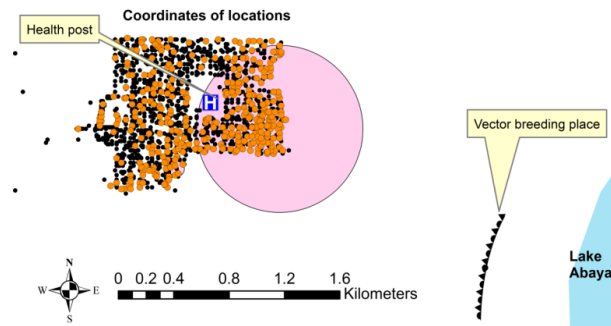
Figure S3

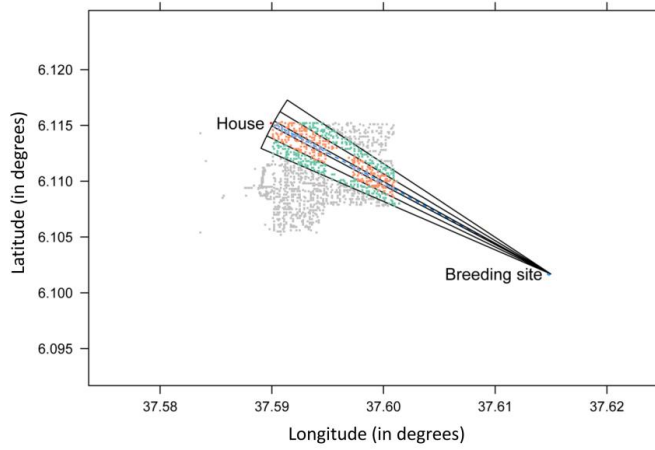**Maximum spatial cluster size: 50 per cent**
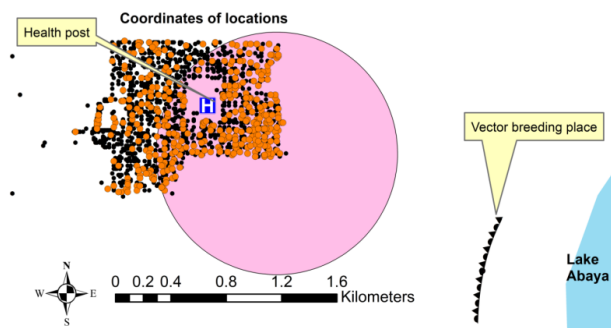
Figure S4

Figure S5

Figure S6

Problem

To make a clear distinction between distance from a breeding site and number of households between a household and the breeding site, some assumptions has to be made about mosquito behaviour and flight patterns. In cases where the terrain is relatively flat, the distance between a breeding site (B) and a household (H) can easily be found using for example Great Circle distance. The counting of number of households can take many forms, and here we show some examples on how this can be calculated.

Methods

Simplistic approach

Let us consider an anopheline breeding site (B) and a household (H). If we assume a mosquito (M) is heading towards the given household, the most simplistic approach would assume the mosquito is flying in a straight line, from B to H, and that the deviation from the line is independent of the distance flown. In this case we can assume flight is restricted inside a rectangular shape, and the number of households inside the rectangular polygon, minus one, is the number of households between B and H. Figure S4 shows an example of the houses which would be counted in case of the simplistic approach.

More realistic approach

To complicate, or maybe making the counting more realistic, we still assume that when leaving the breeding site, the mosquito is heading for the same household. This time, however, we assume the deviation from the line is dependent on the distance own. In this case, the deviation is measured as an angle, A, which is unknown since the true angle has not been measured in the field.

The polygon (a circular sector) can be constructed using an iterative procedure defining the polygon nodes (in addition to the breeding site which is the start and end node). D is equal to the distance from the household (H) to the breeding site (B) in meters. An illustration of the method can be seen in figure S5.

For a search angle A (in deg), the angle at the nodes θ_n is:

$$\theta_n = MOD\left(atan2(H_{lat} - B_{lat}, H_{lon} - B_{lon}) + \frac{A_n \cdot pi}{180}, pi\right) + \frac{pi}{2} \tag{1}$$

with $A_n = -A/2,..., A/2$

Then ΔX and ΔY become

$$\Delta X = D \cdot \cos(\theta_n) \tag{2}$$

$$\Delta Y = D \cdot \sin(\theta_n) \tag{3}$$

and the Δlon and Δlat (lon = longitude, lat = latitude) become

$$\Delta lon = \frac{\Delta X}{f_x \cdot \cos\left(H_{lat} \cdot \frac{pi}{180} \right)} \tag{4}$$

$$\Delta lat = \frac{\Delta Y}{f_y} \tag{5}$$

Where f_x and f_y are flattening factors for the WGS84 ellipsoid

$$f_x = 6378137 \cdot \frac{pi}{180} \tag{6}$$

$$f_y = 6356752.3142 \cdot \frac{pi}{180} \tag{7}$$

and the longitude of the node (N) is $N_{lon} = B_{lon} + \Delta lon$ and the latitude is

$N_{lat} = B_{lat} + \Delta lat$.

A point in polygon operation following Pebesma and Bivand [1] and Bivand et al. [2] is used to identify which houses are in the flight path of the mosquito.

Figure S6 shows an example of the consequences of changing the search angle with 10º (567 houses), 5º (284 houses), and 1º (55 houses).

Discussion

Here we briefly described two methods to calculate the number of houses between a breeding site, B, and a household, H. Since it is not known how the mosquitoes fly in order to reach a house, this document is focusing on the assumptions and methodologies made in order to calculate this index. To construct a robust index, high resolution release-recapture experiments must be carried out. Such experiments were not part of this work.

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IV

RESEARCH   **Open Access**

Freely distributed bed-net use among Chano Mille residents, south Ethiopia: a longitudinal study

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Abstract

Background: A huge discrepancy was reported between ownership *versus* utilization of insecticide-treated bed nets (ITNs). To acquire the benefits of ITNs, households need to use and not merely own them. The objective of this study was to characterize the pattern of, and assess factors related to ITN use in one village in south Ethiopia.

Methods: A prospective cohort study involving 8,121 residents (in 1,388 households) was carried out from April 2009 to April 2011 (101 weeks). Every week, individuals were asked whether they slept under an ITN the night before the interview. Descriptive statistics was used to report the availability and use of ITN. A negative, binomial, probability, distribution model was fitted to find out significant predictors of ITN use. Reasons for not using ITN were summarized.

Results: The total number of ITNs available at the beginning of the study was 1,631 (1.68 ITNs per household). On week 48, 3,099 new ITNs (PermaNet2.0) were distributed freely (2.3 ITNs per household). The number of households who received at least one new ITN was 1,309 (98.4%). The percentage of children <5 years and pregnant women not using ITNs exceeded that of other adults. The mean (range; SD) ITN use fraction before and after mass distribution was 0.20 (0.15-0.27; 0.03) and 0.62 (0.47-0.69; 0.04), respectively. Before mass ITN distribution, the most frequent reason for not using ITN was having worn out bed nets (most complained the bed nets were torn by rats); and after mass ITN distribution, it was lack of convenient space to hang more than one ITN. Males, younger age groups (mainly 15–24 years) and those living away from the vector-breeding site were less likely to use ITN.

Conclusions: The ITN use fraction reached to a maximum of 69% despite near universal coverage (98.4%) was achieved. Gender, age differences and distance from vector breeding site were associated with ITN use. Strategies may need to be designed addressing disproportions in ITN use, lack of convenient space to hang more than one ITN (for those receiving more than one), and measures to prolong usable life of ITNs.

Background

Insecticide-treated bed nets (ITNs) are the tools of malaria control and prevention [1]. The impact of ITNs on reducing malaria episodes is well documented [2,3]. Use of ITNs is one of the major vector control measures in Ethiopia. More than 20 million ITNs were distributed between 2005 and 2007, enabling 68% of the households living in malaria-endemic areas to own at least one ITN [4]. The recent national strategic plan targets that at least 80% of people at risk of malaria shall use ITNs properly and consistently, whereby, 100% of households in malaria-endemic areas should own one ITN per sleeping space by the year 2015. The country aims at malaria elimination in areas with historically low malaria transmission, while achieving near zero malaria transmission in the remaining malarious areas [5]. To achieve such a goal, better understanding of utilization of prevention and control tools, mainly ITNs, is essential.

A huge discrepancy was reported between ownership *versus* use of ITNs [1]. Studies quantified this difference as 95% *vs* 59% (Kenya) [6], 70% *vs* 53.1% (Nigeria) [7] and 90% *vs* 77% (Tanzania) [8]. Misconceptions about prevention of malaria, discomfort, perceived low

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mosquito density, inconvenience to hang the nets, place of residence, economic and educational background, age and gender differences, and colour of nets were among the reported reasons related to ITN utilization [9–15].

To acquire the benefits of ITNs, households need to use, not merely own them [1]. This calls for a need to study ITN use. This study aims to characterize the pattern of ITN utilization and determine associated factors in one malaria-endemic village in south Ethiopia.

Methods

Setting

Chano Mille village is one of the rural, malarious areas near Arba Minch town, 492 km south-west of Addis Ababa. The altitude is 1,206 m above sea level. The village was selected purposively to study malaria epidemiology in detail in the presence of favourable malaria vector breeding site. The presence of Lake Abaya to the south-east of the village resulted in intense malaria transmission since the shore of the lake favoured malaria vector breeding. The incidence rates of falciparum and vivax malaria in the village were 22.9 and 22.2 per 1,000 persons per year, respectively. The distance of the household from the shore of the lake, wealth index, age and gender were found to be significant predictors of malaria infection. In the two-year study period, the government had undertaken indoor residual spraying with insecticides (twice) and mass free ITN distribution (once) as prevention and control measures [16,17].

Study design and data

This was a prospective cohort study that involved all residents of the village. The total number of households was 1,388 and the total number of individuals followed for 101 weeks (from April 2009 to April 2011) was 8,121. Every week, individuals were asked whether they slept under an ITN the night before the interview; and if they did not use the ITN, open-ended question was used to ask the reason why. To maintain a gap of six days between the visits, households were visited on the same day each week. A census was carried out three times to update the denominator: at the start of the study, on week 50, and at the end of the study. For the first four weeks, ITN use data were collected considering vulnerable groups, including children under five years and pregnant women. After week 5, all residents of the village were considered and the name of the individual who slept under an ITN was recorded. Weekly ITN use fraction was calculated by taking the number of individuals who slept under an ITN as numerator and the total population of the week as denominator; this was done for different gender and age categories as well. The number of weekly follow-ups in which ITN use was reported was calculated for each of 8,121 individuals.

The number of bed nets available at each household was recorded at the beginning. In addition, after free mass distribution of ITNs, which was carried out by the government on week 48, ITN coverage survey was carried out on week 50. During the ITN coverage survey, the households were asked if they had usable ITNs in addition to the new ones; and when available, these ITNs were considered old-functional.

The data collectors were recruited from the village having college level diploma.

Data analysis

Summary statistics were used to report the number of bed nets (new and old-functional) available in each household, and the proportion of children aged less than five years, and pregnant women that did not use ITNs. Likewise, the median number of weekly follow-ups in which ITN use was reported was calculated for different population sub-groups. A summary was provided on the reported reasons for not using ITN.

The count data on the number of weekly follow-ups, in which ITN use was reported, was over dispersed while Poisson regression was fitted. The ratio of the deviance over the degree of freedom was 24.4. This value became 1.6 with a negative binomial probability distribution model. As the later model handled the overdispersion problem (since the value was very close to 1), a negative binomial regression model was fitted to the data. The number of weeks an individual was observed was set as a scale weight variable. A fixed value of 1 was used as a scale parameter method and robust estimator was used for the covariance matrix. Exponential parameter estimates were interpreted as incidence rate ratios (IRR). The 95% confidence intervals (CI) for the IRR were also reported. Gender, age, education of the household head, wealth tertiles and distance (in km) from vector breeding site were considered as determinants for ITN use. To construct wealth index, principal component analysis (PCA) was used. The variables included were presence of electricity, watch, TV, radio, mobile phone, refrigerator, separate room used for kitchen, bicycle, agricultural land, livestock, account in bank or credit association and latrine facility. In addition, the main materials of the floor, wall and roof were considered. The details of wealth index construction are reported elsewhere [16]. Distance of each household (in km) from the identified vector breeding site was calculated using proximity analysis tool of ESRI ArcMap 9.3 (Redlands, CA, USA). Statistically significant independent variables during bivariate analyses were used to construct the multivariate model. Pairwise comparison was done for age categories using sequential Sidak as adjustment for multiple comparisons. PASW 18.0 (Chicago, IL, USA) was used for analysis.

Table 1 Number of insecticide-treated bed nets per household according to the first census

Number of ITNs per household (n = 1212)	Number	Percent
0	241	19.9
1	414	34.2
2	463	38.2
3	85	7.0
4	9	0.7
1-4	971	80.1

Ethical clearance

The Southern Nations and Nationalities Regional Health Bureau Ethical Review Committee approved this study. Permission and support letters were obtained from relevant administrative bodies of the area. Informed verbal consent was obtained from each household.

Results

The total population followed was 8,121 in 1,388 households making the average household size 5.9 persons.

ITN coverage

The total number of nets available at the beginning of the study was 1,631 (1.68 ITNs per household). All bed nets were PermaNet2.0 and 241 (19.9%) reported that they did not have any bed nets (Table 1).

According to the ITN coverage survey (carried out on week 50), the households reported that they had 916 old-functional ITNs. On week 48, they received 3,099 new ITNs (PermaNet2.0) for free (2.3 ITNs per household). The number of households receiving at least one new ITN was 1,309 (98.4%), and nearly half, 605 (45.4%) of the households received three ITNs each (Table 2).

ITN use fraction

In the first four weeks of observation, the percentage of ITN use was determined for vulnerable groups. The data
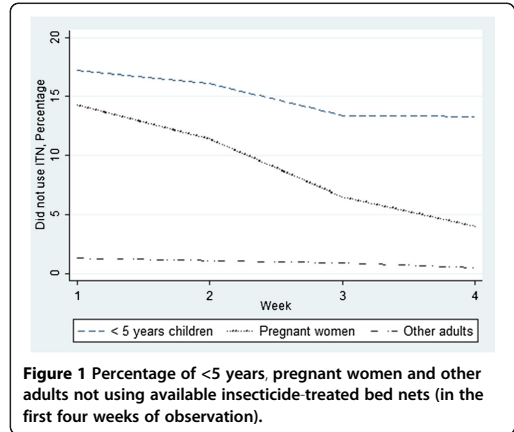
Figure 1 Percentage of <5 years, pregnant women and other adults not using available insecticide-treated bed nets (in the first four weeks of observation).

showed that the percentage of under five years children not using ITNs (followed by pregnant women) exceeded that of other adults (Figure 1).

The mean (range; SD) ITN use fraction before and after mass distribution was 0.20 (0.15-0.27; 0.03) and 0.62 (0.47-0.69; 0.04), respectively. The distribution of ITN use fraction is presented in Figure 2.

The significant increase in ITN use fraction after week 48 indicates the time of free mass ITN distribution. In general, the proportion of females using bed nets exceeded that of males in all weeks of observation (Figure 3). The proportion of adults aged above 24 years (followed by children less than five years old) using ITNs surpassed all other age categories (Figure 4).

Figure 5 shows the ITN use fraction according to age groups and gender. The gap in the level of ITN use fraction between age groups five to 14 and 15 to 24 became wider after free mass ITN distribution, mainly for males (less use among aged 15 to 24 years); and among females, more use was recorded among aged 15 to 24

Table 2 Number of insecticide-treated bed nets according to the second census conducted on week 50

Number of ITNs available per household	Old-functional[¥] n = 1,156 households		New[‡] n = 1,330 households	
	Number	Percent	Number	Percent
0	530	45.8	21[§]	1.6
1	372	32.2	201	15.1
2	223	19.3	468	35.2
3	28	2.4	605	45.5
4	2	0.2	29	2.2
5	0	0.0	5	0.4
6	1	0.1	1	0.1
1-6	626	54.2	1309	98.4

[¥]Distributed three years previously.
[‡]Distributed two weeks previously, the number of households involves both existing and newly added ones.
[§]Four existing households and 17 newly enrolled households.
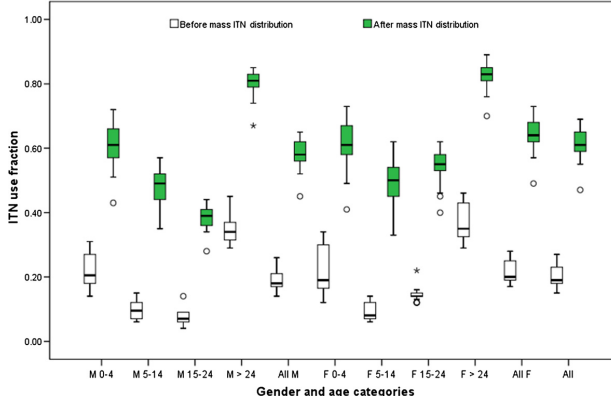
Figure 2 Distribution of insecticide-treated bed net use fraction according to gender and age categories (in year) before and after mass insecticide-treated bed net distribution. M and F refer to males and females, respectively.

years for most of the weeks. For most of the weeks, females under five years had less ITN use fraction compared to the whole population, which was not the case for their male counterparts.

Reasons for not using insecticide-treated bed nets

The most frequent reason for not using an ITN was having worn-out bed nets (most complained that the bed nets were torn by rats). Some did not hang the bed nets because of being dirty, changes in bed arrangement, unsuitable housing structure and considering alternatives such as insecticide sprays. The other reasons were absence of mosquitoes within the house, sleeping in the farm and presence of social gatherings because of death of family member. Some reported that they provided the bed nets to their children as they sent them to other places for schooling. Some reported discomfort (feeling warmth) while sleeping under the net and a few reported

that they used the bed nets as curtain for traditional pit latrines. Meanwhile, after mass ITN distributions, the most frequent reason for not using bed nets was lack of convenient space in the house to hang more than one bed net.

Total number of weekly follow-ups in which insecticide treated bed net use was reported

The median number of weekly follow-ups in which mosquito net use was reported over 97 weeks of observation was higher for females, the age category >24 years, the poor, and residents having secondary level education (Table 3).

Predictors of total number of weekly follow-ups in which insecticide treated bed net use was reported

Wealth tertiles and level of education of the household head did not show statistical significance during
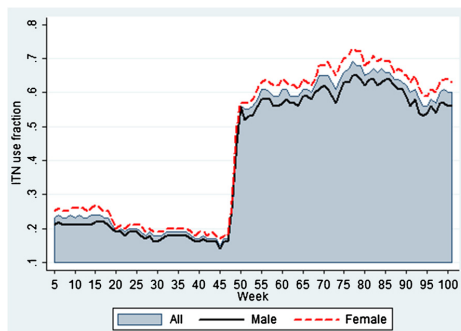
Figure 3 Insecticide-treated bed net use fraction by gender.
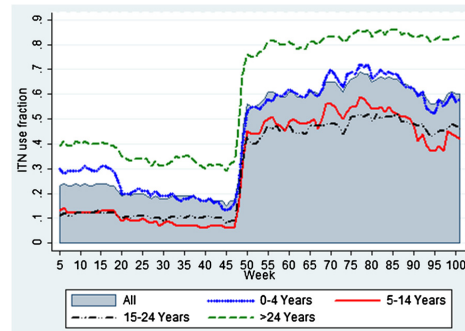
Figure 4 Insecticide-treated bed net use fraction by age group.
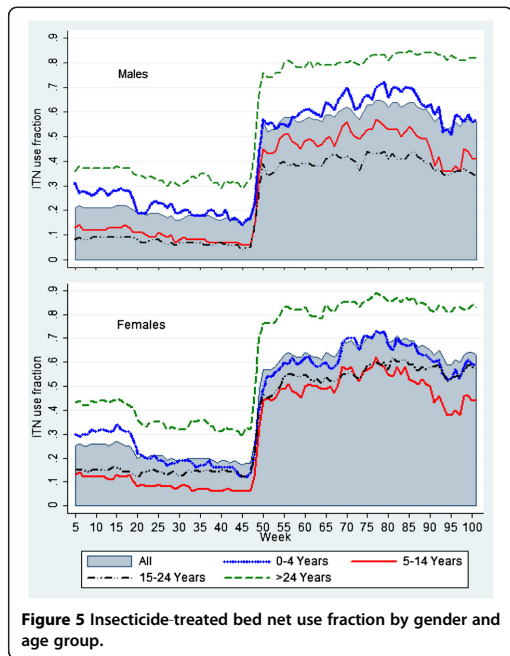
Figure 5 Insecticide-treated bed net use fraction by gender and age group.

Table 3 Median number of weeks in which insecticide treated bed net use was reported over 97 weeks of observation

Variables		N	Median [§]
Gender	Males	4227	26
	Females	3894	34
Age in years	< 5	1067	39
	5–14	2175	25
	15–24	2321	7
	>24	2558	52
Wealth tertiles	Poor	2671	35
	Medium	3168	32
	Rich	2282	19
Education of the household head	No education	4351	25
	Primary	2050	32.5
	Secondary	1616	41
	Above secondary	104	29.5
All		8121	30

[§]Total number of weeks = 97.

bivariate analysis. Controlled for other variables, the rate of total number of weeks spent under ITNs was less by 10% among males. This rate was 54% less for age category 15 to 24 years compared to adults >24 years old. Meanwhile, this rate decreased by 19% for each km distance away from the vector breeding site (Table 4).

The pairwise comparison showed that children under five years old spent more number of weeks under the ITNs compared to age categories five to 14 and 15 to 24 years but less compared to age category >24 years. The least number of weeks spent under ITNs was observed among 15 to 24 years old compared to all other age categories (Table 5).

Discussion

Coverage of new ITN distribution was 98.4% and the maximum ITN use fraction was 69%. The percentage of under five years and pregnant women not using ITNs exceeded that of other adults. Being male, younger, and living farther from the vector breeding site were factors associated with less frequent use of ITNs. Residents in the age range 15 to 24 years were the least users of ITNs. Lack of convenient space to hang the ITN was the prominent reason for not using ITN, despite its availability.

ITN use fraction was calculated based on self-report. It may not be possible to avoid bias with self-report. However, listing the names of household members who slept under ITN the night before the interview was considered to be better than asking a Yes/No question. A similar approach was used in previous studies [10]. The fact that the ITN use fraction did not reach 100% (the maximum was 69%) after mass distribution of ITNs was reassuring in that social-desirability bias did not overwhelm this study.

Distance from the vector breeding site affected use of ITNs. This may support the notion that ITN use is associated with risk perception [10]. This was also indicated by the finding that the number of malaria episodes decreases in the household farther from the vector breeding site [16,17], which might have compelled residents who lived away from the vector breeding site to perceive lower risk of disease and to use ITNs less frequently.

A recent paper showed that before mass ITN distribution, the risk of *falciparum* malaria was higher in the age category <15 years compared to 15 to 24 years. However, this risk shifted to the age category 15 to 24 years after mass ITN distribution [16]. This could be explained by the significant differences in frequency of ITN use among different age categories, whereby less frequent use of ITNs was observed in the category 15 to 24 years. Though this less frequent use (in 15 to 24 years category) had existed before mass ITN distribution, the

Table 4 Factors associated with insecticide-treated bed net use

Variable (n = 8121)		Total number of weeks spent under ITNs	
		Crude IRR (95% CI)	Adjusted IRR (95% CI)
Gender: Male		0.9(0.87-0.93)*	0.9(0.87-0.93) *
Age in years[‡]	< 5	0.71(0.68-0.74) *	0.71(0.68-0.74) *
	5–14	0.51(0.49-0.53) *	0.51(0.49-0.53) *
	15–24	0.46(0.44-0.48) *	0.46(0.43-0.48) *
Wealth tertiles[¥]	Poor	1.04(0.99-1.09)	NA
	Medium	1.04(0.99-1.09)	NA
Education of the household head[§]	No education	0.9(0.77-1.05)	NA
	Primary	0.99(0.85-1.17)	NA
	Secondary	1.12(0.96-1.31)	NA
Distance (km) from vector breeding site		0.84(0.8-0.88) *	0.81(0.77-0.85) *

[‡]Reference category: >24 years.
[¥]Reference category: Rich.
[§]Reference category: Above secondary.
*Significant at 0.05 level.
NA: Not applicable.

increased frequency of ITN use among the younger age categories (<15 years) after mass ITN distribution could move the risk towards 15 to 24 years category.

Educational status and wealth index did not significantly affect ITN use in this study. Some studies reported similar findings [7,18,19], while the others showed significant associations between socio-economic factors and ITN use [11,12]. The presence of prominent vector breeding site yielding varying risk to the households in the study area was worthy of note since the households located closer to the vector breeding site reported more frequent use of ITNs, implying influence of nuisance mosquitoes and/or risk perception might have outweighed factors such as education and wealth, with regard to sleeping under ITNs.

The first four weeks of observation showed adults using ITNs more than the vulnerable groups: under five years and pregnant women. Similarly, during the remaining 97 weeks of follow up, adults used ITNs more

Table 5 Pairwise comparisons of estimated marginal means of age categories based on the original scale of total number of weeks spent under insecticide-treated bed nets

	Category		Mean difference (A-B)	P value (Sequential Sidak)
	A	B		
Age in years[‡]	<5	5-14	Positive	<0.001
		15-24	Positive	<0.001
		>24	Negative	<0.001
	5-14	15-24	Positive	0.002
		>24	Negative	<0.001
	15-24	>24	Negative	<0.001

[‡]Overall test results: Wald Chi-Square = 1036.68 and P value < 0.001.

than the younger (<24 years) residents and this was not expected. This might also have resulted in lower incidence rate of *falciparum* malaria among adults since ITN use at individual level was reported to be protective. Similar speculation could be derived for male study participants, as males used ITNs less frequently and suffered more from *falciparum* malaria [16].

The most frequent reason for not using ITNs before mass distribution was having worn-out bed nets, mainly because the ITNs were ragged by rats. This implies the need to integrate rodent control measures with ITN distribution in areas with a similar problem in order to lengthen the usable life of ITNs, considering cost implications in distributing free ITNs more often.

Except those who did not receive new ITNs, no household reported inadequacy of number of ITNs received during mass distribution; however, frequency of ITN use did not reach to the coverage. The maximum ITN use fraction was 69% while coverage was 98.4%. Studies indicated such discrepancies between bed net coverage and use [6–8]. Quantitative data showed individual differences in frequency of bed net use including age, gender and risk perception. Meanwhile, according to the responses to the open-ended question, the most frequent reason for not using bed nets, while at least one was available, was lack of convenient space to hang the bed nets. This was also the case in the review made on reported reasons for not using ITNs [10]. This implies mere calculation of ratio of number of household members to bed nets (while distributing the nets), without considering the housing structure or helping the household to hang the desired number of bed nets, would not bring this prevention and control measure to the intended level of efficiency and effectiveness. Meanwhile,

the unusual practice of using bed nets for other pur-
poses, including as curtains for traditional pit latrines
(though not frequently reported) should be properly
addressed.

Conclusions

The ITN use fraction reached to a maximum of 69%
despite near universal coverage. Residents aged above 24
years used ITNs more than the younger age categories,
while those aged 15 to 24 years and males were the least
users. Households that were distant from the main vec-
tor breeding site were less likely to use ITNs. After mass
ITN distribution, lack of convenient space to hang more
than one bed net was the most frequently reported rea-
son for not using ITNs. Use of ITNs as malaria preven-
tion and control may benefit from combinations of
strategies to improve ITN use among the younger age
groups, to ensure that each household managed to hang
all the bed nets provided for free, and to incorporate
measures (such as rodent control) that could prolong
the usable life of bed nets. To better understand the rea-
sons for not using ITN, well designed qualitative re-
search approach should be considered.

Authors' contributions
EL conceived the study, collected, analysed and interpreted data, and
prepared the draft manuscript. KT participated in data collection. BL
conceived the study, interpreted data and helped to draft the manuscript. All
authors read and approved the final version of the manuscript.

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Study instruments

Questionnaire to conduct census and gather data on malaria prevention and treatment practices

General Information		
GI1	Household Code	_____ GPS data: Longitude _____ Latitude_____
GI2	Site in which the interview is being conducted	a) Kebele _____ b) Sub-Kebele _____
GI3	Personnel (name and signature)	a) Interviewer_____ b) Field Supervisor_____
GI4	Date of visit	[____|_____|_____] dd | mm| yyyy
GI5	Time at beginning and end of interview	Beginning ____:____ End____:____

Introduction and Consent

My name is_____ and I'm working for Hawassa 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 SNNPR Regional Health Bureau to plan health services. This interview could take less than 15 minutes to complete. Whatever information you provide will be kept strictly confidential 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?

Verbal consent given to interview, check box [_____]

Section 1: Household members' listing and socio-demographic and economic characteristics						
Q101	Total number of household members				Number _____	

Start listing from the respondent him/herself

Q102	**Household Members**	**Age**	**Sex**	**Relationship**	**Educational status**	**Occupation**	**Birth**
1							Birth in the last 1 year:
2							
3							
4							Yes………1
5							No………2
6							
7							If yes, sex:
8							Male…….1
9							Female….2
10							
11							
12							
13							
14							
15							

Sex 1. Male 2. Female	**Relationship** 1. Father; 2. Mother 3. Child; 4. Relative 5. Maid; 6. Other	**Educational Status** *(6 years and above)* • **I=** Illiterate • **RW=** Read and Write only • If formal education, write the highest grade Completed	**Occupation** *(18 years and above)* 1. Employed 2. House wife 3. Farmer 4. Day laborer 5. Trader 6. Fishery 7. Student 8. No job/dependent 9. Housemaid 10. Others

Q103	Does your household have: Electricity? A watch? A radio? A television? A mobile telephone? A non-mobile telephone? A refrigerator? A table? A chair? A bed? An electric mitad? A kerosene lamp/pressure lamp?	Yes No Electricity………………………….1 2 Watch…………………………….1 2 Radio……………………….…......1 2 Television………………………….1 2 Mobile Telephone………….….........1 2 Non-Mobile Telephone…..….…........1 2 Refrigerator……………….………..1 2 Table……………..…………….…..1 2 Chair……………..…………….…..1 2 Bed………………………………...1 2 Electric Mitad…………………...…1 2 Kerosene/Pressure Lamp…………...1 2	
Q104	What type of fuel does your household mainly use for cooking?	Electricity. ……... . . . . 1 Kerosene . ……... . . . . 2 Charcoal . . . . . ……... . . . . 3 Wood . . . . ……. . . . . 4 Animal Dung……..…………….... 5 Other……………………………… 96 (Specify)_____	
Q105	Is the cooking usually done in the house, in a separate building, or outdoors?	In The House . . ….... . . . .1 In a Separate Building…….…. . . . .2 Outdoors. . . . .3 Other..………………………………....96 (Specify) _____	
Q106	Do you have a separate room which is used as a kitchen?	Yes………………………………………1 No………………………………………..2	
Q107	Main material of the floor. ***(Record observation)***	Earth/Dung …………………………….1 Ceramic Tiles………………………….2 Cement…………. . . ..………………3 Other…………………………………96 (Specify)_____	
Q108	Main material of the roof ***(Record observation)***	Thatch/Leaf…………………………….1 Corrugated Iron . . . . ……………....2 Cement/Concrete . . . . . .……….…... . . . 3 Other………………………………96 (Specify)_____	
Q109	Main material of the wall. ***(Record observation)***	No wall………………………………….1 Wood……………………………………2 Wood with mud…………………………3 Wood with mud and cement………………4 Cement blocks……………………………5 Other…………………………………96 (Specify)_____	
Q110	Type of windows ***(Record observation)***	Yes No Any window………….....…………....1 2 Windows with glass…………..……...1 2 Windows with screen/mesh wire……..1 2 Windows with curtains……….……...1 2	
Q111	How many rooms in this household are used for sleeping?	Number of rooms[__|__]	
Q112	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	
Q113	Does any member of this household own any land that can be used for agriculture?	Yes………………………………………1 No………………………………………..2	
Q114	How many (LOCAL UNITS) of agricultural land do members of this household own? ***(If unknown enter 98)***	Local units [___|___] Specify the local unit_____	

Q115	Does this household own any livestock, herds, or farm animals?	Yes…………………………………………1 No…………………………………………2	
Q116	How many of the following animals does this household own? Milk cows, oxen, or bulls? Horses, donkeys, or mules? Goats? Sheep? Chickens? *(If unknown, enter 98)*	Milk cows, oxen, or bulls------ Horses, donkeys, or mules----- Goats----------------------------- Sheep---------------------------- Chickens-------------------------	
Q117	If you have cattle, do they spend the night in the same house with you?	Yes…………………………………………1 No…………………………………………2	
Q118	Does any member of this household have an account with a bank/credit association/micro finance?	Yes…………………………………………1 No…………………………………………2	
Q119	How long does it take you to walk to the nearest health center/health post?	Minutes [___|___] Hours [___|___]	
Q120	What is the main source of drinking water for members of your household? *(Do not read out Responses)*	**Piped (Tap)** Piped into dwelling….…...……1 Piped into compound………....2 Piped outside compound……....3 Covered Well………...….……4 Protected Spring……….……...5 **Open Well/Spring** Open Well……...…….……6 Open Spring……...………..7 **Surface Water** River…………….……….…8 Pond/Lake/Dam…..….……..9 Rainwater…………....……..10 Other….………….……….11 (Specify)_____	
Q121	How long does it take you to go there, get water and come back?	Minutes _____ Hours _____ On premises…………96	
Q122	Do you treat your water in any way to make it safer to drink?	Yes………………….1 No…………………..2	➔ **Skip to Q124**
Q123	If yes, what do you usually do to the water to make it safer to drink? **Probe: Any thing else?** *(Multiple response possible: record all responses)*	(M = Mentioned, N = Not Mentioned) M N Boil…………………………..…………1 2 Add bleach/chlorine……………………..1 2 Strain through cloth………………...……1 2 Use water filter (ceramic, sand, etc…)…....1 2 Store in narrow necked container……….…1 2 Let it to stand and settle…………….....…1 2 Other………………………………...…1 2 Other (specify)_____	
Q124	How far is the nearest irrigation ditch from your house?	Minutes [___|___] Hours [___|___] Very close to the house…..96	

Q125	What kind of toilet facility do most members of your household use? *(observe latrine)*	Flush toilet……………..…………....1 Pit latrine/traditional pit toilet…....…..…2 Ventilated improved pit latrine (VIP) …3 No facility/Bush/Field… ……..………..4 Other……………….…………….…....5 Other(Specify)_____	➔ **Skip to Q201**
Q126	Do you share this facility with other households?	Yes……………1 No..…………..2	

Section 2: Malaria prevention and treatment			
Q201	Does your household have any mosquito net that can be used while sleeping?	Yes……………1 No..…………..2	➔ **Skip to Q209**
Q202	How many mosquito nets do your household use?	Number of Nets _____	
Q203	What type of mosquito net(s) does your household possess? *(Probe by how often net requires treatment)*	**Net #1** Every 6 months………………………1 Every year…………………………2 Permanet……………………………3 Don't Know….………………………8 **Net #2** Every 6 months………………………1 Every year…………………………2 Permanet……………………………3 Don't Know….………………………8	➔ **Skip to Q206** ➔ **Skip to Q206**
Q204	When was your net(s) last treated with a product to kill mosquitoes? *(If less than 1 month, indicate 1 month)*	**Net #1** Months _____ Years _____ Has not been treated……………………97 **Net #2** Months _____ Years _____ Has not been treated……………………97	**If treated, skip to Q206** **If treated, skip to Q206**
Q205	If not treated, how long ago was the net obtained?	**Net #1** Months _____ Years _____ **Net #2** Months _____ Years _____	

Q206	Did anyone sleep under the mosquito net last night?		Yes……………1 No..……………2 Not sure.………3		→ Skip to Q208 → Skip to Q209
Q207	Who slept under this mosquito net last night? (*If more than one under-five child, consider only if all of them slept under the net*)	**Net #1**		**Net #2**	
		Child under 5 years: Yes = 1 No = 2 No under five child in the house = 3 **Pregnant women:** Yes = 1 No = 2 No pregnant women in the house = 3 **Other adult:** Yes = 1 No = 2 No other adult in the house = 3		**Child under 5 years:** Yes = 1 No = 2 No under five child in the house = 3 **Pregnant women:** Yes = 1 No = 2 No pregnant women in the house = 3 **Other adult:** Yes = 1 No = 2 No other adult in the house = 3	
Q208	Why did you not use the bed net?				
Q209	Has your house ever been sprayed with insecticide for malaria prevention by spraymen from the District Health Office?		Yes……………1 No..……………2 Not sure.………3		Skip to Q211
Q210	How many months ago was your house sprayed? (*If less than one month, record 01*)		Months ago [___/___] Not sure…..98		
Q211	Has member of your family been ill with a fever at any time in the last 7 days? (*if more than 3 people, consider those who are younger and pregnant*)	Yes……………1 Q102 number_____ No..……………2 Don't Know…..98	Yes……………1 Q102 number_____ No..……………2 Don't Know…..98	Yes……………1 Q102 number_____ No..……………2 Don't Know…..98	If 2 or 98, skip to Q217
Q212	Did he/she give blood sample from finger tip?	Yes……………1 No..……………2 Don't Know…..98	Yes……………1 No..……………2 Don't Know…..98	Yes……………1 No..……………2 Don't Know…..98	
Q213	Did he or she take antimalarial drug?	Yes……………1 No..……………2 Don't Know…..98	Yes……………1 No..……………2 Don't Know…..98	Yes……………1 No..……………2 Don't Know…..98	If 2 or 98, skip to Q217
Q214	Which antimalarial drug was used? (*Do not read the options*)	Co-Artem.………1 Chloroquine.……2 Don't Know…....98	Co-Artem.………1 Chloroquine.……2 Don't Know…....98	Co-Artem.………1 Chloroquine.……2 Don't Know…....98	If 98, skip to Q217
Q215	How long after the fever started did the diseased individual take the antimalarial drug?	Same day…….…....0 Next day……….…..1 Two days after…….2 Three days after…...3 Four or more days after……………...4 Don't Know……...98	Same day…….…....0 Next day……….…..1 Two days after…….2 Three days after…...3 Four or more days after……………...4 Don't Know……...98	Same day…….…....0 Next day……….…..1 Two days after…….2 Three days after…...3 Four or more days after……………...4 Don't Know……...98	
Q216	For how many days did the diseased individual take the drug?	Days………[_____] Still taking………..96 Don't know.……..98	Days………[_____] Still taking………..96 Don't know.……..98	Days………[_____] Still taking………..96 Don't know.……..98	
Q217	Was there death of family member in the last one year?	Yes……………1 No..……………2	→ When did it occur? _____months ago	Sex Male……1 Female…2	Age _____ **Year/Month**

Chano Mille Malaria research project: Weekly data collection format

House number_____   Date of visit[____|____|____]
                                                    dd | mm| yyyy

Data collector   Name _____ Signature _____

Q01	Did anyone sleep under the bed net last night?	Yes……………1 No...………..2 → **Skip to Q03** Not sure………3 The household doesn't own net…..4 → **Skip to Q04**		
Q02	Who slept under the bed net last night? (*List the names*)	1._____ 2._____ 3._____ 4._____ 5. _____	6._____ 7._____ 8._____ 9._____ 10._____	
Q03	Why did you not use the bed net?			
Q04	Presence of ailments any time in the last 7 days.	**Fever** Yes………1 No……….2	**Cough** Yes………1 No……….2	**Diarrhoea** Yes………1 No……….2	If 2 or Cough/ Diarrhoea only → End
Q05	Description of the individual who had or has fever (*if more than 3 people, use another format and attach*)	Name:_____ _____ Age ____Years/Months Sex Male……1 Female…2	Name:_____ _____ Age ____Years/Months Sex Male……1 Female…2	Name:_____ _____ Age ____Years/Months Sex Male……1 Female…2	
Q06	Did he/she give blood sample from finger tip?	Yes……………1 No...………..2 Don't Know…..98	Yes……………1 No...………..2 Don't Know…..98	Yes……………1 No...………..2 Don't Know…..98	
Q07	Did he or she take antimalarial drug?	Yes……………1 No...………..2 Don't Know…..98	Yes……………1 No...………..2 Don't Know…..98	Yes……………1 No...………..2 Don't Know…..98	If 2 or 98 → Skip to Q11
Q08	Which antimalarial drug was used? (*Don't read the options*)	CoArtem………..1 Chloroquine……2 Quinine………..3 Don't Know…..98	CoArtem………..1 Chloroquine……2 Quinine………..3 Don't Know…..98	CoArtem………..1 Chloroquine……2 Quinine………..3 Don't Know…..98	If 98 → Skip to Q11
Q09	How long after the fever started did the diseased individual take the antimalarial drug?	Same day………..…0 Next day…………....1 2 days after…………2 3 days after………...3 4 or more days after....4 Don't Know………..98	Same day………..…0 Next day…………....1 2 days after…………2 3 days after………...3 4 or more days after....4 Don't Know………..98	Same day………..…0 Next day…………....1 2 days after…………2 3 days after………...3 4 or more days after....4 Don't Know………..98	
Q10	For how many days did the diseased individual take the drug?	Days………[____] Still taking……….96 Don't know………98	Days………[____] Still taking……….96 Don't know………98	Days………[____] Still taking……….96 Don't know………98	
Q11[*]	If there is a member of the family who is febrile during the interview and did not take any medication, take axillary temperature and if it is $\geq 37.5^0$C, record the case's name and house number on your note book, then send the case with a referral slip to HEW as soon as possible. **(Use another format if you got more than one case in the same household and attach)** **(Use separate referral slip for each case)**	Name_____ Age _____Years/Months Sex Male……1 Female…2 Axillary temp. _____ ^{o}C	*Blood sample result* (*To be filled in the laboratory*) P. falciparum………..1 P. vivax……………..2 Mixed infection…….3 Negative …………..4 Other hemoparasite...5 (specify_____)	Parasite density _____ Morphology _____

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[*] *Please confirm the referred case gave blood sample at the health post and if so, request the HEW or the lab technician to have her/his signature on your note book at the end of the day.*
*The HEW or the lab technician is expected to label the slide with **the date** and **the case's first name, age and house number**.*

Re-enumeration of residents of Chano Mille after one year of follow-up

Census conducting week: _____   **HH Number:** _____

New ITN (in number): _____   **Old (functional) ITN (in number):** _____

Q102#	Existing members (from previous census)	(A) Now present	(B) If not present, reason	If not present, month left/deceased
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				

(A)	(B)
1. Yes	1. Left the kebele
2. No	2. Left the HH
	3. Deceased

Register in-migrants or new births in the last one year (after the first census)

Q102#	New comers including births after the last census	Age	Sex	Relationship	Educational status	Occupation	If new comer, when (month)?
21							
22							
23							
24							
25							
26							
27							
28							
29							
30							

Sex
1. Male
2. Female

Relationship
1. Father; 2. Mother
3. Child; 4. Relative
5. Maid; 6. Other

Educational Status
(6 years and above)
- **I**= Illiterate
- **RW**= Read and Write only
- If formal education, write the highest grade Completed

Occupation
(18 years and above)
1. Employed
2. House wife
3. Farmer
4. Day laborer
5. Trader
6. Fishery
7. Student
8. No job/dependent
9. Housemaid
10. Others

Household Identification card –front

DETERMINANTS OF MALARIA TRANSMISSION IN SOUTHERN ETHIOPIA

Eskindir Loha (PhD stud.)
Bernt Lindtjørn (Prof)

House Number/የቤት ቁጥር

Ethiopian Malaria Prediction System (EMAPS)

Phone: +251 913 233294
P.O.Box 111, Awassa, Ethiopia
E-mail: eskindir_loha@yahoo.com

Bergen University, Norway

Household Identification card –back

ይህን ካርድ የወባ ምልክቶች ካለዎት ይዘው ይምጡ

ካርዱ የሚያገለግለው ለእንድ ዓመት ብቻ ነው

የወባ በሽታ የሚተላለፈው በትንኝ አማካኝነት ነው

ትኩሳት" ማንቀጥቀጥ" ራስ ምታት" የመገጣጠሚያ ህመም ወዘተ... የወባ ህመም ምልከቶች ሊሆኑ ይችላሉ

የትንኝ መከላከያ አጎበር በአግባቡ እንጠቀም

የወባ ምልክቶች ሲታዩ ወዲያውኑ ወደ ሕክምና መስጫ ተቋም መሄድና ሕክምናውን በአግባቡ መውሰድ ይኖርብናል

Referral slip

ትኩሳት ያለበት ህፃን ወይም አዋቂ ሲገኝ ወደ ጤና ኬላ መላኪያ ቅፅ

የቤት ቁጥር_____

ስም_____

ዕድሜ_____ ዓመት/ወር

ፆታ: ወንድ....1 ሴት....2

የትኩሳት መጠን_____ °C

የመረኛ ሰብሳቢው ስም:
_____

ፊርማ_____

የደም ናሙና ወሳጅ ስም:
_____

ፊርማ_____ [_|_|_]
                ቀን ወር ዓመት

Format used to collect data on coverage of IRS and practice of re-plastering			
S. No.	**Question**	**Options**	**Skip**
Q1	House Number: _____		
Q2	Was indoor residual insecticide sprayed recently in this house for the purpose of preventing malaria?	1. Yes 2. No ⟶	End here
Q3	Is there any sign of the sprayed insecticide on the wall? (Observe)	1. Yes ⟶ 2. No	End here
Q4	Ask for reasons (including re-plastering): _____ _____		