Bed nets for malaria prevention under field conditions in Ethiopia:

Durability, use, and impact on spatial variation of malaria

Tarekegn Solomon Shanka

Thesis for the degree of Philosophiae Doctor (PhD)
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Dedication

To

Almaz,

Yonatan, Feven and Fikerab

Scientific environment

I joined the Centre for International Health, Department of Global Public Health and Primary Care in the Faculty of Medicine, University of Bergen as a PhD candidate. The entire training component of this PhD was carried out at the Centre for International Health. The research was conducted in Ethiopia. During data collection, I was based at the School of Public Health at Hawassa University in Ethiopia.

Professor Bernt Lindtjørn at the Centre for International Health, University of Bergen supervised this work. Professor Wakgari Deressa at the Department of Preventive Medicine, School of Public Health, Addis Ababa University and Associate Professor Eskindir Loha at the School of Public Health, Hawassa University co-supervised this PhD work.

This research was conducted as part of a large-scale cluster-randomized controlled trial for malaria prevention in Ethiopia (short name 'MalTrials'). The main focus of MalTrials was to provide evidence on the combined use of long-lasting insecticidal nets and indoor residual spraying on malaria prevention by evaluating malaria epidemiology, vector control interventions, entomology, and cost-effectiveness of the interventions.

The MalTrials study was conducted by an interdisciplinary research team from Ethiopian institutions (School of Public Health at Hawassa University and the School of Public Health and Aklilu Lema Institute of Pathobiology at Addis Ababa University) and Norwegian institutions (Centre for International Health at the University of Bergen and Norwegian University of Life Sciences). The team included epidemiologists, entomologists and health economists. Five PhD students and 41 technical staff took part in the research. These institutions have been conducting high-impact research and interdisciplinary studies on malaria in Ethiopia since 2008.

The trial was conducted from October 2014 to January 2017. The Norwegian government funded the project through the Norwegian Research Council (Project number: 220554).

Acknowledgements

I would like to express my deepest appreciation and gratitude to my primary supervisor, Professor Bernt Lindtjørn. You have inspired me in this journey with your timely, constructive critique and advice. You did not 'spoon feed' me, but you gave me direction to tackle each challenge by triggering my critical thinking, independent opinions and independent work. The lessons I learned under your supervision will continue to be invaluable for my independent research work. Now, I have confidence and hope to offer what you have given me to my students. Thank you, Professor Bernt. May God bless you and your family.

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Above all, I praise God Almighty for His mercy, graces and strength throughout my life. You have heard my voice and my prayers in those challenging moments. Thank you Lord for Your daily guidance and the endless blessings showered upon me.

Summary

Background: Despite a remarkable achievement in reducing malaria and in scaling up vector control intervention using long-lasting insecticidal nets (LLINs) in the last 15 years, malaria remains a significant public health problem in Ethiopia. To maximize the benefit of LLINs for malaria reduction, LLINs should provide a serviceable life, and people at risk for malaria should use them. However, field studies show considerable variation in both LLIN serviceable life and utilization in different settings. Moreover, malaria transmission is highly heterogeneous in different geographic locations and over time due to variations in risk factors. To further reduce the malaria burden, it is important to understand the factors that affect malaria clustering. For example, at a micro-geographic scale, it is crucial to target interventions in so-called hotspots (areas with a higher proportion of malaria cases than the overall population). In Ethiopia, several studies have evaluated the spatiotemporal clustering of malaria; however, with the exception of a single study in a southern Ethiopian village with a high malaria infection rate, none quantified the impact of malaria control interventions on the observed clustering. Hence, spatial variation in malaria transmission according to different intervention types in areas with low malaria transmission is not yet fully understood. To fill this knowledge gap, we examined the impact of LLIN use, indoor residual spraying (IRS), and combined LLIN and IRS use on spatial clustering of malaria in settings with low malaria transmission. In summary, evaluating the lifespan of LLINs, the extent of LLIN utilization by people at risk of malaria infection and the impacts of LLIN use alone, IRS use alone or the combination of LLINs and IRS on spatial clustering of malaria, could aid in designing efficient and effective malaria control strategies.

General objective: The overall aim of this study was to assess LLINs in real-life field conditions and their impact on spatial variation of malaria in an area targeted for a cluster-randomized controlled trial for malaria prevention in southern-central Ethiopia.

The specific objectives: 1) To determine the durability of LLINs under field conditions in terms of attrition, physical integrity, functional survival and bio-efficacy; 2) to assess LLIN ownership and use over time and identify factors associated with LLIN use; 3) to assess malaria infection clusters in areas with LLIN use; and 4) to assess malaria infection clusters in relation to IRS alone or a combination of LLIN and IRS interventions.

Methods: We followed up a cohort of 1,532 LLINs every six months from October 2014 to November 2016 to assess their attrition, physical integrity and functional survival under field conditions. In addition, 120 randomly selected LLINs were tested for bio-efficacy (Paper I). In Paper II, we followed a cohort of 17,142 individuals via weekly home visits for 121 weeks to document their LLIN use. We also conducted a survey at 110 weeks after LLIN distribution to determine LLIN ownership. In Paper III, we followed a cohort of 34,548 persons every week from October 2014 to January 2017 and used active and passive case detection mechanisms to identify clinical malaria episodes. A discrete Poisson model was used to identify high rates of spatial, temporal and spatiotemporal clustering of malaria using SaTScan software v9.4.2 (Paper III).

Results: In Paper I, we observed high attrition and low functional survival of LLINs in the study period. LLIN attrition was mainly due to disposal. The median functional survival time of the LLINs was only 12 months. The PermaNet ® 2.0 LLIN met the criteria of effective bio-efficacy up to 24 months after distribution.

In Paper II, we observed low LLIN ownership and use during the study period. After 110 weeks, only 8% of households owned at least one LLIN. The median proportion of LLIN use per individuals was only 14%. More frequent LLIN use was reported by older age groups, compared with children under five years old. Similarly, people residing in houses whose heads of household had better educations reported more frequent LLIN use, compared with those with higher rates of illiteracy. Having a family size of more than five persons was associated with less frequent use of LLINs, compared with smaller family sizes.

In Paper III, we observed spatial, temporal and spatiotemporal clustering of malaria infections in the study area and period. The spatial clustering of malaria at the household level was detected in all study arms (LLIN + IRS, LLIN alone, IRS alone and routine arms) with no significant difference in the risk of clustering between the arms. The risk of malaria clustering was high among households located closer to potential vector breeding sites. Moreover, an overlap was observed between clusters with low rates of LLIN use and clusters with high rates of malaria

infection. The risk of malaria infection among people living in clusters with low LLIN use was 2.20 times higher than for people living in areas outside of these clusters (adjusted hazard ratio = 2.20, 95% confidence interval (CI): 1.80–2.60).

Conclusion: The bed nets given for malaria prevention did not last long and were utilized as expected under field conditions in southern-central Ethiopia. Therefore, strategies are needed to address these problems and maximize malaria control efforts. We demonstrated variations in the risk of malaria infection across micro-geographic areas. None of the malaria control interventions (LLIN alone, IRS alone or combined LLIN and IRS) affected the occurrence of spatial clustering of malaria. The risk of malaria clustering was high in areas nearer to potential malaria vector breeding sites and thus prioritizing malaria control intervention in these locations could optimize resources. However, locations with low rates of LLIN use also exhibited high rates of malaria infection. Reducing variations in LLIN use in different locations thus also could reduce the risk of infection.

Trial registration: PACTR 201411000882128 (8 September 2014)

List of original papers

This thesis is a synthesis of the following three original research papers, which I will refer to by their respective Roman numerals I–III.

I. Solomon T, Loha E, Deressa W, Balkew M, Gari T, Overgaard HJ, Lindtjørn B. Bed nets used to protect against malaria do not last long in a semi-arid area of Ethiopia: a cohort study. Malaria J (2018) 17:239.

II. Solomon T, Loha E, Deressa W, Gari T, Overgaard HJ, Lindtjørn B. Low use of long-lasting insecticidal nets for malaria prevention in south-central Ethiopia: A community-based cohort study. PLoS ONE (2019) 14 (1): e0210578.

III. Solomon T, Loha E, Deressa W, Gari T, Lindtjørn B. Spatiotemporal clustering of malaria in southern-central Ethiopia: A community-based cohort study. PLoS ONE (2019) 14 (9): e0222986.

Abbreviations

ACT	Artemisinin Combination Therapy
AIDS	Acquired Immune Deficiency Syndrome
CI	Confidence Interval
DDT	Dichlorodiphenyltrichloroethane
HIV	Human Immunodeficiency Virus
IRR	Incidence Rate Ratio
IRS	Indoor Residual Spraying
ITN	Insecticidal-Treated Net
LLIN	Long-Lasting Insecticidal Net
OR	Odds Ratio
RDT	Rapid Diagnostic Test
RR	Relative Risk
UNICEF	United Nations Children's Fund
USD	United States Dollar
WHO	World Health Organization

List of content

Introduction

What is this thesis about?

A team of researchers from the University of Bergen, the Norwegian University of Life Sciences, Hawassa University and Addis Ababa University conducted a large-scale, cluster-randomized controlled trial for malaria prevention in southern-central Ethiopia. The study was conducted from October 2014 to January 2017 and focused on three areas: the epidemiological, entomological and economic benefits of the combined or individual use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). The primary objective of the trial was to determine whether the combined use of LLINs and IRS with propoxur provides more protection against clinical malaria among all age groups than LLINs or IRS alone. The secondary objectives were as follows:

1. To estimate the costs of LLINs + IRS, LLINs alone or IRS alone and compare them to current routine practice and to evaluate the incremental costs, effects and cost-effectiveness of these interventions
2. To assess whether LLINs + IRS reduce entomological parameters, such as human biting rates, mosquito resting density, longevity, sporozoite rates and the entomological inoculation rate inside houses and compare those assessments with those for LLINs alone or IRS alone
3. To determine whether LLINs + IRS improve haemoglobin concentrations and reduce anaemia rates among children under five years of age more than LLINs alone or IRS alone

The study protocol and results of the trial are published elsewhere [1, 2]. A total of 13 papers have been published from the results of the trial [2-14].

I joined the trial team in 2014 as a researcher from Hawassa University. I was actively involved in the study, facilitating supplies and equipment for the trial intervention, supervising the overall data collection process and cleaning data for the epidemiological parts of the study. I also co-authored several related publications [2, 7, 8, 13]. My thesis focused on the epidemiological aspects of the trial, particularly LLIN intervention.

In Paper I, we assessed the durability of LLINs in terms of attrition, physical integrity, functional survival and bio-efficacy under field conditions. Currently, the recommended serviceable life of

an LLIN is three years under field conditions [15]. However, studies show considerable variation in this estimate, from less than two years to more than four years [16-18]. Therefore, the information from this paper could inform local decision-makers regarding how often LLINs should be distributed. It also could guide communication interventions related to behavioural changes associated with LLIN lifespans.

In Paper II, we evaluated LLIN use among the study participants over the entire trial period. To achieve the goals and targets for malaria reduction, consistent use of LLINs by people at risk of malaria must be maintained. Therefore, understanding LLIN use over time could be useful in evaluating strategies and LLIN distribution campaigns.

In Ethiopia, several studies have evaluated spatiotemporal clustering of malaria [19-22]. However, these studies did not investigate the impact of malaria vector control interventions on spatial clustering of malaria. To fill this gap in the literature, the main purpose of Paper III was to examine the impact of LLINs alone, IRS alone, or a combination of LLINs and IRS on spatial clustering of malaria and associated risk factors. The results, such as how LLIN use affects spatial variation of malaria on a micro-geographic scale, could improve the understanding and efficacy of vector control interventions. Moreover, the information from this study could help target areas with the highest risk of malaria transmission. In conclusion, this thesis addresses some important issues regarding LLIN durability, LLIN use, and the impact of malaria vector control interventions on spatial variation of malaria in southern-central Ethiopia.

General overview of malaria

Malaria has been known since an ancient times [23, 24]. Evidence suggests that people have been suffering from malaria for the past 5,000 years [23]. The first evidence of fever caused by malaria occurred in China during the Xia Dynasty in about 2700 BC [23]. Malaria also was endemic in ancient Egypt [24]. The name malaria derives from the Italian words '*mal*' and '*aria*', meaning 'bad air' [23]. In 1880, the French scientist Charles Louis Alphonse Laveran discovered the etiologic agent of malaria [23]. Nearly two decades later, the British scientist Ronald Ross identified the *Anopheles* mosquito as a vector of malaria [25].

Clinical features of malaria can vary by severity of infection (uncomplicated or complicated). Non-specific symptoms include fever, a vague absence of wellbeing, headache, fatigue, muscle aches and abdominal discomfort [26, 27]. In addition to these, chills, sweats, backache, joint pain, nausea, vomiting and malaise occur frequently in uncomplicated malaria infection. Rarely, uncomplicated malaria can show a palpable spleen, enlarged liver (especially in young children) and mild jaundice (in adults) after several days of infection. In stable malaria transmission regions, recurrent malaria infection in young children can cause chronic anaemia and splenomegaly [26]. Severe (complicated) malaria, mainly due to *Plasmodium falciparum* infection, can cause impaired consciousness, generalized seizures, coma (cerebral malaria), severe anaemia, acidosis, respiratory distress, severe jaundice and kidney failure [26, 28, 29].

Malaria is a protozoan infectious disease caused by a single-celled organism belonging to the genus *Plasmodium.* More than 100 *Plasmodium* species have been identified, of which five are known to infect humans: *P. falciparum, P. vivax, P. malariae, P. ovale* and *P. knowlesi* [30-32]. *P. falciparum* is the most dominant species found in tropical and subtropical areas, especially in Africa [33, 34]. *P. falciparum* also causes the most dangerous form of malaria, which is responsible for severe infection and death [35, 36]. *P. vivax* is the second-most dominant species, mostly prevalent in south-eastern Asia, Latin America and Ethiopia, and it too causes considerable morbidity [34]. *P. falciparum* (60%) and *P. vivax* (40%) are the two main malaria parasites in Ethiopia [37, 38].

Human malaria is transmitted from person to person by female *Anopheles* mosquitoes. Of the more than 480 species of *Anopheles* mosquitoes worldwide, about 70 transmit human malaria in nature [39, 40]. The following *Anopheles* species transmit malaria in Africa: *An. gambiae*, *An. arabiensis* and *An. Funestus* [39, 41]. *An. arabiensis* is the dominant malaria vector in Ethiopia [37, 38]. In the Asia-Pacific region, more than 16 *Anopheles* species co-dominate. *An. freeborni* is the dominant vector in Latin America [39, 41]. Temperature, precipitation and humidity are important environmental factors in the life cycle of the malaria vector. *Anopheles* mosquitoes breed in marshy, stagnant water in warm, humid climates [42]. The small, temporal, clean, sunlit and shallow freshwater pools of Ethiopia thus are ideal breeding sites for *An. Arabiensis* [43-45].

The malaria parasite life cycle involves two hosts, the human and the female *Anopheles* mosquitoes that feed on human blood for egg production (Figure 1). The male and female forms of the parasite gametocytes mate in the gut of infected mosquitos via sexual reproduction. After 10–18 days, the sporozoites migrate from the mosquito's gut to the salivary gland [26, 46]. When an infected *Anopheles* mosquito takes a blood meal from a human host, it inoculates the host with its own anticoagulant saliva and the malaria sporozoites [46, 47]. The sporozoites then migrate to the human liver, where they grow and multiply in the liver cells by asexual reproduction and then travel to the red blood cells. The daughter parasites, or merozoites, are released from the red blood cells after destroying the cells in the process of growth. The merozoites continue to invade and destroy other red blood cells, and the cycle continues in the infected human host unless interrupted.

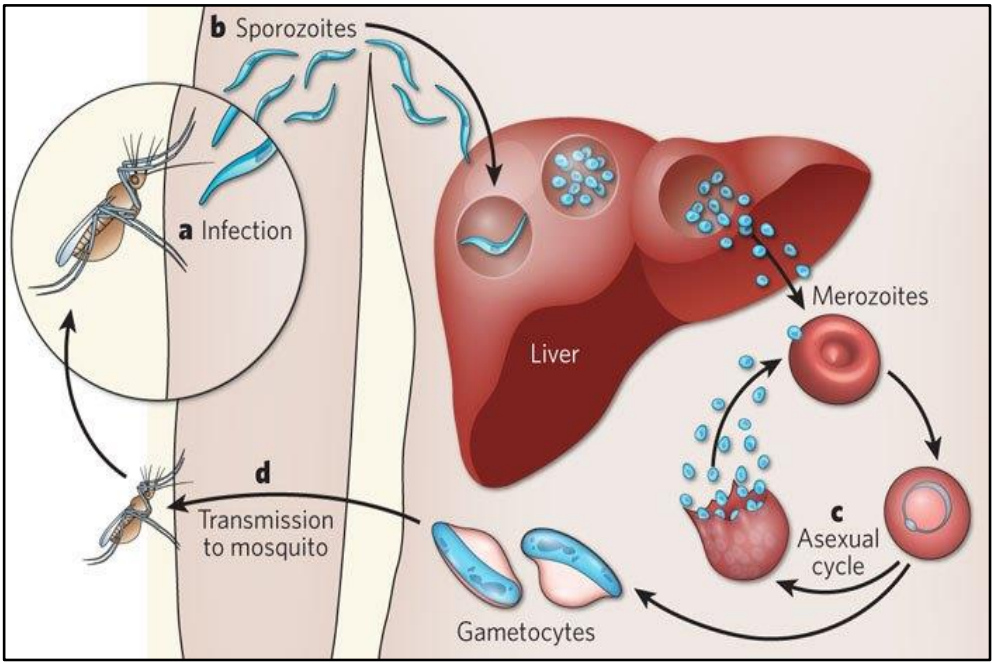

Figure 1: Life cycle of *Plasmodium falciparum* malaria parasite (adopted from Michalakis and Renaud [48]). (A) Infected mosquito inoculating sporozoites into human host. (B) Sporozoites infect, multiply and convert to merozoites in liver cells, then infect red blood cells. (C) Merozoites multiply via asexual reproduction, bursting the cell and infecting new blood cells. (D) Gametocytes form from merozoites and are transmitted to mosquitoes from the human host via mosquito bites.

Burden of malaria

Global

The world has made remarkable progress in reducing malaria rates in the past two decades. According to the World Malaria Report, global malaria incidence has fallen by 41%, and malaria-specific mortality decreased by 62% between 2000 and 2015 [49]. The number of countries with endemic malaria decreased from 108 in 2000 to 91 at the beginning of 2016 [49]. These dramatic reductions can be attributed to the scale-up of effective malaria control tools, such as vector control interventions, improved diagnosis and treatment services, along with

renewed political leadership and financial commitment [50]. An estimated 663 million clinical cases of malaria were averted by malaria control interventions, of which insecticide-treated nets (ITNs) averted 68% of cases, artemisinin combination therapies (ACTs) averted 22% of cases and IRS averted 10% of cases [51].

Despite substantial progress, an estimated 1.2 billion people remain at high risk of malaria infection worldwide [52]. In 2018, 228 million malaria cases and 405,000 related deaths occurred globally, mostly among children under five years old in Africa [53]. Sub-Saharan Africa accounted for 93% of all cases and had the largest malaria case incidence rate, 94% of all malaria deaths, in 2018 [53]. Six countries in sub-Saharan Africa (Nigeria, Democratic Republic of Congo, Uganda, Cote d'Ivoire, Mozambique and Niger) accounted for over 50% of global malaria cases in 2018; Nigeria alone accounted for 25% [53]. Figure 2 shows the malaria case incidence rates in 2018.

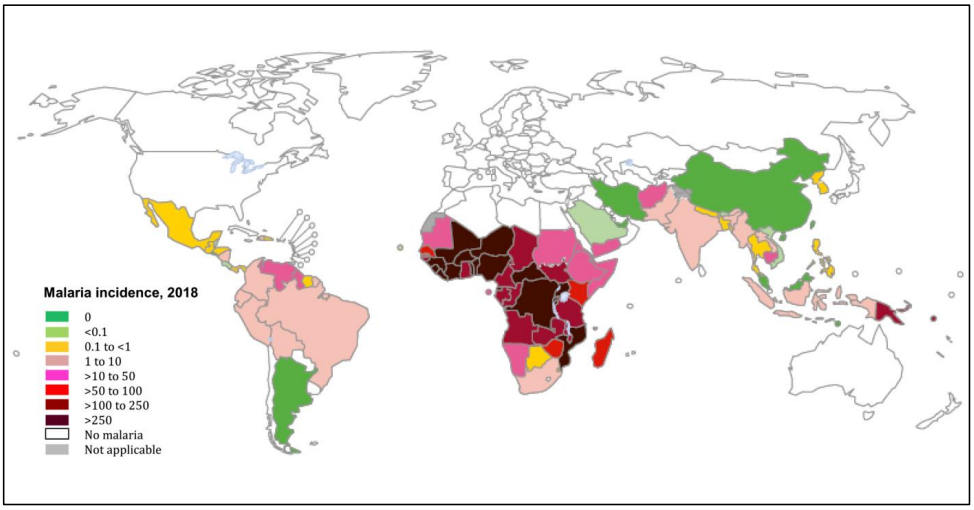

Figure 2: Map of 2018 malaria case incidence rates (cases per 1,000 people at risk), by country (adopted from the World Malaria Report 2019 [53])

After 2014, malaria cases increased globally, particularly in Africa [53], as shown in Figure 3. For example, in 2018, Ghana experienced 0.5 million more cases and Nigeria 3.2 million more cases, compared with 2017. Rwanda reported 2.5 million in 2015, up from 4.2 million in 2018,

an increase of 68%. Similarly, Madagascar reported a 30% increase and Mozambique a 20% increase from 2015 to 2018. Inadequate vector control, climatic factors and improved reporting were mentioned as causes for these increases [53]. Furthermore, residual transmission may be a contributing factor for the observed increases of malaria cases [53].

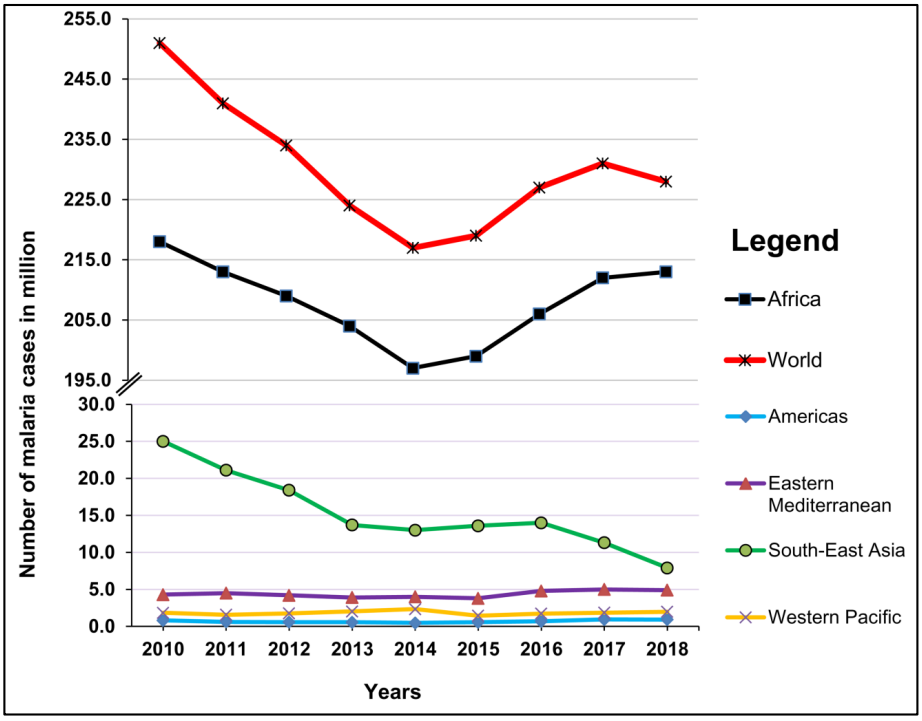

Figure 3: Global trends of malaria cases between 2010 and 2018 (data adopted from the World Malaria Report 2019 [53])

Currently, only four counties in Africa, including Ethiopia, Rwanda, Zambia and Zimbabwe, are on track for a 40% reduction in the incidence of malaria by the end of 2020. Ethiopia reported a decrease in malaria cases from 3.6 million in 2015 to 2.3 million in 2018, about a 36% reduction [53]. However, the most recent local data for 2019 shows an increase in some parts of the country [54]. These increases or plateaus in malaria cases in many Africa countries underscore the need for continued efforts aimed at malaria prevention and control.

Ethiopia

According to the Ministry of Health, 68% of Ethiopia's land mass is favourable for malaria transmission, and 60% of the population is at risk of malaria infection [38]. The transmission of malaria is seasonal and unstable in many parts of the country [38]. The main malaria transmission season is from September to December, following monsoons in July and August. Another minor malaria transmission occurs between May and June, following rainfalls in March and April [55].

The risk of malaria epidemics is high in the highland fringe areas above 2,000 meters above sea level, where 17% of Ethiopia's population lives. These populations have poor immunity against malaria infection. Therefore, people living in these areas are at high risk of epidemics after aberrations in climatic conditions or when they travel to lowland areas with endemic malaria [38].

According to the World Malaria Report, the incidence of malaria in Ethiopia decreased by more than 50% between 2000 and 2015 [56]. The last three national malaria indicator surveys also show low prevalence of malaria: 1.0% in 2007 [57], 1.3% in 2011 [58] and 0.5% in 2015 [59] in areas below 2,000 meters above sea level. The reasons for these reductions are believed to be the scale-up of vector control interventions, such as LLINs and IRS, and improved malaria diagnosis techniques, such as the rapid diagnostic test (RDT), and treatments, such as artemether-lumefantrine [55].

Despite these reductions, malaria was a top-five reason for seeking treatment at an outpatient department in Ethiopia in 2015 [60], and more than 2.3 million malaria cases and 4,757 malaria-specific deaths were reported in 2018 [53]. Recent data also shows an increase in malaria cases in some parts of Ethiopia [54]. According to the Regional Health Bureau of the Southern Nations, Nationalities, and Peoples' Region, 87,021 malaria cases were confirmed between July 2018 and January 2019, and this number increased a year later to 203,328 between July 2019 and January 2020. This 134% increase represents an additional 116,307 cases (Figure 4).

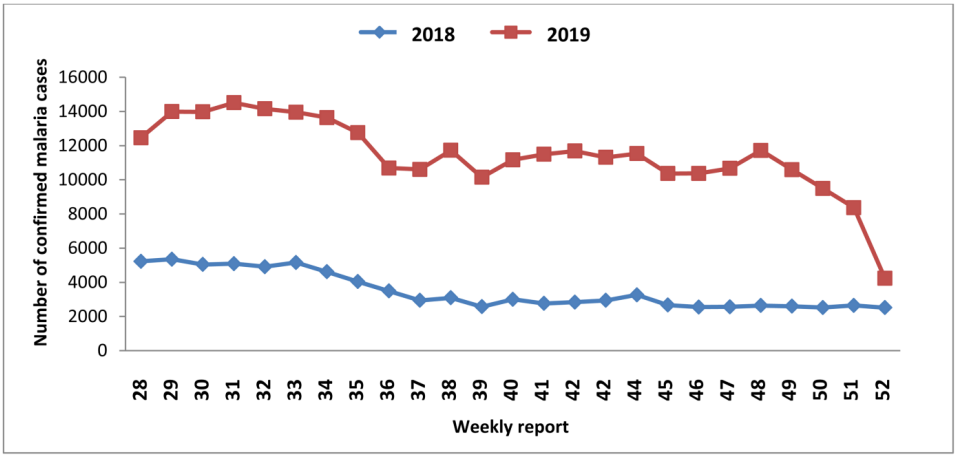

Figure 4: Weekly distribution of confirmed malaria cases in the second half of 2018 and of 2019 in the Southern Nations, Nationalities, and People's Region (data from the Regional Health Bureau Malaria Report 2019 [54])

Among 25 high-burden districts and city administrations in the region, nine reported a malaria outbreak in the second half of 2019, and the annual parasite incidence ranged from 27 to 249 cases per 1,000 people in 2019 [54]. These increases in malaria cases may be partially due to political instability and regional conflicts, which prevent health systems from conducting routine surveillance and control activities. In 2018, ethnic and border-based conflicts displaced more than 3 million people internally in Ethiopia [61]. These internally displaced people are at increased risk of malaria infection, as observed in a study conducted in the Democratic Republic of Congo, which found that internally displaced children living in a camp were at higher risk of malaria infection than those in a neighbouring village [62]. Therefore, additional efforts are needed to sustain the achievements of the past few decades and further reduce, if not eliminate, this disease.

Variation in transmission of malaria

Researchers use the Geographic Information System, which is based on geo-statistics, to understand and explain interactions between malaria parasites, vectors and environmental and human factors in relation to time and space [63]. Several studies have demonstrated significant

variations in malaria transmission by time and place and at different geographic scales (macro- or micro-geographic) due to complex interactions among parasites, vectors and hosts [19, 64, 65]. Malaria transmission can vary over short distances between neighbouring villages, and even within a single village, due to small variations in risk factors [20, 66, 67]. Changes in time and place can create a non-random distribution of parasites and vectors. For example, mosquitoes require high temperatures, high humidity and suitable aquatic habitats (e.g. dams, irrigation canals, wetlands, man-made pools, rain pools, shoreline floods and agricultural field puddles) to complete their pre-adult life cycles [68-70]. Thus, a better understanding of the micro-geographic spatial and temporal patterns in the risk of malaria transmission and identification of the responsible determinants factors are useful to facilitate targeted malaria control interventions [63, 71].

Economic burdens of malaria

Malaria can negatively affect economic productivity and growth in countries with high transmission rates. Each year, malaria costs Africa more than United States Dollar (USD) 12 billion due to health care costs, working and education days lost due to sickness, decreased productivity and loss of investment and tourism [72, 73]. The World Malaria Report estimates that USD 6.5 billion will be required annually to achieve the 2030 malaria elimination targets [56]. These costs represent heavy economic strains in malaria-endemic countries.

A macro-level economic model found that malaria is associated with losses in gross domestic product growth [74, 75]. For example, malaria reduces economic growth in some African countries by 1.3% per year, compared with non-endemic countries and after controlling for other variables that influence economic growth [74]. At the micro-economic level, malaria can affect household wealth by increasing out-of-pocket health care costs, such as transportation to a health facility, antimalarial drugs and other laboratory and health service fees [76, 77]. Moreover, these households lose working days due to recovery time, providing care to sick household members and the premature deaths of productive household members [77-79]. As malaria transmission is seasonal in Ethiopia and coincides with major agricultural activities from September to December [55] and as agriculture is the dominant source of employment and income in Ethiopia

[80], malaria substantially affects household wealth by reducing farmers' labour productivity [9, 81].

Risk factors for malaria infection

Immunity

The degree of acquired immunity against malaria depends on the extent of exposure to malaria infection and the individuals' immunological responses [82]. Acquired immunity can be either active or passive [83]. Active acquired immunity boosts the defence mechanism of the human host as a result of repeated exposure to the malaria parasite [35, 83, 84]. People who are frequently exposed to malaria infection can develop antibodies against antigens of different stages of the malaria parasite, including the sporozoite, liver-stage, blood-stage or sexual-stage [83-86]. Passive acquired immunity is the prenatal or postnatal transfer of antibodies from mother to child [83]. Babies born with passive acquired immunity to malaria can be protected in the first few months after birth, which is particularly beneficial in areas with high transmission [87, 88]. This passive acquired immunity in newborns gradually declines, however, eventually putting young children at risk of malaria infection [88, 89].

In sub-Saharan Africa, where rates of malaria transmission are high, most adults have active acquired immunity and thus do not develop overt disease [83]. However, several populations remain at high risk, including pregnant mothers, especially those who are primigravidae [90]; young children [83]; people without previous exposure to malaria [91] and people living in areas where malaria control interventions have reduced the exposure level below that required to maintain active acquired immunity [92].

Demographic and socio-economic factors

Studies show that some population groups are at greater risk of contracting malaria infection than others [93-97]. In areas with stable malaria transmission, malaria incidence and deaths are high among children between one and five years old [34, 98, 99]. This risk decreases as age increases due to repeated exposure to malaria. In epidemic-prone or low-transmission areas, the risk of infection is similar across all age groups due to the absence of acquired immunity [100]. The risk

of malaria infection among males and females is inconsistent. Some studies report that males are at increased risk of infection [93, 101, 102], whereas others document similar risks for both sexes [103]. Pregnancy increases the risk of infection and death from malaria [90]. Other factors, such as poor-quality housing [97], low socioeconomic status [104] and HIV/AIDS infection [105], also increase the risk of malaria infection.

Climate and malaria

Climatic factors, such as temperature, rainfall and relative humidity, play important roles in the transmission and distribution of malaria [46, 106-109]. For example, air temperature and relative humidity influence mosquito abundance, development, biting rate and survival [46, 110]. Air temperature also plays an important role in *Plasmodium* development within the mosquito [46, 111]. Rainfall strongly influences seasonal variations in malaria by creating temporal habitats for vector breeding and larval development [109, 112]. Any changes in temperature and rainfall patterns thus could greatly influence the transmission of malaria infection [113-116]. For example, periods of long-term drought can reduce malaria transmission [115, 116], whereas unusually high rainfall, warmer temperatures or altered relative humidity can increase or modify the distribution and duration of malaria transmission [115, 116]. Indeed, areas that were previously malaria-free due to their low temperatures, such as highland areas, are now at increased risk of malaria epidemics due to global warming [117, 118].

Environmental changes

The environmental changes resulting from both human activity and natural causes can create favourable conditions for breeding of malaria vectors and increased risk of malaria transmission [119]. Human activity, such as dam construction, deforestation and road construction, are all associated with increased malaria vectors and risk of malaria transmission [120-123], whereas some man-made environmental changes (e.g. urbanization) are associated with reduced malaria transmission [124]. In Ethiopia, dams for electricity production and irrigation increase the density of vector populations and thus are associated with increased risk of malaria in nearby villages [68, 125, 126].

Population movement

Human populations move from their usual residential places to other places for various reasons, including natural disasters (flooding, drought and famine), shortage of land for agriculture (high population pressure), conflict, wars and seasonal agriculture harvesting [127]. When people move from malaria-free or low-transmission areas to malaria-endemic areas, they are more at risk of infection and death than the resident population, which has higher rates of acquired immunity. If these immigrants then return to their malaria-free areas, they can initiate malaria outbreaks by exposing previously malaria-free local vectors to the infection [128, 129]. In Ethiopia, some studies have documented that people who move from highland (malaria-free) areas to lowland (malaria-endemic) areas are at high risk of malaria infection [129, 130].

Historical overview of malaria prevention and control

Global

The World Health Organization (WHO) launched the Global Malaria Eradication Program in 1955 to eradicate malaria from all endemic areas [131], using chloroquine to treat cases and dichlorodiphenyltrichloroethane (DDT) for vector control [132]. Malaria eradication is a permanent reduction to zero of worldwide incidence of infection caused by human malaria parasites as a result of deliberate activities. Interventions are no longer needed once eradication has been achieved [133]. Although most of Africa was excluded from this campaign due to logistical difficulties, Ethiopia launched its own national malaria eradication campaign in the 1960s, which was unfortunately not successful [131]. The Global Malaria Eradication Program successfully eliminated malaria from 37 out of 143 endemic countries in 1950, mainly in Europe, North America, the Caribbean and parts of Asia, South America and Central America [134, 135]. Malaria elimination involves interrupting local transmission of a specified malaria parasite in a defined geographical area as a result of deliberate activities, leading to zero incidences of the disease, but continued measures are required to prevent re-establishment of transmission [133].

Despite declines in malaria morbidity and mortality, including in Ethiopia, the WHO suspended the Global Malaria Eradication Program in 1969 due to growing drug and insecticide resistance and funding shortages [132]. The World Health Assembly instead recommended malaria-control

strategies to those countries that failed to achieve malaria elimination [136]. In contrast to eradication or elimination, the WHO defines malaria control as the reduction of disease incidence, prevalence, morbidity or mortality to a locally acceptable level as a result of deliberate efforts [133]. Again, continued interventions are required to sustain this control. Unfortunately, in the 1970s and 1980s, a global resurgence of malaria in some countries led to increased malaria morbidity and mortality [137, 138].

The endorsement of the Global Malaria Control Strategy in 1992 [139], the Roll Back Malaria initiative in 1998 [140] and the Millennium Development Goals in 2001 [141] again prioritised financial investment in malaria control [142]. The Global Malaria Control Strategy involved a primary health care approach wherein a decentralized programme for disease control was tailored for local contexts [143]. The Roll Back Malaria initiative addressed growing concerns about increases in malaria cases and deaths in developing countries, mainly in Africa, and aimed to decrease the malaria burden by half in 2010 [144]. On 8 September 2000, all 189 member states of the United Nations adopted eight Millennium Development Goals, including Goal 6 to combat HIV/AIDS, malaria and other diseases [141].

With these three global initiatives, investment in worldwide malaria control increased from USD 960 million to USD 2.5 billion annually between 2005 and 2014 [136]. Highly innovative and effective malaria control tools, such as LLINs, RDTs and ACTs were implemented and scaled up [136]. These initiatives were achieved through financial support from the Global Fund to Fight AIDS, Tuberculosis, and Malaria; the United States President's Malaria Initiative; the Bill and Melinda Gates Foundation and domestic sources [142]. As a result, malaria incidence declined by an estimated 37%, and malaria deaths fell by 60% between 2000 and 2015 [36].

To sustain these gains and realize a malaria-free world in the coming decades, continued political commitment, sustainable financial investment in research and development and increased regional and global collaboration are recommended [145]. To facilitate worldwide malaria reductions, WHO member states endorsed a new 15-year Global Technical Strategy in 2015, which aims to reduce malaria cases and deaths by 90% from 2015 to 2030 and to eliminate the disease from at least 35 countries [145]. To achieve this goal, the WHO articulated three working

pillars [145]. Pillar 1 ensures universal access to malaria prevention, diagnosis and treatment. Pillar 2 accelerates efforts towards elimination and attainment of malaria-free status. Pillar 3 transforms malaria surveillance into a core intervention. Ethiopia is among the countries planning to eliminate malaria from low-transmission districts by 2020 and from the whole country by 2030 by using and scaling up existing malaria control interventions [146]. However, current trends indicate that the 2030 goal is unlikely to be attained [53]. Since 2015, malaria infections and deaths have held steady and even reversed in many malaria-endemic countries [53].

Despite these current trends, malaria eradication remains a goal, even if experts disagree on the timelines. For example, the Lancet Commission on malaria eradication, established in 2017 and comprising a diverse range of malariologists, biomedical scientists, economists and other health policy experts, suggests that malaria can be eliminated outside of Africa by 2030 and worldwide by 2050 if the correct steps are followed (e.g. improved management and operations, better use of existing technology, development of new technologies and sufficient funding) [147]. In contrast, the WHO Strategic Advisory Group on Malaria Eradication, established in 2016 and comprising scientists and public health experts who advise the WHO on malaria and its eradication, believes timelines are unrealistic and infeasible with the currently available tools and drugs, citing the failed Global Malaria Eradication Program that ended in 1969 [148]. Both the WHO and Lancet commissions agree on the need for new tools, however, and on the possibility of global eradication [147, 148].

Ethiopia

General background of Ethiopia

Ethiopia is a geographically diverse country with altitudes ranging from 125 meters below sea level to 4,550 meters above sea level. Its climate is mainly affected by altitudinal limits, which are used to describe the climate zones: the *kola* zone includes high-temperature lowlands less than 1,500 meters above sea level with mean annual temperatures ranging from 23–33 °C; the *weyna dega* zone includes areas at 1,500 to 2,400 meters above sea level with mean annual temperatures ranging from 16–29 °C; and the *dega* zone includes low-temperature highlands at 2,400 meters or more above sea level with mean annual temperatures ranging from 10–16 °C.

According to the National Strategic Plan 2014–2020, areas below 2,000 meters above sea level are classified as malaria endemic and targeted for malaria control interventions; areas between 2,000 and 2,200 meters above sea level are categorized as highland fringe areas with unpredicted patterns of malaria transmission and thus are targeted for malaria elimination interventions; and areas above 2,200 meters above sea level are classified as malaria free and will not receive any interventions [146]. Accordingly, the *kola* and most of the *weyna dega* zones are classified as malaria transmission areas.

Rainfall distribution correlates with altitude and thus varies across Ethiopia. The main rainy seasons are from June to August in most of the country, along with March to May in the southern and south-eastern parts of the country. December, January and February are generally dry, but this dry period lasts only few weeks in some areas. Some areas receive rainfall for several months, and others for only a few months. These variations in topography, altitude and rainfall lead to well-known and recurrent droughts, famine and epidemics of infectious diseases, including malaria, in several parts of the country [38, 55].

As of 2019, Ethiopia is the second-most populous country in Africa after Nigeria, with an estimated 112 million people [149]. Ethiopia's population skews young: 43.2% are younger than 15 years old, and the median age is about 18 years. In 2018, the population growth rate was 2.83% and total fertility rate (children born per woman) was 4.91 [150]. The estimated life expectancy at birth was 63.7 years for men and 67.3 years for women in 2016 [151]. Approximately 80% of the population lives in rural areas [150], and most engage in rain-fed agriculture. Agriculture accounted for 37% of the country's gross domestic product in 2016 and 73% of employment in 2013 [80]. For more than a decade, the country's gross domestic product grew at a rate between 8% and 11% annually, making it one of the fastest growing economies in the world. Yet, Ethiopia remains one of the poorest countries in the world. In 2018, it ranked 173 out of 189 countries on the human development index [152]. The same year, its per capital income of USD 772.3 fell below the average for sub-Saharan Africa[153]. About one-quarter (23.5%) of the population lives in absolute poverty [154].

Health services in Ethiopia

Medical treatment was introduced to Ethiopia in the 16th century during the regime of Emperor Libne Dingel (1508–1540). The first Ministry of Public Health and National Health Service was established in 1947 during the regime of Emperor Haile Selassie (1930–1974) [155]. Ethiopia accepted and implemented the Alma-Ata Declaration on Primary Health Care in 1978 with the aim to provide health for all by the year 2000 [155].

Ethiopia's current health service delivery structure has three tiers (primary, secondary and tertiary) of care [156]. Primary health care comprises primary hospitals, health centres and health posts and provides preventive, promotive and basic curative services. A primary hospital provides inpatient and outpatient services to about 100,000 people, as well as emergency surgical services. A health centre serves approximately 25,000 people in rural areas and 40,000 in urban areas. These centres deliver both preventive and curative services, with an inpatient capacity of about five beds. Health posts provide services in *kebeles*, which are the lowest administrative structures in the country. A health post serves about 5,000 people and is staffed by two health extension workers. Secondary health care includes a general hospital serving 1–1.5 million people. These hospitals also make referrals to primary-level hospitals. Tertiary health care comprises a specialized hospital serving 3.5–5.0 million people. These hospitals make referrals to general hospitals. Specialized care is provided at general and specialized hospitals, which have appropriate personnel and diagnostic and treatment facilities [156].

The Ethiopian health policy prioritises infectious disease prevention and control, including malaria, as infectious diseases account for 60–80% of the health problems in the country [157, 158]. Since 1997/1998 and in line with the objectives of the health policy, Ethiopia has been implementing the Health Sector Development Programme to achieve universal access to and utilization of health services [159]. In 2002, at the end of the Health Sector Development Programme-I, the country introduced the Health Extension Programme to enhance primary health care services at the community level [160]. This Programme focuses on preventive and promotive aspects of health care under four key components: hygiene and environmental sanitation, disease prevention and control, family health services and health education and communication [161]. Over the last two decades, health facilities and human resource have been

scaled up, resulting in improved access to universal coverage of basic health services at the grassroots level. Specifically, 311 hospitals, 3,547 health centres and 16,440 health posts were built in Ethiopia, and about 38,000 health extension workers were deployed nationwide [156]. Despite this remarkable progress, the country still has high rates of morbidity and mortality from preventable infectious diseases [158].

History of malaria prevention and control in Ethiopia

Before the 1930s, the epidemiology of malaria was not well known in Ethiopia. However, surveys conducted between 1936 and 1941 provide important information on geographic locations of dominant vectors and prevalence of infection [38]. In 1955, the first organized malaria eradication projects were established in different parts of the country. These projects involved IRS with DDT and training local staff on vector control methods [38]. In 1958, a severe malaria epidemic occurred in the central highland provinces of Ethiopia, with over 3.5 million cases and about 150,000 deaths [162]. In response, the Malaria Eradication Training Centre was established in 1959 [38, 163]. By 1966, intensive malaria eradication activity began with the goal to eradicate it from Ethiopia by 1980 using DDT [163]. These efforts protected millions from the disease [163], but in 1970, an independent review team concluded that Ethiopia could not achieve malaria eradication in the specified period [38].

The civil war between 1974 and 1991 changed Ethiopia's political landscape and further complicated eradication efforts. Social and economic development halted, and the health system suffered [163]. In 1977, based on recommendations from an independent international review team [38, 163], Ethiopia scaled back its eradication efforts to focus on malaria control. Control activity was integrated into basic health services and focused on reducing malaria morbidity, mortality and people's inability to work.

In 2000, Ethiopia signed the Abuja Declaration to halve malaria mortality by 2010 [164]. It endorsed four national strategic plans to support implementation of the Abuja Declaration and facilitate elimination of malaria between 2001 and 2015 [146, 165-167]. In this period, the Global Fund to Fight AIDS, Tuberculosis and Malaria was awarded USD 400 million to support implementation of the strategic plans and Millennium Development Goal 6 [38]. Other

significant funders included the United States President's Malaria Initiative, UNICEF, and World Bank. The WHO supported malaria control efforts both financially and technically [38]. These funds and additional financial sources from the government of Ethiopia helped promote effective case management using drugs (e.g. ACTs), RDTs, LLINs and implementation of targeted IRS [167-169]. Following these intensive malaria control measures, malaria morbidity and mortality decreased in Ethiopia [37, 170].

Ethiopia has a unique malaria ecology in terms of parasites, vectors and transmission risk [171]. Two malaria parasites, *P. falciparum* (60%) and *P. vivax* (40%), are equally important in malaria epidemiology in Ethiopia [38], whereas *P. falciparum* accounts for 99.7% of malaria infection in the rest of sub-Saharan Africa [34]. The relatively high prevalence and complex biology of *P. vivax* poses challenges to malaria elimination targets [172]. During the liver stage, *P. vivax* can lie dormant, leading to relapses after treatment. It also can develop in vectors at lower ambient temperatures, enabling it to survive in more ecologically diverse areas than *P. falciparum. P. vivax* also can infect at a lower multiplicity of infection than *P. falciparum*. Thus, RDTs may not detect *P. vivax* infections, which can lead to missed diagnoses and delayed treatment [172, 173]. Another unique aspect of malaria in Ethiopia is that *An. arabiensis* is the dominant malaria vector, whereas *An. Gambiae* is the major malaria vector elsewhere [38]. Because of the outdoor and early evening feeding and zoophagic behaviour of *An. arabiensis*, reducing its vector density via currently recommended malaria control tools, such as LLINs and IRS, is difficult [5, 174, 175]. Supplementary vector control interventions are needed to reduce outdoor malaria exposure. Finally, malaria transmission in Ethiopia is relatively low and unstable but relatively high and stable in most other Sub-Saharan African countries [38]. This instability in transmission leads to frequent epidemics, mainly in the highland fringe areas, where 17% of the at-risk population lives [38]. Aberrations in climatic variables, such as rainfall and temperature, can further exacerbate malaria transmission in these areas, mainly due to the lack of acquired immunity against the disease [117].

In 2014, the Ethiopia Federal Ministry of Health revised the malaria risk stratification to use an annual parasite incidence per 1,000 people [146]. In 2017, the stratification was updated again to improve target interventions. Areas were classified per 1,000 people per year as follows:

malaria-free (annual parasite incidence = 0), low (annual parasite incidence > 0 to < 5), moderate (annual parasite incidence ≥ 5 to < 100), or high (annual parasite incidence ≥ 100) [176]. The most densely populated highland areas, including the capital city, were classified as malaria-free, and lowland areas in the western part of the country, particularly areas bordering South Sudan and Sudan, were classified as high risk (see Figure 4).

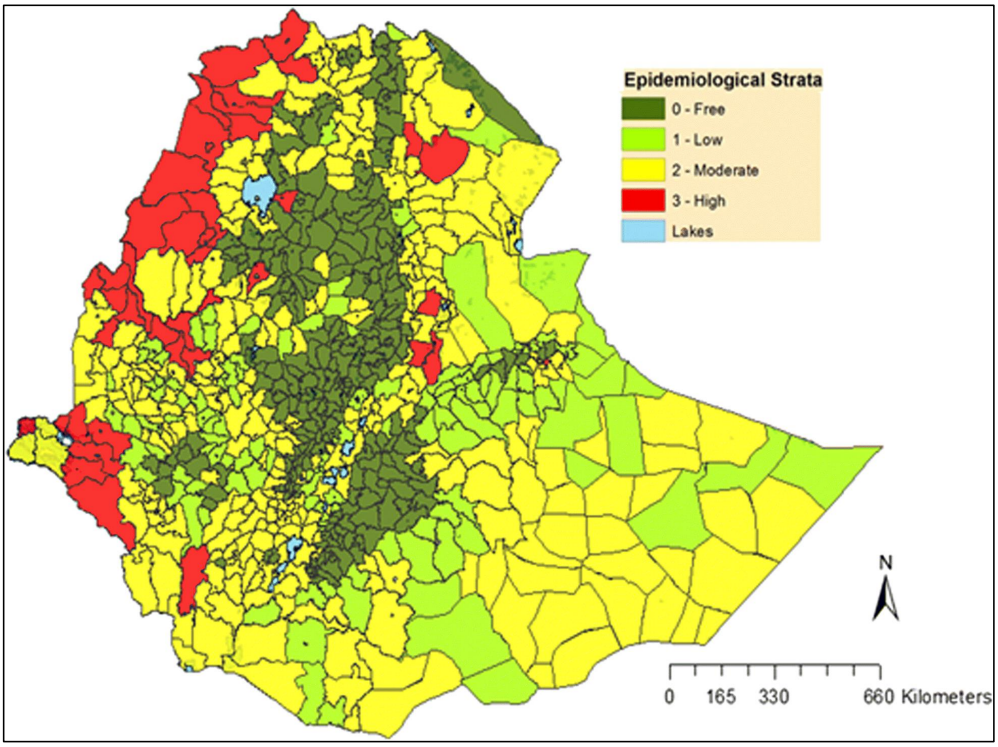

Figure 4: Malaria risk map, categorized by annual parasite incidence in Ethiopian districts in 2017 (adopted from the Ethiopian Federal Ministry of Health, 2017 [176])

The stratification was used to allocate malaria prevention and control intervention efforts. For example, no interventions were allocated to malaria-free areas, and LLINs and IRS were distributed in high-transmission areas [176], as shown in Table 1.

Table 1: Malaria risk stratification and planned interventions based on annual parasite incidence in different Ethiopian districts 2017 (adopted from the Ethiopian Federal Ministry of Health, 2017 [176])

Malaria strata	Annual parasite incidence (case/ 1,000)	Elevation (meters above or below sea level)	Population (2017)	% Pop.	# Woredas	% Woredas	Intervention					
							Insecticide-treated nets	Indoor residual spraying	Larval control	Case Managements	Surveillance	Information, Education and Communication; Behaviour Change Communication
FREE	0	≥2000	37,083,083	40.3	280	33.1	-	-	-	X	X	X
LOW	>0 to <5	<2000	17,115,269	18.6	146	17.3	X	X*	WA	X	X	X
MODERATE	≥5 to <100		34,782,644	37.8	365	43.2	X	X**	WA	X	X	X
HIGH	≥100		3,036,580	3.3	54	6.4	X	X	WA	X	X	X
Total			92,017,576	100	845	100						

Notes: *Only 32% of at-risk population in highland fringe/epidemic-prone areas covered by indoor residual spraying; **only 14.8% of districts at high-stratum boundary covered by moderate indoor residual spraying; WA: where applicable

The existing malaria prevention and control strategies

In May 2015, after the end of Millennium Development Goal 6, the World Health Assembly agreed on the importance of scaling up the malaria response towards elimination by 2030. The WHO member states endorsed a new target to reduce the global malaria burden by 90% from 2015 to 2030 [145]. To achieve this target, the WHO described a package of core interventions comprising vector control, chemoprevention, diagnostic testing and treatment [145].

Vector control

Vector control measures target mosquitoes that transmit malaria parasites to human hosts. Insecticide-based methods, such as conventional ITNs, LLINs and IRS, are proven to reduce or interrupt malaria transmission when coverage is sufficiently high [177]. The WHO recommends that malaria-endemic countries use both LLINs and IRS, where appropriate, to protect at-risk populations [34, 178]. In specific settings and under special circumstances, other supplemental methods, such as larval source management and improved housing, can be implemented [178].

Long-lasting insecticidal nets

Both conventional ITNs and LLINs are the main malaria prevention tools used against indoor resting and biting mosquitoes [179]. Both provide a physical barrier between humans and

mosquitoes, as well as insecticide that repels, disables or kills mosquitoes [180, 181]. Conventional ITNs, introduced in the 1970s, must be re-activated by dipping them in insecticide after three washes, whereas LLINs, introduced in the 2000s, incorporate insecticide within the fibres so that the insecticide lasts for the life of the net [51, 180]. Currently, LLINs are widely used, and universal coverage is recommended in all malaria-endemic countries [182]. They provide population-wide protection against malaria in communities with high LLIN access and usage [183, 184]. Several studies in sub-Saharan Africa have documented the effectiveness of LLINs against malaria when used consistently [179, 185, 186]. According to the WHO 2015 World Malaria Report [187] and Bhatt et al. [51], LLINs contributed to more than 60% of averted malaria incidences and deaths between 2000 and 2015. However, in some settings, community-level protection has not been observed [188, 189].

A rapid scale-up of LLINs was documented between 2000 and 2015 in sub-Saharan Africa, including Ethiopia [36]. LLIN use in children under five years old in sub-Saharan Africa increased from 2% in 2000 to 68% in 2015 [36]. In 2017, 50% of people at risk of malaria in Africa slept under an LLIN and 56% had access to an LLIN [34]. About 40% of households had at least one LLIN for every two people in 2017 [34]. These coverage and use rates show that malaria control programmes still must work hard to achieve universal coverage [145].

Indoor residual spraying

IRS is a core malaria prevention and control method that has been used for several decades in malaria-endemic countries, following the discovery of DDT in the 1940s [190]. IRS is mainly used to kill or incapacitate adult mosquitoes by spraying insecticide on the walls, ceilings and other indoor resting places of mosquitoes [191]. IRS using DDT eliminated malaria from several countries in Europe, the Americas, Asia and in Australia, but it was not successful in Africa due to political conflicts, transportation difficulty in rainy seasons, weak health infrastructures and lack of trained personnel [192]. It was introduced to Ethiopia in 1959 as a pilot project of a malaria eradication campaign and widely used for malaria control until the late 2000s [38].

In 2009, deltamethrin replaced DDT due to widespread DDT resistance among *Anopheles* mosquitoes in the country [193]. Carbamate soon replaced deltamethrin, because *An. arabiensis*

mosquitoes in Ethiopia developed resistance to pyrethroid-based insecticides [38]. Although IRS has averted 10% of clinical cases of malaria between 2000 and 2015 [51], the percentage of at-risk populations in Africa protected by IRS declined from 10.1% in 2010 to 6.6% in 2017 [34]. Globally, only 3% of at-risk populations were protected by IRS in 2017. According to the World Malaria Report 2018, reasons for this declining coverage may be the shift to more expensive insecticides in response to widespread pyrethroid resistance or changes in operational strategies, such as declining at-risk populations in countries aiming for malaria elimination [34].

Combined use of indoor residual spraying and long-lasting insecticidal nets

Several studies have shown that IRS and LLINs are effective against malaria infection when applied independently [179, 194, 195]. In recent years, some malaria-endemic countries have used both IRS and LLINs in combination to suppress malaria transmission [196]. This combined use may delay the emergence of insecticide resistance, because different classes of insecticide are used in each intervention [197]. However, observational and interventional studies have produced conflicting results on the added protection of this combined method, compared to the use of IRS alone or LLINs alone [198-200]. Only one interventional study has demonstrated added protection against malaria infection when IRS and LLINs are used in combination, compared to individually [201]. Other cluster-randomized controlled trials have shown no such effect [2, 202-206]. Thus, it is not yet possible to draw any conclusions about the efficacy of combined use of IRS and LLINs.

Larval source management

Larval source management is the control of potential aquatic habitats of *Anopheles* mosquito larvae to prevent development of the immature stages (eggs, larvae and pupae) [133]. Larval source management involves four methods: 1) habitat modification (permanently destroying breeding sites), 2) habitat manipulation (temporarily making the breeding sites unstable), 3) larvicide control (applying chemical or biological insecticides to aquatic habitats), and 4) biological control (introducing biological predators, such as larvivorous fish) [190]. When it is feasible and cost-effective, such as areas where the habitats are few, fixed and findable, larvicide is recommended as a supplementary intervention together with the core interventions (LLINs or IRS) [178].

In the early twentieth century before the discovery of DDT, larval source management using larvicide and environmental management were the only tools available to control malaria [178]. For example, *An*. mosquitoes were greatly reduced using a well-targeted larvicide, Paris green, on breeding sites in Brazil [207], the United States [208] and other parts of the world [209, 210]. In Ethiopia, larvicides (e.g. temephos) and habitat manipulation (e.g. draining stagnant water bodies) are considered effective deterrents in military camps, resettlement villages and urban settings [211]. Larval source management thus can play a significant role in combatting insecticide-resistant mosquitoes by killing the resistant vector in its aquatic stages [212].

Malaria and housing

Several studies have shown that modern, well-built and improved housing is associated with reduced risk of malaria infection by interrupting human–vector contact [213-215]. Improved construction includes physical barriers, such as doors and window screens [214], and iron sheet roofs rather than thatch roofs, which impair parasite development in the gut of mosquitoes due to high indoor daytime temperatures [216, 217]. Major human exposure to malaria vectors occurs indoors in Africa [218], and improving housing can prevent their entry. For example, in Gambia, the modification of houses reduced the entry of *An. Gambiae* by 78%–80% [219]. A two-arm randomized control trial conducted in southwest Ethiopia showed that screening doors and windows reduced the indoor density of *An. arabiensis* by 48% and offered protective efficacy of 61% against incidence of *P. falciparum* infection [220]. Thus, housing could be an important supplementary tool to be used in conjunction with other core vector control interventions (LLINs and IRS) for malaria control and elimination.

Preventive chemotherapy

Preventive chemotherapy is the use of antimalarial drugs either alone or in combination to prevent malaria infection and its consequences [133]. Preventive chemotherapy comprises intermittent preventive treatment in pregnancy and in infants, chemoprophylaxis, seasonal malaria chemoprevention and mass drug administration [133]. The WHO recommends intermittent preventive treatment using sulfadoxine-pyrimethamine for pregnant women and infants in African countries with moderate and high malaria transmission [221]. Studies in sub-

Saharan Africa have shown that this treatment reduces rates of placental malaria, maternal anaemia, low birth weight and perinatal mortality [222-224]. In infants, it protects against clinical malaria and anaemia [225-227]. Despite the efficacy of intermittent preventive treatment, coverage remains low. Only about 22% of eligible pregnant women in Africa receive the recommended doses [34].

In areas with seasonal transmission of malaria, chemoprevention is recommended for children aged 3–59 months [228]. In areas with seasonal high malaria transmission in sub-Saharan Africa, chemoprevention with amodiaquine plus sulfadoxine-pyrimethamine reduces the incidence of all malaria episodes by approximately 75% and severe malaria episodes by approximately 75% and mortality by about 1 in 1,000 in children aged 3–59 months [229]. In 2017, 15.7 million children in 12 countries were protected by seasonal malaria chemoprevention. However, approximately 13.6 million children were not covered by intervention due to lack of funding [34]. Preventive chemotherapy is not included in the malaria prevention and control package in Ethiopia because of the high resistance of *P. falciparum* to sulfadoxine-pyrimethamine and unstable malaria transmission in the country [55].

Malaria diagnosis and treatment

Early and accurate diagnosis of malaria is crucial for effective management and treatment of the disease [230]. Diagnostic tests (e.g. light microscopy and RDT), molecular diagnostic methods (e.g. polymerase chain reaction) or clinical diagnosis (e.g. based on symptoms or signs) can be used to diagnose malaria [231]. Since 2010, the WHO has recommended only confirmatory diagnostic tests, such as microscopy and RDT, to diagnosis malaria prior to treatment [232].

Light microscope is the gold standard due to its ability to visualize parasites in thick and thin blood smears [233]. Thick blood smears are used to determine presence or absence of the parasite and thin smears identify the species [231, 234]. Moreover, light microscope is useful to determine circulating parasite stages (e.g. distinguishing asexual parasite stages from gametocytes) and parasite density (e.g. level of parasitaemia) in the patient's blood [231, 234]. Despite the advantages of this test, it may not be available in remote rural areas where most malaria transmission occurs due to poor infrastructure, inadequate equipment, lack of reagents,

intermittent electricity or lack of trained or skilled laboratory staff [234, 235]. In Ethiopia, health centres and hospitals use light microscopes to diagnose malaria [55].

Consequently, RDTs have become a good alternative to the light microscope in most malaria-endemic countries [236]. Indeed, demand for RDTs has grown in the last 10 years from 46 million tests sold in 2008 to 412 million tests sold in 2018 [53]. Malaria RDTs are lateral-flow immune-chromatographic tests that identify specific parasite antigens (proteins) in the whole or peripheral blood [237]. RDTs do have some limitations. They may not detect malaria in the bloodstream at levels below 200 parasites/μL [238]. They cannot quantify the proportion of red blood cells infected by malaria parasites. If administered within two weeks after treatment, they can produce false positive results even if parasites are totally cleared from the bloodstream. RDTs also can be damaged by heat and humidity if not properly stored and handled [230, 239, 240]. Moreover, field studies show variable performance of RDTs, which may be due to poor manufacturing quality, incorrect storage and handling, poor preparation and interpretation of the results or even problems arising from the studies themselves, such as poor methods, analysis and reporting [241-244]. In Ethiopia, most health posts use RDTs to diagnose malaria [55].

The polymerase chain reaction-based malaria test is highly sensitive and specific in its detection of malaria parasites, even at lower parasitaemia levels than detected by microscopy and RDTs. However, this test is very expensive, because it requires considerable start-up costs and highly skilled laboratory staffs. For these reasons, it is not used for routine diagnosis of malaria in resource-poor countries, where most malaria occurs [232].

ACTs are the drugs of choice for uncomplicated *P. falciparum* infections around the world. The WHO recommends five ACT combinations: artemether plus lumefantrine, artesunate plus amodiaquine, artesunate plus mefloquine, dihydroartemisinin plus piperaquine and artesunate plus sulfadoxine-pyrimethamine [230]. Intravenous artesunate is recommended for severe *P. falciparum* infections. Although chloroquine is the preferred treatment for *P. vivax, P. ovale* and *P. malariae* infections, ACTs are used to treat chloroquine-resistant *P. vivax*. In Ethiopia, artemether plus lumefantrine has been used since 2004 as a first-line drug to treat uncomplicated *P. falciparum* infections, whereas chloroquine is the drug of choice for *P. vivax* infections [55].

Challenges for future malaria control and elimination

Insecticide resistance

LLINs protect against malaria by killing mosquitoes and reducing vector contact [179]. LLIN effectiveness depends on the mosquitoes' susceptibility to the insecticides impregnated in the fibres. Insecticide resistance remains a major challenge for global malaria control and elimination efforts [34]. The 2018 WHO global report on insecticide resistance revealed that malaria vectors have developed resistance to four classes of insecticides (pyrethroids, organochlorines, carbamates and organophosphates) in all ongoing malaria transmission regions [245]. Among 80 malaria-endemic countries that provided data between 2010 to 2017, 68 reported resistance to at least one insecticide class and 57 reported resistance to two or more [34]. Widespread resistance to pyrethroids, the only insecticide class currently used in ITNs and LLINs, thus could dramatically reverse the progress made towards controlling and eliminating the disease [246, 247]. The WHO report showed that more than two-thirds of sites that tested the efficacy of pyrethroids showed documented resistance in at least one malaria vector. Mathematical models further predict that a drop in effectiveness of the insecticide could lead to increases in malaria incidence [248]. Despite widespread resistance to pyrethroids, ITNs continue to be effective for malaria prevention [249].

Drug resistance

Antimalarial drug resistance is the ability of a malaria parasite to survive or multiply, despite absorption of the recommended or higher than recommended dose of an antimalarial [133]. Drug resistance mainly results from genetic changes in the parasites due to mutations or gene amplification [133]. Several factors affect antimalarial drug resistance, including unusual genetic structure of the parasite, substandard antimalarial drug treatments, unregulated or poor administration of the drug and the use of artemisinin without a complementary combination treatment, such as lumefantrine [250].

Resistance to antimalarial drugs threatens the control and elimination of malaria [230]. *P. falciparum's* resistance to chloroquine was first documented in the late 1950s in south-eastern

Asia, around the Thai-Cambodian border [251]. Eventually, the resistant strain spread to Africa [252], where more than 90% of the malaria burden occurs each year [34]. After *P. falciparum* in Africa developed resistance to chloroquine, hospital admissions, transmission intensity and mortality all increased [253, 254]. As monotherapies became less effective, a combination of sulfadoxine-pyrimethamine was used to treat *P. falciparum*, parasites in several malaria-endemic countries quickly developed resistance [255]. Increasing resistance to artemisinin in south-eastern Asia also threatens to reverse malaria reductions due to use of ACTs [256-258].

Residual malaria transmission

Residual malaria transmission is defined as all forms of malaria transmission that persist after achieving full universal coverage with LLIN and IRS interventions [259]. Residual transmission is likely to become a serious challenge to malaria control and elimination [174, 259]. An estimated 10.6 million additional cases of malaria occur in Africa due to outdoor malaria transmission in areas with universal LLIN and IRS coverage [174]. Increases in malaria cases since 2014 in some parts of Africa also may be associated with residual transmission [174].

A study of mosquito feeding behaviour and its influence on residual transmission of malaria across Africa showed that 21% of mosquito biting occurs before bedtime, which is higher than the previous estimation of 10% [174]. Outdoor biting was observed in Ethiopia and Eritrea, where the predominant malaria vector is *An. Arabiensis* [174]. This shift in biting behaviour may be associated with a behavioural adaptation (i.e. vectors avoid resting on LLINs or walls sprayed with insecticide), leading to early evening biting and outdoor biting. The study also predicted that a 20% increase in mosquito survival in areas with high outdoor biting will likely result in a 10% or greater reduction in effectiveness of LLINs, irrespective of overall mosquito susceptibility, whereas this impact on effectiveness of LLINs will be negligible in countries with low outdoor biting [174].

Evidence also has shown possible residual transmission of malaria in some parts of Ethiopia [2, 5, 174, 260], which threatens elimination targets. An entomological study of *An. arabiensis* in the same area as the MalTrials study in southern-central Ethiopia showed higher outdoor than indoor biting and more early-evening indoor biting than late-night indoor biting [5]. These

findings suggest a high outdoor malaria transmission potential in the study area. Another entomological study conducted in the same population as the MalTrials study showed significant reductions in indoor densities and human biting rates of *An. arabiensis* in the intervention arms (LLIN + IRS, IRS alone, LLIN alone), compared with the control arm [14]. These results suggest that application of indoor vector control interventions were effective against *An. arabiensis* in the study area. However, none of the intervention arms demonstrated reduced incidence of malaria or prevalence of anaemia, compared with each other or with the control arm [2], which could be due to residual transmission in the study area. Similarly, a study from southern Ethiopia reported residual malaria transmission in settings with high indoor vector control interventions [260].

Both human and vector behaviours are responsible for residual malaria transmission. For example, people who go outdoors at night could be exposed to outdoor mosquito bites, and people who are indoors could be exposed to indoor biting in the early evening before bed. It also is possible that *Anopheles* mosquito, particularly *An. Arabiensis,* could avoid entering to houses with effective indoor vector control intervention [133].Therefore, in addition to the core indoor vector control interventions, strategies such as mass drug administration or additional outdoor vector control interventions (e.g. larvicide, environmental management, toxic sugar bait traps, spatial repellents and insecticides or ivermectin against mosquitoes that feed on cattle) must be considered to achieve malaria elimination targets in areas with high potential for residual malaria transmission [259, 261, 262].

Misuse of long-lasting insecticidal nets

To maximize the effect of LLINs against mosquitoes, universal access to and consistent use of LLINs must be maintained by people at risk of malaria infection [182]. Misuse of LLINs could hinder this effort [263]. Studies from sub-Saharan Africa have shown considerable LLIN misuse, such as for fishing, transporting and storing maize and making rope, blankets, bed sheets and mattress covers [13, 263-265]. This misuse could affect future malaria elimination activities. Therefore, efforts to follow up after mass campaigns and enhance community awareness on the proper use of LLINs are crucial.

Rationale for this thesis

Between 2000 and 2015, the overall global burden of malaria decreased, particularly in sub-Saharan Africa, where more than 90% of malaria cases and deaths occur each year [36]. This reduction is mainly attributed to scaling up of malaria control interventions such as LLINs and IRS, prompt diagnosis via RDT and treatment with ACTs [50, 51]. To sustain this success and eliminate the disease, the World Health Assembly approved a new global technical strategy, to be implemented between 2016 and 2030, which aims to reduce malaria cases and deaths by 90% by 2030 and to eliminate the disease from at least 35 countries [145].

Ethiopia aims to eliminate malaria in selected low-transmission areas by 2020 and to eliminate malaria from the entire country by 2030 [146]. However, there are no official reports tracking the 2020 goal. Since 2015, malaria infections and deaths have held steady and even reversed in some sub-Saharan African countries [53]. Although the World Malaria Report 2019 mentions Ethiopia as one of the few countries to achieve a 40% reduction in incidence of malaria between 2015 and 2020 [53], the most recent local data shows an increase in malaria cases in some parts of Ethiopia in 2019 [54].

To overcome the emerging problems related to plateaus and increases in malaria cases, continuous efforts are needed to strengthen the health system, to scale up existing vector control tools, to improve diagnostic and treatment services and to develop new tools, such as vaccines. In the meantime, existing resources and tools must be implemented efficiently and effectively [145]. Moreover, enhanced malaria interventions should be deployed to areas with increased risk of malaria transmission [63, 71].

LLINs are the most widespread vector control intervention and the largest contributor to malaria reductions between 2000 and 2015 [51, 56]. To maximize their impact, the WHO recommends that LLINs should be serviceable for at least three years under field conditions [15] and universally accessible to and consistently used by people at risk of malaria infection [182]. However, several studies have shown large variations in the lifespan of LLINs in different settings [16-18, 266, 267], and ownership and use of LLINs are lower than expected [34]. Local data are needed to assess the serviceable life and use of LLINs [268]. Understanding these

factors could help guide communication interventions for behavioural change and to evaluate existing strategies and subsequent LLIN distribution campaigns.

Previous studies from Ethiopia have investigated physical integrity and bio-efficacy of LLINs [266, 269, 270], as well as the level of LLIN utilization [57, 59, 271, 272], using cross-sectional designs. However, these studies did not assess attrition, functional survival or potential causes of poor physical integrity of LLINs. Moreover, because of the nature of the cross-sectional designs, they failed to show trends in LLIN use over time after mass LLIN distribution campaigns. To fill this knowledge gap, we conducted a study (Paper I) to assess durability of LLINs in terms of attrition, physical integrity, functional survival and bio-efficacy. In Paper II, we conducted a prospective cohort study to evaluate trends in LLIN use over more than two years.

Because malaria transmission is highly heterogeneous across geographic locations and time due to variations in risk factors [19, 64, 65], understanding its spatiotemporal distribution at the micro-geographic scale and determinants of this heterogeneity can help target high-risk locations and optimize interventions [63, 71]. In Ethiopia, previous studies have examined the spatiotemporal distribution of malaria [19, 21, 22, 273]. However, these studies did not investigate the role of vector control interventions, such as LLINs alone, IRS alone or LLINs + IRS, on the spatial clustering of malaria, with the exception of a single publication in the southern part of the country in a village with a high malaria infection rate [20]. To fill this knowledge gap, in Paper III we examined the impact of using LLINs alone, IRS alone or a combination of LLINs and IRS on spatial clustering of malaria. We also assessed the risk factors for the observed spatial clustering in areas with low malaria transmission.

Objectives

General objective

The overall aim of this study was to assess LLINs under real-life field conditions and their impact on spatial variation of malaria in an area targeted for a cluster-randomized controlled trial for malaria prevention in southern-central Ethiopia.

Specific objectives

Objective number	Objective	Paper
1	To determine the durability of LLINs under field conditions in terms of attrition, physical integrity, functional survival and bio-efficacy	Paper I
2	To determine LLIN ownership and use over time and to identify factors associated with LLIN use	Paper II
3	To assess malaria infection clusters in areas with LLIN use	Paper III
4	To assess malaria infection clusters in relation to use of IRS alone or a combination of LLIN and IRS interventions	Paper III

Methods

Study locations

The study area is in the Oromia Regional State, which is one of the nine regional states in Ethiopia. Oromia is the largest and most populous state in Ethiopia, covering about one-third of the total area of the country, with an estimated population of 35.5 million in 2017. Administratively, the region has 20 zones, 356 districts and 6,300 rural and 303 urban *kebeles*. A *kebele* comprises about 1,000 to 5,000 people. Each *kebele* is further divided into smaller villages, called '*gare'* in the local *Affan Oromo* language. A *gare* comprises about 35 households. According to a Ethiopian Ministry of Health report, the region has 84 functional hospitals, 1,396 health centres and 6,962 health posts, as of 2019 [274].

All studies in this thesis were conducted in Adami Tullu District in the East Shewa Zone of the Oromia Regional State (see Figure 5). The district is in the Great Rift Valley, 160 km south of Addis Ababa (the capital city of Ethiopia). The district has an elevation ranging 1,500 to 2,300 meters above sea level, with a mean annual temperature ranging from 14.5 ˚C to 27.7 ˚C [1]. The average annual precipitation of the district is 700 mm, which peaks during the heavy rainy season in July and August. The district population was projected to be 190,000 in 2017 [275]. The largest ethnic group is the Oromo, most of whom follow the Islam religion. Most live in rural areas, and their main means of livelihood are rain-fed agriculture and livestock rearing. The district is divided into 48 *kebeles*.

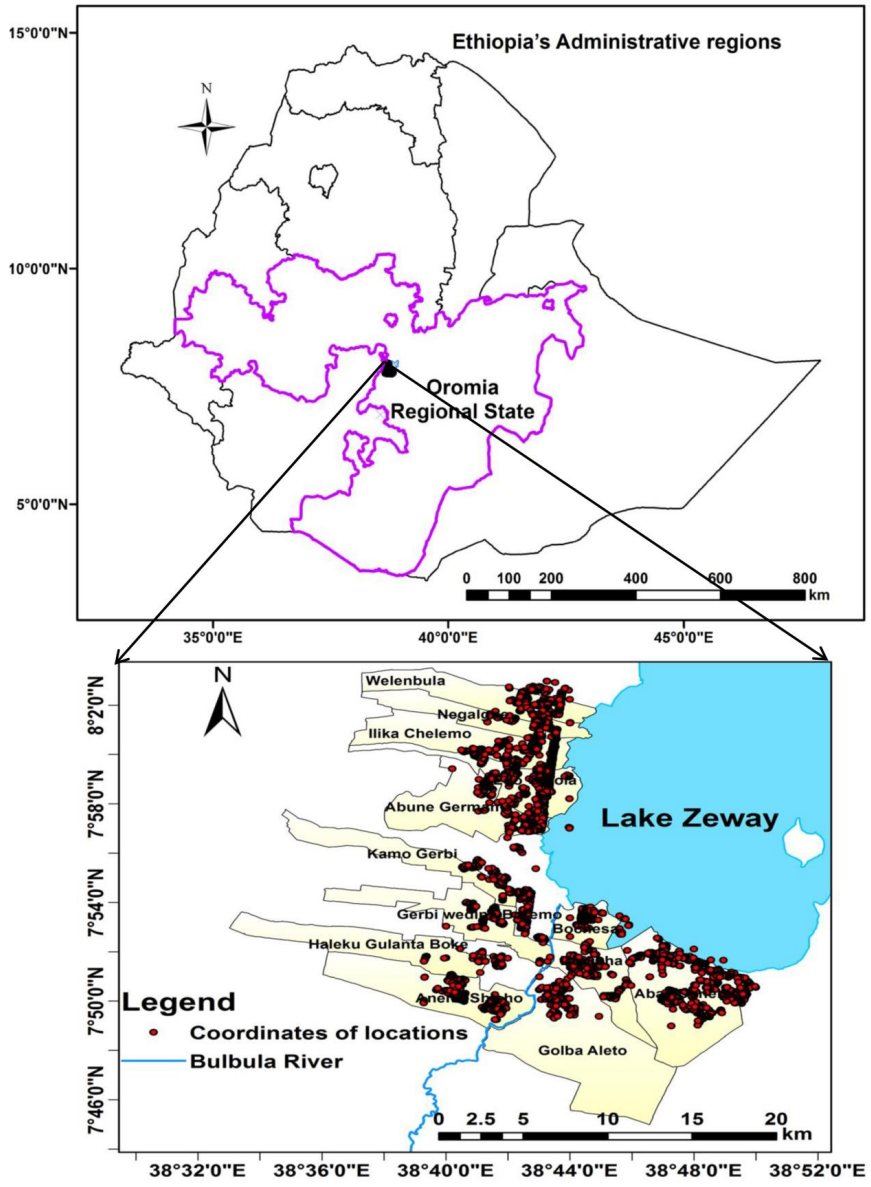

Figure 5: Map of Ethiopia showing the geographic coordinates of the study locations

As in other lowland areas of the country, malaria is the major health problem of the district. Transmission peaks from September to December, following heavy rains in July and August. As of 2014, the district was served by two hospitals (one public and one non-governmental), nine public health centres and 43 health posts. Each *kebele* has at least one health post, run by two health extension workers. Among other services, these workers provide malaria-preventive services, such as distribution of LLINs and supervision of IRS operations [146]. They also can diagnose malaria with RDTs and treat uncomplicated cases using antimalarial drugs (artemether plus lumefantrine or chloroquine).

Study design and data

All three studies in this thesis are part of a malaria prevention trial called MalTrials, which was conducted in southern-central Ethiopia from October 2014 to January 2017 [1, 2].

MalTrials project

The primary objective of the MalTrials project was to compare effects of combined use of LLINs and IRS with propoxur, the use of LLINs alone, the use of IRS alone and routine intervention against clinical malaria among all age groups of the population. The trial had three secondary objectives: 1) to compare cost-effectiveness of the interventions with routine practices, 2) to determine the effect of the interventions on entomological parameters, such as biting rates, resting density, longevity, sporozoite rate and entomological inoculation rate and 3) to assess the effect of interventions on haemoglobin concentrations and anaemia among children under five years old.

A pilot study was conducted between August and December 2013 to estimate the sample size of the trial [3]. A baseline census was conducted from June to July 2014 to collect data on demographic and socio-economic characteristics, LLIN ownership and use, IRS coverage, malaria treatment practices, history of illness and health-seeking behaviour. Pre-tested, interviewer-administered questionnaires were used to collect baseline data. A unique identification number was given to each household, and a metal plate with that number was affixed to the main entrance of the house. The geographic coordinates of each household were recorded using a hand-held global positioning system device. Each household member was

assigned a unique personal identification number corresponding to their household number. Two subsequent censuses were conducted in July 2015 and July 2016 to update for births and in- and out- migration.

To collect baseline, updated census and weekly follow-up data, 24 field data collectors with college diplomas were recruited from the respective *kebeles*. To diagnose and treat malaria, 13 nurses (one nurse per health post per *kebele*) were recruited and assigned to each health post, after receiving training on how to collect blood samples for the RDT and document results. The overall data collection process and data quality were monitored daily by three field supervisors. The preferred respondent for all interviews were heads of households, followed by family members aged 18 years or older.

The implementation of interventions was conducted in 13 *kebeles* within 5 km of Lake Zeway or the Bulbula River in early October 2014. A total of 176 villages (44 villages per arm) were selected, based on the calculated sample size for the trial. A village contained approximately 35 households and 196 people. A total of 34,548 people living 6,071 households participated. The unit of randomization was villages, which were randomly assigned to one of four intervention arms: LLINs + IRS, LLINs alone, IRS alone and Routine (standard Ethiopian malaria prevention).

In early October 2014, 7,740 LLINs were distributed free of charge to 3,006 households in the LLINs + IRS and LLINs alone arms. Distribution of LLINs was conducted based on National Malaria Guidelines [55]. A 'hang-up' campaign (door-to-door visit of households to assist LLIN recipients to hang-up their LLINs in sleeping spaces), including education on LLIN handling and proper use, was conducted two weeks after LLIN distribution. Households eligible for IRS were sprayed with propoxur, free of charge, in September 2014, July 2015 and July 2016.

In the subsequent follow-up period, each household was visited weekly on the same day of the week to maintain a seven-day gap between visits. These visits were conducted early in the morning to observe whether LLINs were hung in the sleeping spaces. Names of individuals who used an LLIN the night before the home visit were recorded to evaluate trends in weekly LLIN

use over the study period. Persons with current fever or fever within the past 48 hours were referred to the health post with a referral card for malaria testing (i.e. active case detection). Persons with fever occurring on days between weekly home visits were advised to visit the health posts on their own (i.e. passive case detection).

Study design and data in this thesis (Papers I–III)

Using the malaria trial framework, community-based prospective cohort studies were conducted to assess the durability of LLINs (Paper I), LLIN use (Paper II) and spatiotemporal clustering of malaria (Paper III). For Paper I, we followed 1,532 randomly selected LLINs in 659 households to assess attrition and functional survival and a sub-sample of 833 LLINs to assess physical integrity using follow-up surveys that were conducted every six months from November 2014 to November 2016. We also tested 120 LLINs (30 LLINs every six months) to evaluate their bio-efficacy over two years. In Paper I, we followed LLINs until the end of the study or until a participant stopped using the LLIN as intended. Reasons for discontinuing use included discarding, damage, repurposing for another use (e.g. fish net), giving it away, selling it, stolen or participant lost to follow-up.

For Paper II, we conducted a cross-sectional survey at the 110th week after LLIN distribution to document ownership of LLINs among all households that received LLINs at baseline.
To quantify weekly and overall LLIN use, we followed 17,142 people in 3,006 households who received LLINs as part of the MalTrials in October 2014. Weekly follow-up was conducted for 121 weeks from October 2014 to January 2017.

For Paper III, we followed 34,548 people to document episodes of malaria and to evaluate spatiotemporal clustering of malaria in the study area from October 2014 to January 2017. In Papers II and III, we followed study participants on a weekly basis for the duration of the study period unless they were lost to follow-up, moved to another location, refused to participate or died. People who joined the cohorts as new household members and children born during the study period were included in these two studies.

Table 2 summarizes the design, study population, sample size and data sources used in the studies in this thesis.

Table 2: Study design, participants and data collection methods

Paper	Design	Participants and sample size	Data
Bed nets used to protect against malaria do not last long in a semi-arid area of Ethiopia: a cohort study (Paper I)	Cohort and cross-sectional	Newly distributed LLINs in randomly selected households (1,532 LLINs for attrition and functional survival, 833 LLINs for physical integrity, 120 LLINs for bio-efficacy)	Interview with heads of households; observation for LLIN status; testing susceptible, 2- to 5-day-old, non-blood fed female *An. arabiensis* for bio-efficacy
Low use of long-lasting insecticidal nets for malaria prevention in south-central Ethiopia: A community-based cohort study (Paper II)	Cohort and cross-sectional	Residents in LLIN + IRS and LLIN alone arms (17,142 people in 3,006 households)	Interview with heads of households, observation for LLIN status
Spatiotemporal clustering of malaria in southern-central Ethiopia: A community-based cohort study (Paper III)	Cohort	All age groups from 13 *kebeles* and all intervention arms (34,548 people in 6,071 households)	Interview with heads of households, rapid diagnostic test for malaria diagnosis

Notes: IRS = indoor residual spaying; LLIN = long-lasting insecticidal net

Assessment of exposure and outcome variables

Tables 3 and 4 show the outcome and main exposure variables used in this thesis and their respective definitions.

Table 3: Definitions of outcome variables used in this thesis

Name of Variable	Definition	Paper
Attrition of LLIN	The proportion of LLINs no longer in use due to a known outcome (e.g. discarding, destruction or used for other purposes) or unknown outcome (e.g. given away, used in a different location, stolen, sold or lost to follow-up, such as family moved to another location or not at home). We calculated attrition as the number of LLINs lost due to known outcome or torn divided by all LLINs enrolled at baseline by excluding LLINs lost due to unknown outcome.	I
Physical integrity of LLIN	The number, size and location of holes in an LLIN, used to estimate the protective ability of the net against mosquito bites. We determined the value by dividing the number of LLINs with a hole size 0.5 cm or larger by the total number of LLINs found and assessed in the surveyed households. Another definition of physical integrity of LLINs was the number of torn LLINs divided by all LLINs assessed for holes.	I
Functional survival of LLIN	The serviceable condition of the LLIN at a given time after distribution. We defined it as the number of LLINs in serviceable condition divided by all nets present in surveyed households and nets lost due to a known outcome or torn. To categorize LLINs as serviceable or torn, we used the proportional hole index method: LLINs with holes were categorized in 'good condition' (no reduction of efficacy compared to an undamaged net; proportional hole index 0–64), 'acceptable condition' (effectiveness reduced but still significantly more protective than no net; proportional hole index 65–642) or 'torn or poor physical integrity' (protective efficacy in serious doubt and LLIN should be replaced as quickly as possible; proportional hole index ≥ 643). LLINs in serviceable condition included LLINs in 'good' or 'acceptable' condition.	I

Name of Variable	Definition	Paper
Bio-efficacy of LLIN	The ability of a net to incapacitate or kill susceptible *Anopheles* mosquitoes after contact with the LLIN. LLINs fulfilled the criteria of effective bio-efficacy if ≥ 95% knockdown or ≥ 80% mortality of susceptible *Anopheles* mosquitoes occurred after exposure to the LLIN. The proportion of effective bio-efficacy was determined by dividing the number of effective LLINs by the total number of LLINs tested.	I
Ownership of LLIN	The number of households with at least one LLIN divided by the total number of households enrolled in the study at baseline excluding the number of households lost to follow-up.	II
Proportion of individuals using LLIN per week	The total number of individuals in all households who used an LLIN the night before the day of the interview divided by the total population in all households of interviewed that week.	II
The proportion of LLIN use per each individual per the whole study period	The total number of weeks in which LLIN use was reported by each individual during the study period divided by the total number of weeks an individual stayed in the study area.	II
Spatial clustering	Areas with unusually high rates of malaria infection, as obtained by scan statistics.	III
Spatiotemporal clustering	Areas with unusually high rates of malaria infection, by place and time, as computed by scan statistics.	III
Temporal clustering	A period with unusually high rates of malaria infection, as identified by scan statistics.	III
Malaria cases in spatial clusters or outside of the cluster	Indicating whether a malaria case is present within the identified clusters (yes/no). A malaria case is a study participant who presented to the health post with symptoms of malaria (fever, chills, malaise, headache or vomiting) and who had a positive RDT for *P. falciparum*, *P. vivax* or mixed infection.	III

Notes: LLIN = long-lasting insecticidal net; RDT = rapid diagnostic test

Table 4: Definitions of exposure variables used in this thesis

Name of Variable	Level	Definition	Papers
Age	Individual	Proportion of different age groups	II and III
Gender	Individual	Proportion of male and female	I, II and III
Educational status of the head of the household	Individual	Assessed from the question that asked the highest education level attained by the head of household and categorized as illiterate (cannot read and write), can read and write, primary or secondary and above	I, II and III
Occupational status of the head of the household	Individual	Head of household's job or profession, grouped into farmers and others	II and III
Family size	Household	The number of persons living together in one house, categorized as ≤5 persons or >5 persons	I, II and III
Household wealth index	Household	Constructed using principal component analysis of household assets related variables and ranked into three categories in Papers I and II (poor, medium and rich) and into five categories in Paper III (poorest, poor, medium, rich and richest)	I, II and III
Open eave gaps	Household	Open holes in the eaves of a house in which malaria mosquitoes could enter the house	I
Type of bed	Individual	Bed types included wooden, stick or iron bedframe, mattress (with no bed frame) or mat	I
LLIN use	Individual	Self-report of sleeping under a bed net the night before an interview	I
Net washing	Individual	Measured by frequency of washing in last 6 months	I
Hygienic condition of LLIN	Individual	Cleanliness assessed by observation of the LLIN and categorized as 'clean' or 'dirty'	I

Number of sleeping spaces	Household	Number of spaces used for sleeping on the night before an interview	II
Distance from vector breeding sites	Household	Distance from Lake Zeway or Bulbula River, calculated from geographic coordinates taken using a geographic positioning system	I, II and III
Intervention	Household	Intervention type assigned to each household (LLIN + IRS, LLIN alone, IRS alone or routine)	II and III

Notes: IRS = indoor residual spraying; LLIN = long-lasting insecticidal net

Statistical analysis

Data were entered into SPSS version 20.0 (IBM Corp., Armonk, NY, USA) and analysed using both SPSS and STATA version 15 (StataCorp, College Station, TX, USA) for Papers I, II and III. SaTScan v9.4.4 was used for spatial, temporal and space-time statistical analysis (Paper III). ESRI ArcMap 10.3.1 (ESRI, Redlands, CA, USA) was used to create maps, visualize data and calculate distances between households and nearest potential vector breeding sites (Papers I, II and III) and between households and nearest health facilities (Paper III). Principal component analysis was used to compute the relative household wealth index [276, 277] by combining 14 variables related to household fixed assets, type of house, building material and land ownership (Papers I, II and III).

Descriptive statistics, such as means, standard deviations, medians, interquartile ranges, proportions and rates, were used to present descriptive data (Papers I, II and III). A Kaplan-Meier survival analysis was applied to estimate the median survival time of functional LLINs (Paper I). A Cox regression model was fitted to the dataset to investigate the predictors of physical integrity and functional survival of LLINs (Paper I). A mixed-effect multilevel negative binomial regression model was implemented to investigate the predictors of LLIN use (Paper II). Kulldorff's spatial and space-time scan statistics were applied to assess the presence of spatial and temporal heterogeneity of malaria infection in the study area and period. A retrospective purely spatial, purely temporal and space-time Poisson probability model was used to identify

locations and periods of significantly high rates clustering of malaria (Paper III). To identify the contributing factors for the observed spatial clustering of malaria, we compared malaria cases within identified spatial clusters with malaria cases outside of clusters by applying a mixed-effect multilevel logistic regression model. Exposure variables with a $P<0.25$ in bivariate analysis were included in the multivariate analysis to identify the independent predictors of outcome variables in Papers I, II and III. All statistical tests performed in Papers I, II and III were two-tailed, and the level of statistical significance was set at $P<0.05$. Table 5 summarizes the major statistical methods and approaches used to analyse data in this thesis.

Table 5: Summary of major statistical methods used for data analysis in this thesis

Statistical method	**Papers**
Descriptive statistics	I, II, III
Principal component analysis	I, II, III
Kaplan-Meier survival analysis	I
Cox regression model	I
Mixed effect multilevel negative binomial regression model	II
Mixed effect multilevel logistic regression model	III
Kulldorf's spatial and space-time scan statistics	III

Study context

The studies in this thesis were conducted in Adami Tullu district as part of the MalTrials project [1]. The capital of the district is Zeway (Batu), which is located approximately 160 km south of the Addis Ababa with an elevation of 1,640 m above sea level. The studies were conducted in 13 rural *kebeles* within 5 km of Lake Zeway. Similar to most rural Ethiopians, the study population primarily depended on rain-fed agriculture and livestock rearing for livelihood. Maize, wheat and sorghum were the main crops, though residents also practiced irrigation activity and fishing. Most houses in the district were small with mud or cement walls and thatched or iron roofs.

Malaria is a leading health problem in the district. Malaria surveys confirm a high prevalence of malaria, 6.8% in 1994 [278] and 4.8% in 2006 and 2007 [279], though more recent studies have

shown much lower prevalence and incidence rates [2, 3]. Transmission of the disease occurs in seasonal and unstable forms in the district, peaking between September and December following monsoon rains in July and August [55]. The swampy areas and irrigation lands around Lake Zeway also contribute to mosquito breeding [68, 280]. The main malaria parasites are *P. falciparum* and *P. vivax*, and the major malaria vector is *An. Arabiensis* [278]. Insecticide susceptibility tests from a pilot study of the MalTrials shows that *An. arabiensis* is highly resistant to deltamethrin, lambda-cyhalothrin, permethrin and alphacypermethrin, with mortality ranging from 0.8 to 16.8%, but they are susceptible to propoxur and bendiocarb, with 100% mortality [3].

The Adami Tullu district has been affected by repeated droughts and famines over the past decades [281, 282]. During our study period, in 2015 and early 2016, the district was affected by severe drought and food shortages following the El Nino effect from the Indian Ocean [283]. From 2014 to 2015, the annual maximum temperature increased by 2 °C and annual rainfall decreased by 60% [284]. During this period, the government of Ethiopia classified the district as one of most severely affected by drought in the country. As a result, mass food distribution and emergency relief was conducted in the study area. A decline in mosquito populations and reduced malaria incidence also were observed in the study area and period [2, 14], probably due to dry and warm weather conditions following the 2015 El Nino effect [283].

Ethical considerations

Ethical clearance was obtained from the National Ethics Committee of the Ethiopian Ministry of Science and Technology (Ref: 3.10/446/06), the institutional review board of the College of Health Sciences at Addis Ababa University and the Regional Committee for Medical and Health Research Ethics, Western Norway (Ref: 2013/986/REK vest). The MalTrials protocol was registered at the Pan African Clinical Trials Registry under the number PACTR 201411000882128 on 8 September 2014.

Written permission to conduct the study was obtained from the Oromia Regional State Health Bureau, East Shewa Zonal Health Department and Adami Tullu District Health Office. Sensitization meetings were conducted with community elders and leaders on the objectives,

randomization procedures, implementation, follow-up and expected outcomes of the study. Informed verbal consent was obtained during data collection from household heads or members older than 18 years. For minors, consent was obtained from parents or caretakers. A standard information sheet was used to explain the purpose of the study, and participants were informed that their participation was voluntary and that they could refuse or withdraw from the study at any time. People with malaria were treated at health posts with anti-malaria drugs according to the Ethiopian national malaria treatment guidelines [55]. In cases of severe illness, patients were referred to the nearest health centre for further investigation and treatment.

Results

Paper I: Durability of long-lasting insecticidal nets

In Paper I, we aimed to determine the durability of LLINs under field conditions in southern-central Ethiopia in terms of attrition, physical integrity, functional survival and bio-efficacy. A cohort of 1,532 LLINs in 659 households was followed every six months from November 2014 to November 2016 to assess attrition and functional survival. A sub-sample of 833 LLINs were followed to assess physical integrity at six months post distribution, and 120 LLINs (30 LLINs per survey per six months) were tested for bio-efficacy. In the two-year follow-up period, the overall attrition of LLINs was 96%, most of which were thrown away due to damage (64.2%), torn (21.9%) or used for other purposes (13.9%). Regarding physical integrity, the proportion of LLINs with a hole size in categories 1–4 increased from 35.8% to 79.5% during 6–24 months post distribution. The proportion of torn LLINs increased from 14.8% to 23.1% after 24 months. By the 24th month, only 4% of LLINs met the criteria for functional survival. The median functional survival time of LLINs was 12 months. The PermaNet®2.0 LINN met the WHO pesticide evaluation criteria of effective bio-efficacy for up to 24 months after distribution. Sixty two (4%) of LLINs were lost to follow-up in the entire follow-up period.

Good physical integrity of LLINs was associated with use of it on the night before data collection and cleanliness. Poor physical integrity was associated with households located more than 1 km away from mosquito breeding sites. Longer functional survival of LLINs was associated with cleanliness, whereas shorter functional survival was associated with households located more than 1 km away from the mosquito breeding sites.

Paper II: Utilization of long-lasting insecticidal nets

In Paper II, we aimed to determine LLIN ownership and use over time and to identify factors associated with LLIN use in southern-central Ethiopia. A cohort 17,142 people in 3,006 households were followed to assess their LLIN use for 121 weeks from October 2014 to January 2017. At baseline, all households received LLINs according to national malaria guidelines. After two years, at the 110th week, only 8% of households still owned at least one LLIN. Most (76%) were thrown away due to damage. During the 121 weeks of follow-up, the median proportion of

LLIN use per individual was only 14% (interquartile range of 4.1% to 29.8%). During the first year (weeks 1–52) of follow-up, the mean proportion of LLIN use per individual was 36%, which decreased to 4.6% in the second year (weeks 53–104) of follow-up.

Those aged 5–14 years (adjusted incidence rate ratio (IRR) = 1.13, 95% CI: 1.04–1.22), 15–24 years (adjusted IRR = 1.33, 95% CI: 1.23–1.45), and ≥25 years (adjusted IRR = 1.99, 95% CI: 1.83–2.17) were more likely to use LLINs than those aged <5 years. Similarly, people whose heads of households could read and write (adjusted IRR = 1.17, 95% CI: 1.09–1.26), had a primary education (adjusted IRR = 1.20, 95% CI: 1.12–1.27) or had a secondary or higher education (adjusted IRR = 1.20, 95% CI: 1.11–1.30) were more likely to use LLINs than people whose heads of households were illiterate. A family size larger than five persons (adjusted IRR = 0.78, 95% CI: 0.73–0.84) was associated with less frequent use of LLINs, compared with smaller family sizes.

Paper III: Spatiotemporal clustering of malaria

In this paper, we aimed to assess malaria infection clusters in areas of southern-central Ethiopia with LLIN use and to assess whether malaria infection clustered in areas with IRS alone or a combination of LLIN and IRS interventions. We followed 34,548 people in 6,071 households from October 1, 2014, to January 31, 2017, to document episodes of malaria. During the study period, 1,183 episodes of clinical malaria were recorded among 1,059 study participants. Most (55.1%) malaria cases were due to *P. falciparum* infection, followed by *P. vivax* infection (25.3%) and mixed *P. falciparum* and *P. vivax* infection (19.6%). The overall incidence of malaria in the study period was 16.5 episodes per 1,000 person-year observations.

In this analysis, we confirmed spatial, temporal and spatiotemporal clustering of malaria, rather than random distribution. We found spatial clustering of malaria at the *kebele*, village and household levels. For all types of malaria infection, the most likely significant spatial clusters were found in two of the 13 *kebeles*. The risk of contracting malaria in these two *kebeles* was 3.30 times higher than in the other *kebeles* (relative risk (RR)=3.30, P<0.001). At the village level, people living within the most likely significant spatial clusters were 3.55 times more likely to develop malaria than those outside of the cluster (RR=3.55, P<0.001). At the household level,

households within the most likely significant spatial clusters were 4.75 times more at risk of contracting malaria than those outside the cluster (RR=4.75, P<0.001). The household-level analysis also showed that space-time clustering of malaria occurred in the same areas as the spatial clusters. For all types of malaria infection, the most likely significant spatiotemporal cluster lasted for 12 out of the 28 months of the study period. In this high-risk area and period, the risk of contracting malaria was 4.30 times higher than in the non-cluster area and period (RR=4.30, P<0.001). In the purely temporal analysis, we identified a high-risk malaria transmission period lasting three months, from September 1, 2015, to November 30, 2015. The risk of contracting malaria during this temporal clustering was 2.25 times more likely than during the rest of the study period.

To understand the underlying risk factors for the observed spatial clustering of malaria, we compared cases within spatial clusters with those outside the clusters at the household and village levels. In both analyses, living close to potential vector breeding site was the only factor independently associated with spatial malaria clustering. At the household level, living 1 km closer to a potential vector breeding site increased the odds of being in a spatial cluster by 41.32 fold (adjusted odds ratio (OR) = 41.32, 95% CI: 3.79–138.89). At the village level, this risk increased by 1.50 fold (adjusted OR = 1.50, 95% CI: 1.15–1.93).

We separately analysed the malaria episodes data for each of the four study arms of the trial to understand whether spatial clustering of malaria occurred at the household level. The results revealed that all four study arms (LLIN + IRS, LLIN alone, IRS alone and routine) exhibited spatial clustering, with no significant difference in the risk of clustering between study arms. Moreover, to evaluate whether the identified clusters of high malaria infection overlapped with the clusters of low LLIN use, we conducted a purely spatial scan analysis to identify areas with low LLIN use among households who received LLINs. The analysis showed that clusters of low LLIN use indeed overlapped with clusters of increased malaria infections. The risk of malaria infection among people living in clusters with low LLIN use was 2.20 times higher than it was for people living in areas outside of these clusters, after adjusting for distance from potential vector breeding site (adjusted hazard ratio = 2.20, 95% CI: 1.80–2.60).

Discussion

Methodological discussion

Study design

In this thesis, we used a prospective cohort study design in Papers I, II and III and cross-sectional study design in Papers I and II. All three studies in the thesis were part of a cluster-randomized, controlled malaria prevention trial, MalTrials. The main focus of MalTrials was to evaluate the effect of combining LLINs and IRS on malaria prevention in Ethiopia [2].

Cluster-randomized trials are experiments in which communities or groups are randomly allocated to different arms of the trial [285]. When appropriately designed and implemented, cluster-randomized designs can reduce selection bias and confounding (both known and unknown). However, cluster-randomized trials also are more complex to design, and they require more complex analysis and more study participants to obtain the same statistical power as individually randomized trials [285]. As all studies in our thesis were part of the cluster-randomized controlled trial, the role of selection bias and confounding should be minimal. We also discuss the issue of confounding in the final report [2].

Cohort studies are an analytical design of observational studies in which groups of people are identified based on their exposure status and then followed up to assess the outcome of interest [286]. Cohort studies are useful when interventional studies are not feasible or ethical. Unlike other observational study designs (e.g. case-control, cross-sectional and ecological), cohort studies can establish a temporal sequence between the exposure and outcome by allowing measurement of incidence rates of diseases and by quantifying the relationship between exposure and outcome in terms of relative risk, hazard ratio and attributable risk. Additionally, they are useful for studying rare exposure and multiple outcomes and for documenting changes in exposure variables over time [286]. Cohort studies also are less susceptible to survival and recall biases than other observational study designs [286, 287]. Cohort studies do have limitations, however. They require a large sample size, which may be difficult to manage, expensive and time consuming. They are prone to loss to follow-up, which can lead to differential (unequal) loss to follow-up among exposed and unexposed people. They cannot be used to study rare

outcomes, such as diseases with low prevalence and incidence. Finally, they are more susceptible to selection bias and confounding than randomized controlled trials [287].

The prospective follow-up nature of our study designs allowed us to quantify changes over time in the durability of LLINs, such as attrition, physical integrity, functional survival and bio-efficacy (Paper I), and in LLIN use (Paper II). It also helped us to assess the impact of malaria control interventions on spatial distribution of malaria (Paper III). Conversely, the longitudinal nature of the studies, which required frequent follow-up visits, may have influenced LLIN users to keep their LLINs longer because they were being observed (Papers I and II). However, this effect was unlikely, because we observed high attrition rates and low use rates of LLINs in the study area and period. In the LLIN durability study (Paper I), we conducted follow-up surveys every six months, which may have overestimated the functional survival time of LLINs because LLINs could be lost at any time during the six-month follow-up periods. However, this overestimation also is unlikely, as we found that functional survival of LLINs was lower than expected.

Cross-sectional studies measure exposure and outcome status at the same point in time [287]. They may have two purposes: descriptive studies describe the prevalence or proportion of the outcome of interest, and analytical studies describe the association between the exposure and outcome. The analytical part of cross-sectional studies cannot be used to draw causal inferences, as it does not show whether the exposure happened before, during or after onset of the outcome [288]. In this thesis, we used the cross-sectional design only for descriptive purposes to evaluate bio-efficacy of LLINs (Paper I) and to assess LLIN ownership (Paper II).

Sample size

An adequate sample size is crucial for the credibility of study findings, particularly to assess the primary objective of a study. The sample size influences the precision of the study estimates and the power of the study to draw conclusions [289]. A small sample size is less likely to reveal a true difference between study groups, leading to false negative results (a type II error) and a waste of time and money [290].

To estimate attrition rates, physical integrity and functional survival of LLINs, the WHO recommends at least 250 LLINs in the study cohort at baseline [291]. However, in Paper I, we included a larger sample size (1,532 LLINs in 659 households) to estimate attrition, physical integrity and functional survival by considering a higher attrition rate that found in a study in Benin [17]. After selecting a random sample of 659 households, we further included all 1,532 LLINs that were available in the selected households to avoid selection bias. To evaluate physical integrity, we included a sub-sample of 833 LLINs after six months of follow-up, which was far larger than the 75 LLINs recommended by the WHO [291]. For the bio-efficacy test, 120 LLINs (30 nets per survey per six months) were included according to WHO recommendations [291]. Therefore, the sample size in Paper I should be adequate to address the research question in the study.

We also used sufficient sample sizes with adequate follow-up in the studies in Papers II and III. We did not calculate the sample size for these studies; however, we included a large sample estimated for the main trial [1, 2]. In Paper II, we included all 3,006 households and their 17,142 occupants to evaluate LLIN use over time. In Paper III, we included all 6,071 households and their 34,548 occupants to perform spatial analysis. Hence, the sample sizes in the main trial [2] and in the three papers in this thesis should be adequate, as they produce narrow confidence intervals for the most expected exposure variables associated with the respective outcomes.

Internal validity

Validity refers to the degree of closeness between a measured value and the true value of what is being measured [287]. There are two types of validity, internal and external. Internal validity is the degree to which a study is free from bias, or the ability of a study to measure what it intended to measure for the particular group of people in the study [292]. Internal validity depends on the methods used to select study participants, collect data, measure exposures and outcomes and conduct the analysis. The observed association between exposure and outcome must be valid for any individual in the study and not due to another explanation [287], such as selection bias, information bias, confounding and chance (random error).

Selection bias

Bias is a systematic error in a study that results in an incorrect estimate of association between exposure and outcome of interest and undermines its internal validity [293]. Selection bias can result from the procedures used to select study participants or from differential loss to follow-up in longitudinal studies [293]. This bias leads to systematic differences between those who participated in the study and those who refused to participate (non-response bias) or those who were lost to follow-up due to dropouts or deaths (loss to follow-up bias) and those who remained in the study [286, 293].

The impact of non-response bias in the studies in this thesis was minimal. In Paper I, all randomly selected households and all LLINs found in these households were included in the study. Similarly, non-response was negligible in Papers II and III, because we used data collected for the MalTrials, for which allocation of the intervention was done by randomization and all residents voluntarily participated in the study for the entire period. The results in these studies also were less prone to loss to follow-up bias. For example, in Paper I, only 62 (4%) of LLINs were lost to follow-up in the entire follow-up period.

Information bias

Information bias is a systematic error in the information collected from study participants due to differences in the way data was obtained on the exposure, outcome or both. It also occurs when data are recorded inaccurately. Such errors can originate from study participants, observers or data collection instruments [293]. Information bias can lead to misclassifications, such as miscategorising study participants [292].

In this thesis, there may be social desirability bias if study participants deliberately adapted their answers to align with what is socially acceptable in their communities [293]. In Papers I, II and III, we collected data on LLIN use via self-reporting using ‘Yes/No’ questions, which may have led to social desirability bias if participants preferred a ‘Yes’ response. To reduce this bias, the respondents also were asked to list the names of household members who used an LLIN the night before the date of the interview. Data collectors further confirmed this information by observing whether LLINs were hung over the household beds. The results indicate that social

desirability bias was unlikely, because the median LLIN use per individual was only 14%, despite 100% LLIN coverage at baseline.

In Papers II and III, interviewer fatigue may have led to reporting bias due to frequent weekly visits for a relatively long period (121 weeks) leading them to anticipate the next week's visits. Such bias could inflate LLIN use more than would be expected without intensive follow-up. However, because LLIN use was much lower than expected, this potential bias is unlikely to have influenced the results. Recall bias also could have influenced the causes of LLIN loss if respondents did not accurately remember what happened to their LLINs over the past six months (Paper I) and two years (Paper II) after LLIN distribution. Other recall bias could be related to the ages of study participants (Papers II and III), as some may not know their exact dates of birth or their children's. To minimize this bias, interviewers were trained to use local calendars of main historical events known in the specific settings.

In these studies, a measurement error could have originated from data collection instruments or documenting the exposure and outcome variables. We attempted to minimize such bias by training data collectors and supervisors on data collection and recording and handling techniques and by facilitating close supervision during the entire study period. Additionally, we used pre-tested [3], validated [294], interviewer-administered, structured questionnaires to collect census and weekly follow-up data (Papers II and III). For malaria diagnosis in Paper III, we used RDTs according to the manufacturer's recommended standard procedures, and the test kits were stored properly in accordance with the standards.

Confounding

Confounding refers to the mixing of the effect of an extraneous or a third variable with the effect of the exposure and outcome of interest, thereby leading to overestimation or underestimation of an effect or even reversing the direction of an effect [287]. Confounding can be controlled at the design stage or at analysis stage [292].

During the design stage, confounding can be minimized using randomization, restriction or matching. To minimize selection bias in the MalTrials study, randomization was conducted

during the design stage in Bergen, Norway, by a researcher not involved in the study [2]. The studies in this thesis were part of the trial, so this randomization helped minimize the role of confounding in the studies' results.

During the analysis stage, confounding can be controlled using standardization, stratification or a multivariate analysis. To control for potential confounding at the analysis stage, we applied multivariate analysis using regression models such as Cox regression (Paper I), multilevel negative binomial regression (Paper II) and multilevel logistic regression (Paper III). For example, to identify the independent predictors of physical integrity or functional survival of LLINs in Paper I, we adjusted for socio-demographic variables, wealth status, net condition (washing, use and cleanness), presence of rats or cats and distance from potential mosquito breeding site. In Paper II, socio-demographic and economic characteristics, distance from potential mosquito breeding site and intervention groups (LLIN + IRS and LLIN alone) were adjusted to identify factors independently associated with LLIN use. In the process of controlling for confounding, we identified confounder variables in Papers I and II. For example, in Paper I, presence of rats or cats in the households was confounding with net use, having a clean net, distance from potential mosquito breeding site and physical integrity of LLINs. In Paper II, the number of sleeping spaces in the households was confounding with age group, education of head of household and family size. In Paper III, the effect of distance from potential mosquito breeding site on the spatial clustering of malaria was adjusted for socio-demographic and economic characteristics and intervention groups. Other unknown (residual) confounding factors or effect modifications in these studies also could affect the findings and be considered limitations of our studies. For instance, in Paper III, we did not include possible risk factors for malaria clustering, such as climate variables (e.g. temperature, relative humidity) and irrigation-related vector breeding sites.

Chance

A random error (chance) can arise from the sampling variability of a study when an investigator draws an inference about the entire population based on a sample of the population. Chance cannot be avoided without including the whole population, but it can be reduced by increasing the sample size [295]. It also can be estimated by performing appropriate statistical tests using P-

values and confidence intervals [287]. If the P-value is low (e.g. <0.05), it is unlikely that the observed results are caused by chance alone. If it is high, it is more likely that the results are due to chance. The confidence interval provides a range of values in which the true estimated effect is likely to lie, with a certain degree of assurance [296]. Confidence intervals are more informative than P-values, because they provide a range of magnitude of the effect and variability in the estimate due to sample size [287]. In our studies in this thesis, we evaluated the role of chance using appropriate statistical models by applying both P-values (<0.05) and 95% confidence intervals.

External validity

External validity is the generalizability of the results to people not in the study or outside the study area [287]. If the finding is to be generalized to the wider population, it must have adequate internal validity [287]. Field trials can suffer from poor external validity if they include study sites with better access to and availability of health care services, if they use restrictive inclusion and exclusion criteria and if they artificially enhance compliance that may not be applicable to or representative of a wider population [285].

The studies in this thesis quantified the durability and use of LLINs over time (Papers I and II) and evaluated the impact of malaria control interventions on spatial variation of malaria (Paper III) in a typical rural community of Ethiopia. The study area shares similarities with many parts of the country, including socioeconomic factors, ecological factors, access to health facilities and extent of malaria transmission and malaria control interventions. Moreover, as part of the MalTrials, the studies were based on a random selection of villages and large sample sizes with adequate power and adequate follow-up periods. For these reasons, our findings can be extrapolated to many rural parts of Ethiopia that exhibit conditions similar to those in the study site. However, the generalizability of our results also might be affected by the context of the study period, which included an unexpected severe drought in 2015 and early 2016 as a result of the El Nino effect [283]. Entomological indictors such as mosquito abundance and human biting rates declined [14], as did malaria incidence (only 37% of pre-intervention incidence) [2]. These unexpected conditions might have affected typical LLIN use due to low perceived risk and

nuisance from mosquitoes and malaria infection and thus should be considered when interpreting our results.

Discussion of the main findings

In this section, we discussed the key findings from the studies in this thesis. The overall aim of these studies was to assess LLIN use under real-life field conditions and the impact on spatial variation of malaria in an area targeted for a cluster-randomized controlled trial assessing malaria prevention in southern-central Ethiopia. The findings show that LLINs did not last long: only 4% remained in serviceable condition after two years. Having a clean LLIN was associated with longer service life, whereas LLINs in households located more than 1 km from potential mosquitoes breeding sites were less likely to survive. We found low LLIN ownership and use in subsequent follow-up periods, despite 100% coverage at the beginning of the study. More frequent LLIN use was observed among people in older age groups and among those whose heads of household had better educations. Having a family size of over five persons was associated with less frequent use of LLINs. We found a large variation in the risk of malaria infection at the *kebele*, village and household levels. None of the malaria control interventions (LLIN + IRS, LLIN alone or IRS alone) affected the occurrence of spatial clustering of malaria. Proximity to the nearest potential vector breeding site increased the risk of being in an identified spatial cluster. People living in areas with spatial clusters of low LLIN use were at increased risk of contracting malaria.

Using a prospective cohort study design, we monitored durability of the PermaNet® 2.0 LLIN for two years (Paper I) and weekly LLIN use for 121 weeks (Paper II). In the durability study, among the cohort nets with known outcomes, 96% were lost within two years, largely due to disposal or poor physical integrity. As a result, only 4% of LLINs survived in serviceable condition after two years, with an estimated median survival of one year (Paper I). This finding was much lower than the expected 75% functional survival for a three-year lifespan, as predicted by NetCALC [297]. Our results also were lower than results reported by similar studies using comparable methodology and the same LLIN brand [16, 298]. For example, median functional survival was 2.5 years in Zambia [16] and 3.5 years in Cambodia [298].

Despite universal net coverage at the beginning of the study, LLIN use was much lower than expected (Paper II). The median proportion of LLIN use per individual during the study period was only 14%, which is lower than the 80% bed net use target set by the Ethiopian Ministry Health by 2015[146] and lower than studies from Ethiopia and other sub-Saharan African countries [294, 299-301]. For instance, a study conducted in southern Ethiopia using similar methodology reported higher LLIN use than our study, with a mean net use of 62% over 49 weeks of follow-up after 98.4% initial net coverage [294].

Two main factors may have played a role in the observed high attrition, poor physical integrity, low functional survival and low use of LLINs (Papers I and II). The first factor was the unusually dry and warm weather conditions in the study area in 2015 and early 2016 following the El Nino effect in the Indian Ocean [283]. As a result, the study area experienced severe drought and food shortages [284]. A subsequent decline in mosquito abundance, human biting rates and malaria incidence was documented in the study area and period [2, 14]. The highest loss (40%) of LLIN was documented during the drought between April and September 2015. These unexpected weather conditions may have affected LLIN durability and use if people perceived a lower risk of malaria infection. The second factor relates to misperceptions among net users about the usable lifespan of LLINs. A qualitative study conducted at two years after LLIN distribution on the same population as our study showed that many believed that LLINs are not serviceable for more than one year [13]. Thus, after one year, most LLINs were repurposed for grain storage and transportation from the field, rope making, toilet covers, blankets, bed sheets, mattress covers and other uses [13]. This finding implies that distribution of LLINs alone without proper education on their handling and effective use will not be sufficient to meet malaria control and elimination goals. Strategies also should include regular monitoring of distributed LLIN in terms of durability and utilization patterns.

In Paper I, we conducted a net-level analysis of LLIN use (i.e. whether a net was used the night before the first follow-up survey at six months). Controlling for other variables, net use was associated with good physical integrity, perhaps because net users protected their in-use nets from physical damage or they preferred to use intact nets more often than damaged ones. Having

a clean net was a predictor of both good physical integrity and longer functional survival. This finding could relate to the behaviour of net owners: keeping an intact net clean for prolonged use by default increases its functional survival. Furthermore, LLINs in households located more than 1 km from potential vector breeding sites were more likely to be damaged and less likely to survive, compared with nets in households within 1 km of breeding sites. This result also is logical, as people in more distant locations might not properly handle their nets for prolonged use due to low perceived risk of mosquito bites and malaria infection [93]. Further evidence from the qualitative study showed that net owners who lived far from potential mosquito breeding sites were likely to handle them improperly and use them for other purposes [13].

In the LLIN use study (Paper II), different determinants of LLIN use were identified. During the study period, the older residents of the study district used LLINs more frequently than children under five years old, despite the latter age groups being at increased risk of malaria infection due to lack of acquired immunity [83]. Similar findings have been observed in other parts of Ethiopia [294, 302]. The underlying causes for the observed lower use of LLINs among more vulnerable age groups are unclear and warrant further investigation. However, focusing on education and promotion of LLIN use, increased net access and improved net conditions for children under five years old are vital approaches to ensure maximum benefits of LLIN interventions. Although LLIN distribution in this study was conducted based on family size, having a large family size was associated with less frequent use of LLINs. Similar findings have been reported in southwest Ethiopia [303]. The reason for this could be a lack of convenient spaces to hang enough LLINs for all household members [294] or an inadequate number of LLINs in the household due to the high attrition of LLINs observed in our study area (Paper I).

In Paper III, we observed specific areas, at increased risk of malaria infection. For example, just 15% of households in the identified clusters accounted for 50% of all malaria episodes in the study area and period. We found spatial clustering of malaria at micro-geographic scales, such as *kebele*, village and household levels. Identifying such small areas will provide important input for geographically targeted interventions and optimization of resources, thus improving coverage and effectiveness of malaria elimination programmes [71].

We evaluated whether malaria control interventions (LLIN use, IRS use or combined LLIN and IRS use) could affect spatial clustering of malaria infection at the household level. Spatial clustering of malaria occurred in all intervention groups, and the risk of clustering was similar across study groups. We observed no significant difference in the incidence of malaria infection across intervention arms in the main trial [2]. However, we found that the most likely cluster of malaria was completely imbedded within the cluster for low use of LLINs, thus the risk of malaria infection was high in these clusters. This result suggests that maintaining high LLIN utilization could reduce the risk of infection.

Several reasons might explain the apparent failure of any intervention to prevent spatial clustering and the lack of difference in the risk of spatial clustering across the study groups. First, we observed high LLIN attrition and low LLIN use in the subsequent follow-up periods in the study area (Papers I and II), probably due to severe drought and declines in malaria incidence, which could have influenced the apparent effect of LLINs on interrupting spatial clustering. Second, it is possible that the interventions, all of which primarily acted indoors, did not prevent residual malaria transmission due to outdoor and early evening indoor biting behaviour of *An. Arabiensis* [5]. As a result, none of the interventions may prevent human–vector contact to the extent required to interrupt local transmission in this low malaria transmission setting. Outdoor vector control interventions may be needed in addition to core vector control interventions to interrupt local transmission in areas with high potential of residual malaria transmission. These interventions include larvicide and environmental management, toxic sugar baits traps, spatial repellents, insecticides and ivermectin treatment for mosquitoes that feed on cattle [259, 261, 262]. Third, the decline in efficacy of LLINs could be due to possible resistance in wild *An. arabiensis* populations to deltamethrin, an insecticide impregnated in LLINs, in the study area [3]. Fourth, we cannot rule out a spill-over effect between villages of each intervention arm, which may have diluted any difference in the risk of malaria clustering.

Spatial clustering of malaria may appear either due to the underlying aggregation of risk factor(s) among at-risk populations or to spatial dependency, which is a tendency of disease cases to occur near other cases rather than homogeneously within at-risk populations [304]. Understanding the risk factor(s) for the observed spatial clustering may help to predict clusters and thus target

interventions [63]. Previous studies have reported several risk factors for heterogeneous transmission of malaria, including proximity to vector breeding sites [20, 305], wind direction [306], housing type [307], behavioural factors [305, 307], human genetic factors [305, 308] and human movement [309]. In the present study, distance to potential vector breeding sites was associated with identified malaria clusters at the household and village levels. Therefore, targeting interventions at households or villages located closer to potential mosquitoes breeding sites could further reduce the burden of malaria infection.

The one-size-fits-all approach to malaria control and elimination may not be appropriate in settings with heterogeneous transmission. Instead, a problem-based solution that addresses local operational challenges may be best. One such approach is the identification and targeting of spatial malaria clusters at the *kebele* or village level. Targeting these spatial clusters of malaria can optimize resources and improve effectiveness [71], but some important practical challenges must be considered before implementation. For instance, to identify spatial clusters at the household or higher geographic scale, data are needed on the incidence or prevalence of malaria cases, family size and geographic coordinates of respective households. This approach also requires a person who is familiar with spatial statistical methods and software, data handling, analysis and result interpretation. We believe that acquiring relevant spatial data and skilled personnel could be manageable at the district level in Ethiopia for the following reasons: (1) Health extension workers can register household information, including family size in each *kebele*, as part of their routine activity; (2) health extension workers can be trained to document geographic position data of respective households; (3) health extension workers already diagnose and treat malaria, so these cases could be used to capture incidence rates and to link household members' information and geographic coordinates; and (4) district malaria focal persons can be empowered and trained on spatial data handling, software, data analysis and interpretation of results and then lead the implementation of targeted interventions in their respective districts. This approach seems achievable at the district level in Ethiopia, but further implementation studies are needed to assess its applicability, efficiency and effectiveness in reducing malaria transmission.

Conclusions and recommendations

Conclusions

Based on the findings from the studies in this thesis, we present conclusions for each main objective:

Objective 1. We aimed to determine the durability of LLINs under field conditions in terms of attrition, physical integrity, functional survival and bio-efficacy. For this specific objective, we made the following conclusions:

- The bed nets given for malaria prevention did not last as long as expected under field conditions, mainly due to high loss and poor physical integrity.
- The occurrence of severe drought in the study period and misperceptions about serviceable lifetime of the nets could explain the observed high net loss and poor physical integrity.
- The assumption of a three-year functional survival for LLINs under real-life conditions is unrealistic in the study community, as most LLINs survived only one year.
- Cleanliness of LLINs was associated with increased serviceable life, but LLINs in households located more than 1 km from a potential mosquito breeding sites were less likely to survive.
- The PermaNet ® 2.0 LLIN showed sufficient insecticidal effectiveness for at least two years after distribution.

Objective 2. We aimed to assess LLIN ownership and use over time and to identify factors associated with LLIN use. Our conclusions were as follows:

- Despite universal coverage of LLIN, low LLIN ownership and use was observed during the study period.
- Age differences, family size and educational status of household heads were associated with LLIN use.

Objective 3. We aimed to assess malaria infection clusters in areas with LLIN use, and we reached the following conclusions:

- The risk of malaria infection varied significantly between *kebeles*, villages and households and exhibited spatial, temporal and spatiotemporal clustering in the study area and period.
- The LLIN interventions had no impact on the occurrence of spatial clustering of malaria. However, the risk of malaria infection was high in clusters with low LLIN use.

Objective 4. We aimed to assess malaria infection clusters in areas with IRS alone or a combination of LLIN and IRS interventions. We concluded the following:

- Neither IRS alone nor a combination of LLINs and IRS interventions interrupted the occurrence of spatial malaria clusters.
- The risk of malaria clustering was high in areas close to potential malaria vector breeding sites.

Recommendations

Based on the findings of studies in this thesis, we make the following operational, policy and research recommendations for each main objective.

Operational and policy recommendations

Objective 1. We aimed to determine the durability of LLINs under field conditions in terms of attrition, physical integrity, functional survival and bio-efficacy. Based on the results, we make the following recommendations:

- Stronger and more efficient LLINs may be required to prolong the useful life of LLINs for conditions observed in the study area.
- Implementation of a regular monitoring system to evaluate the durability of LLINs at the district or *kebele* level may help to prolong the serviceable lifetime of LLINs. This system should integrate malaria control efforts with the existing health extension programme.
- Behaviour change communication messaging should be provided so that net users know how to handle, hang and use LLINs for their intended purpose.

Objective 2. We aimed to assess LLIN ownership and use over time and to identify factors associated with LLIN use. Based on the results, we make the following recommendations:

- Distribution of LLINs should be complemented with regular follow-up on their utilization.
- As more than 90% of LLINs were lost within two years after LLIN distribution, a replacement strategy should be implemented to ensure maximal health benefits to the community.
- Behaviour change communication messaging should focus on equitable use of available LLINs by all household members, including prioritisation of vulnerable populations, such as children under five years old, especially in cases of net shortages.

Objective 3. We aimed to assess whether malaria infection was clustered in areas with LLIN use. Based on the results, we recommend the following:

- Maintain a high level of LLIN use among all at-risk populations to reduce variations in net use and the overall risk of malaria infection.

Objective 4. We aimed to assess whether malaria infection was clustered in areas with IRS alone or a combination of LLIN and IRS interventions. Based on the results, we recommend the following:

- Malaria prevention and control programmes at the district level should consider identification and targeting of high-risk malaria clusters at the micro-geographic scale to support malaria elimination efforts.
- For accelerated reduction of malaria, strategies must target interventions to high-risk households and villages close to mosquito breeding sites and ensure more efficient and effective utilization of resources.

Recommendations for future research

Based on the studies' findings in this thesis, we recommend pursuing the following research areas:

- Our durability findings were limited to one brand of LLIN, PermaNet ® 2.0, and were based in a single area. Other LLIN brands could perform differently under similar or different field

conditions. Therefore, more research is needed to assess different brands of LLINs in diversified ecological, socioeconomic and cultural contexts.

- Further research should investigate the causes of low LLIN utilization, especially among vulnerable age groups.
- Future studies should include potential confounders for clustering of malaria, such as temporal vector breeding sites that can occur due to wet seasons, irrigation activities and population movement (in- or out-migration of at-risk populations).
- Further studies using a robust design are required to compare the applicability and impact of targeting interventions to high-risk malaria clusters at the *kebele* or village level with ongoing malaria intervention strategies.

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Original articles I-III and Appendices

I

Paper I

Solomon T, Loha E, Deressa W, Balkew M, Gari T, Overgaard HJ, Lindtjorn B. Bed nets used to protect against malaria do not last long in a semi-arid area of Ethiopia: a cohort study. Malar J. 2018;17:239.

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RESEARCH

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Bed nets used to protect against malaria do not last long in a semi-arid area of Ethiopia: a cohort study

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Abstract

Background: Long-lasting insecticidal nets (LLINs) are a key tool for malaria prevention and control. Currently, the recommended serviceable life of an LLIN is 3 years under field conditions. However, field studies show considerable variation in LLIN lifespan, from less than 2 years to more than 4 years. This study aimed to determine the attrition, physical integrity, functional survival, and bio-efficacy of LLINs under field conditions in south-central Ethiopia.

Methods: In October 2014, 7740 LLINs (PermaNet® 2.0) were distributed to 3006 households. Among the distributed LLINs, a cohort study involving 1532 LLINs in 659 households was carried out from October 2014 to November 2016. Data were collected every 6 months by observation, and through interviews with the heads of households. The proportional hole index was used to categorize LLINs as either serviceable or torn. In addition, 120 randomly selected LLINs were tested for bio-efficacy.

Results: The overall attrition of LLINs was 96% (n = 993) during the study period. The nets' attrition was mainly due to disposal (64.2%; n = 638). The proportion of LLINs with a hole size 0.5 cm or larger was 79.5% after 24 months. The use of the net on the previous night and having a clean net were associated with a good physical integrity. However, living in a household more than 1 km away from the mosquitoes' breeding site was associated with poor physical integrity. By the 24th month, only 4% of the nets met the criteria for functional survival. The median functional survival time of the nets was 12 months. A longer functional survival was associated with having a clean net, and shorter survival was associated with living in a household more than 1 km away from the mosquitoes' breeding site. The PermaNet® 2.0 met the criteria of effective bio-efficacy up to month 24 after distribution.

Conclusions: The study showed that the median serviceable life of LLINs is only 12 months. However, the bio-efficacy of the LLINs is acceptable for at least 24 months. Therefore, stronger and more efficient LLINs need to be developed for conditions similar to those studied here.

Keywords: Durability, Long-lasting insecticide nets, Attrition, Physical integrity, Functional survivorship, Ethiopia

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Background

Globally, the burden of malaria has declined in the past 15 years with the scaling-up of cost-effective vector control interventions, diagnosis, and treatment [1]. The reduction in the global incidence of malaria is estimated to be 37%, and the decline in malaria-specific mortality is estimated to be 60% [1]. Similar reductions have also been observed in Ethiopia [2], but the incidence of malaria is still high; it is estimated that 2,588,000 cases of malaria and 5000 malaria-specific deaths occurred in 2016 [3].

Vector control through the use of long-lasting insecticidal nets (LLINs) is a widely implemented tool for the prevention of malaria [4, 5]. To maximize the impact of the intervention, universal access to and use of LLINs by people at risk for malaria must be maintained [6]. However, access to LLINs remains lower than expected [3]. For example, in sub-Saharan Africa, only 43% of people had access to sufficient LLINs (a net for two people), and only 54% people slept under LLINs in 2016 [3]. According to a 2015 national malaria indicator survey, 64% of Ethiopian households own at least one LLIN, and 32% have one LLIN for every two persons. The same survey reported that only 40% of the population at risk slept under a LLIN the night before the survey [7].

The LLIN interventions have a limited service life because they become worn out or are lost. The most common causes for the short service life of LLINs are a high attrition rate and physical damage [8–12]. Moreover, care and repair of bed nets, usage pattern, washing frequency, and type of LLIN all have potential impacts on the length of the service life of an LLIN [8, 13–15]. The World Health Organization (WHO) recommends that LLINs should be serviceable for at least 3 years under field conditions, with adequate insecticidal activity [16]. However, studies show considerable variation in the length of an LLIN' serviceable life, ranging from less than 2 years to more than 4 years [8, 13, 17–19]. Furthermore, it cannot be assumed that an LLIN product that is durable in one setting will last in other settings. Thus, there is substantial need for regional data to assess the durability of LLINs [20, 21]. Such data could inform decision-makers regarding how often bed nets should be distributed. Furthermore, understanding the factors that lead to a shortened LLIN service life could help guide communication interventions for behavioural change [20].

Previous studies from Ethiopia have investigated physical integrity and bio-efficacy of LLINs using cross-sectional study design [17, 21, 22]. However, these studies did not consider the attrition rate, functional survival, or potential causes of poor physical integrity of LLINs. To fill this knowledge gap, this study used a cohort design to determine the durability of LLINs under user conditions in the Adami Tullu district, south-central Ethiopia, in terms of attrition, physical integrity, functional survival, and bio-efficacy.

Methods

Study setting

This study was carried out in the Adami Tullu district in south-central Ethiopia (Fig. 1) from October 2014 to November 2016. The district is located approximately 160 km south of Addis Ababa. The study population was primarily composed of the Oromo ethnic group, who follow the religion Islam. This rural population primarily engages in farming, livestock, and fishing. Based on the 2007 national census, approximately 190,000 people lived in the district in 2017 [23]. The district has 48 *kebeles* (the lowest government administrative unit), each with an average population ranging from 1000 to 5000 people [23].

Malaria is among the leading causes of illness in the Adami Tullu district, sometimes occurring as an epidemic [24]. The shores of Lake Zeway and irrigated areas serve as mosquito breeding sites in the district [25, 26]. *Anopheles arabiensis* is the main malaria vector, whereas *Plasmodium falciparum* and *Plasmodium vivax* are the main parasites of malaria in the district [27, 28]. The district is a drought-prone area, and is characterized by a semi-arid climatic condition [29]. The district was affected by a severe drought that occurred in 2015 following the El Nino [30]. Effects of the drought included food shortages, a decline in annual rainfall (by 60% in 2015) and an increase in the average maximum temperature (2 °C above normal) [31].

Study design and participants

This study was part of a cluster-randomized controlled trial that aimed at quantifying the combined effect of indoor residual spray (IRS) and LLINs against clinical malaria, compared with LLINs or IRS alone or routine intervention (the MalTrials project) [32]. The trial had 176 study clusters (44 clusters per arm). The LLIN + IRS arm contained 1619 households and 8216 people; the LLIN-alone arm contained 1387 households and 7288 people; the IRS-alone arm contained 1530 households and 7753 people; and the routine arm contained 1544 households and 8038 people. In early October 2014, 7740 LLINs (purchased in June 2014 from the Vestergaard Frandsen Group) were distributed to 3006 households, both in combination and in the LLIN-alone arm. They had a light blue colour and rectangular shape, with a size of 160 cm width, 180 cm length, and 150 cm height [32]. The distribution of LLINs was conducted based on the National Malaria Guidelines: one net for a family with 1–2 persons; two nets for a family with 3–5 persons;

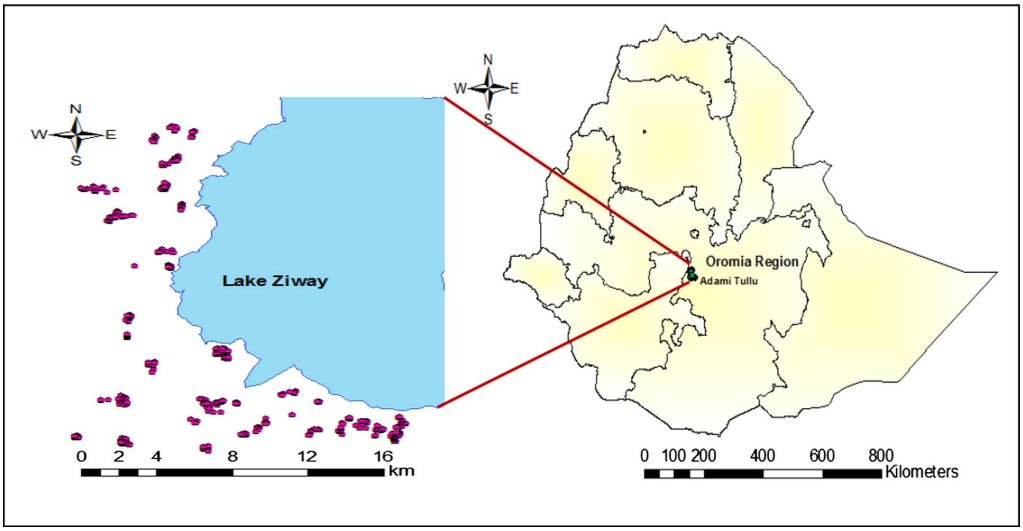

Fig. 1 Map of the study area showing the location of selected households in south-central Ethiopia

three nets for a family with 6–7 persons; and four nets for a family with ≥ 8 persons [33]. A "hang-up" campaign and net tracking activities were carried out after distribution by putting a unique identification number on each LLIN with indelible ink.

Using the malaria trial framework [32], a cohort study was conducted among households with newly distributed LLINs to assess attrition, physical integrity, and functional survivorship. Four follow-up surveys were conducted every 6 months. The first survey was conducted in April 2015, the second in October 2015, the third in April 2016, and the fourth in early November 2016. The LLINs were followed until one of the following outcomes: LLIN loss due to discarding, distraction, used for other purposes, given away to other users, sold, stolen, lost to follow up, torn, or to the end of the study. Moreover, every 6 months cross-sectional surveys were carried out to evaluate the ability of LLINs to knockdown (KD) or kill susceptible *Anopheles* mosquitoes (bio-efficacy).

Sample size estimation

The sample size was calculated based on the findings from a study in Benin, in which 48% of the LLINs were in poor condition (torn) after 1.5 years of use [13]. Using a single-population proportion formula (with OpenEpi software), and assuming a 4% margin of error, a 95% confidence level at $\alpha = 5\%$, and a 10% non-response rate, a total sample size of 659 LLINs was calculated. The households were randomly selected from a sampling frame of the LLIN-alone arm of the trial. Computer-generated random numbers were used to select random samples of LLINs using IBM SPSS version 20.0 (Armonk, NY: IBM Corp. USA). To avoid selection bias, all LLINs in the selected households were included in the study. A total of 1532 LLINs in 659 households were enrolled at baseline for evaluation of attrition and functional survivorship. A sub-sample of 833 LLINs were enrolled at the sixth month and followed for assessment of physical integrity. According to WHO recommendations [34], a total of 120 LLINs (30 LLINs per survey) were evaluated for bio-efficacy over a 2-year period. The LLINs were collected for the test based on the eligibility criteria of being used for sleeping during data collection. One LLIN per household was considered for the test. The LLINs that were taken for analysis were immediately replaced with new LLINs.

Data collection

Baseline and follow-up data on household characteristics and net status were collected using structured, pretested and interviewer-administered questionnaires. The questionnaires were prepared in English and then translated into the local language, *Afan Oromo*. The USAID-supported Malaria Consortium NetWorks training guideline was used to train data collectors for 2 days on the LLIN hole assessment technique [35]. All data collectors were diploma graduate personnel. Three teams of data collectors, each of which was comprised of three members (one supervisor and two data collectors in each

team), were involved in data collection. During data collection, heads of households or competent family members (age ≥ 18 years) were interviewed about the status of their LLINs. If LLINs were not found or used for other purposes, the respondents were asked why and how nets were lost or damaged or used for other purposes. If the visited house was closed or no competent (age ≥ 18 years) respondent was present, the house was revisited at least three times within a week. If the house was closed or no competent respondent was present after three visits, LLINs were considered lost to follow-up.

Definition and follow up of outcome variables

Attrition

Attrition was defined as the proportion of LLINs no longer in household use [34]. Attrition of LLINs was categorized as "attrition for known outcome" and "attrition for unknown outcome".

Attrition for known outcome

Net lost from household due to discarding, destruction, or used for other purposes.

Attrition for unknown outcome

Net lost from household due to being given away for others to use, used in different location, stolen, sold or lost to follow up (due to family moving to other location or not at home).

Physical integrity

The physical integrity of the LLINs was defined considering the number, size, and location of holes to estimate the protection ability of the net against mosquito bites. For nets presented and used for sleeping during data collection, inspections were carried out for the presence, type, location, and size of holes. A rectangular metal frame with a size of 165 cm width, 185 cm length, and 160 cm height was used to hang and inspect each net for holes. Hole categories recommended by the WHO were used to determine hole size [20]. Hole-size categories were defined as follows: hole size 1, 0.5–2 cm (smaller than a thumb); hole size 2, 2–10 cm (larger than a thumb, but smaller than a fist); hole size 3, 10–25 cm (larger than a fist, but smaller than a head); and hole size 4, larger than 25 cm (larger than a head). Holes smaller than 0.5 cm were not counted. Moreover, the causes of holes were identified and evidence of repair was recorded. The proportional hole index (pHI) was used to group LLINs into serviceable or torn categories. The pHI for each LLIN was calculated by weighting each hole by its size (size 1–4) and totaling up the weighted number of holes as described elsewhere [34]. The LLINs with holes were categorized into one of the following groups: pHI 0–64, "good condition": no reduction of efficacy compared to an undamaged net; pHI 65–642, "acceptable condition": effectiveness somewhat reduced, but still provides significantly more protection than no net at all; and pHI ≥ 643, "torn" or poor physical integrity condition: the protective efficacy is in serious doubt, and the LLIN should be replaced as quickly as possible. The number of combined LLINs in "good" and "acceptable" condition represented the number of LLINs in "serviceable" condition or in good physical integrity condition [34].

Functional survivorship

Functional survival was defined as the proportion of LLINs in serviceable ("good" + "acceptable") condition at a given time point after LLIN distribution. Both attrition with known outcome and LLINs in serviceable or torn conditions were used to evaluate functional survival [36].

Bio-efficacy

The ability of a net to incapacitate or kill susceptible *Anopheles* mosquitoes after contact with the insecticide on the LLIN. For the bio-efficacy test, five samples from each LLIN measuring 30 cm × 30 cm were cut according to the guideline [34]. Each piece of the net section was labelled with a unique identification number by combining the household number and a sample location. The samples were then wrapped in a foil and placed in a black plastic bag for storage until the test. In the laboratory, 10 susceptible, 2- to 5-day-old, non-blood fed female *An. arabiensis* mosquitoes were exposed for 3 min on each piece of sample according to the WHO cone bioassay test procedure [12]. Control tests were carried out each day immediately before and after exposure of mosquitoes to experimental LLINs. The LLINs fulfilling the criteria of ≥ 95% KD or ≥ 80% mortality using susceptible *Anopheles* mosquitoes were considered effective [34].

Statistical analysis

Data were entered into and analysed by IBM SPSS version 20.0. For non-normally distributed continuous variables, medians and the interquartile range (IQR) were calculated. The dependent variables of the study were attrition, physical integrity, functional survival, and the bio-efficacy of LLINs. The exposure variables were gender and educational status of the head of the household, family size, wealth status, the presence of open eave gaps in the house, type of bed, status of net use, status of net washing, hygienic condition of the LLIN, presence of rodents or cats in the household and distance of household from vector breeding sites.

The household wealth index was calculated using principal component analysis (PCA) [37, 38]. Fourteen household assets were used in the calculations,

including presence of electricity, ownership of television, radio, mobile telephone, chair, table, bed, bicycle, land, separate kitchen from living house, animal and animal cart, and types of roof and walls. A wealth index was constructed from the first principal component for each household, and then categorized into three relative measures of socioeconomic class (poor, middle, and rich). The Kaiser-Meyer-Olkin (KMO) measure of sample adequacy was 0.77. The total variance explained by the first principal component was 23.8%, with a corresponding Eigen value of 3.33.

The attrition rate of the LLINs was calculated as the number of LLINs lost with known outcome of attrition or torn, divided by all LLINs enrolled at baseline. However, the LLINs lost with unknown outcome of attrition were excluded from the denominator. The physical integrity of the net was determined using two measurements. The first measurement was the proportion of LLINs with a hole size 0.5 cm or larger divided by the total number of coded LLINs found and assessed in the surveyed households. The second measurement was the proportion of torn nets divided by all nets assessed for holes. To estimate the proportion of functionally surviving LLINs, the nets in "good" and "acceptable" condition were used as a numerator, and all nets present in surveyed households and nets lost due to "known outcome of attrition" + torn were used as a denominator. The proportion of functionally surviving nets was compared against reference survival curves provided by the WHO [36]. A Kaplan–Meier survival analysis was used to estimate the median survival time of functionally surviving LLINs. The proportion of LLINs with effective bio-efficacy was calculated as the number of effective LLINs ($\geq$ 95% KD or $\geq$ 80% mortality) divided by the total number of LLINs tested. The LLINs were considered effective against malaria-transmitting *Anopheles* mosquitoes if at least 80% of the sampled LLINs fulfilled the criteria of $\geq$ 95% KD or $\geq$ 80% mortality after at least 20 washes and 3 years of use [34].

To investigate the predictors of physical integrity and functional survival of LLINs, a proportional Cox regression model was fitted to the dataset. The failure endpoint for physical integrity was defined as an LLIN in torn condition. And, for functional survival, the failure endpoint was either the LLIN having the known outcome of attrition or being in torn condition. The LLINs in the unknown outcome of attrition category were censored at the time of net loss. Variables having a P value <0.25 in bivariate analysis were included in the multivariate analysis to identify independent predictors. A P value <0.05 was considered statistically significant.

Ethical considerations

Ethical clearance was obtained from the Ethiopian Ministry of Science and Technology (Ref: 3.10/446/06), Institutional Review Board of the College of Health Sciences at Addis Ababa University and the Regional Committee for Medical and Health Research Ethics, Western Norway (Ref: 2013/986/REK vest). Also, permission letters were obtained from the Oromia Regional Health Bureau, East Shewa Zonal Health Department, and Adami Tullu District Health Office. Information about the study objectives, procedures and benefits were clearly explained to the study participants. Written consent was not obtained because the majority of the study participants could not read or write [32]. Therefore, verbal informed consent was obtained from study participants during data collection.

Results

Characteristics of study households

A total of 659 households were included in this study. The majority of heads of households were male (407; 62%) and illiterate (369; 57%). About 331 (50%) of study households had a family size of more than five individuals. Approximately one-third of households (202; 31%) lived within 1 km from a potential vector breeding site (Table 1).

Table 1 Characteristics of households with long-lasting insecticidal nets assessed for durability in Ethiopia

Variable	n (%)
Gender of head of household	
Male	407 (61.8)
Female	252 (38.2)
Educational status of head of household (n = 647)	
Illiterate	369 (57.0)
Read and write	59 (9.1)
Primary	162 (25.0)
Secondary and above	57 (8.8)
Wealth status (n = 622)	
Poor	230 (37.0)
Middle	198 (31.8)
Rich	194 (31.2)
Family size	
≤ 5	328 (49.8)
>5	331 (50.2)
House with open eave gap (n = 615)	
Yes	99 (16.1)
No	516 (83.9)
Distance from mosquito breeding site (km)	
≤ 1	202 (30.7)
>1	457 (69.3)

Table 2 Reasons for loss of long-lasting insecticide nets over a 2-year follow-up period in Ethiopia

Reason for LLIN loss	6 months n (%)	12 months n (%)	18 months n (%)	24 months n (%)	Total n (%)
Known outcome of attrition or torn					
Thrown away	102 (40.0)	301 (71.8)	173 (74.9)	62 (70.5)	638 (64.2)
Used for something else	30 (11.8)	58 (13.8)	28 (12.1)	22 (25.0)	138 (13.9)
Torn (pHI > 643)	123 (48.2)	60 (14.3)	30 (13.0)	4 (4.5)	217 (21.9)
Total	161 (100)	331.1 (100)	149 (100)	12 (100)	993 (100)
Unknown outcome of attrition					
Given away	260 (76.7)	44 (45.8)	11 (20.0)	1 (12.5)	316 (63.5)
Lost to follow-up[a]	52 (15.3)	43 (44.8)	26 (47.2)	5 (62.5)	126 (25.3)
Stolen	8 (2.4)	9 (9.4)	3 (5.5)	2 (25.0)	22 (4.4)
Unknown reasons	6 (1.8)	0 (0.0)	1 (1.8)	0 (0.0)	7 (1.4)
Other[b]	13 (3.8)	0 (0.0)	14 (25.5)	0 (0.0)	27 (5.4)
Total	461 (100)	558.2 (100)	153 (100)	(168) (100)	498 (100)

LLIN long-lasting insecticidal net

[a] Family moved to other location, family not at home, refusal to participate

[b] Sold or destroyed by fire

Enrollment of LLINs and study completion

At the start of the study, 1532 LLINs were included in the study for attrition and functional survival assessment. Out of this number, 1061 at 6 months (T_6), 517 at 12 months (T_{12}), 198 at 18 months (T_{18}), and 56 at 24 months (T_{24}) were available for examination.

Attrition

A total of 1491 LLINs were lost during the 2-year follow-up period. Among the lost LLINs, 993 (67%) were lost due to a known outcome or torn, and 498 (33%) were lost due to an unknown outcome (Table 2). The attrition for known outcomes or torn of LLINs increased more rapidly over time (Fig. 2). The overall attrition for known outcomes or torn from the beginning to the end of the study was 96% (95% CI 94.7–97.1; n = 993; N = 1034). The reasons for this attrition were as follows: being thrown away because of damage (638; 64.2%), torn (217; 21.9%), and being used for other purposes (138; 13.9%; Table 2).

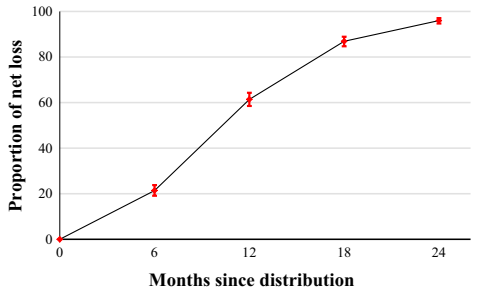

Fig. 2 Proportion of lost LLINs due to known reasons of attrition or torn over a 2-year-period. The error bars indicate the 95% confidence interval

Physical integrity

The number of eligible LLINs, and those included for physical integrity evaluation at 6 months, as well as the number of LLINs found in the households during follow up are summarized in Fig. 3. The proportion of LLINs with a hole corresponding to the size categories 1–4 was 35.8% (298 of 833) after 6 months. This proportion increased to 79.5% (31 of 39) after 24 months of follow up. When the locations of holes were considered, the mean number of holes of any size was found to be higher in the lower half of the LLIN compared with the upper half or the roof. The median pHI increased from month 6 to 18, whereas the pHI decreased slightly at 24 months (Table 3).

The proportions of LLINs in the "good" and "acceptable" categories decreased with age, whereas LLINs in the "torn" category increased with age. The proportion of torn LLINs increased from 14.8% (123 of 833) to 23.1% (9 of 39) between 6 and 24 months. Only 39 LLINs were identified during follow up visits. Among these, only one LLIN was torn at 6 months, and the number of torn LLINs increased to nine (23.1%) after 24 months (Table 4).

Predictors of physical integrity of LLINs

A bivariate proportional Cox regression analysis showed that using the LLIN during the night before the day of the survey, having a clean LLIN, and the presence of a cat in the house were all associated with

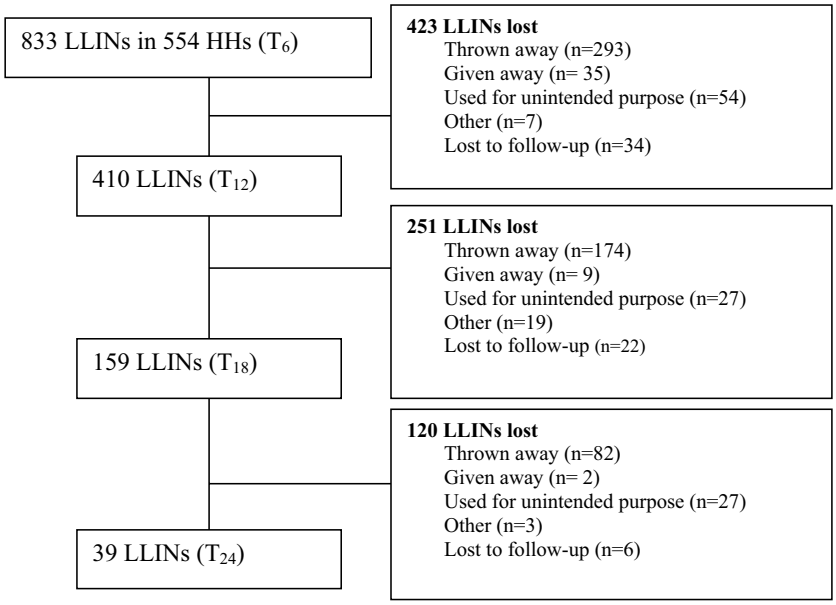

Fig. 3 Flow diagram shows the number of LLINs enrolled at 6 months in south-central Ethiopia

Table 3 Proportion of long-lasting insecticide nets with holes, mean number of holes by location, and proportional hole index over time in Ethiopia

Characteristic	6 months N = 833	12 months N = 410	18 months N = 159	24 months N = 39
Holes (size categories 1–4), n (%)	298 (35.8)	204 (49.8)	107 (67.3)	31 (79.5)
Mean (SD) number of holes				
Lower half segment of nets	3.7 (12.7)	4.2 (12.0)	5.7 (13.3)	4.4 (6.6)
Upper half segment of nets	2.1 (8.1)	2.2 (4.6)	3.7 (8.1)	5 (9.2)
Roof segment of nets	1.2 (4.9)	1.8 (4.6)	2.6 (4.5)	3.3 (3.8)
GM pHI (95% CI)[a]	216 (167–279)	251 (195–323)	316 (234–427)	211 (117–378)
Median pHI (IQR)[a]	270 (48–993)	275 (88–843)	422 (173–775)	296 (77–604)

N: The total number of LLINs available for evaluation at each data collection period

CI Confidence interval, *IQR* interquartile range, *LLIN* long-lasting insecticidal net, *SD* standard deviation, *GM* geometric mean, *pHI* proportional hole index

[a] Number of LLINs evaluated (n = 274 at 6 months, n = 170 at 12 months, n = 85 at 18 months, and n = 28 at 24 months)

the good physical integrity of LLINs. The presence of rats in the house and a household location of more than 1 km from a mosquito breeding site were associated with poor physical integrity. Multivariate analysis indicated that using a LLIN during the previous night (adjusted hazard ratio [HR] = 0.7; 95% CI 0.50–0.98), having a clean LLIN (adjusted HR = 0.4; 95% CI 0.30–0.60), and being in a household more than 1 km away from a mosquito breeding site (adjusted HR 1.8; 95% CI 1.2–2.6) were independent predictors of the physical integrity of LLINs (Table 5).

Table 4 Proportion of long-lasting insecticide nets in good, acceptable, or torn condition over time, as defined by the proportional hole index in Ethiopia

Category defined by pHI	6 months N = 833 n (%)	12 months N = 410 n (%)	18 months N = 159 n (%)	24 months N = 39 n (%)
Good (0–64)	610 (73.2)	243 (59.3)	63 (39.6)	14 (35.9)
Acceptable (65–642)	100 (12.0)	82 (9.8)	45 (28.3)	16 (41.0)
Torn (> 643)	123 (14.8)	85 (20.7)	51 (32.1)	9 (23.1)
LLINs present at all follow-up visits, n = 39				
Good (0–64)	34 (87.2)	29 (74.4)	19 (48.7)	14 (35.9)
Acceptable (65–642)	4 (10.3)	6 (15.4)	13 (33.3)	16 (41.0)
Torn (> 643)	1 (2.6)	4 (10.3)	7 (17.9)	9 (23.1)

N: The total number of LLINs available for evaluation at each data collection period

LLIN long-lasting insecticidal net, *pHI* proportional hole index

Functional survival

Observed functional survivals at different time points, compared with the reference NetCALC loss prediction curves, are shown in Fig. 4. Six months after distribution, 78.6% (95% CI 76.2–80.9) of the LLINs were functionally surviving. However, by month 24, only 4% (95% CI 2.9–5.4) had survived. The median (95% CI) survival time (time since distribution, in which 50% of LLINs were in a serviceable condition) was 12 (11.6–12.4) months. The observed functional survival was less than the 3-year serviceable model, being closer to the 1-year serviceable model (Fig. 4).

Predictors of functional survivorship of LLINs

A multivariate proportional Cox regression model showed that having a clean LLIN (adjusted HR = 0.8; 95% CI 0.6–0.9) was an independent predictor of longer functional survival, whereas the distance of a household 1 km from a mosquito breeding site (adjusted HR = 1.3; 95% CI 1.1–1.6) was associated with the shorter functional survival of LLINs (Table 6).

Bio-efficacy

A total of 120 LLINs were tested using WHO cone bioassays over a 2-year period. The GM of 60-min KD rates was greater than 90% in all four surveys. The GM of 24-h mortality rates was below 80% in the second year with 76.6% (95% CI 71.0–82.6) at 12 months and 69.4% (95% CI 59.4–80.9) at 24 months (Table 7). Statistically significant differences were observed both in 60-min KD rates, and 24-h mortality rates between 12 and 18 months and between 12 and 24 months (Table 8).

The proportion of LLINs meeting the WHO pesticide evaluation scheme criteria at different time points is presented in Table 9. At 6 months, the proportion of LLINs meeting the criteria of effective bio-efficacy was 90% (95% CI 72.5–96.8), and this proportion decreased to 80% (95% CI 61.5–90.9) at 24 months. However, LLINs met the criteria of effective bio-efficacy in all study periods.

Discussion

Low functional survivorship of LLINs was observed in south-central Ethiopia. The data show that most LLINs survive for approximately 1 year. High attrition rates due to discarding and the poor physical integrity of LLINs were the major causes of low functional survivorship. The LLINs were found to be effective against malaria-transmitting mosquitoes and met the criteria of optimal effectiveness of bio-efficacy up to month 24.

Previous studies have used cross-sectional study designs to evaluate the durability of LLINs in Ethiopia [17, 21]. Because of the design, these studies could not quantify attrition, functional survivorship, and changes in the physical integrity of LLINs over time. The current study has addressed these limitations. Unlike previous follow-up studies [8, 13, 39], this study followed all LLINs in selected households to avoid selection bias and potential observer effects (Hawthorne effect) in which users might treat the net under observation differently than nets not under observation.

This study had some limitations. The prospective nature of the study may have influenced the user to keep their LLINs longer because they were being observed. However, because the attrition rate was much higher than expected, this potential limitation is less likely to have influenced the results. The functional survival time of LLINs may have been overestimated because LLINs could be lost at any time during the 6-month follow-up period. There may have also been recall bias, as people may not have correctly remembered what had happened to their LLINs over the previous 6 months. Furthermore,

Table 5 Predictors of physical integrity of long-lasting insecticide nets over a 2-year follow-up period in Ethiopia

Variables	Net months observation	Number of torn LLINs	IR/100 NMO (95% CI)	Crude HR (95% CI)	Adjusted HR (95% CI)
Gender of head of household					
Male	5250	138	2.6 (2.2–3.1)	1.00	1.00
Female	2958	60	2.0 (1.5–2.5)	0.8 (0.6–1.0)	0.8 (0.6–1.3)
Educational status of head of household (n = 816)					
Illiterate	4458	108	2.4 (2.0–2.9)	1.0	NA
Read and write	822	19	2.3 (1.4–3.5)	1.0 (0.6–1.6)	
Primary	2010	44	2.2 (1.5–2.8)	0.9 (0.6–1.3)	
Secondary and above	786	18	2.3 (1.2–3.3)	1.0 (0.6–1.6)	
Wealth status					
Poor	2826	77	2.7 (2.1–3.3)	1.0	1.0
Middle	2640	62	2.3 (1.8–2.9)	0.9 (0.6–1.2)	1.0 (0.7–1.5)
Rich	2742	59	2.2 (1.6–2.7)	0.8 (0.6–1.1)	0.9 (0.6–1.4)
Type of bed					
Wooden bedframe	3786	86	2.3 (1.8–2.8)	1.0	NA
Stick or iron bedframe	924	23	2.5 (1.5–3.5)	1.1 (0.7–1.8)	
Mattress (with no bed frame)	2340	56	2.4 (1.8–3.0)	1.1 (0.8–1.5)	
Mat	1158	33	2.8 (1.9–3.8)	1.2 (0.8–1.9)	
LLIN hung up					
No	2358	64	2.7 (2.0–3.4)	1.0	NA
Yes	5850	134	2.3 (1.9–2.7)	0.8 (0.6–1.1)	
House has open eave gap (n = 820)					
No	6888	160	2.3 (2.0–2.7)	1.0	1.0
Yes	1158	38	3.3 (2.2–4.3)	1.4 (0.98–2.0)	1.0 (0.6–1.4)
LLIN used last night					
No	2496	75	3.0 (2.3–3.7)	1.0	1.0
Yes	5712	123	2.2 (1.8–2.5)	0.7 (0.5–0.96)*	0.7 (0.5–0.96)*
LLIN ever washed					
No	4992	109	2.2 (1.8–2.6)	1.0	1.0
Yes	3216	89	2.8 (2.2–3.3)	1.3 (1.0–1.7)	1.2 (0.9–1.7)
LLIN was clean					
No	4440	156	3.5 (3.0–4.1)	1.0	1.0
Yes	3768	42	1.1 (0.8–1.5)	0.3 (0.2–0.4)*	0.4 (0.3–0.6)*
Rats present in the house (n = 658)					
No	3900	73	1.9 (1.4–2.3)	1.0	1.0
Yes	3186	82	2.6 (2.0–3.1)	1.4 (1.02–1.92)*	1.1 (0.8–1.5)
Cat present in the house (n = 658)					
No	3648	94	2.6 (2.1–3.1)	1.0	1.0
Yes	3438	61	1.8 (1.3–2.2)	0.7 (0.5–0.9)*	0.8 (0.5–1.1)
Distance from mosquito breeding site (n = 833) (km)					
≤ 1	2844	51	1.8 (1.3–2.3)	1.0	1.0
> 1	5364	147	2.7 (2.3–3.1)	1.5 (1.1–2.1)*	1.8 (1.2–2.6)*
Family size (n = 833)					
≤ 5	3594	90	2.5 (2.0–3.0)	1.0	NA
> 5	4614	108	2.3 (1.9–2.8)	0.9 (0.7–1.2)	

NMO net months of observation, *IR* incidence rate, *LLIN* long-lasting insecticidal net, *HR* hazard ratio, *NA* not applicable when P < 0.25, *statistically significant at P < 0.05

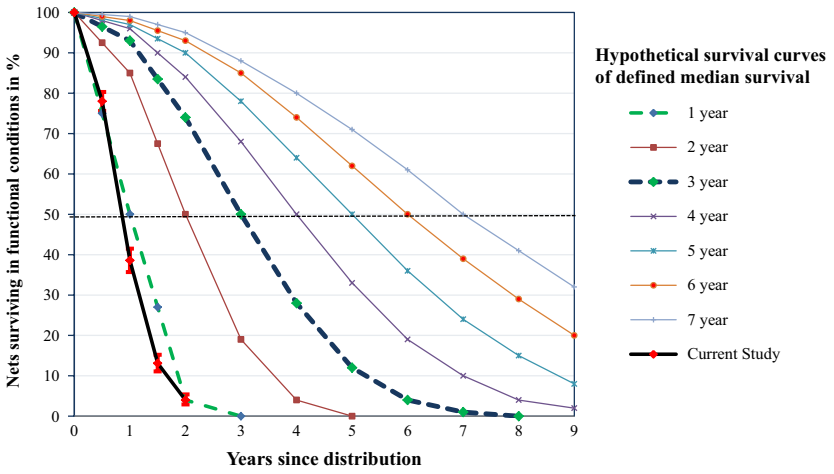

Fig. 4 Functional survival of LLINs in south-central Ethiopia. The error bars indicate the 95% confidence interval

it was difficult to trace the reason for LLIN loss when more than one LLIN was lost within the same household.

As expected, the physical integrity of LLINs deteriorated over time. The proportion of LLINs with a hole size 0.5 cm and larger (36–80% of LLINs) between 6–24 months was comparable to other studies in Ethiopia (54.5–85.5%) between 6–20 months [17], and in Zambia (60.2–87.2%) between 12–24 months [8]. The observed high number of holes in the lower half of the nets was also consistent with the findings of previous studies [8, 10, 17, 40]. The previous study reported that using nets over a reed mat was significantly associated with larger holes in the lower half of the nets [8].

Using the LLIN the night before the survey was associated with the good physical integrity of LLINs. Net use and having good physical integrity might have a bi-directional association. The users might keep their in-use nets from physical damage. Conversely, the users might prefer to use intact nets more than damaged nets. Having a clean LLIN was another predictor of the good physical integrity of LLINs in this study. This finding also might be due to the tendency of users to keep an intact net clean for prolonged use compared with damaged nets. Moreover, the presence of kitchens inside the house or using firewood as a cooking fuel could make the nets dirty [13]. Thus, dirty nets may be frequently washed, and could lose their physical integrity. Proximity to a mosquito breeding site was a significant predictor of physical integrity. The LLINs in households that lived more than 1 km away from potential vector breeding sites were more likely to be damaged than households located within a 1 km radius. This finding could be explained by nets being less valued in areas with a lower perceived risk of mosquito bites and malaria infection. Evidence from the qualitative study showed the tendency of owners far from potential mosquito breeding sites to misuse nets [14].

Six months after distribution, the functional survivorship of LLINs was 78.6%. This percentage is lower than that reported in a study in Benin (93%) [13]. One potential explanation for this difference is that all LLINs presented in the household, including torn nets, were considered to have survived in the Benin study. Moreover, the percentage of surviving nets in this study is lower than the NetCALC model 3-year serviceable prediction value of 96.5% [41]. After 12 months, net survivorship further decreased to 38.6%, lower than the 72% reported from Benin [13] and 90.4% in Zambia [8]. In our area, the greatest loss (40%) occurred during the 6- to 12-month period after LLIN distribution, and is probably related to the unusually dry and warmer weather that followed the El Nino in 2015 [30]. Moreover, the marked decline in the incidence of malaria in the study area (only 37% of pre-distribution incidence) [42] could have indirectly affected the survivorship of LLINs by decreasing the perceived risk of malaria infection. After 24 months, the functional survival was only 4%, which is substantially lower than the expected 75% by the NetCALC 3-year serviceable prediction model [41]. In general, the functional survival of LLINs in the current study is comparable to a 1-year serviceable prediction model, in which 4% of LLINs are predicted to survive after 2 years [36, 41].

Table 6 Predictors of functional survival of long-lasting insecticidal nets over a 2-year follow-up period in Ethiopia

Variable	Net month observation	Number of lost LLINs	IR/100 NMO (95% CI)	Crude HR (95% CI)	Adjusted HR (95% CI)
Gender of head of household (n = 1193)					
Male	9930	660	6.6 (6.1–7.2)	1.0	NA
Female	5262	333	6.3 (5.6–7.0)	1.0 (0.8–1.1)	
Educational status of head of household (n = 1170)					
Illiterate	8472	567	6.7 (6.1–7.2)	1.0	1.0
Read and write	1554	99	6.4 (5.1–7.6)	0.9 (0.7–1.1)	0.9 (0.7–1.3)
Primary	3606	227	6.3 (5.5–7.1)	0.9 (0.8–1.1)	1.0 (0.9–1.3)
Secondary and above	1308	79	6.0 (4.7–7.4)	0.9 (0.7–1.1)	0.9 (0.7–1.2)
Wealth status (n = 1184)					
Poor	5244	363	6.9 (6.2–7.6)	1.0	1.0
Middle	4872	312	6.4 (5.7–7.1)	0.9 (0.8–1.0)	1.0 (0.8–1.2)
Rich	4968	312	6.3 (5.6–7.0)	0.9 (0.8–1.0)	0.9 (0.8–1.2)
Open eave (n = 1176)					
No	12,480	819	6. 6 (6.1–7.0)	1.0	NA
Yes	2460	166	6.7 (5.7–7.8)	1.0 (0.9–1.2)	
LLIN used last night (n = 833)					
No	3468	237	6.8 (6.0–7.7)	1.0	1.0
Yes	7746	459	5.9 (5.4–6.5)	0.8 (0.7–0.97)*	1.0 (0.7–1.0)
LLIN ever washed (n = 833)					
No	6876	423	6.2 (5.6–6.7)	1.0	
Yes	4338	273	6.3 (5.5–7.0)	1.0 (0.9–1.2)	NA
LLIN was clean (n = 833)					
No	6012	418	7.0 (6.3–7.6)	1.0	1.0
Yes	5202	278	5.3 (4.7–6.0)	0.7 (0.6–0.8)*	0.8 (0.6–0.9) *
Rats present in the house (n = 858)					
No	6462	382	5.9 (5.3–6.5)	1.0	1.0
Yes	5316	334	6.3 (5.6–7.0)	1.1 (1.0–1.3)	1.1 (0.9–1.3)
Cat present in the house (n = 858)					
No	6102	378	6.2 (5.6–6.8)	1.0	
Yes	5676	338	6.0 (5.3–6.6)	1.0 (0.8–1.1)	NA
Distance from mosquito breeding site (n = 1193) (km)					
≤ 1	5058	311	6.1 (5.5–6.8)	1.0	1.0
> 1	10,134	682	6.7 (6.2–7.2)	1.2 (1.1–1.3)*	1.3 (1.1–1.6)*
Household population size (n = 1193)					
≤ 5	6264	392	6.3 (5.6–6.9)	1.0	1.0
> 5	8928	601	6.7 (6.2–7.3)	1.1 (1.0–1.3)	1.1 (0.9–1.3)

NMO net month observation, *IR* incidence rate, *LLIN* long-lasting insecticidal net, *HR* hazard ratio, *NA* not applicable when P < 0.25, *statistically significant at P < 0.05

Table 7 Geometric means of 1-h knockdown and 24-h mortality of mosquitoes in Ethiopia

Survey (months)	Number of LLINs	60-min KD GM (95% CI)	24-h mortality GM (95% CI)
6	30	94.1 (87.1–100)	81.1 (67.7–97.0)
12	30	99.9 (99.7–100)	89.5 (87.2–91.8)
18	30	93.9 (90.0–98.1)	76.6 (71.0–82.6)
24	30	94.1 (91.3–97.1)	69.4 (59.4–80.9)

CI confidence interval, *GM* geometric mean, *KD* knockdown, *LLIN* long-lasting insecticidal net

In addition to the unexpected weather conditions and a decline in the incidence of malaria, the behaviour of the net users could play a role in high attrition rate and low functional survivorship. A qualitative study done on the same households as our study showed that many informants believed that the LLINs would not serve more than 1 year. The users claimed the LLINs could lose their insecticidal effect after 6 months by mentioning that the nets "stopped killing bugs." Washing LLINs several times was also believed to cause a loss of insecticides [14]. As

Table 8 Mean differences in the proportions of knockdowns and mortality of mosquitoes in Ethiopia

Variable	Between months	Mean difference (95% CI)[a]	P value
Knockdown	12 and 18	5.4 (0.4–10.4)	0.029
	12 and 24	5.5 (1.7–9.3)	0.002
Mortality	12 and 18	11.7 (4.1–19.2)	0.001
	12 and 24	15.4 (3.1–27.8)	0.008

CI confidence interval

[a] One-way ANOVA assuming unequal variance used

Table 9 Proportion of long-lasting insecticidal nets meeting WHO pesticide evaluation scheme criteria effective (1-h knockdown ≥ 95% or 24-h mortality ≥ 80%) in Ethiopia

Age of LLINs (months)	Number evaluated	n (%), 95% CI
6	30	27 (90.0), 72.5–96.8
12	30	30 (100)
18	30	25 (83.3), 65.1–93.1
24	30	24 (80.0), 61.5–90.9

CI confidence interval, *LLINs* long-lasting insecticidal nets, *WHO* World Health Organization

explored by this qualitative study, after 1 year most of the LLINs were misused. However, this finding was not supported by the current study, as 64.2% of reported net loss was due to disposal. There could be a possible social desirability bias, because people did not report the misuse of LLINs in the current study. There is also a possibility that the LLINs were used for agricultural purposes, such as grain storage and transportation from the field, as well as the separation of grains from their chaffs, before being discarded as explored by the qualitative study [14]. Moreover, a low level of knowledge and a low positive perception towards net care and repair in Ethiopia may have also played a role in the observed high attrition, poor physical integrity and lower functional survival of LLINs [15].

In this study, having a clean LLIN was found to be associated with a longer functional survival time. This could be due to the behaviour of the owners, who would like to use LLINs for a prolonged time and thus keep the nets clean. A result from a qualitative study showed that nets become dirty from excessive smoke from indoor cooking stoves or fires, which leads the users to discard the nets prematurely or misuse them [14]. The LLINs in households living more than 1 km from potential vector breeding sites were less likely to survive. This could be related to a higher perceived risk of mosquito bites and malaria infection among net owners living closer to a vector breeding site [43, 44]. In this study, neither using the net the night before the survey nor having ever washed the net was associated with functional survival of LLINs. However, a previous study observed an association between using the net the night before the survey and a longer survival time, and an association between having ever washed the net and a shorter survival time [8].

Previous studies have reported that the bio-efficacy of the LLIN is correlated with the concentration of the insecticide [8, 21]. In the current study, PermaNet® 2.0 LLINs met the WHO pesticide evaluation scheme criteria of bio-efficacy (at least 80% of the sampled LLINs effective in a WHO cone test) after 24 months [34], which was in agreement with other similar studies [21, 39] and higher than a result reported by Tan et al. [8].

In general, our results suggested that the survivorship of LLINs after 2 years was low compared with the prediction of the NetCALC model (4% vs 75%). This finding raises a serious concern about the programmatic assumption of the 3-year LLIN replacement cycle. Therefore, we suggest that nationally representative LLIN durability studies should be conducted to estimate the correct LLIN replacement cycle. Meanwhile, national malaria control programs should closely work with manufacturers to develop stronger and more durable LLIN products. Moreover, strengthening the behaviour change communication messages on net care and repair, as well as the proper use of LLINs, may help to improve the durability of LLINs.

Conclusions

The study results suggested that the serviceable time of LLINs is 1 year, as a "3-year" serviceable assumption was unrealistic in this study community. Consequently, stronger and more efficient LLINs need to be developed for conditions similar to those studied here. After all, many parts of Ethiopia exhibit conditions similar to those at this study site. Because this study was conducted on one brand of LLIN and in one area only, the findings may not be extrapolated to other brands and people living in different topographic and socioeconomic settings. Therefore, more research still needs to be conducted to generalize the findings to the country level.

Abbreviations

AHR: adjusted hazard ratio; CI: confidence interval; GM: geometric mean; KD: knockdown; IQR: interquartile range; IRS: indoor residual spray; LLIN: long-lasting insecticidal net; MD: mean difference; PCA: principal component analysis; pHI: proportional hole index; WHO: World Health Organization.

Authors' contributions

TS, EL, WD, MB, HJO, and BL conceived and designed the study, and designed data collection tools. TS, EL, and TG facilitated the data collection and supervised the field work. TS and MB conducted the bio-efficacy test. TS and BL analysed and interpreted the data and drafted the manuscript. All authors

reviewed and edited the draft manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Consent for publication
Not applicable.

Ethics approval and consent to participation
This study was approved by the Ethiopian Ministry of Science and Technology (Ref: 3.10/446/06) and the Regional Committee for Medical and Health Research Ethics, Western Norway (Ref: 2013/986/REK vest).

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Malaria Journal

CORRECTION

Open Access

Correction to: Bed nets used to protect against malaria do not last long in a semi-arid area of Ethiopia: a cohort study

Tarekegn Solomon[1,5*], Eskindir Loha[1], Wakgari Deressa[2], Meshesha Balkew[3], Taye Gari[1,5], Hans J. Overgaard[4] and Bernt Lindtjørn[5]

Correction to: Malar J (2018) 17:239
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Following publication of the original article [1], the author has flagged errors that affect the scientific content of the article.

The errors concern Table 2 (page 6), Table 5 (page 9) and Table 6 (page 11) of the published article.

Firstly, with concern to Table 2, the errors concern the 'Totals' provided in the table. Please find below (the correct version of) the table, with the corrected 'Totals' data highlighted in italics:

Table 2 Reasons for loss of long-lasting insecticide nets over a 2-year follow-up period in Ethiopia

Reason for LLIN loss	6 months n (%)	12 months n (%)	18 months n (%)	24 months n (%)	Total n (%)
Known outcome of attrition or torn					
Thrown away	102 (40.0)	301 (71.8)	173 (74.9)	62 (70.5)	638 (64.2)
Used for something else	30 (11.8)	58 (13.8)	28 (12.1)	22 (25.0)	138 (13.9)
Torn (pHl > 643)	123 (48.2)	60 (14.3)	30 (13.0)	4 (4.5)	217 (21.9)
Total	*255* (100)	*419* (100)	*231* (100)	*88* (100)	993 (100)
Unknown outcome of attrition					
Given away	260 (76.7)	44 (45.8)	11 (20.0)	1 (12.5)	316 (63.5)
Lost to follow-up[a]	52 (15.3)	43 (44.8)	26 (47.2)	5 (62.5)	126 (25.3)
Stolen	8 (2.4)	9 (9.4)	3 (5.5)	2 (25.0)	22 (4.4)
Unknown reasons	6 (1.8)	0 (0.0)	1 (1.8)	0 (0.0)	7 (1.4)
Other[b]	13 (3.8)	0 (0.0)	14 (25.5)	0 (0.0)	27 (5.4)
Total	*339* (100)	*96* (100)	*55* (100)	*8* (100)	498 (100)
Overall total	*594*	*515*	*286*	*96*	*1491*

LLIN long-lasting insecticidal net

[a] Family moved to other location, family not at home, refusal to participate

[b] Sold or destroyed by fire

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Regarding **Tables 5 and 6**, the error on the footnotes of the published tables is: "**NA not applicable when P < 0.25".**

The footnotes on **Tables 5 and 6** should read as: "**NA not applicable when P > 0.25".**

The original article can be found online at https://doi.org/10.1186/s12936-018-2391-5.

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II

Paper II

Solomon T, Loha E, Deressa W, Gari T, Overgaard HJ, Lindtjorn B. Low use of long-lasting insecticidal nets for malaria prevention in south-central Ethiopia: A community-based cohort study. PLoS ONE. 2019;14:e0210578.

PLOS ONE

RESEARCH ARTICLE

Low use of long-lasting insecticidal nets for malaria prevention in south-central Ethiopia: A community-based cohort study

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Abstract

Introduction

A decline in malaria morbidity and mortality has been documented in Ethiopia since 2005 following a scale-up of the distribution of long-lasting insecticidal nets (LLINs). However, universal access to LLINs ownership and use has not yet been achieved. This study aimed to determine ownership and use of LLINs over time in south-central Ethiopia.

Methods

A cohort of 17,142 individuals residing in 3,006 households was followed-up from October 2014 to January 2017 (121 weeks). New PermaNet2.0 LLINs were given to households in October 2014. Once per week, the LLIN use status was documented for each individual. A survey was conducted after 110 weeks of LLIN distribution to determine LLIN ownership. A multilevel negative binomial regression model was fitted to identify significant predictors of LLIN use.

Results

At baseline, the LLIN ownership was 100%. After 110 weeks only 233 (8%) of the households owned at least one LLIN. The median proportion of LLIN use per individuals during the study period was only 14%. During the first year (week 1–52) the average LLIN use per individuals was 36% and during the second year (week 53–104) it was 4.6%. More frequent LLIN use was reported among age group [5–14 years (adjusted IRR = 1.13, 95% CI 1.04–1.22), 15–24 years (adjusted IRR = 1.33, 95% CI 1.23–1.45), ≥25 years (adjusted IRR = 1.99, 95% CI 1.83–2.17)] compared to <5 years, and household head educational status [read and write (adjusted IRR = 1.17, 95% CI 1.09–1.26), primary (adjusted IRR = 1.20, 95% CI 1.12–1.27), secondary or above (adjusted IRR = 1.20, 95% CI (1.11–1.30)] compared to illiterate. Having a family size of over five persons (adjusted IRR = 0.78, 95% CI

0.73–0.84) was associated with less frequent use of LLINs compared to a family size of ≤5 persons.

Conclusions

The study showed a low LLIN ownership after 110 weeks and a low LLIN use during 121 weeks of follow-up, despite 100% LLIN coverage at baseline. The study highlights the need to design strategies to increase LLIN ownership and use for setting similar to those studied here.

Introduction

A decline in malaria morbidity and mortality has been documented in sub-Saharan Africa since 2000, where an estimated 90% of global malaria cases and deaths have occurred [1, 2]. The use of the long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are considered the two main vector control interventions that played a role in the reduction of the malaria burden. Studies from sub-Saharan Africa showed that the use of LLINs alone has reduced malaria incidence rates by 50% and malaria mortality rates by 55% in children under the age of 5 years [3, 4]. Moreover, in the past 15 years increases in LLIN coverage and use have been documented in sub-Saharan Africa [2]. Although a decline in malaria burden and increases in LLIN access and use were documented, the use of LLINs by people at risk remained lower than expected [5]. For example, in 2016, only 43% of people had access to sufficient LLINs (one net for two people), and only 54% of people at risk for malaria used LLINs [5]

In Ethiopia, where 60% of the population are at risk of malaria infection, and 68% of the country's area is favourable for malaria transmission, a decline in malaria morbidity and mortality has been observed since 2005 [6]. A scaling-up of anti-malaria interventions, such as LLINs, IRS, malaria diagnoses using a rapid diagnostic test (RDT) and prompt treatment using artemether-lumefantrine, are believed to be the primary reasons for these achievements [7, 8]. An estimated 64 million LLINs were distributed within the country through periodic mass campaigns between 2005 and 2015 [9], with an additional 29.6 million LLINs distributed in 2015 [10]. Moreover, Ethiopia had set a target to achieve 100% of LLIN coverage (at least one LLIN per sleeping space in malaria-endemic areas) and 80% of use (people at risk of malaria shall use LLINs properly and consistently) by the year 2015 [11]. However, the three national malaria indicator surveys (MIS) have shown that universal access to LLIN ownership and use has yet to be achieved. The households with at least one LLIN were 65% in 2007, 55% in 2011 and 64% in 2015 [12–14]. Furthermore, households with at least one LLIN for every two persons were as low as 37% in 2007, 24% in 2011 and 32% in 2015 [12–14]. The overall LLIN use rates were 32% in 2007 and 40% in 2015 MIS surveys [12, 14]. The commonly reported barriers to the use of LLINs in Ethiopia include worn out LLINs, a lack of space to hang the LLINs, living away from vector breeding sites, discomfort, a low-risk perception of malaria, saving nets for future use, and a lack of awareness and perception of low efficacy to prevent malaria [15–17].

To achieve the goals and targets for reducing the malaria burden, consistent use of LLINs by people at risk of malaria must be maintained. Therefore, understanding the level of LLINs ownership and use over time is helpful to evaluate existing strategies and subsequent LLINs distribution campaigns. Previous studies in Ethiopia evaluate the LLIN ownership and use

using cross-sectional study designs [12, 14, 16, 18]. Because of the nature of the designs, they fail to show trends of LLIN use over time after mass LLIN distributions campaigns. To fill this knowledge gap, a prospective cohort study design was used to evaluate LLIN use of each study participant for more than two years (121 weeks). The weekly follow-up started in October 2014 immediately after distribution of LLINs free of charge according to the National Malaria Guidelines [19]. Therefore, the aim of this study was to determine the LLIN ownership and use over time, and to identify factors associated with LLIN use in south-central Ethiopia.

Methods

Ethical clearance

Ethical clearance was obtained from Ethiopian Ministry of Science and Technology (Ref: 3.10/446/06), Institutional Review Board (IRB) of the College of Health Sciences of Addis Ababa University, and the Regional Committee for Medical and Health Research Ethics, Western Norway (Ref: 2013/986/REK vest). Permission letters were obtained from the Oromia Regional Health Bureau, the East Shewa Zonal Health Department and the Adami Tullu District Health Office. Before the start of the study, the community elders, *Kebele* and village leaders were sensitized about the study objectives, implementation processs and expected outcomes of the study. Verbal informed consent was obtained from the head of household or members of the household older than 18 years in the absence of the head of household. We opted to take verbal informed consent because we had a challenge to get written consent as the majority of the study participants could not read and write [20]. We used a standard information sheet to explain the purpose of the study. The participants were informed that participation was voluntary and they had the right to withdraw from the study at any time. The information was read to study participants using the information sheet in their own language (*Afan Oromo*), and their consent was recorded using check mark.

Study setting

This study was conducted in the Adami Tullu District of Oromia Regional State in south-central Ethiopia (Fig 1) from October 2014 to January 2017. The district is situated in the East African Great Rift Valley, approximately 160 km south of Addis Ababa. Based on the 2007 national census, approximately 190,000 people lived in the district in 2017 [21]. The majority of the population live in rural areas, and are engaged in subsistence farming and livestock rearing. The district is characterized by a semi-arid climatic condition, with a total annual precipitation of 700 mm, an average minimum temperature of 14.5 ˚C and a maximum temperature of 27.7 ˚C [20].

Malaria is among the leading causes of morbidity and mortality in the district. Malaria transmission is seasonal, and peaks during the months from September to December following the monsoon rains in July and August [19]. The shores and irrigated areas around Lake Zeway serve as mosquito breeding sites [22, 23]. The primary malaria vector is *Anopheles arabiensis*. *Plasmodium falciparum* and *Plasmodium vivax* are the two main malaria parasites causing malaria infection [24, 25]. During the study period, a 63% decline in malaria incidence was reported [26]. In this period, the district experienced a severe drought and food shortage following the El Nino of 2015 [27].

Study design and participants

This study was part of a cluster-randomized controlled trial that aimed to determine whether the combined use of LLINs and IRS with propoxur provides additional protection against

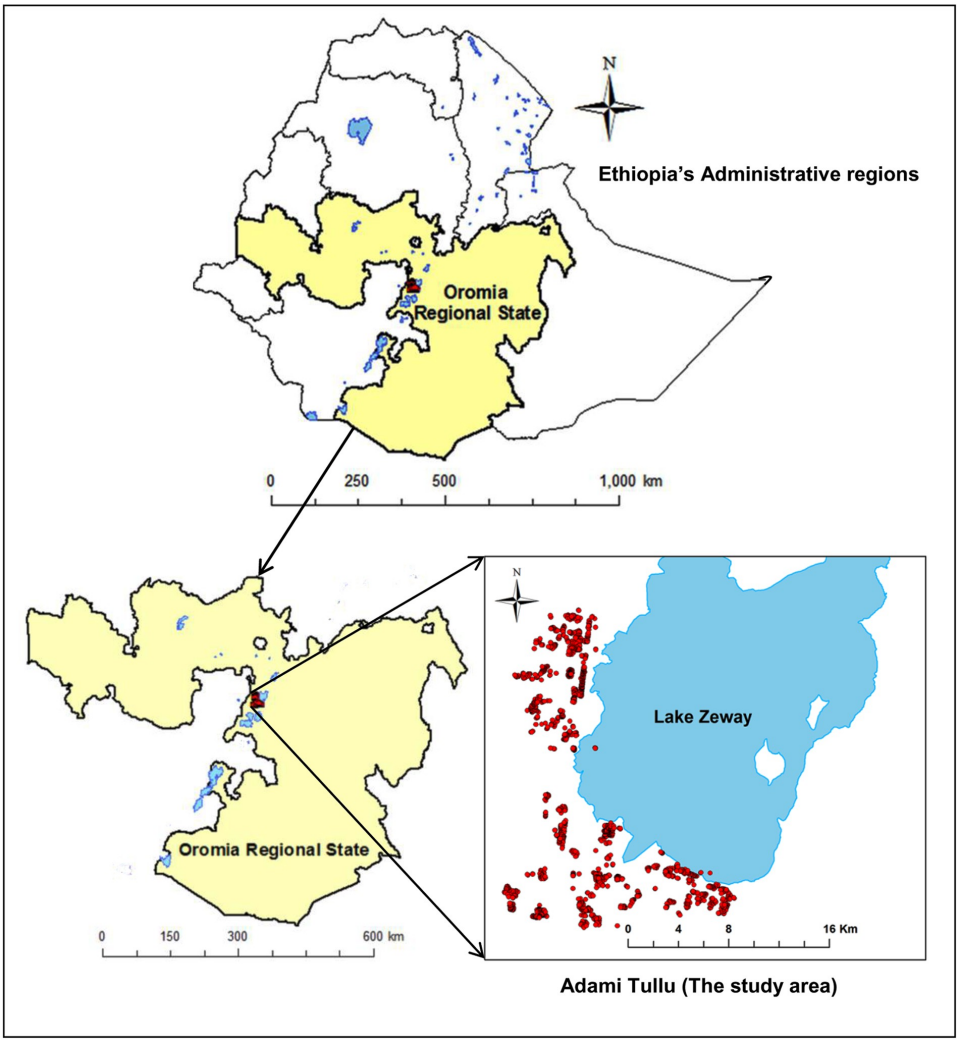

Fig 1. Map of the location of the study households in the Adami Tullu District in south-central Ethiopia.

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malaria (*Plasmodium falciparum* and/or *Plasmodium vivax*) among all age groups compared to LLINs or IRS alone (the Maltrials project). The trial is described in more detail in the published protocol [20]. Briefly, the unit of randomization was villages (clusters) that contained approximately 35 households and 196 people in each cluster. A total of 176 clusters (44 clusters per arm) from 13 kebeles (the lowest government administrative unit) were included in the trial. Clusters were identified based on the rate of malaria transmission and located within 5 km from Lake Zeway. The trial distributed in all 7,740 PermaNet2.0 LLINs (Vestergaard

Frandsen Group) free of charge to 3,006 households (4,157 LLINs in the combined LLIN+IRS arm and 3,583 LLINs in the LLIN arm), with an average of 2.57 LLINs per household. The LLINs had a light blue colour and rectangular shape, with a width of 160 cm, a length of 180 cm and a height of 150 cm. Although we planned for 104 weeks (two complete years) of follow-up, the period was extended to 121 weeks to add one additional malaria transmission season; thus including three complete transmission seasons. The reason for adding the third season was to increase the number of cases, because malaria was lower than expected, potentially due to a draught caused by El Nino of 2015 [27].

The number of LLINs distributed to each household was recorded at baseline. Following the National Malaria Guidelines [19] one LLIN was given to a family of 1–2 persons; two LLINs to a family of 3–5 persons; three LLINs to a family of 6–7 persons and four LLINs to a family with $\geq$ 8 persons. Two weeks after LLINs distribution, a "hang-up" campaign was carried out by fieldworkers, which consisted of education on LLIN handling and proper use.

A cohort study was conducted among 17,142 people in the 3,006 households of the LLIN +IRS and LLIN arms to quantify the LLIN use. All study participants were followed on a weekly basis for 121 weeks, from October 2014 to January 2017. All study participants were followed until the end of the study or until they were lost to follow-up. Newcomers (individuals who joined a cohort as new household members) and newborns during the study period were included in the study (Fig 2). A cross-sectional survey was carried out at the 110th week post-distribution to assess LLIN ownership among all households that received LLINs at baseline and to validate the results of LLIN use. A parallel follow-up study was conducted from October

Randomized	
(n=88 clusters, 3,006 households, 17,142 people to LLIN alone or IRS+LLIN arm)	
Allocation	
IRS + LLIN	**LLIN**
Allocated for intervention (N=44 clusters, 1,618 households)	Allocated for intervention (N=44 clusters, 1,388 households)
Received allocated intervention (LLINs) (N=1,618 households)	Received allocated intervention (LLINs) (N= 1,388 households)
Did not receive allocated intervention for LLIN (N=0 households)	Did not receive allocated intervention (N=0 households)
Follow-up	
Total follow-up period for LLIN use (121 weeks)	Total follow-up period for LLIN use (121 weeks)
Lost to follow up (N= 143 household, 682 people)	Lost to follow up (N=121 household, 658 people)
Newly joined: 897 people	Newly joined: 740 people
A survey on LLIN ownership at week 110: Only 7% households owned at least one LLIN	A survey on LLIN ownership at week 110: Only 10% households owned at least one LLIN
Analysis	
Analysed for LLIN use (N=1,618 households, 9,104 people)	Analysed for LLIN use (N= 1,388 household, 8,038 people)
Excluded from analysis (N=0)	Excluded from analysis (N=0)

Fig 2. Flow chart of the study for weekly long-lasting insecticidal nets use in Adami Tullu District south-central Ethiopia, October 2014 to January 2017.

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2014 to November 2016 in the sampled households aiming to assess attrition, physical integrity, functional survival and bio-efficacy of the LLINs. The results of the parallel study are reported elsewhere [28]

Data collection

A baseline census was conducted in August 2014 using pre-tested interviewer-administered questionnaires containing socio-demographic and economic variables (see S1 File). The questionnaires were prepared in English, and then translated into *Afan Oromo* (local language). Two more such censuses were carried out to update the follow-up population at weeks 51 and 103.

We carried out weekly home visits to record the LLIN use of the study participants (see S2 File). Each week, the heads of households or family members aged ≥18 years were asked whether any household members used an LLIN the night before the day of the interview. The names and codes of the individuals who used the LLIN were recorded. If the visited houses were closed, or if heads of households or family members aged ≥18 years were absent, the data collectors visited the house at least three more times within the same week. If one or more or all of the household members had left the study area during the study period, the individuals were considered lost to follow-up. In subsequent follow-ups, the households were visited on the same day of the week to maintain a seven-day gap between visits. The visits were carried out early in the morning to observe whether the LLINs were hung in the sleeping space. Moreover, during weekly follow-up for the trial study [20], data collectors identified and referred people with a history of fever over the past 48 hours to health post for malaria diagnosis. The families were advised to visit the health post if any family member developed fever between the dates of home visits. Individuals who were found to be positive for malaria parasites were treated according to national guidelines [19].

For the LLIN ownership survey, respondents were asked if they had useable LLINs in their household. The presence of usable LLINs was verified in the visited household by observation. If the LLINs were lost, the reasons for the loss were asked.

Twenty-four data collectors having a college diploma were recruited from the respective kebeles. Three supervisors were recruited to monitor the overall data collection process, and to control data quality. The data collectors and supervisors were trained for five days on the use of questionnaires, interviewing techniques, household visits and supervision. The data collection questionnaire for weekly LLIN use was adopted from a longitudinal study from southern Ethiopia [15] and from a pilot study in the study area [25]. The questionnaires were cross-checked for their reliability with source households by the supervisors.

Statistical analysis

A total of 17,142 study participants in 3,006 households were included in the analysis. For non-normally distributed continuous variables, medians and interquartile range (IQR) were calculated. The ownership of LLINs after two years was calculated by taking the number of households with at least one LLIN as the numerator and the total number of households enrolled in the study at baseline as the denominator, excluding the number of households lost to follow-up from the denominator. To calculate the proportion of individuals using LLIN per week, we used the total number of individuals in all households who used an LLIN the night before the day of the interview as the numerator and the total population in all households of

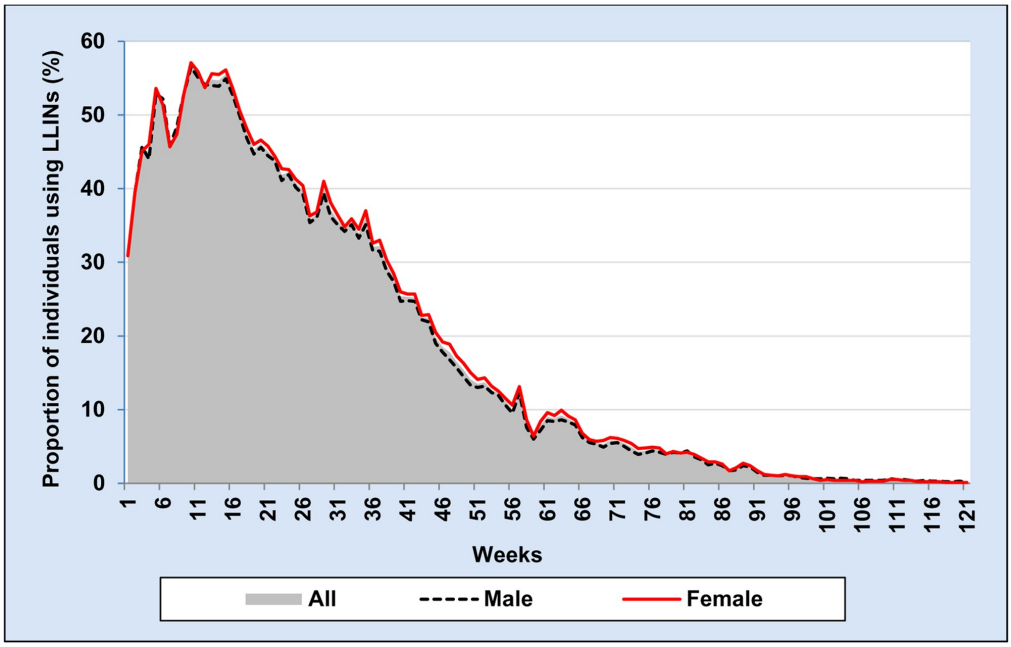

Fig 3. Weekly proportion of individuals using long-lasting insecticidal net by gender during 121 weeks from October 2014 to January 2017.

https://doi.org/10.1371/journal.pone.0210578.g003

that week as the denominator as shown in the following formula.

$$\begin{aligned} &Poportion\ of\ individuals\ using\ LLIN\ per\ week \\ &Total\ number\ of\ individuals\ slept\ under \\ &= \frac{LLINs\ in\ each\ week}{Total\ population\ of\ the\ week} \times 100\% \end{aligned}$$

The proportion of individuals using LLIN per week was calculated for each week of 121 weeks and stratified by gender, age groups and distance of household from potential vector breeding sites (see Figs 3, 4 and 5). To calculate the proportion of LLIN use per each individual per the whole study period, we used the total number of weeks in which LLIN use was reported by each individual during the study period as the numerator and the total number of weeks an individual stayed in the study area as the denominator as shown in the following formula.

$$\begin{aligned} &Proportion\ of\ LLIN\ use\ per\ individual \\ &Total\ number\ of\ weeks\ an\ individual\ reported \\ &= \frac{LLIN\ use\ during\ the\ study\ period}{Total\ number\ of\ weeks\ an\ individual\ stayed\ in\ the\ study\ area} \times 100\% \end{aligned}$$

The median and interquartile range (IQR) proportion of LLIN use per individuals were calculated and reported to all 17,142 study participants.

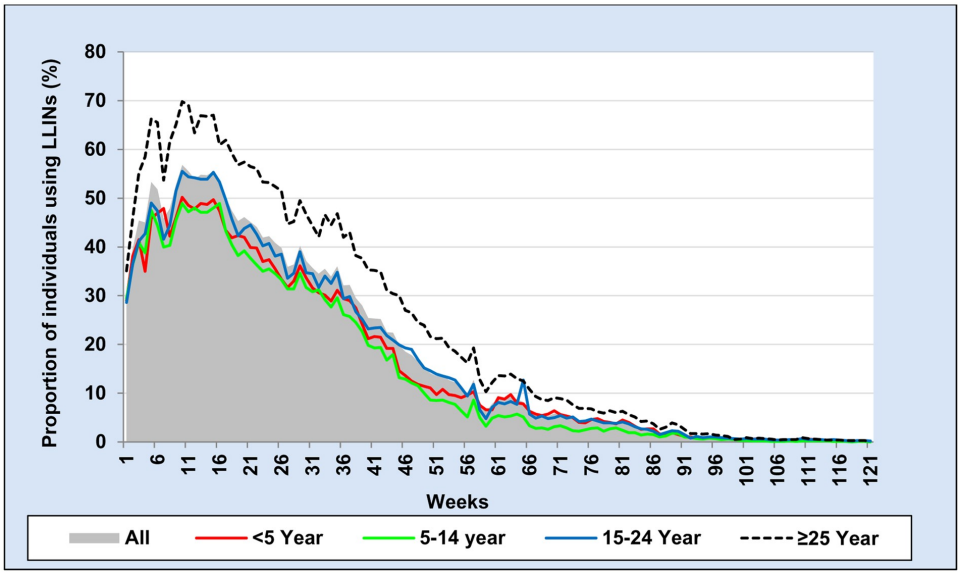

Fig 4. Weekly proportion of individuals using long-lasting insecticidal net by age group during 121 weeks from October 2014 to January 2017.

https://doi.org/10.1371/journal.pone.0210578.g004

We used weekly malaria episodes collected as part of the main trial [20], rainfall and proportion of individuals using LLINs per week data to construct a sequence chart, similar to that of Loha et al. [29], to show seasonal patterns of proportion of individuals using LLINs per week (lagged by 2 weeks—considering the incubation period of malaria infection) compared with malaria episodes and rainfall (lagged by 6 weeks—as in the model published from relatively similar setup) (see Fig 6).

A principal component analysis (PCA) was used to calculate the household wealth index [30, 31]. Fourteen household assets were included in the calculation: presence of electricity, ownership of a television, radio, mobile telephone, chair, table, bed, bicycle, land, a separate kitchen from the house, livestock and cart, as well as type of roof and wall. The first principal component was taken to construct the wealth index. Next, the index values were categorized into three relative measures of socioeconomic classes, poor, middle-class and rich. The details of wealth index calculations are reported elsewhere [32]. Distance from potential vector breeding sites to a household (in km) was calculated using proximity analysis in ESRI ArcMap 10.3 (CA, USA). The buffer option under proximity analysis was used to categorize the distance of households into $\leq$1 km and $>$1 km from the border line around Lake Zeway or the Bulbula River.

To investigate the predictors of LLIN use, we ran both a Poisson regression and a negative binomial regression models separately. When the Poisson regression model was fitted to the count data of total number of weeks in which LLIN use was reported, the ratio of the deviance over the degree of freedom was 13.8. This value became 1.2 when a negative binomial regression model was fitted. Since the latter model handled the problem of overdispersion, we used negative binomial regression model as the final model. Furthermore, to account for clustering

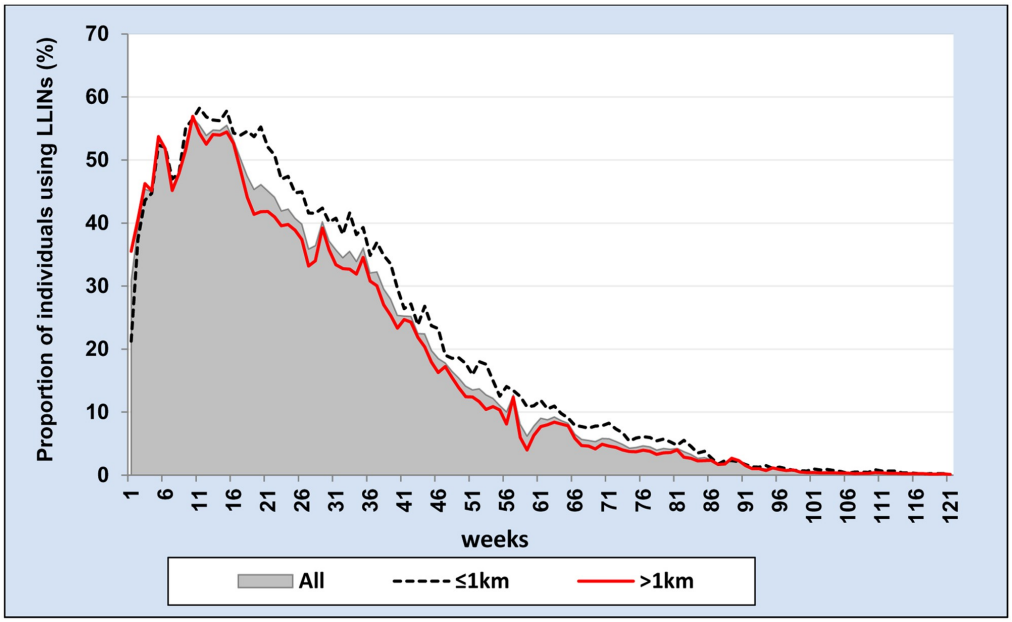

Fig 5. Weekly proportion of individuals using long-lasting insecticidal net by distance from potential vector breeding site during 121 weeks from October 2014 to January 2017.

https://doi.org/10.1371/journal.pone.0210578.g005

effect of LLIN use within individual, household and village level, we used a multilevel negative binomial regression model. We assumed that the predictors of LLIN use were clustered at three levels. Individuals (first level) were nested within households with the assumption that individuals have similar LLIN use within a household, but different LLIN use between households. Similarly, households (second level) were nested within villages (third level) with the assumption that LLIN use was similar among households within a village, but different between villages. Based on these assumptions, the presence of clustering was checked before fitting a multilevel negative binomial regression model. The following steps were used to check clustering: first, a null single level (standard) negative binomial regression model was fitted to the data, and then a null multilevel negative binomial regression with the random household and village effect was fitted. The estimated variance for village level random effect was 0.58 (SE = 0.24); for household level 0.24 (SE = 0.06). The calculated likelihood ratio test statistics showed strong evidence of village and household effect on the LLIN use (Chi-square = 5627.38, $P<0.001$). Therefore, to account for the clustering effect, we used a multilevel negative binomial regression model to estimate unadjusted incidence rate ratio (IRR) and adjusted IRR of LLIN use with 95% confidence interval (CI). To construct the model, the following parameters were specified: total number of weeks in which study participants reported LLIN use as dependent variable; total number of weeks study participants stayed (followed-up) in the study area as exposure variable. The covariance structure of the random effects was unstructured and standard error type was robust. Age, gender, educational and occupational status of the head of the household, household wealth status, household size, number of sleeping spaces in the

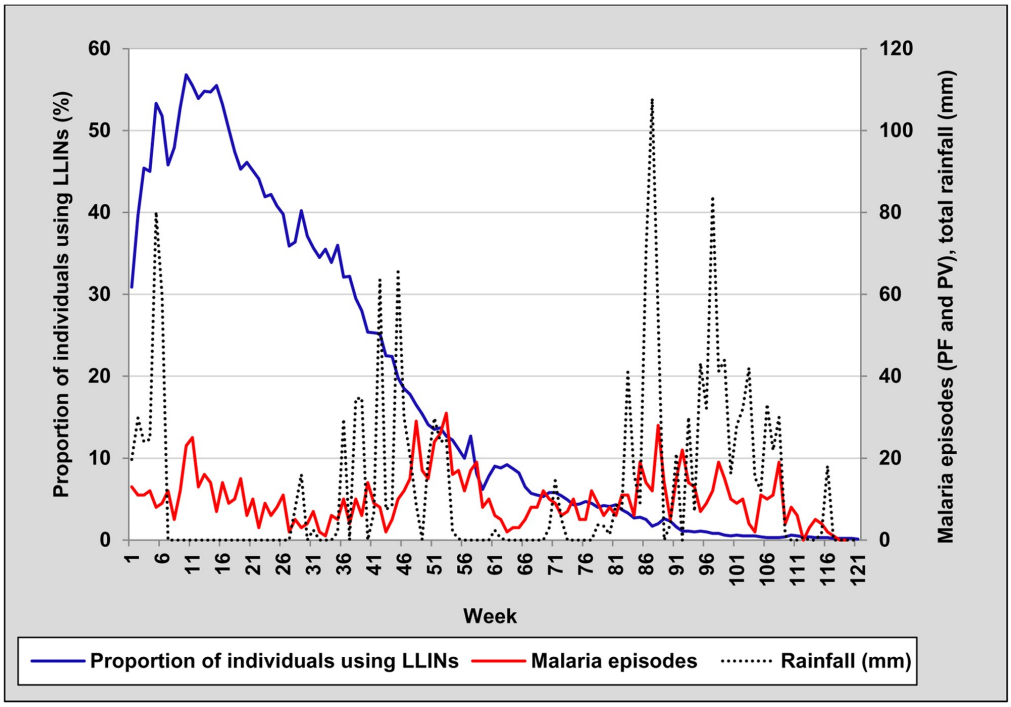

Fig 6. Sequence chart of weekly proportion of individuals using long-lasting insecticidal nets (LLINs) (lagged by 2 weeks), malaria episodes [plasmodium Falciparum (PF) and P. Vivax (PV)], and total rainfall (lagged by 6 weeks), south-central Ethiopia, October 2014-January 2017.

https://doi.org/10.1371/journal.pone.0210578.g006

household, study arm, and distance from a lake or river were considered as independent variables for LLIN use. Independent variables having a P-value <0.25 in bivariate analysis were included in the multivariate to identify significant predictors of LLIN use, adjusting for other variables. The level of statistical significance was set as a P-value <0.05. Data were entered into SPSS version 20.0 (Armonk, NY: IBM Corp. USA) and, analyzed using both SPSS and STATA version 15 (StataCorp, Texas, USA).

Results

Characteristics of the study participants

The median follow-up time was 121 weeks, and the median population size was 6.0 (IQR: 4–7) with a range of 1 to 18 people per household. Approximately 1,650 (54.9%) of the head of households were illiterate. Approximately one-third, 1,006 (33.5%) of the households were located within 1 km from potential vector breeding sites close to Lake Zeway or the Bulbula River (Table 1).

Long-lasting insecticidal net ownership

In October 2014, a total of 7,740 PermaNet2.0 LLINs were distributed to 3,006 households free of charge by the Maltrials project (with an average of 2.57 LLINs per household). After two

Table 1. Characteristics of the study participants and their households, Ethiopia.

Variable	n (%)
Gender (n = 17,142)	
Male	8,618 (50.3)
Female	8,524 (49.7)
Age in years (n = 17,132)	
<5	3,196 (18.7)
5–14	5,557 (32.4)
15–24	3,396 (19.8)
≥25	4,983 (29.1)
Educational status of head of household[a]	
Illiterate	1,650 (54.9)
Can read and write	274 (9.1)
Primary	759 (25.2)
Secondary and above	323 (10.7)
Occupational status of head of household[a]	
Farmer	2,278 (75.8)
Others	728 (24.2)
Household size[a]	
≤5 persons	1,449 (48.2)
>5 persons	1,557 (51.8)
Wealth status[a]	
Poor	1,153 (38.4)
Medium	978 (32.5)
Rich	875 (29.1)
Intervention arm[a]	
LLIN+IRS	1,618 (53.8)
LLIN alone	1,388 (46.2)
Distance from lake or river[a]	
≤1km	1,006 (33.5)
>1km	2,000 (66.5)

[a] calculated for household characteristics (n = 3006 households)

https://doi.org/10.1371/journal.pone.0210578.t001

years in November 2016 (week 110), 2,788 (93%) households were surveyed to determine whether the distributed LLINs were available or not. In that survey, 218 (7%) households were not evaluated, as the houses were closed or the household members had moved to other locations. Only 233 (8%) of the interviewed households had at least one LLIN. The remaining 2,555 (92%) households had lost their LLINs (Table 2).

Reasons for long-lasting insecticidal net loss

The most common reason for LLIN loss (76%; n = 4713) was that the LLINs were thrown away due to damage. The second most common (12%; n = 750) reason for LLIN loss was the misuse of LLINs for other purposes, such as grain transportation from the field, grain storage at home and toilet cover. Some (9%; n = 554) reported that they gave the LLINs to their children when they sent them to school or gave them to relatives living in other places (Table 3).

Table 2. Number and percentage of households with long-lasting insecticidal nets at baseline and after 110 weeks, Ethiopia November 2016.

Number of LLINs available per household	Week 1 (baseline) (n = 3,006 households)		Week 110 (n = 2,788 households)*		LLINs ownership at week 110 compared to the baseline
	Number	Percent	Number	Percent	P-value‡
0	0	0.0	2,555	91.6	
1	423	14.1	182	6.5	<0.001
2	1,275	42.4	45	1.6	<0.001
3	799	26.6	6	0.2	<0.001
4	509	16.9	0	0.0	<0.001
1–4	3,006	100.0	233	8.4	<0.001

*218 households were not evaluated at survey in week 110 due to being closed or moved to other location.

LLINs = long-lasting insecticidal nets

‡Z-test was used to compare proportions of LLIN ownership

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Trend of long-lasting insecticidal net use

During 121 weeks of follow-up, the median proportion of LLIN use per individual was 14.0% (IQR: 4.1–29.8%) (Table 4). The mean proportion of LLIN use per individual during the first year (week 1–52) was 36% and in the second year (week 53 to 104) it was just 4.6%. The proportion of individuals using LLIN per week among females was slightly higher than males (Table 4 and Fig 3). In general, weekly proportion of individuals using LLIN was higher among age group 15–24 years and older than 25 years compared to other age groups (Table 4 and Fig 4). Individuals who were living in the wealthier households reported higher proportion of LLIN use per individual compared with the poor (Table 4). Similarly, people who were living within 1 km from potential mosquito breeding sites reported a higher weekly proportion of LLIN use compared to those who were living further away than 1 km (Table 4 and Fig 5). However, gender, intervention arm, wealth index and distance from potential vector breeding site were not significantly associated with the total number of LLIN use report during the study period after multilevel analysis, adding random effect variables at household and village level (Table 5).

Fig 6 shows the pattern of LLIN use, malaria episodes and total rainfall in the follow-up period. Our study covered three main malaria transmission seasons which include: October—December 2014, September—December 2015 and September—December 2016. The

Table 3. Reported causes for long-lasting insecticidal net loss after two years of post-distribution, Ethiopia November 2016.

Reason for LLIN loss	Number	Percentage
Thrown away	4,713	75.7
Used for something else	750	12.0
Given away	554	9.0
Stolen	84	1.3
Other*	73	1.2
Don't know	52	0.8
Total	6,226	100.0

*reported as not receiving the LLIN at all or sold

LLIN = long-lasting insecticidal net

https://doi.org/10.1371/journal.pone.0210578.t003

Table 4. The median (IQR) proportion of weeks individuals used an LLIN during 121 weeks from October 2014 to January 2017.

Variables	n	Median (IQR)	t- test*	P-value
Gender				
Male	8,618	13.2 (4.1–28.9)	Ref	
Female	8,524	14.0 (4.1–30.6)	1.97	0.048
Age in years				
<5	3,196	9.1 (0.8–24.0)	Ref	
5–14	5,557	9.9 (3.3–24.8)	1.84	0.066
15–24	3,396	13.2 (4.1–28.1)	8.29	<0.001
≥25	4,983	23.1 (10.7–35.5)	30.67	<0.001
Intervention arm				
LLIN+IRS	9,104	13.2 (4.1–29.8)	Ref	
LLIN alone	8,038	14.0 (4.1–29.8)	1.97	0.049
Wealth index				
Poor	6,058	12.4 (2.5–28.9)	Ref	
Medium	5,671	14.9 (5.8–30.6)	3.28	0.001
Rich	5,413	14.8 (5.8–30.6)	4.86	<0.001
Distance from vector breeding				
≤1 km	5,602	17.4 (3.3–30.6)	-11.93	<0.001
>1 km	11,540	12.4 (4.1–28.5)	Ref	
All	17,142	14.0 (4.1–29.8)¥		

* Test statistics was calculated using median regression model.

IQR = interquartile range, IRS = indoor residual spray, LLIN = long-lasting insecticidal net

¥ Overall mean (standard deviation) LLIN use = 17.8 (16.0), mean proportion of LLIN use per individuals during the first year (week 1–52) = 36%, and during the second year (week 53 to 104) = 4.6%.

https://doi.org/10.1371/journal.pone.0210578.t004

proportion of individuals using LLIN per week was consistently declining in spite of seasonal variation of malaria and rainfall.

Predictors of LLINs Use

Table 5 shows the association between the total number of weeks in which LLIN use reported and some explanatory variables. LLIN use was significantly higher in the age group from 5–14 years (adjusted IRR = 1.13, 95% CI 1.04–1.22), 15–24 years (adjusted IRR = 1.33, 95% CI 1.23–1.45) and ≥25 years (adjusted IRR = 1.99, 95% CI 1.83–2.17) compared with the age group <5 years. Similarly, LLIN use was higher among people whose heads of households could read and write (adjusted IRR = 1.17, 95% CI 1.09–1.26), had attended primary education (adjusted IRR = 1.20, 95% CI 1.12–1.27) and secondary or higher education (adjusted IRR = 1.20, 95% CI 1.11–1.30), compared with households where the household head was illiterate. On the other hand, people living in households with family size of more than five people (adjusted IRR = 0.78, 95% CI 0.73–0.84) were less likely to have used an LLIN compared to people living in households having a family size of five or less. In this study, gender, occupational status of head of household, number of sleeping spaces in household, household wealth status, distance from lake or river and interventional group did not show any significant difference.

Discussion

A low LLIN use was observed in a semi-arid area of south-central Ethiopia. The median proportion of nights individuals used an LLIN during the 121 weeks follow-up period was only

Table 5. Multilevel negative binomial regression for predictors of long-lasting insecticidal net use, during 121 weeks from October 2014 to January 2017.

Variables	n (%)	Unadjusted IRR (95% CI)	P-value	Adjusted IRR (95% CI)	P-value
Gender					
Male	8,618 (50.3)	1			
Female	8,524 (49.7)	1.02 (0.99–1.04)	0.112	1.01 (0.98–1.03)	0.580
Age (years) group					
<5	3,196 (18.7)	1		1	
5–14	5,557 (32.4)	1.11 (1.02–1.20)	0.012	1.13 (1.04–1.22)	0.003
15–24	3,396 (19.8)	1.35 (1.24–1.46)	<0.001	1.33 (1.23–1.45)	<0.001
≥25	4,983 (29.1)	1.99 (1.83–2.16)	<0.001	1.99 (1.83–2.17)	<0.001
Educational status of head of household					
Illiterate	9,479 (55.3)	1		1	
Read and write	1,774 (10.3)	1.09 (1.02–1.17)	0.017	1.17 (1.09–1.26)	<0.001
Primary	4,281 (25)	1.15 (1.08–1.22)	<0.001	1.20 (1.12–1.27)	<0.001
Secondary and above	1,608 (9.4)	1.21 (1.11–1.32)	<0.001	1.20 (1.11–1.30)	<0.001
Occupational status of head of household					
Other	3,629 (21.2)	1			
Farmer	13,513 (78.8)	0.96 (0.91–1.01)	0.133	0.99 (0.93–1.05)	0.679
Household size					
≤5	5,212 (30.4)	1		1	
>5	11,930 (69.6)	0.72 (0.68–0.77)	<0.001	0.78 (0.73–0.84)	<0.001
Number of sleeping spaces in household					
1	3,843 (22.4)	1		1	
2	9,716 (56.7)	0.84 (0.78–0.89)	<0.001	0.94 (0.88–1.00)	0.054
≥3	3,583 (20.9)	0.81 (0.74–089)	<0.001	0.97 (0.89–1.05)	0.390
Household wealth index					
Poor	6,056 (35.3)	1		NA	
Medium	5,672 (33.1)	1.01 (0.93–1.10)	0.818		
Rich	5,414 (31.6)	1.01 (0.93–1.09)	0.895		
Intervention arm					
IRS+LLIN	9,104 (53.1)	1		NA	
LLIN alone	8,038 (46.9)	089 (0.65–1.22)	0.461		
Distance from lake or river					
≤1km	5,602 (32.7)	1		NA	
>1km	11,540 (67.3)	0.98 (0.72–1.35)	0.906		

IRR = incidence rate ratio, IRS = indoor residual spray, LLIN = long-lasting insecticidal net, NA = not applicable (P > 0.25 in bivariate analysis)

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14%. This low LLIN use could be explained by a high attrition rate of LLINs due to disposal, and misuse [33] of the LLINs for other purposes. Moreover, a low mosquito density and malaria incidence that occurred after a severe drought in 2015 in the study area could play an important role in the low use of LLINs [26, 34], since people may perceive lower risk of malaria infection and tend to use LLINs less likely in this condition.

This study was part of a large cluster-randomized control trial, in which we followed a large cohort of people for 121 weeks in a rural community of Ethiopia. Unlike cross-sectional studies on LLIN use, the weekly evaluation of LLIN use in this study gives the real LLIN use per week over the study period and during different seasons. Since the study population was randomly selected from a source population in a semi-arid area of south-central Ethiopia our

findings can be generalized to many parts of Ethiopia that exhibit conditions similar to those in the study site.

In the current study, the ownership of LLINs was low despite universal LLIN coverage at baseline. Two years after distribution of LLINs, only 8% of the households owned at least one LLIN. LLIN ownership was lower compared with other studies in the area showing 27% ownership in 2013, 12% in 2014, and 31% in 2016 [25, 35, 36] and much lower than the 2015 national MIS, which reported 64% LLIN ownership [14]. Ownership was also lower than the study findings from Uganda [37, 38], Tanzania [39] and Madagascar [40]. The time interval between the LLIN distribution mass campaigns and data collection for each survey may be a reason for the observed difference in the level of LLIN ownership. Furthermore, a decline in mosquito population and malaria incidence that was observed in 2015 after a severe drought in the study area [26, 34], could be a potential reason for low LLINs ownership since people may perceive lower risk of malaria infection in such conditions.

In the current study, 92% of the households reported that they lost all the LLINs after two years of the distribution (Table 2). The most common reasons for LLIN loss were throwing away due to damage and using the LLINs for other purposes. A previous study in the same study area revealed that 21% of LLINs were lost after 6 months, 61% after 12 months and 96% after 24 months due to disposal because nets were damaged, torn or used for other purposes [28]. Studies from sub-Saharan African countries also reported similar reasons for LLINs loss [41, 42].

The observed mean LLIN use (36% over 52 weeks and 18% over 121 weeks of follow-up after 100% LLIN coverage) was lower than that of a previous longitudinal study from the Arba Minch area in southern Ethiopia (62% LLIN use over 49 weeks of follow-up after 98.4% LLIN coverage) [15]. The reasons for this difference may be related to the burden of malaria infection (14.7 malaria episodes per 1,000 persons per year in our study area versus 45.1 per 1,000 persons per year in the Arba Minch study area) [43]. Additionally, the observed first year mean LLIN use (36%) in our study was comparable with that of the national MIS LLIN use report (40% in the 2015 MIS survey) [14]. However, the overall mean LLIN use (18%) was lower than that of national MIS LLIN use report, which may be due to the difference in study designs. The MIS survey was a cross-sectional study, and did not provide LLIN use information over time. The mean LLIN use over the study period was also much lower than findings from studies in several sub-Saharan African countries [37, 38, 40].

The observed low LLIN use in our study could be related to a reported high attrition rate of the LLINs in the study area [28]. Our previous quantitative study on durability of the same LLINs as this study showed that 61% of LLINs were lost after one year and 96% after two years of follow-up [28]. Two main factors were mentioned as possible causes for this high attrition rate of LLINs. The first was unexpectedly dry and warmer climatic conditions following the El Nino effect in 2015 [27], manifested by a decline in annual rainfall (by 60% in 2015) and an increase in average maximum temperature (2˚C above normal) [44]. At this period a parallel study showed low mosquito abundance and low human biting rates [45]. In addition to this, the positivity rate for *Plasmodium* species was zero for mosquito specimen tested for sprozoites [45]. A decline in malaria incidence (only 37% of pre-distribution incidence) was also documented in the study area [26]. This may lead LLIN users to a lower perceived risk of nuisance from mosquitoes and malaria infection. The second reason was the LLIN users' behaviour and perception toward the serviceable life cycle of the LLINs [33]. A qualitative study on the same LLINs of this study showed that many informants believe that the LLINs would not serve more than one year, by claiming the LLINs lose their insecticide effect after six months (by mentioning the "nets stopped killing bugs") [33]. Moreover, they mentioned that after one year most of

the LLINs were used for other purposes, such as grain storage and transportation from the field, toilet covers, blankets, bed sheets and mattress covers [33].

In this study, the age-specific difference in LLIN use was observed and remained unchanged over the study period. The older age groups over 25 years were using LLINs more often than those of more vulnerable age groups, such as children less than five years, as has been observed elsewhere in Ethiopia [15, 46]. The reasons for a lower LLIN use by more vulnerable age groups need to be further investigated. Unlike our findings, a Ugandan study reported a higher LLIN use among vulnerable age groups [47]. This may be due to a higher malaria prevalence and incidence rate among children in Uganda [48, 49] compared with our study area [34].

The wealth status of households was not significantly associated with LLIN use. This finding is similar to the study findings from southern Ethiopia, northern Nigeria and Uganda [15, 50, 51]. Some studies show a significant association between wealth status and LLIN use in which people living in wealthy households were more likely to use LLINs compared to the poor [52, 53]. In contrast to this, some other studies have shown that people living in poor households were more likely to use the LLINs compared to the wealthy [54, 55]. The lack of significant associations between the wealth status and LLIN use in our study may be influenced by context of the study as a decline in mosquito populations and malaria incidence following unexpected dry and warm weather condition in the study period may commonly affect all households irrespective of their wealth status.

A study from southern Ethiopia reported that LLIN use decreased with an increasing distance from the vector breeding site [15]. However, we did not found a significant difference between residents who lived within and more than 1 km away from Lake Zeway or the Bulbula River, which have been identified as the locations where most breeding sites are found [22, 23]. Having a large family size was associated with a lower use of LLINs, with a similar finding observed in southwest Ethiopia [56]. The reason for this could be an inadequate number of LLINs for households with a large family size due to a high attrition of LLINs in our study area [28].

Achieving malaria elimination in Ethiopia in 2020 requires maintaining high coverage and consistent use of LLINs throughout all season of the year. However, our study findings suggest that mere universal LLIN coverage immediately post-distribution of LLINs does not guarantee for required level of LLIN use. Therefore, the national public health policymakers may use these findings to revise ongoing LLIN distribution schedules and communication and advocacy activity regarding LLIN ownership and use. The behavioural aspects that determined the ownership and use of LLINs should be taken into consideration during communication and advocacy activity. Behaviour change communication (BCC) messages should be provided on how to handle, hang and use LLINs for only intended purpose. Moreover, children under five years need special attention. The national malaria control programme should encourage the consistent use of the LLIN among under five children by involving children's parents and caregivers even in areas or seasons of low mosquitos and malaria transmission.

Our study had a number of limitations: 1) The LLINs use data were collected based on self-reporting. This may have led to social desirability bias as people prefer a "yes" response. To minimize the bias, the respondents were asked to list the name of the household members who used a LLIN the night before the date of interview. Furthermore, the data collectors observed whether the LLINs were hung over the bed or not. 2) There may be possible interviewer fatigue and reporting bias due to frequent weekly visits for relatively longer period of time by anticipating the next week visit. This may increase the actual LLIN use than would be expected without intensive follow-up or it would be still possible social desirability bias as people prefer to report use instead of reporting non-use. However, because the LLIN use was much lower than expected in the study, this potential limitation is less likely to have influenced the results. 3)

There may also be a possible recall bias on the causes of LLIN loss since the data on the reasons for LLIN loss was collected after two years of LLIN distribution. 4) LLIN use was not evaluated for pregnant women, and reasons for not using the available LLINs were not investigated.

Conclusion

In conclusion, the data showed that despite universal LLIN coverage, a low LLIN ownership and use was observed during 121 weeks of follow-up in the study area. A decline in mosquito populations and malaria incidence following unexpected dry and warm weather condition in the study period could indirectly affect the ownership and LLIN use by decreasing the perceived risk of mosquito bites and malaria infection. However, a high attrition rate of LLINs is the primary reason for an observed low LLIN ownership and use. Meanwhile, age groups, educational status of the head of the household and family size were the main predictors of LLIN use. Consequently, addressing the causes of early loss of LLINs from the household would help to maximize LLIN ownership and use. Since more than 90% of LLINs were lost within two years after LLIN distribution, LLIN replacement strategies should be strengthened to ensure maximal health benefits to the community. Last, the reasons for lower LLIN use by more vulnerable age groups need to be investigated.

Supporting information

S1 File. Questionnaire used to conduct census on selected socio-demographic variables and to gather data on malaria prevention and treatment practices in Adami Tullu District in south-central Ethiopia.
(PDF)

S2 File. Questionnaire used to conduct weekly LLIN use data collection in Adami Tullu District in south-central Ethiopia, October 2014 –January 2017.
(PDF)

S3 File. Raw data used to construct Figs 3, 4, 5 and 6 in Adami Tullu District in south-central Ethiopia, October 2014 –January 2017.
(XLSX)

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Supplementary information

Paper II

S1 File. Questionnaire used to conduct census on selected socio-demographic variables and to gather data on malaria prevention and treatment practices in Adami Tullu District in south-central Ethiopia.

QUESTIONNAIRE TO CONDUCT CENSUS ON SELECTED SOCIO-DEMOGRAPHIC VARIABLES AND TO GATHER DATA ON MALARIA PREVENTION AND TREATMENT PRACTICES

General Information		
GI1	Household number	_____
GI2	Site in which the interview is being conducted	a) Kebele _____b)Zone_____ c)Gare _____
GI3	Personnel (name and signature)	a) Interviewer_____ b) Supervisor_____
GI4	Date of visit	[_____|_____| _____| dd	mm| yyyy

Introduction and Consent

My name is_____ and I'm working for Hawassa University and Addis Ababa 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 Oromia 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. There will be weekly visit for the next 2 years.

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											
Q102a		**102b**	**102 c**	**102d**	**102e**	**102f**	**102g**	**102h**	**102i**	**102j**	**102k**
Individual ID	**Household Members**	**Age**	**Sex**	**Relationship to head of household**	**Educational status**	**Marital Status**	**Current pregnancy status 1. Yes 2. No**	**Duration of pregnancy in months**	**Occupation**	**Ethnicity**	**Religion**
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											

Sex
1. Male
2. Female

Relationship
1. head
2.Wife or husband
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

Marital Status(15years & above)
1.Maried
2.Living together
3.Divorced or separated
4.Widowed
5. Naver married/naver lived together

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

Ethnicity
1.Oromo
2. Amhara
3. Gurage
4. Other (Specify__)

Religion
1.Orthodox
2.Muslim
3.Protestant
4.Other (Specify__)

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	Do you have a separate room which is used as a kitchen?	Yes……1 No……2	
Q105	Main material of the floor. ***(Record observation)***	Earth/Dung ……1 Ceramic Tiles……2 Cement……3 Other……96 Specify_____	
Q106	Main material of the roof ***(Record observation)***	Thatch/Leaf……1 Corrugated Iron ……2 Cement/Concrete ……3 Other……96 (Specify)_____	
Q107	Main material of the exterior wall. ***(Record observation)***	No wall……1 Wood……2 Wood with mud……3 Wood with mud and cement……4 Cement blocks……5 Other……96 (Specify)_____	
Q108	How many rooms in this household are used for sleeping?	Number of rooms[__|__]	
Q109	How many sleeping spaces such as mats, rugs, mattresses or beds are used in this household?		
Q110	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	
Q111	Does any member of this household own any land that can be used for agriculture?	Yes……1 No……2 → **Skip to Q113**	
Q112	How many (LOCAL UNITS) of agricultural land do members of this household own? ***(If unknown enter 98)***	Local units [___|___] Specify the local unit_____	
Q113	Does this household own any livestock, herds, or farm animals?	Yes……1 No……2	
Q114	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---------- |||	
Q115	Does any member of this household have an account with a bank/credit association/micro finance?	Yes……1 No……2	

Q116	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_____	
Q117	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**
Q118	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 Q211**
Q202	How many mosquito nets do your household have?		Number of Nets _____		
Q203	Ask respondent to show you the net(s) in the household.	NET #1 _____ Observed ..... 1 Not observed..... 2	NET #2 _____ Observed.....1 Not observed 2	NET #3 _____ Observed.....1 Not observed.. 2	
Q204	How long ago did your household obtain the mosquito net?	____ ____ Months ago	____ ___ Months ago	____ ____ Months ago	
Q205	Where did you obtain the net?	Government Clinic/hospital Health extension worker……1 Retail shop Pharmacy……2 Workplace……3 Other (specify)_____.....4 Don't know.....98	Government Clinic/hospital Health extension worker……1 Retail shop Pharmacy……2 Workplace……3 Other (specify)_____....4 Don't know…….98	Government Clinic/hospital Health extension worker……1 Retail shop Pharmacy……2 Workplace……..3 Other (specify.....4 Don't know…….98	
Q206	Did you purchase the net?	YES.....1 NO.....2 Not sure..... 8	YES.....1 NO.....2 Not sure..... 8	YES.....1 NO.....2 Not sure..... 8 →	skip to 208

Q207	How much did you pay for the net when it was purchased?	_____ birr	_____ birr	_____ birr	
Q208	Did anyone sleep under the mosquito net last night?	Yes……1 No..……..2 Not sure……8	Yes……1 No..……..2 Not sure……8	Yes……1 No..……..2 Not sure……8	**Skip to Q210**
Q209	Who slept under this mosquito net last night?	Individual ID 1. _____ 2. _____ 3. _____ 4. _____	Individual ID 1. _____ 2. _____ 3. _____ 4. _____	Individual ID 1_____ 2_____ 3_____ 4_____	
Q210	Why did no-one sleep under this mosquito net last night?	No malaria…….. 1 No nuisance/insects… 2 No space for net …….3 Irritation ……4 Suffocation / too hot ….5 Difficult hanging net ….6 Shape ……..7 Absence from home …..8 Other..... 9 Don't know……....98	No malaria……. 1 No nuisance/insects.. 2 No space for net ….3 Irritation ……4 Suffocation / too hot ..5 Difficult hanging net ..6 Shape ……..7 Absence from home ..8 Other..... 9 Don't know…….98	No malaria….. 1 No nuisance/insects.. 2 No space for net …3 Irritation ……4 Suffocation / too hot ...5 Difficult hanging net ..6 Shape ……..7 Absence from home…..8 Other..... 9 Don't know…….98	
Q211	Has your house ever been sprayed with insecticide for malaria prevention by spraymen from the District Health Office?			Yes……1 No..……..2 Not sure..……8	**Skip to Q215**
Q212	How many months ago was your house sprayed? ***(If less than one month, record 0)***			Months ago [___/___] Not sure…..8	
Q213	At any time in the past 12 months, have the walls in your dwelling been plastered or painted?			Yes..... ...1 No..... 2	
Q214	How many months ago were the walls plastered or painted? *If less than one month, record 0.*			MONTHS AGO , _____ _____	
Q215	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**

Health service seeking and utilization									
ID	**Q216**	**Q217**	**Q218**	**Q219**	**Q220**	**Q221**	**Q222**	**Q223**	**Q224**
	Have any of your family members faced any health problem during the **last 2** months? **Yes.....1** **No.....2 ► Q222**	What was the sickness/ injury faced? Malaria.....1 Diarrhea.....2 Injury.....3 Dental.....4 Opthalmic.....5 Skin disease.....6 Ear/nose/throt (ENT)....7 Tuberclosis.....8 Other (specify)_____	For how many days were he/she absent from usual activity due to the health problem during the **last 2** months?	Has he/she received medical assistance or consulted from health institutions or traditional healers during the **last 2** months? **Yes.....1** **No.....2 ► Q221**	Where did he/she receive or consult medical assistance primarily? Hospital.....1 Health center.....2 Health post.....3 Private Clinics.....4 Private Pharmacy.....5 Traditional healer.....6 Religious/spiritual.....7 Other (specify).....8	What was the main reason for he/she not to consult health institutions/ traditional healer during the **last 2** months? Lack of money.....1 Expensive.....2 Too far .....3 Do not believe in medicine.....4 Lack of health professional.....5 Poor quality/ service.....6 Did not require medical assistance. 7 Other (specify).....8	Have any of your family member consulted any medical assistance during the **last 12** months? (Regardless of whether sick or not)? **Yes.....1** **No.....2**	How many times have he/she consulted any medical assistance during the **last 12** months?	Has member of your family been ill with a fever at any time in the last 7days? **Yes.....1** **No.....2**
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									

GAAFFIIWWAN QORANNOO LAKKOOBSA UUMMATAA HAWAASAA FILATAMANII FI RAGAALEE ITTISAA FI YAALII DHUKKUBA BUSAA QORACHUUF QOPHAA'E

Odeeffannoo Walii Galaa		
GI1	Lakk. (Koodii) Mana	_____
GI2	Dirree itti gaaffii fi deebiin geggeeffame	a) Ganda _____ b) Zone_____ c)Garee _____
GI3	Personeelii (Maqaa fi Mallattoo)	a) Gaaffii kan gaafate _____ b) To'ataa Dirree Supervis_____
GI4	Guyyaa Hordoffii	[_____|_____| _____| Guyyaa | Ji'a | Bara

Seensaa fi Walii Galtee

Maqaan koo _____ jedhama. Hojjaa kanaaYuunivarsiitii Addis Ababaa fi Hawwaasaa finii hojjedha. Waajjira fayyaa aanaa keessanii wajjin wal ta'uudhaan qorannoo dhukkuba busaa irratti hojjechaa jira. Qorannoo kana irratti hirmaannaa gootaniif dursinee isin galateeffanna. Bu'aan qorannoo kanaa Biiroon Eegumsa Fayyaa Oromia karoorsanii tajaajila adda addaa kennuuf gargaara.

Gaaffii fi deebiin kun daqiiqaa15 keessatti xumurama. Odeeffannoon isin nuu kennitan kamiyyuu icciitii cimaadhaan kan eegamuu fi qaama kamitti iyyuu kan hin agarsiifane ta'a. Hirmmaannaan isin gaaffii if deebii kana irratti gootan fedha keessan irratti kan hundaa'e yammuu ta'u gaaffii kamiyyuu filattanii deebisuu yookin gaaffii hunda iyyuu deebisuu dhiisuudhaaf mirga guutuu qabdu. Haa ta'u iyyuu malee deebiin isin nuu keennitan qorannoo kanaaf baayyee murtteessaa waan ta'eef gutumaan guutuutti gaaffii fi deebii kana irratti hirmaattu jennee abdii guddaa qabna.

Akkasumas Waggaa lamaan dhufanniif torban torbaniin du'annaan kan deemsifamu ta'a. Qorannaa kana ilaalchisee gaaffii qabduu?Gaaffii fi deebii keenya itti fufuu dandeenyaa?

Gaafi gaafatamu deebi kennudhaf eyyamamadha yoo ta'e, sanduqa kessatti mallato godhii ☐

Kutaa1: - Ibsa Haala Hawaas-Dinagdee , Halaa Uummataa fi Miseensota Maatii											
Q101	**Baayyina Miseensota Maatii**	**Baayyina _____**									
Nama Gaafatamaa jiru irraa eegalaatii tarreessaa											
Q102a		**102b**	**102c**	**102d**	**102e**	**102f**	**102g**	**102h**	**102i**	**102j**	**102k**
Kodii dhunfaa	**Miseensota Maatichaa**	**Umurii**	**Saala**	**Walitti dhufeenya**	**Sadarkaa barnootaa**	**Sadarka matii 1.Eyee 2. Lakki**	**Ulfa yeroo amma/kan ulfa tee jirra**	**Ji'a meqaaf ulfofte**	**Hojii**	**Lammii/ Qomoo**	**Amantii**
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											

Saala
1. Dhiira
2. Dhalaa

Walitti dhufeenya
1. Abbaa waara /hadha waara
2. Haadha waara
3. Mucaa
4. Fira
5. Hojjettuu manaa
6. Kan biroo

Sadarkaa barnootaa (waggaa 6 fi ol)
- **I=** Hin baranne
- **RW=** Barreessuu fi dubbisuu
- Barnoota idilee yoo ta'e sadarkaa isaa barreessi

Sadarka matii(waggaa 15 ol)
1.ka fudhe
2.wajjin kanjiratan
3. Walihikan/gare gare kanbaan
4. ka iradue
5. ka hinfune;

Hojii (waggaa 18 fi ol)
1. Hojj. Mootummaa
2. Haadha manaa
3. Qotee bulaa
4. Hojii oolmaa
5. Daldalaa
6. Qurxummii qabduu
7. Barataa
8. Hojii hin qabuu
9. Hojjettuu manaa
10. Kan biroo

Lammii/ Qomoo
1.Oromoo
2. Amaara
3. Guraagee
4. Kan biroo (Ibsi__)

Amantii
1. Ortodoksii
2. Islaama
3. Pirotestantii
4. Kan biroo (Ibsi__)

Q103	Mana keessanitti tajaajila armaan gadii qabduu? Elektirikaa? Sa'atii? Televizhinii? Raadiyoo ? Mobaayilii? Bilbila manaa? Firiijii? Teessuma? Miinjaala Siree ciisichaa? Eelee elektirikaa? Buttaa gaazii?	Eyyee Lakkii Elektirikaa ……..……..……1 2 Sa'atii ……..……1 2 Televizhinii……...……1 2 Raadiyoo ……...……1 2 Mobaayilii ……..……..1 2 Bilbila manaa ……..…1 2 Firiijii.. …..……1 2 Miinjaala..……..……1 2 Teessuma ……..……...1 2 Siree ciisichaa ……..……1 2 Eelee elektirikaa ……...……..…1 2 Buttaa gaazii ……...…...1 2	
Q104	Mana nyaata itti bilcheeffattan kophaatti qabduu	Eyyee ……1 Lakkii ……2	
Q105	Lafti mana keessanii maal irraa hojjeteme? (Waan daawwatte sana waraabi)	Biyyoo……...1 Seraamikii… ……2 Simmintoo ….. . . .. ……..3 Kan biroo ……96 (Kan biroo Ibsaa)_____	
Q106	Guutuun (Xaaraan) mana keessanii maal irraa hojjeteme? (Waan daawwatte sana waraabi)	Baala/ fi Citaa……1 Qorqorroo …… . . . . . ……... ……...2 Simmintoo…… . . . . ……... . . . . 3 Kan biroo ……..……..96 (Kan biroo Ibsaa)_____	
Q107	Dhaabin mana keessanii (Girgiddaa) maal irraa hojjeteme? (Waan daawwatte sana waraabi)	Hin qabu ……..1 Muka ……2 Mukaa fi dhoqqee ……3 Muka ,dhoqqee fi simmintoo ……..4 Bilookeetii simmintoo ……...5 Bilookeetii Biyyoo……..6 Kan biroo ……..……..96 (Kan biroo Ibsaa)_____	
Q108	Mana keessan keessaa kutaa ciisicha meeqa qabdu?	Lakkoobsaa kutaa[_____|_____]	
Q109	Maatiin kun edoo ciisicha kan akka firashii, siree, minxaafii yookiin boraatii meeqatti fayyadama?	Lakkoobsaa edoo ciisicha [_____|_____]	
Q110	Maatii mana kanaa keessaa namni armaan gadii kana qabu jiraa: Saayikilii? Dhokdhokkee? Gaarii? Konkolaataa?	Eyyee Lakkii Saayikilii ……...1 2 Dokdhoqqee ……..……1 2 Gaarii ….……..1 2 Konkolaataa ……..…...1 2	
Q111	Maatii mana kanaa keessaa namni lafa qonnaa qabu jiraa?	Eyyee ……1 Lakkii ……2 →	**Q113tti ce'i**
Q112	Maatiin kun lafa qonnaa safartuu hammame qabu? *(*Yoo hinbeekamu ta'e "98" barreessi)	Safartuu lafaa naannoo kanaatti fayyadamuudhaan ibsaa _____ Safartuu lafaa gargaramitan ibsa _____	
Q113	Maatiin kun horii manaa qabuu?	Eyyee ……1 Lakkii ……2	

Q114	Maatiin kun horii manaa armaan gadii meeqa qabu? Loon annanii, Qotiyyoo, Horii foonii? Farda, Harree, Gaangee? Re'ee? Hoolota? Lukkuuwwan? (Yoo hinbeekamu ta'e "98" barreessi)	Loon annanii, Qotiyyoo, Horii foonii------- Farda, Harree, Gaangee --------- Re'ee ---------- Hoolota ---------- Lukkuuwwan ----------	
Q115	Maatii kana keessaa namni herrega qusannaa baankii qabu jiraa?	Eyyee ……1 Lakkii ……2	
Q116	Maddi bishaan dhugaatii maatii keessanii maali? (Deebicha hin dubbisin)	*Boombaa* Boombaa mana keessatti ……1 Boombaa mooraa keessatti ……2 Boombaa mooraa alatti ……3 Biirii eegumsa qabu ……4 Burqituu eegumsa qabu ……5 *Biirii banamaa/burqituu* Biirii banaa ……6 Burqituu banaa ……7 *Bishaan Yaa'u* Bishaan lagaa ……8 Kuufama/haroo ……9 Bishaan bokkaa ……10 Kan biroo ……11 (Kan biroo Ibsaa)_____	
Q117	Maatiin keessan mana fincaanii attamii gargaaramu? (Mana fincaanichaa daawwaadhu)	Boolla fincaanii bishaannin kan hojetu (Flush toilet)……1 Boolla fincaanii kan duri /traditional pit toilet……2 Mana fincaanii (Ventilated improved pit latrine) -3 Hin qabu / Bosona keessatti /Bakkeetti.....4 Kan biraa ……5 Kan biraa (ibsaa)_____	→ **Q201tti ce'i**
Q118	**Mana fincaanii kana maatii biraa waliin gargaaramtuu?**	**Eyyee ……1** **Lakkii ……2**	

Kutaa: 2ffaa Ittisaa fi Yaalii Dhukkuba Busaa

Q201	Mana kana keessaa tajajila kenu kandandu agoobara qabduu?		Eyyee ……1 Lakkii ……2		→ **Q211 itti ce'i**
Q202	Agoobara meeqa qabduu?		Lakkobsaa Agoobaraa_____		
Q203	Agoobara mana keessa jiru akka agarsiisaniif gaafatamtoota gaafadhu.	Agoobara #1 Ilaalameera ..... 1 Hin ilaalamne..... 2	Agoobara #2 Ilaalameera ....1 Hin ilaalamne 2	Agoobara #3 Ilaalameera .....1 Hin ilaalamne 2	
Q204	Maatiin kun agoobara bookee busaa ittisu yoom argate?	_____ _____Ji'a	____ ___ Ji'a	____ ____ Ji'a	
Q205	Agoobaricha eessaa argattan?	Mootummaa Kilniika/ Hospitaala Hojjettuu Eksteenshenii Fayyaa.....1 Faarmaasii dhuunfaa.....2 Bakka hojii……3 Kan biroo (Ibsi).....4 Hinbeeku……98	Mootummaa Kilniika/ Hospitaala Hojjettuu Eksteenshenii Fayyaa…..1 Faarmaasii dhuunfaa....2 Bakka hojii……3 Kan biroo (Ibsi).....4 Hinbeeku……98	Mootummaa Kilniika/ Hospitaala Hojjettuu Eksteenshenii Fayyaa…..1 Faarmaasii dhuunfaa...2 Bakka hojii……3 Kan biroo (Ibsi).....4 Hinbeeku……98	

Q206	Agoobaricha ni bittanii?	Eyyee.....1 Lakkii.....2 Sirritti hin beeku...8	Eyyee.....1 Lakkii.....2 Sirritti hin beeku..8	Eyyee.....1 Lakkii(8).....2 Sirritti hin beeku..... 8	Q208 tti darbi
Q207	Agoobaricha qarshii meeqaan bittan?	Qarshii _____	Qarshii _____	Qarshii _____	
Q208	Galgala darbe/eda namni agoobara hidhatee rafe jiraa?	Eyyee …….1 Lakkii……...2 Sirritti hin beeku...8	Eyyee …….1 Lakkii……...2 Sirritti hin beeku..8	Eyyee …….1 Lakkii……...2 Sirritti hin beeku...8	**Q210 ce'e**
Q209	Eenyutu agoobara hidhatee rafe?	Koddi dhunfa 1._____ 2._____ 3._____ 4._____	Koddi dhunfa 1._____ 2._____ 3._____ 4._____	Koddi dhunfa 1._____ 2._____ 3._____ 4._____	
Q210	Kaleessa halkan (eda galgala) maatii kana keessaaa namni kamiyyuu agoobara maaliif hin huffanne?	Busaan waan hinjirreef..... 1 Ilbiisonni/bookeen waan hinjirreef..... 2 Agoobaraaf bakki waan hinjirreef .....3 Nama rifachiisa ….....4 Namatti o'a/ bulluqa ...5 Fannisuuf waan ulfaatuuf .....6 Bochi isaa hin mijatu...7 Mana keessa agoobarri waan hin jirreef…....8 Sababa biroo..... 9 Hin beeku…….....98	Busaan waan hinjirreef..... 1 Ilbiisonni/bookeen waan hinjirreef..... 2 Agoobaraaf bakki waan hinjirreef .....3 Nama rifachiisa.....4 Namatti o'a/ bulluqa.....5 Fannisuuf waan ulfaatuuf ….....6 Bochi isaa hin mijatu..7 Mana keessa agoobarri waan hin jirreef…....8 Sababa biroo..... 9 Hin beeku.....98	Busaan waan hinjirreef..... 1 Ilbiisonni/bookeen waan hinjirreef.....2 Agoobaraaf bakki waan hinjirreef .....3 Nama rifachiisa .....4 Namatti o'a/ bulluqa .....5 Fannisuuf waan ulfaatuuf ….....6 Bochi isaa hin mijatu..7 Mana keessa agoobarri waan hin jirreef.....8 Sababa biroo..... ...9 Hin beeku…….....98	

Q211	Hojjettoota fayyaa aanaa keessaniin qorichi farra bookee busaa mana keessanitti ni biifamaa?	Eyyee …….1 Lakkii……...2 Hin yaadadhu(Hin beeku)……3	**Q215 ti darbi**
Q212	Ji'a meeqa dura qorichi kun kan isinii biifame? (Ji'a tokko gadi yoo ta'e waraabi 01)	Ji'a [___/___] Hin yaadadhu/…..98	
Q213	Batiiwwan 12 darban kam iyyuu keessatti dhabaani /Girgida/ mana keessanii dibamee yookiin lastikiin itti godhamee beekaa?	Eyyee.....1 Lakkii.....2	
Q214	Batiiwwan meeqa dura dhabaani /Girgida mana keessanii kan dibame yookiin lastikiin kan itti godhame? (Ji'a tokko gadi yoo ta'e waraabi 01)	Ji'ota/baatiiwwaniin dura , _____ _____	

Q215	Waggaa kana keessa mana kana keessaa namni boqate/ du'e jiraa?	Eyyee ….1 Lakkii ……2	→ Yoom boqote? Ji'a meeqa dura ____	Saala Dhira …1 Dhala ……2	**Umurii** _____ **Waggaa / Ji'a**

Fedhii fi itti fayyadama tajaajila fayyaa									
kodii	216	217	218	219	220	221	222	223	224
	Baatiwwan 12 darban keessatti namni maatii keessan keessaa dhibeen fayyaa isa mudate jiraa Eyyee……1 Lakkii…2 ► **Q222**	Dhibee yookin balaa /madaa akkamiitu mudate? Busaa…….1 Garaa kaasaa……..2 Balaa /madaa ……...3 Dhibee ilkaanii.....4 Ija…….....5 Dhukuba gogaa…..6 Gurra/funyaan/liqimsituu(ENT)…….....7 Dhukkuba sombaa…….....8 Kan biroo(ibsi)_____	Baatii lamaan darban keessatti namni dhukabsate sun guyyaa meeqaaf hojii irraa hafe?	Baatii lamaan darban keessatti namni dhukabsate sun dhabbilee fayyaa irraa yookiin aadaadhaa iraa gargaarsa fayyaa argatee jiraa? Eyyee……1 Lakkii…2► **Q221**	Gargaarsa fayyaa kana adda dureedhaan eessa irraa argata/ti? Hospitaala irraa.....1 Buufata fayyaa irraa.....2 Kellaa fayya.....3 Kilnika dhuunfaa irraa.....4 Faarmaasii dhuunffaa.....5 Qoricha aadaa.....6 Dhaabbata amantii.....7 Kan biroo(ibsi)……...8	Baatii lamaan darban keessatti namni dhukabsate sun dhabbilee fayyaa yookiin aadaa iraa gargaarsa/ gorsa fayyaa akka hin arganneef sababni guddaan maal ture? Maallaqa dhabuu.....1 Gatiin yaala qaala'uu /miyaa'uu isaaa....2 Baayyee fagoo .....3 Qorichatti hin amanu/tu.....4 Ogummaa fayyaa dhabuu.....5 Tajaajila qulqulina hin qabne....6 Gargaarsi fayyaa waan hin barbaachifneef. .....7 Kan biroo(ibsi)_____..8	Baatii 12 darban keessatti maatii kana keessaa namni gargaarsi faayyaa kennameef jiraa?(Dhukkubsates dhukkubsachuu baatus) Eyyee….1 Lakkii….2	Baatii 12 darban keessatti ala meeqa gargaarsa fayyaa argate/te?	Guyyaa torban darban keessatti maatii kana keessa namni dhukuba ho'aa qaaman qabamee jiraa? Eyyee….1 Lakkii…2
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									

S2 File. Questionnaire used to conduct weekly LLIN use data collection in Adami Tullu District in south-central Ethiopia, October 2014 –January 2017.

<table>
<tr><th colspan="5">MalTrials Project: Weekly malaria data collection format</th></tr>
<tr><td colspan="2">Kebele_____
Gare_____
House number_____</td><td colspan="3">Date of visit[_____|_____|_____]
dd | mm| yyyy</td></tr>
<tr><td colspan="2">Data collector</td><td colspan="2">Name _____ Signature _____</td><td></td></tr>
<tr><td>Q01</td><td>Did anyone sleep under the bed net last night?</td><td colspan="3">Yes…….1
No.……2
Not sure……3
The household doesn't own net…..4
Skip to Q03</td></tr>
<tr><td>Q02</td><td>Who slept under the bed net last night?
(List the names)</td><td colspan="3">1._____ 6. _____
2._____ 7._____
3._____ 8. _____
4._____ 9. _____
5. _____ 10. _____</td></tr>
<tr><td>Q03</td><td>Presence of fever any time in the last 48 hours</td><td>Yes……1
No…….2</td><td colspan="2">If 2 ⟶ End</td></tr>
<tr><td>Q04*</td><td>If there is a member of the family who is febrile during the interview and did not take any medication, take axillary temperature and record the case's name and house number on your note book, then send the case with a referral slip to nurse as soon as possible.
(Use another format if you got more than three case in the same household and attach) (Use separate referral slip for each case)</td><td>Name_____
Age _____Years/Months
Sex
Male……1
Female…2</td><td>Name_____
Age _____Years/Months
Sex
Male……1
Female…2</td><td>Name_____
Age _____Years/Months
Sex
Male……1
Female…2</td></tr>
</table>

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

Projektii 'MalTrials': Unka Galmee Ragaa Dhukkuba Busaa Torbanii (Afan Oromo)				
Ganda _____ Zone_____ Garee _____ Lakkophsa manaa_(koodi)_____			Guyyaa Hordoffii [_____|_____|_____] Guy | ji'a | bara **Maqaa Nama Gaafatamee** _____	
Ragaa kan Funaanu		Maqaa _____	Mallattoo _____	
Q01	Galgala darbe namni agoobara hidhaatee rafe jiraa?	Eyyee……1 Lakkii……..2 Hin beeku……3 Agoobara hin qaban…..4	→ **Q03 darbi**	
Q02	Eenyutu agoobara hidhatee rafe (*maqaa tarreessaa*)	**1.**_____ **2.**_____ **3.**_____ **4.**_____ **5.** _____	**6.**_____ **7.**_____ **8.**_____ **9.**_____ **10.**_____	
Q03	Sa'ati 48 darbe kessaatii namni dhukkuba ho'aa qamaan qabamee jira?	**1.** Eyyee **2.** Lakkii	→ Ta'e dhabii	
Q04[*]	Yeroo gaaffii fi deebii geggeessitan yoo namni dhukuba ho'aa qaaman qabamee fi Qoricha kan hin gargaaraminii isin quunname galmeessaatii gabaasaa. (Yoo bayyatan 3ol ta'an unka biraaa gargaaramaa)	Maqaa:_____ _____ Umr ____ wagaa/ji'a **Salaa** Dhira.....1 Dhala.....…2	Maqaa:_____ _____ Umr ____ wagaa/ji'a **Salaa** Dhira.....1 Dhala.....…2	Maqaa:_____ _____ Umr ____ wagaa/ji'a **Salaa** Dhira.....1 Dhala.....…2

[*] *Nami kara keellaa fayyaatti dhiiga kennuf ergame, dhiiga kennu isaa mirkaneefadhu. Yoo dhiigni kennameera ta'e, Nurseichi akka kitaaba yadanno kee irratti simalateesu goodhi Nursein slidi irratti gayyaa,maqaa, umuri, salaa fi lakkoofsa mana haa barressu.*

S3 File. Raw data used to construct Figs 3, 4, 5 and 6 in Adami Tullu District in south-central Ethiopia, October 2014 –January 2017.

Week	Proportion of individuals using LLINs per week (%)*	Raw data for Figure 3		Raw data for Figure 4				Raw data for Figure 5		Raw data for Figure 6	
		Male	Female	<5 Year	5-14 year	15-24 Year	>24 Year	≤1km	>1km	Malaria (PF and PV) episodes	Rainfall (mm)
1	30.9	30.9	30.9	29	29.4	28.6	35.1	21.28	35.51	13	19.7
2	39.6	39.6	39.6	38.1	36.4	36.5	45.9	37.47	40.57	11	29.8
3	45.4	45.7	45.1	41.5	40.9	41.2	55.2	43.59	46.25	11	24
4	45	44	46	35	38.8	42.7	58.5	44.71	45.13	12	24.6
5	53.3	52.9	53.6	45.9	47.5	49	66.3	52.36	53.7	8	80.1
6	51.8	52.2	51.4	46.9	44.4	47.4	65.5	52.03	51.75	9	59.7
7	45.8	45.8	45.7	47.9	40	41.6	53.7	46.98	45.17	12	0
8	47.9	48.4	47.4	42.2	40.3	44.4	61.6	47.97	47.88	5	0
9	52.8	52.8	52.8	46	45.5	51.5	65.1	55.12	51.65	12	0
10	56.8	56.5	57.1	50.2	48.9	55.5	69.8	56.49	56.91	23	0
11	55.5	55.1	55.9	48.5	47.2	54.4	69.1	58.23	54.25	25	0
12	53.9	54.1	53.7	47.8	48	54.2	63.4	56.83	52.52	13	0
13	54.8	54	55.6	48.9	47.1	53.9	66.9	56.35	54.03	16	0
14	54.7	53.9	55.5	48.7	47.1	53.9	66.8	56.24	53.96	14	0
15	55.5	54.9	56.1	49.7	48	55.3	67	57.8	54.43	7	0
16	53.2	52.7	53.6	47.4	48.9	53.3	60.9	54.27	52.63	14	0
17	50.2	49.9	50.6	43.4	43.3	49.7	61.9	53.9	48.52	9	0
18	47.4	46.8	48.1	41.9	40.4	45.7	59.2	54.57	44.07	10	0
19	45.3	44.7	46	42.3	38.2	42.3	56.8	53.72	41.39	15	0
20	46.1	45.6	46.6	42	39.2	43.8	57.4	55.21	41.8	6	0
21	45.1	44.5	45.8	39.9	37.7	44.5	56.5	52.04	41.83	10	0
22	44.1	43.8	44.4	39.8	36.3	42.6	56.1	50.76	40.98	3	0
23	41.9	41.1	42.7	37	35	40.2	53.3	46.94	39.56	9	0
24	42.2	41.9	42.6	37.4	35.5	40.7	53.2	47.39	39.79	6	0
25	40.8	40.2	41.3	35.5	34.5	38.1	52.4	44.78	38.87	8	0
26	39.8	39.2	40.4	33.4	33.3	38.5	51.5	44.95	37.4	11	0
27	35.9	35.4	36.3	31.7	31.4	33.6	44.7	41.57	33.2	2	0
28	36.4	36.1	36.8	33	31.4	34.6	45.2	41.53	34.02	5	7.7
29	40.2	39.4	41	36.1	34.6	39	49.5	42.37	39.19	3	16
30	37.1	36.2	38.1	33.7	31.7	34.7	46.7	40.15	35.72	4	0
31	35.7	35.1	36.4	31.6	30.8	34.5	44.3	40.75	33.38	7	2.5
32	34.5	34.2	34.8	30.6	31.2	31.7	42.1	38.21	32.75	2	0
33	35.5	35.1	35.9	30.1	29.2	34	46.6	41.57	32.68	1	0
34	33.9	33.3	34.5	28.9	27.7	32.5	44.5	38.18	31.9	6	0
35	36	35.1	37	31.1	29.6	34.8	46.8	39.28	34.53	5	1.9
36	32.1	31.6	32.6	29.4	26.1	29.4	42	34.85	30.8	10	29.6
37	32.2	31.5	33	29	25.7	29.8	43	36.95	30.03	4	0
38	29.5	28.8	30.3	27.5	24.5	26.7	38.2	34.87	27.03	10	34.5
39	28	27.4	28.5	24.2	22.7	25.2	37.7	33.5	25.35	6	34.7

S3 file continued

Week	Proportion of individuals using LLINs per week (%)*	Raw data for Figure 3		Raw data for Figure 4				Raw data for Figure 5		Raw data for Figure 6	
		Male	Female	<5 Year	5-14 year	15-24 Year	>24 Year	≤1km	>1km	Malaria (PF and PV) episodes	Rainfall (mm)
40	25.4	24.7	26	21.2	19.8	23.2	35.3	29.65	23.33	14	0
41	25.3	24.8	25.7	21.6	19.3	23.4	35.2	26.43	24.7	9	6.7
42	25.2	24.7	25.7	21.5	19.4	23.5	34.9	27.16	24.26	8	63.6
43	22.5	22.2	22.8	19.2	16.8	21.8	31.1	23.83	21.84	2	7.4
44	22.4	21.9	22.9	19.2	17.9	20.9	30.4	26.78	20.37	5	8.6
45	19.8	19	20.5	14.6	13.2	19.9	30	23.7	17.93	10	65.5
46	18.5	17.8	19.2	13.6	12.9	19.3	27	23.25	16.27	12	30
47	17.8	16.8	18.9	12.5	12.1	19	26.5	19.05	17.24	15	20.6
48	16.5	15.7	17.3	11.8	11.5	16.9	24.5	18.53	15.51	29	8.7
49	15.4	14.5	16.3	11.4	10	15.2	23.9	18.64	13.88	17	0.1
50	14.1	13.3	15	11.1	8.6	14.6	21.6	17.72	12.44	15	19.7
51	13.5	13	14.1	9.7	8.5	13.9	21.2	15.9	12.42	24	29.8
52	13.7	13.2	14.3	10.8	8.6	13.5	21.3	18.01	11.68	26	24
53	12.7	12.3	13.2	9.7	8.1	13.2	19.4	17.59	10.45	31	24.6
54	12.2	11.9	12.5	9.5	7.7	12.7	18.6	15.02	10.87	16	2.1
55	11.1	10.6	11.5	9.1	6.4	11.1	17.4	12.53	10.36	17	0.3
56	10	9.5	10.6	9.6	5.1	9.4	16.2	14.06	8.14	12	0
57	12.7	12.3	13.1	10.3	8.6	11.8	19.3	13.44	12.38	17	0
58	8.1	7.6	8.6	7.5	5	6.6	12.8	12.47	5.98	19	0
59	6.2	6	6.4	6.6	3.2	4.8	10.3	10.86	4	8	0
60	7.8	7.2	8.4	6.6	4.9	7.1	12.2	11.02	6.29	10	0
61	9	8.5	9.6	9.1	5.4	8.1	13.6	11.86	7.7	6	2.4
62	8.8	8.4	9.2	8.8	5.1	7.8	13.5	10.51	7.99	5	0.4
63	9.2	8.6	9.9	9.7	5.3	8.3	13.9	10.98	8.41	2	0
64	8.7	8.3	9.1	8	5.7	7.7	13	9.81	8.13	3	0
65	8.2	7.9	8.6	7.8	5.1	12.7	12.5	9.09	7.83	3	0
66	6.5	6.2	6.8	6.3	3.3	5.7	10.8	7.92	5.85	5	0
67	5.7	5.5	5.9	5.7	2.8	4.9	9.3	7.7	4.72	8	0
68	5.5	5.3	5.7	5.4	2.9	5.3	8.7	7.45	4.61	8	0
69	5.3	4.9	5.8	5.7	2.6	4.8	8.5	7.8	4.15	12	0
70	5.8	5.4	6.2	6.4	3.1	5	9.1	7.82	4.89	10	3
71	5.8	5.5	6.1	5.6	3.3	5.4	8.9	8.26	4.61	9	14.5
72	5.4	5	5.8	5.3	2.9	4.9	8.6	7.35	4.43	6	5.1
73	4.9	4.4	5.4	5	2.3	5.1	7.6	6.73	4.02	7	0
74	4.3	3.9	4.7	4	2.2	4.2	6.9	5.39	3.76	10	0
75	4.4	4.1	4.8	3.9	2.5	4.3	6.9	5.89	3.71	5	0
76	4.7	4.4	4.9	4.6	2.8	4.7	6.8	6.12	3.96	5	0
77	4.5	4.2	4.8	4.8	2.9	4.3	6.2	5.97	3.8	12	0
78	4	3.9	4	4.2	2.2	3.9	5.9	5.46	3.29	9	3.7
79	4.2	4.2	4.3	4	2.7	3.9	6.4	5.72	3.53	6	3.4

S3 file continued

Week	Proportion of individuals using LLINs per week (%)*	Raw data for Figure 3		Raw data for Figure 4				Raw data for Figure 5		Raw data for Figure 6	
		Male	Female	<5 Year	5-14 year	15-24 Year	>24 Year	≤1km	>1km	Malaria (PF and PV) episodes	Rainfall (mm)
80	4.1	4.1	4.1	3.7	2.9	3.8	6	5.28	3.57	8	1.4
81	4.3	4.4	4.2	4.5	2.4	4.1	6.3	4.73	4.02	6	8.7
82	3.7	3.6	3.9	4	1.9	3.7	5.6	5.54	2.86	11	7.55
83	3.3	3.2	3.4	3.2	1.9	3.1	5.1	4.58	2.7	11	41
84	2.7	2.5	2.9	2.6	1.4	2.7	4.2	3.52	2.28	6	16
85	2.8	2.7	2.9	2.7	1.6	2.6	4.3	3.8	2.32	19	9.1
86	2.5	2.4	2.6	2.7	1.5	2.2	3.7	2.76	2.36	14	70.3
87	1.7	1.7	1.7	1.6	1	1.5	2.6	1.76	1.68	12	107.8
88	2	1.8	2.1	1.7	1.2	1.9	3	2.29	1.79	28	54.1
89	2.6	2.4	2.7	1.9	1.8	2.3	3.9	2.3	2.68	14	0
90	2.3	2.2	2.4	1.5	1.7	2.2	3.5	2.15	2.33	5	3.9
91	1.6	1.5	1.7	1.1	1	1.5	2.6	1.68	1.54	15	21.1
92	1.1	1.1	1.2	1	0.9	0.8	1.7	1.34	1.04	22	0
93	1.1	1.1	1.1	0.8	0.7	1.1	1.7	1.28	1.03	14	30
94	1	1	1	0.8	0.6	0.9	1.6	1.51	0.76	13	7
95	1.1	1.1	1.2	1	0.8	1	1.7	1.07	1.15	7	43.2
96	1	1	1	1	0.7	1	1.4	1.3	0.9	9	32.1
97	0.8	0.8	0.9	0.6	0.5	0.9	1.3	1.02	0.73	12	84
98	0.8	0.7	0.9	0.7	0.6	0.8	1.1	0.77	0.82	19	41.2
99	0.6	0.6	0.6	0.6	0.5	0.7	0.5	0.72	0.48	15	44
100	0.5	0.6	0.4	0.6	0.3	0.6	0.6	0.68	0.41	10	16.2
101	0.6	0.7	0.5	0.6	0.3	0.7	0.9	0.98	0.4	9	28
102	0.5	0.6	0.4	0.6	0.2	0.6	0.7	0.83	0.36	10	32
103	0.5	0.7	0.4	0.7	0.2	0.6	0.8	0.92	0.36	4	42
104	0.5	0.6	0.4	0.6	0.2	0.6	0.7	0.74	0.38	2	15.3
105	0.4	0.4	0.4	0.4	0.1	0.4	0.6	0.57	0.32	11	12.1
106	0.3	0.4	0.2	0.4	0.1	0.4	0.4	0.28	0.34	10	33.2
107	0.3	0.4	0.3	0.4	0.1	0.4	0.5	0.51	0.25	11	21.9
108	0.3	0.4	0.3	0.3	0.2	0.5	0.5	0.49	0.28	19	30
109	0.4	0.4	0.3	0.4	0.1	0.5	0.5	0.45	0.31	4	1
110	0.6	0.6	0.5	0.5	0.2	0.7	0.9	0.87	0.41	8	0
111	0.5	0.5	0.5	0.6	0.2	0.5	0.7	0.66	0.37	6	0
112	0.4	0.5	0.4	0.5	0.2	0.5	0.6	0.68	0.3	0	0.7
113	0.4	0.4	0.4	0.5	0.2	0.5	0.5	0.68	0.29	3	0
114	0.3	0.3	0.2	0.3	0.2	0.4	0.4	0.36	0.25	5	0
115	0.3	0.4	0.2	0.4	0.1	0.5	0.4	0.38	0.27	4	1.7
116	0.3	0.3	0.2	0.2	0.1	0.4	0.4	0.3	0.25	2	18.1
117	0.2	0.3	0.2	0.1	0.1	0.3	0.3	0.15	0.25	1	0
118	0.2	0.2	0.1	0.2	0	0.2	0.3	0.23	0.15	0	0
119	0.2	0.2	0.1	0.2	0.1	0.3	0.3	0.27	0.15	0	0
120	0.2	0.3	0.1	0.2	0.1	0.3	0.3	0.23	0.19		0
121	0.1	0.1	0.1	0.2	0	0.2	0.1	0.13	0.12		0

* Proportion of individuals using LLINs per week (%) was used to construct Figs 3, 4, 5 and 6.

III

Paper III

Solomon T, Loha E, Deressa W, Gari T, Lindtjorn B. Spatiotemporal clustering of malaria in southern-central Ethiopia: A communitybased cohort study. PLoS ONE. 2019;14 e0222986.

PLOS ONE

RESEARCH ARTICLE

Spatiotemporal clustering of malaria in southern-central Ethiopia: A community-based cohort study

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Abstract

Introduction

Understanding the spatiotemporal clustering of malaria transmission would help target interventions in settings of low malaria transmission. The aim of this study was to assess whether malaria infections were clustered in areas with long-lasting insecticidal nets (LLINs) alone, indoor residual spraying (IRS) alone, or a combination of LLINs and IRS interventions, and to determine the risk factors for the observed malaria clustering in southern-central Ethiopia.

Methods

A cohort of 34,548 individuals residing in 6,071 households was followed for 121 weeks, from October 2014 to January 2017. Both active and passive case detection mechanisms were used to identify clinical malaria episodes, and there were no geographic heterogeneity in data collection methods. Using SaTScan software v 9.4.4, a discrete Poisson model was used to identify high rates of spatial, temporal, and spatiotemporal malaria clustering. A multilevel logistic regression model was fitted to identify predictors of spatial malaria clustering.

Results

The overall incidence of malaria was 16.5 per 1,000 person-year observations. Spatial, temporal, and spatiotemporal clustering of malaria was detected in all types of malaria infection (*P. falciparum*, *P. vivax*, or mixed). Spatial clustering was identified in all study arms: for LLIN + IRS arm, a most likely cluster size of 169 cases in 305 households [relative risk (RR) = 4.54, P<0.001]; for LLIN alone arm a cluster size of 88 cases in 103 households (RR = 5.58, P<0.001); for IRS alone arm a cluster size of 58 cases in 50 households (RR = 7.15, P<0.001), and for control arm a cluster size of 147 cases in 377 households (RR = 2.78, P<0.001). Living 1 km closer to potential vector breeding sites increased the odds of being in spatial clusters by 41.32 fold (adjusted OR = 41.32, 95% CI = 3.79–138.89).

Conclusions

The risk of malaria infection varied significantly between *kebeles*, within *kebeles*, and even among households in areas targeted for different types of malaria control interventions in low malaria transmission setting. The results of this study can be used in planning and implementation of malaria control strategies at micro-geographic scale.

Trial registration

PACT R2014 11000 882128 (8 September 2014).

Introduction

Malaria is a major global public health problem. In 2017, there were about 219 million malaria cases and 435,000 related deaths worldwide [1]. Among these, an estimated 92% of cases of malaria and 93% of deaths occurred in Sub-Saharan Africa [1]. In Ethiopia, 60% of the population is at risk, and 68% of the land is favorable for malaria transmission [2]. *Anopheles arabiensis* is the main malaria vector, and *Plasmodium falciparum* (60%) and *Plasmodium vivax* (40%) are the main malaria parasites in Ethiopia [2, 3]. Malaria transmission is seasonal and unstable in many parts of the country [2, 4, 5], occurring mostly between September and December, following the July and August rainfalls. Another smaller peak occurs in May and June, following short rains [6].

Over the last 15 years, considerable efforts (e.g., increased vector control, improved diagnosis and treatment) have led to a decline in malaria morbidity and mortality. The overall reduction in the global incidence of malaria is estimated at 37%, and the reduction in malaria-specific mortality is estimated at 60% [7]. Similar reductions have been observed in Ethiopia [3, 8]. However, despite these gains, control efforts remain inadequate, and malaria continues to be a major health problem [9].

Studies suggest that additional steps can be taken to further reduce malaria infection [10, 11], such as a more targeted intervention using available, though limited, resources in low to moderate malaria transmission areas [10, 12]. Studies have shown that 20% of a source population for infectious diseases could contribute to 80% of cases in the wider population, and such transmission often occurs in aggregate (clusters) [10, 13]. Woolhouse and colleagues suggest that this 20/80 rule may be useful for improving control of diseases such as malaria, which are transmitted heterogeneously and occur in clusters [14]. In other words, targeting the 20% source population could be more effective than targeting the whole population. Moreover, programs that fail to reach this clustered source population are less effective in reducing infection in the wider population [11, 14].

To facilitate targeted malaria control in high-risk populations [10, 11], understanding the epidemiological and spatiotemporal transmission of the disease is helpful. Malaria transmission is highly heterogeneous across geography and time due to complex interactions among parasites, vectors, and hosts [12, 15, 16]. The physical and seasonal environments directly influence spatial patterns of malaria transmission by creating nonrandom pathogen and vector distributions. Several studies have shown that mosquito distribution, prevalence, and incidence of malaria can vary over short distances between high-elevation and low-elevation areas, between neighboring villages, and even within a single village, due to small variations in risk factors [17–21]. For example, malaria is uncommon in high-elevation areas, because

mosquitoes require high temperatures, high humidity, and suitable aquatic habitats to complete their pre-adult life cycles [22]. Conversely, areas with dams, irrigation canals, wetlands, man-made pools, rain pools, shoreline floods, and agricultural field puddles can influence the spatiotemporal pattern of malaria transmission [23, 24]. Transmission also is affected by proximity to mosquito breeding sites and the type of malaria control [19]. In the past decade, several studies have examined the spatiotemporal distribution of malaria in Ethiopia [16, 19, 25, 26]. However, these studies did not investigate how malaria interventions affect the heterogeneity of malaria transmission and the underlying risk factors for malaria clustering. Only one study tried to quantify the relationship between malaria transmission patterns and malaria intervention by assessing the use of insecticide-treated nets and indoor residual spraying (IRS) in a southern Ethiopian village with a high malaria infection rate [19]. Variation in malaria transmission according to different types of malaria control interventions (long-lasting insecticidal nets (LLINs) alone, IRS alone, a combination of LLINs and IRS) in areas of Ethiopia with low transmission rates has not yet been fully explored.

To fill this gap in the literature, we assessed the spatiotemporal patterns of malaria transmission in the presence of different malaria controls in a low-transmission area of southern-central Ethiopia. This study was a part of the cluster- randomized controlled trial utilizing the data collected for primary analysis published in elsewhere [27].We followed a large cohort of 34,548 people from October 2014 to January 2017 (121 weeks) in 13 *kebeles* (the lowest government administrative unit) that were targeted for the trial [27, 28]. The objectives of this study were to assess whether malaria infection were clustered in areas with LLINs alone, IRS alone, a combination of LLINs and IRS interventions, and to determine the risk factors for the observed clustering. The findings will help improve understanding of malaria distribution and prevention methods on a local scale.

Materials and methods

Ethical statement

The National Ethics Committee of the Ethiopian Ministry of Science and Technology (Ref: 3.10/446/06) and Institutional Review Board of the College of Health Sciences of Addis Ababa University approved the study protocol. We also obtained approval from the Regional Committee for Medical and Health Research Ethics, Western Norway (Ref: 2013/986/REK vest). Permission letters from the Oromia Regional State Health Bureau, East Shewa Zonal Health Department, and Adami Tullu District Health Office were written to the local administrators. Before implementing the study, a consultative meeting was conducted with representatives from each of these three organizations.

Sensitization meetings were conducted with the community elders and with *kebele* and village leaders to discuss the objectives, randomization procedures, implementation, follow-up, and expected outcomes of the study. Because most of the study population could not read and write, we obtained verbal informed consent from the heads of households or other household members older than 18 years. We used a standard information sheet to explain the purpose of the study. The participants were informed that their participation was voluntary and that they could refuse or withdraw from the study at any time. The participants were assured that refusal to participate in the study would not affect their right to use health services in the health posts. The information about the study was read to the study participants using an information sheet written in their language (Afan Oromo). Consent was recorded using a checkmark. As previously described, all participants who tested positive for *P. falciparum* or *P. vivax* on a rapid diagnostic test (RDT), a product of Premier Medical Corporation Limited, India, were treated at the health post with anti-malaria drugs according to national malaria treatment guidelines

[6]. Individuals with severe illness were referred to the nearest health center for further investigation and treatment.

Study area

The study was conducted in the Adami Tullu district of the Oromia Regional State, located approximately 160 km south of Addis Ababa, the capital city of Ethiopia (Fig 1). The district is in the Great Rift Valley, with altitudes ranging from 1500 m to 2300 m. The climate is semi-arid, with an average annual precipitation of 700 mm, which peaks during the rainy season in July and August. The annual rainfall of the district was 813 mm in 2014, 471 mm in 2015, and 890 mm in 2016. The average maximum temperature was 27˚C in 2014, 29˚C in 2015, and 28˚C in 2016 [29]. The majority of the population lives in rural areas. Economic activity in the district is limited to subsistence farming, livestock rearing, and to a lesser extent, fishing in Lake Zeway. Houses consist of mud walls and thatched or corrugated iron roofs. The Oromo is the largest ethnic group in the district. Based on the 2007 national census, approximately 173,000 people lived in the district in 2014 [30]. The district has 48 *kebeles*, each with an average population of 1,000 to 5,000 people [30]. In 2014, there were two hospitals (one public and one non-governmental), nine public health centers, and 43 health posts in the district. Each *kebele* has at least one health post staffed by two health extension workers who report to the health center.

As a major health problem in the study area, malaria transmission is seasonal and unstable [31]. Most transmission occurs between September and December, following the monsoon rains in July and August [6]. A smaller peak of malaria transmission occurs between May and June, following rains in March and April [6]. Moreover, the shores and irrigated areas around Lake Zeway serve as potential mosquito breeding sites [23, 32]. The principal malaria vector in this area is *An. arabiensis*, and the two main malaria parasites are *P. falciparum* and *P. vivax* [33, 34]. During the study period, a severe drought occurred in the area following the El Nino effect in 2015 [35].

Study design and participants

This study was part of a larger study, MalTrials, which aimed to evaluate whether the combined use of LLINs and IRS with propoxur provides additional protection against *P. falciparum* and *P. vivax* among all age groups, compared with LLINs alone or IRS alone [27, 28]. MalTrials was conducted in 13 *kebeles* adjacent to Lake Zeway. It used a 2x2, factorial, cluster-randomized, controlled design with four arms: LLIN + IRS; LLIN alone; IRS alone; and routine (control), which received standard Ethiopian malaria prevention. The unit of randomization was villages (clusters) that contained approximately 35 households and 196 people. The sample included 176 clusters within 5 km of Lake Zeway. In October 2014, eligible study participants received new PermaNet 2.0 LLINs free of charge. Based on national malaria guidelines [6], 7,740 LLINs were distributed to 3,006 households in the two eligible study arms (LLIN alone and LLIN + IRS). Eligible households (IRS alone and LLIN + IRS) received IRS with propoxur free of charge in September 2014, July 2015, and July 2016. See the MalTrials protocol and results for a detailed description of the study [27, 28].

This cohort study included all age groups and was conducted for 121 weeks, from October 2014 to January 2017. We recruited 24 field data collectors with college diplomas from the respective *kebeles* to conduct the baseline and update censuses and weekly follow-up data collection. Three supervisors were recruited to monitor the overall data collection process and data quality. All received five days of training on the use of questionnaires, interviewing techniques, household visits, and supervision. All study participants were followed on a weekly

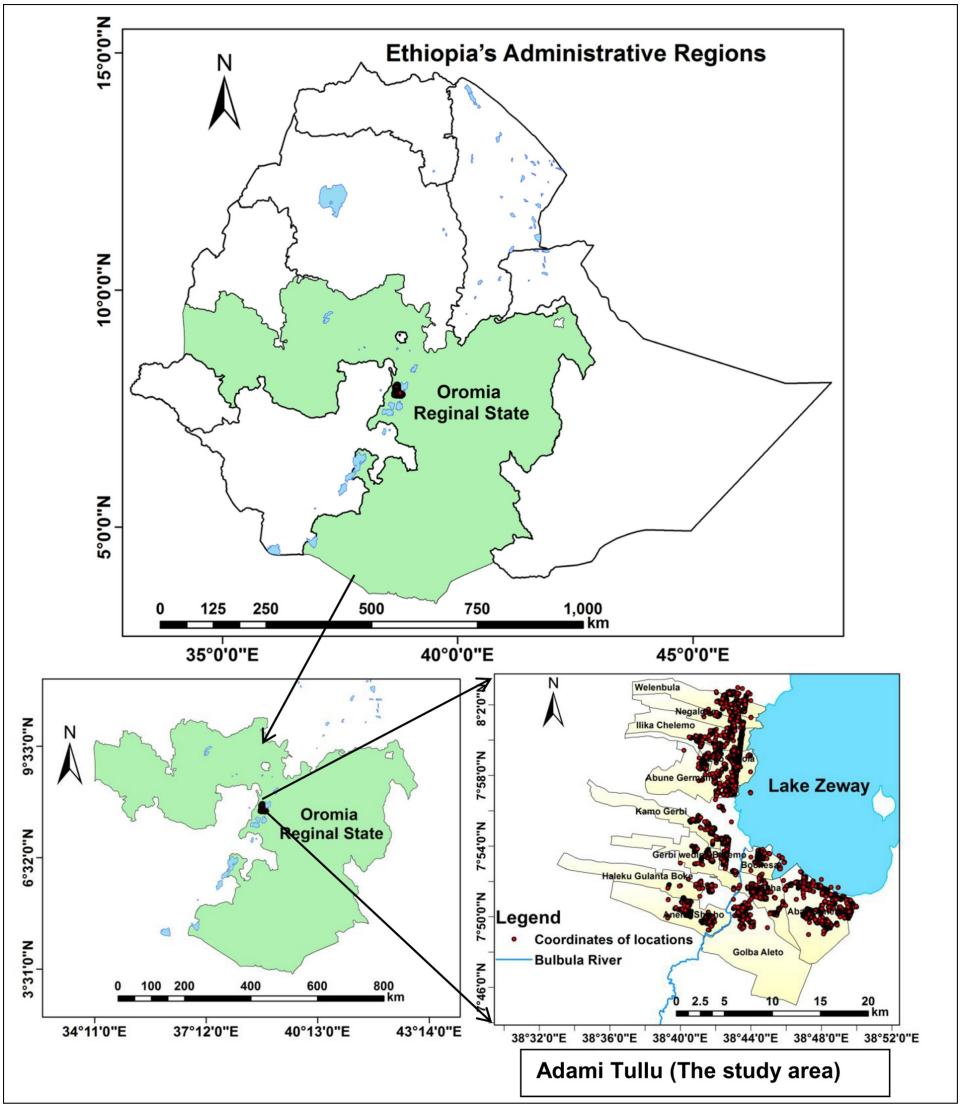

Fig 1. Map of Ethiopia, including the study location in the Adami Tullu district in southern-central Ethiopia. Red dots indicate households participating in the study.

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basis for the duration of the study period unless they were lost to follow-up (e.g., moved to another location, refused to participate, or died). Newcomers (individuals who joined a cohort as new household members) and children born during the study period also were included. The flow diagram illustrating the follow-up of study participants reported elsewhere [27]. Thirteen nurses (one nurse per health post per *kebele*) were recruited and trained on blood sample

collection for the RDT, malaria diagnosis and treatment, and documentation of data. To ensure accurate data collection, refresher trainings were conducted in July 2015 and July 2016.

We assigned each household a metal plate with a unique identification number, and data collectors affixed the plate to the main entrance of the house. We also gave a unique identification card with a number corresponding to the unique number posted on the metal plate on the main entrance of each house. We advised the residents to come to the health posts with the unique identification card if they got febrile in the days between the weekly home visits. Study households were geo-referenced using a hand-held global positioning system (GPS) device (Garmin GPSMAP 60CSx, Garmin International Inc., Olathe, KS, USA).

Baseline and subsequent censuses of the target population

We conducted a baseline census survey in July 2014 to collect individual- and household-level data. Individual-level data included age, sex, education status, religion, marital status, occupation, ethnicity, and morbidity. Household-level data included availability of household assets (e.g., television, radio, telephone, bed, chair, table, bike, animal cart, motor bike, car), access and types of latrine, source of drinking water, possession of land or animal, and type of construction material of the house. In July 2015 and July 2016, subsequent censuses were conducted to update for births, and in- and out-migration. To collect this information, the interviewer used a pre-tested questionnaire that was adopted from a pilot study of the trial [34]. The questionnaire was developed in English and then translated into the local language, Afan Oromo.

Weekly follow-up data collection

Malaria episodes were identified using both active and passive case detection mechanisms. At weekly home visits, study participants with history of fever in the last 48 hours were registered and referred to a health post for malaria testing (active case detection). On days between weekly visits, the study participants were advised to report to the health post if they became febrile (passive case detection). At the weekly home visits, the names of the individuals who used the LLIN the night before the date of the visit were recorded.

Heads of households were the preferred respondent to all questions during data collection. In the absence of a head of household, family members $\geq$18 years old were asked to respond to questions. If no such person was available, the data collectors visited the house at least three more times within the same week.

Malaria diagnosis and patient management

A malaria diagnosis was carried out at the health posts using a RDT. For the RDT, a nurse performed a single finger prick to collect a sample from the febrile patient and tested the sample. An individual with more than one positive RDT within a 30-day period was considered a single episode of malaria.

Based on the RDT results, patients with *P. falciparum* or mixed infection were treated with artemether-lumefantrine (Coartem), and patients with *P. vivax* infection were treated with chloroquine according to national malaria treatment guidelines [6]. Three health centers and one hospital were quarterly visited by field supervisors to collect data about malaria cases among study participants who visited the health facilities but did not report to our field workers. A malaria case was defined as a study participant who presented to the health post with symptoms of malaria (fever, chills, malaise, headache, or vomiting) and who had a positive RDT for *P. falciparum*, *P. vivax*, or mixed infection.

Data analysis

Data were visualized using ESRI ArcMap 10.3.1 (ESRI, Redlands, CA, USA) software. The World Geodetic system 1984 and Universal Transverse Mercator Zone 37˚N were used to define the coordinates' projection. Three Microsoft Excel files (case, population, and coordinate) were prepared as input data for the Poisson probability model. Kulldorff's spatial and space-time scan statistics were used to identify statistically significant retrospective clusters (purely spatial, purely temporal, and space-time) of high malaria rates using a Poisson probability model. SaTScan version 9.4.4 software was used to identify locations and periods of statistically significant clusters. The scan statistics computed data gradually across space and time to identify the number of observed and expected observations within each scanning window at each location and time. The scanning window shapes included a circle for space, an interval for time, and a cylinder with a circular base for space-time. In the space-time analysis, a circular geographic base represented space and corresponding height represented the time in months.

We used spatial scan statistics with circular windows of varying sizes from zero to a maximum radius of less than 50% of the total population at risk, allowing relocation across the study area. An unlimited number of overlapping circles of different sizes were obtained, and each circular window was a possible cluster. The corresponding log likelihood ratio (LLR) and relative risk (RR) were calculated for each circular window. The window with the maximum LLR was defined as the most likely cluster if the P-value <0.05. A criterion of "no geographic overlap" was used to report secondary clusters [36].

We applied space-time scan statistics using cylindrical windows with circular bases and heights corresponding to monthly timescale. The radius of each circular base allowed variation from zero to a maximum size of 50% of the total population, and the height of the cylinder varied in size from zero to 50% of the study period within one month. An infinite number of overlapping cylinders with different dimensions were obtained, and each cylinder was a candidate cluster. For each possible space-time cluster, the LLR and RR were calculated, and the most likely cluster was defined as the cylinder with the highest LLR having a P-value <0.05 [36]. The statistical significance of the clusters was tested using 999 Monte Carlo simulations. The P-value was obtained using a combination of the Monte Carlo, sequential Monte Carlo, and Gumbel approximations [36].

Spatial malaria clusters may appear due to underlying aggregation of one or more known risk factors within cluster areas. A non-random distribution of unstudied risk factors and spatial dependence could explain the lack of difference in known risk factors between a cluster and non-cluster area [37]. Tobler's first law of geography on spatial dependency states that "everything is related to everything else, but nearby objects are more related than distant objects" [37]. Thus, to identify the underlining contributing factors for spatial malaria clustering observed in the study area, we compared malaria cases within identified spatial clusters (most likely and secondary) with malaria cases outside of the clusters. We applied a multilevel logistic regression model to account for malaria clustering effect within a group at the individual and village levels. Individual malaria cases (first level) were nested within the village (second level), assuming a difference in risk of spatial clustering of malaria between villages but a similar risk within a village.

Based on this assumption, the presence of clustering was checked before fitting the model. First, a null, single-level (standard), logistic regression model was fitted to the data. Then, a null, multilevel, logistic regression with the random village effect was fitted. The calculated likelihood ratio test statistics showed strong evidence of a village effect on the status of spatial clustering of malaria (Chi-square = 1024.50, $P<0.001$). Thus, to account for the clustering

effect, we used a multilevel, logistic, regression model to estimate unadjusted and adjusted odds ratio (OR) with a 95% confidence interval (CI). The dependent variable is a binary variable and shows whether a malaria case was present within the identified spatial clusters or not (yes/no). We considered the following potential predictor variables based on their risk for malaria infection [13, 19, 34, 38–40]: age (<5, 5–14, 15–24 or >24 years), sex (male or female), family size (≤5 persons or >5 persons), educational status of head of household (illiterate, can read and write, primary, or secondary and above), occupational status of head of household (farmer or others), wealth index (poorest, poor, medium, rich or richest), intervention group (LLIN + IRS, LLIN only, IRS only or routine (control) arm), and distance from a lake or river (km) used as a continuous variable. Independent variables having P-values <0.25 in bivariate analyses were included in the multivariate logistic regression model for identifying independent risk factors of spatial malaria clustering, adjusting for other variables. Since the intervention group was our main variable that we wanted to test its effect on the final model, we included it in the multivariate logistic regression model irrespective of the P-value result in bivariate analysis. All tests were two-tailed, and the level of statistical significance was set at $P<0.05$.

We used principal component analysis (PCA) to construct a relative household wealth index [41, 42]. Fourteen household asset variables were included in the PCA model: presence of electricity and ownership of a television, radio, mobile telephone, chair, table, bed, bicycle, land, separate kitchen, livestock, livestock cart, types of roof (corrugated iron sheet vs. thatch) and wall (wood with mud/wood with mud and cement vs. no wall/only wood). The variables were dichotomized and coded as "1" if the household owned the asset or "0" if not. The Kaiser-Meyer-Olkin measure of sample adequacy was 0.79. A factor score derived from the first PCA was used to construct the wealth index. It represented 23.6% of the variance in the sample, with an Eigen value of 3.3. For descriptive purposes, the resulting index scores were used to assign households into quintiles: poorest, poor, medium, rich, and richest (see S1 File for the details).We used a proximity analysis tool in ESRI ArcMap 10.3.1 to calculate the distance (in km) between a household and the nearest potential vector breeding site from the border of Lake Zeway or the Bulbula River, and the nearest health facilities.

Results

Characteristics of the study population

The study comprised 34,548 people in 6,071 households. One-fifth, or 6,488 (18.8%), of the study participants were children younger than five years. Half, or 17,227 (50.2%), were male. More than half, or 3,345 (55.9%), of heads of households were illiterate, and 4,436 (74.5%) were farmers. Approximately half, or 3,106 (51.2%), of study households had a family size greater than five persons. One-third, or 2,051 (33.8), were located within 1 km of a potential mosquito breeding site. Table 1 describes the baseline study characteristics.

Incidence of malaria

From October 1, 2014, to January 31, 2017, we documented 1,183 episodes of malaria in the study area. Of these, 652 (55.1%) were due to *P. falciparum* infection, 299 (25.3%) due to *P. vivax* infection, and 232 (19.6%) were mixed *P. falciparum* and *P. vivax* infections. Of the 34,548 people under follow-up during the 121 weeks, 1,059 (3.1%) developed at least one clinical episode of malaria with a range of 1 to 5 episodes. Similarly, of the 6,071 households, 812 (13.4%) had at least one malaria episode. Within the study period, the overall incidence of malaria was 16.5 episodes per 1,000 person-year observations (PYOs). These rates were 9.1

Table 1. Baseline characteristics of study participants and their households, southern-central Ethiopia, October 2014 to January 2017.

Variable	n (%)
Age in years (n = 34548)	
<5	6488 (18.8)
5–14	11136 (32.2)
15–24	6822 (19.8)
>24	10102 (29.2)
Sex (n = 34548)	
Male	17327 (50.2)
Female	17221 (49.8)
Educational status of head of household (n = 5981)[a]	
Illiterate	3345 (55.9)
Can read and write	560 (9.4)
Primary	1487 (24.9)
Secondary and above	589 (9.8)
Occupational status of head of household (n = 5956)[a]	
Farmer	4436 (74.5)
Others	1520 (25.5)
Family size[a]	
≤5 persons	2965 (48.8)
>5 persons	3106 (51.2)
Wealth index[a]	
Poorest	1216 (20.0)
Poor	1199 (19.8)
Medium	1229 (20.2)
Rich	1206 (19.9)
Richest	1221 (20.1)
Intervention arm[a]	
LLIN + IRS	1618 (26.7)
LLIN only	1388 (22.9)
IRS only	1527 (25.2)
Routine (control)	1538 (25.3)
Distance from lake or river[a]	
≤1 km	2051 (33.8)
>1 km	4020 (66.8)

[a] Household-level characteristics (n = 6071 households, unless otherwise specified), LLIN = long-lasting insecticidal nets, IRS = indoor residual spraying

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episodes per 1,000 PYOs for *P. falciparum*, 4.2 per 1,000 PYOs for *P. vivax*, and 3.2 per 1,000 PYOs for mixed infection. Table 2 shows the results.

Spatial clustering of malaria

We found areas with higher risk of malaria infection than in the underlying at-risk populations at the *kebele*, village, and household levels. The most likely and secondary significant spatial clusters for all malaria types (*P. falciparum*, *P. vivax*, or mixed) were identified at each geographic scale. The most likely cluster for each type occurred in the northern part of the study area, with the same geographic area at each geographic scale. The most likely clusters of *P.*

Table 2. Malaria incidence rate per 1,000 person-year observations, southern-central Ethiopia, October 2014 to January 2017.

Variable	Person years	*Plasmodium falciparum*		*Plasmodium vivax*		Mixed		Total	
		Episodes	IR	Episodes	IR	Episodes	IR	Episodes	IR
Total population	71862	652	9.1	299	4.2	232	3.2	1183	16.5
Age in years									
<5	12742	150	11.8	69	5.4	51	4.0	270	21.2
5–14	23727	192	8.1	99	4.2	84	3.5	375	15.8
15–24	14000	69	4.9	47	3.4	32	2.3	148	10.6
>24	21393	241	11.3	84	3.9	65	3.0	390	18.2
Sex									
Male	36179	331	9.1	146	4.0	115	3.2	592	16.4
Female	35683	321	9.0	153	4.3	117	3.3	591	16.6
Educational status of head of household									
Illiterate	40028	333	8.3	165	4.1	112	2.8	610	15.2
Read and write	7396	80	10.8	44	5.9	47	6.4	171	23.1
Primary	17518	184	10.5	67	3.8	53	3.0	304	17.4
Secondary and above	5999	49	8.2	21	3.5	18	3.0	88	14.7
Occupational status of head of household									
Farmer	55156	499	9.0	256	4.6	199	3.6	954	17.3
Others	15434	146	9.5	39	2.5	31	2.0	216	14.0
Family size									
≤5 persons	21672	195	9.0	84	3.9	65	3.0	344	15.9
>5 persons	50190	457	9.1	215	4.3	167	3.3	839	16.7
Wealth index									
Poorest	14316	152	10.6	73	5.1	37	2.6	262	18.3
Poor	14406	153	10.6	61	4.2	42	2.9	256	17.8
Medium	14247	118	8.3	61	4.3	61	4.3	240	16.8
Rich	14390	115	8.0	52	3.6	55	3.8	222	15.4
Richest	14503	114	7.9	52	3.6	37	2.6	203	14.0
Intervention arm									
LLIN + IRS	18713	180	9.6	86	4.6	57	3.0	323	17.3
LLIN only	17244	173	10.0	69	4.0	36	2.1	278	16.1
IRS only	17153	153	8.9	68	4.0	68	4.0	289	16.8
Routine (control)	18752	146	7.8	76	4.1	71	3.8	293	15.6
Distance from lake or river									
≤1 km	22723	251	11.0	135	5.9	115	5.1	501	22.0
>1 km	49139	401	8.2	164	3.3	117	2.3	682	13.9

IR = Incidence rate, LLIN = long-lasting insecticidal nets, IRS = indoor residual spraying

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falciparum and *P. vivax* did not overlapped geographically at household level. However, there was complete overlap in the secondary significant clusters of *P. falciparum* and *P. vivax* (Fig 2).

Moreover, a spatial clustering of malaria was detected among children 1 to 15 years and adults greater than 15 years in a separate analysis at household level. Despite variations in size of the clusters, all the identified significant clusters were overlapped among children 1 to 15 years and adults greater than 15 years (S1 Table and S1 Fig).

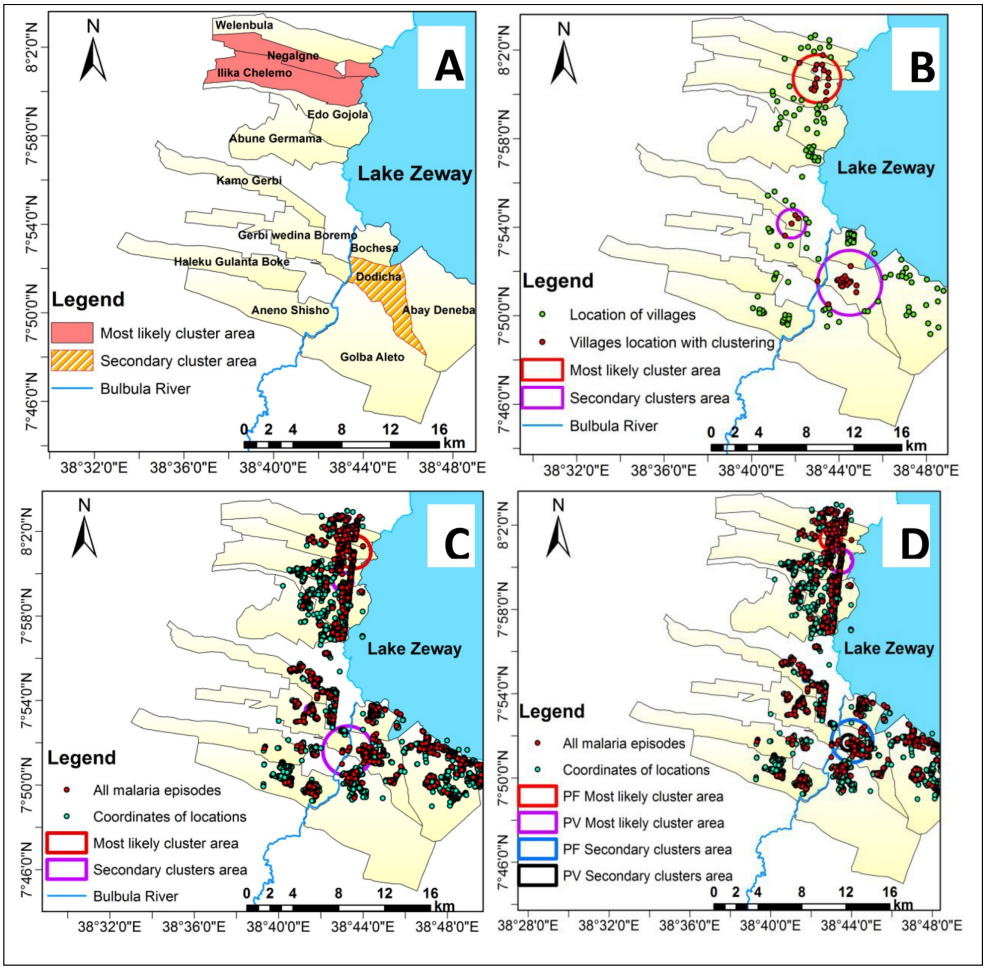

Fig 2. Most likely cluster and secondary clusters of all malaria types in southern-central Ethiopia at different scales using purely spatial scan statistics, October 2014 to January 2017. Panel A shows clustering at the *kebele* level, panel B at the village level, panel C at the household level, and panel D shows clustering of *Plasmodium falciparum* and *Plasmodium vivax* species at the household level.

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We conducted purely spatial scan analysis to identify areas with low rate of LLIN use using a discrete Poisson model. For this analysis, we used the average household level LLIN use both in LLIN alone and LLIN+IRS arms. Low LLIN use clusters were defined as areas having significantly lower average LLIN use than the underlining study area during the study period. Therefore, households or study participants in the study area were grouped into two categories: 1) households or study participants within low LLIN use clusters (clusters of significantly lower than expected LLIN use); and, 2) households or study participants in non-cluster (all other households or study participants outside the identified low LLIN use clusters). The analysis revealed the presence of significantly low LLIN use in the northern and southern parts of

the study area. Meanwhile, the identified most likely high rate malaria cluster overlapped with the cluster of low rate of LLIN use (S2 Fig). Moreover, the risk of malaria infection in the identified low LLIN use clusters was significantly higher than non-cluster area by adjusting for distance from potential breeding site. People living in low LLIN use clusters were 2.20 times at increased risk of malaria infection than those living in non-cluster area (adjusted Hazard Ratio = 2.20, 95% CI = 1.80–2.60). See the S2 Table for details.

For all types of malaria episodes, the most likely significant cluster was identified in two of the 13 *kebeles* (Ilka Chalemo and Negalign), and a significant secondary cluster was detected in one *kebele* (Dodicha). Compared with people living in the other *kebeles*, those living in Ilka Chalemo and Negalign were 3.30 times more likely and those in Dodicha were 2.25 times more likely to contract malaria. This risk was 6.80 for *P. falciparum* in Negalign and 2.83 for *P. vivax* in Ilka Chalemo and Negalign. Table 3 shows the results.

People in villages within the most likely significant cluster area were 3.55 times more at risk of contracting all types of malaria than those living outside the cluster area. This risk was 8.69 for *P. falciparum* and 3.25 for *P. vivax* malaria infections. At the village level, each malaria type had two significant secondary clusters. Table 4 shows the results.

Households within the most likely significant cluster were 4.75 times more at risk of contracting all types of malaria than households outside the cluster. This risk was 9.19 for *P. falciparum* and 5.79 for *P. vivax* malaria infection. At the household level, all malaria types had five secondary clusters, and the *P. falciparum* and *P. vivax* malaria species each had two secondary clusters. Table 5 shows the results.

In a separate analysis for each study arm at the household level for all malaria types, all four study arms (LLIN + IRS, LLIN alone, IRS alone, and routine) had most likely clusters. Except for the LLIN + IRS arm, all other arms had two secondary clusters. Households within the most likely cluster in the LLIN + IRS arm were 4.54 times more at risk of contracting all types of malaria infections than households outside the cluster in the same intervention arm. This risk was 5.58 within the LLIN alone arm, 7.15 within the IRS alone arm, and 2.78 within the routine arm. See the S3 Table for details.

Spatiotemporal clustering of malaria

We analyzed space-time scan statistics at the household level. In the study district, both most likely and secondary spatiotemporal clusters were identified for *P. falciparum* and *P. vivax*

Table 3. Purely spatial scan statistics of the most likely cluster and secondary clusters of malaria episodes at the *kebele* level, southern-central Ethiopia, October 2014 to January 2017.

Cluster	*Kebele*	Pop.	# episodes	Expected cases	Annual episodes per 1000	RR	LLR	P-value
All malaria types*								
Most likely	Ilka Chalemo, Negalign	3654	332	125.1	38.9	3.30	138.8	<0.001
Secondary	Dodicha	3360	231	115.1	29.4	2.25	51.6	<0.001
Plasmodium falciparum								
Most likely	Negalign	1132	122	21.4	46.1	6.80	120.4	<0.001
Secondary	Dodicha	3360	143	63.4	18.2	2.61	42.3	<0.001
Secondary	Qamo Garbi	1442	55	27.2	16.3	2.12	11.5	<0.001
Plasmodium vivax								
Most likely	Ilka Chalemo, Negalign	3654	75	31.6	8.8	2.83	25.1	<0.001
Secondary	Dodicha	3360	62	29.1	7.9	2.43	16.1	<0.001
Secondary	Garbi Widena	1617	26	14.0	6.9	1.94	4.4	0.047

* *Plasmodium falciparum*, *Plasmodium vivax*, or mixed, RR = Relative risk, LLR = Log likelihood ratio

https://doi.org/10.1371/journal.pone.0222986.t003

Table 4. Purely spatial scan statistics of the most likely cluster and secondary clusters of malaria episodes at the village level, southern-central Ethiopia, October 2014 to January 2017.

Cluster	# villages	Coordinates	Radius (km)	Pop.	# episodes	Expected cases	Annual episodes per 1000	RR	LLR	P-value
All malaria types*										
Most likely	17	8.012083 N, 38.716507 E	2.03	3605	346	123.44	41.05	3.55	159.3	<0.001
Secondary	19	7.858422 N, 38.741448 E	2.69	3055	246	104.60	34.30	2.69	77.7	<0.001
Secondary	4	7.902991 N, 38.697144 E	1.16	568	58	19.46	43.67	3.08	25.5	<0.001
Plasmodium falciparum										
Most likely	5	8.022632 N, 38.716322 E	0.95	927	126	17.49	58.13	8.69	150.1	<0.001
Secondary	11	7.863378 N, 38.737913 E	0.73	1637	103	30.89	26.91	3.77	56.28	<0.001
Secondary	6	7.920306 N, 38.692410 E	1.83	971	49	18.32	21.58	2.81	18.28	<0.001
Secondary	2	8.027165 N, 38.691838 E	1.28	246	17	4.64	29.55	3.73	9.83	0.004
Plasmodium vivax										
Most likely	18	8.006982 N, 38.724748 E	2.15	3602	82	31.17	9.74	3.25	33.63	<0.001
Secondary	1	7.893858 N, 38.692012 E	0.0	228	19	1.97	35.64	10.21	26.5	<0.001
Secondary	15	7.871003 N, 38.742309 E	1.83	2219	55	19.20	10.60	3.28	24.5	<0.001

* *Plasmodium falciparum*, *Plasmodium vivax*, or mixed, RR = Relative risk, LLR = Log likelihood ratio

https://doi.org/10.1371/journal.pone.0222986.t004

infections. Each type had two secondary spatiotemporal clusters. Fig 3. Shows the identified most likely cluster and secondary clusters.

For all malaria types, the most likely spatiotemporal cluster lasted for 12 out of the 28-month study period, with varying start and end times, and clustering started on November 1, 2014. For *P. falciparum*, clustering began on December 1, 2014. For *P. vivax*, it began on October 1, 2014. The coverage area for all types of malaria (2.53 km) was larger than that for *P. falciparum* (1.49 km) and *P. vivax* (1.04 km). However, the relative risk of infection was highest

Table 5. Purely spatial scan statistics of the most likely cluster and secondary clusters of malaria episodes at the household level, southern-central Ethiopia, October 2014 to January 2017.

Clusters	# locations	Coordinates	Radius (km)	Pop.	# episodes	Expected cases	Annual episodes per 1000	RR	LLR	P-value
All malaria types*										
Most likely	330	8.0175 N, 38.7262 E	1.5	1881	254	64.4	57.8	4.75	176.0	<0.001
Secondary	412	7.8606 N, 38.7213 E	2.2	2515	220	86.1	37.4	2.91	81.0	<0.001
Secondary	31	7.893 N, 38.6914 E	0.4	189	32	6.5	72.4	5.05	25.9	<0.001
Secondary	5	7.9122 N, 38.6949 E	0.2	26	10	0.9	164.5	11.32	15.1	<0.001
Secondary	123	7.9937 N, 38.7173 E	0.9	680	50	23.3	31.5	2.20	11.8	0.017
Secondary	28	7.954 N, 38.7132 E	0.2	225	24	7.7	45.6	3.16	11.1	0.027
Plasmodium falciparum										
Most likely	146	8.0232 N, 38.7161 E	1.0	828	120	15.6	62.0	9.19	149.3	<0.001
Secondary	443	7.8629 N, 38.7339 E	1.9	2716	136	51.3	21.4	3.09	54.2	<0.001
Secondary	7	7.9118 N, 38.6952 E	0.2	42	12	0.8	122.2	15.40	21.5	<0.001
Plasmodium vivax										
Most likely	156	8.0052 N, 38.7247 E	1.0	847	38	7.3	19.2	5.79	33.5	<0.001
Secondary	28	7.8927 N, 38.6914 E	0.4	174	19	1.5	46.7	13.41	31.2	<0.001
Secondary	187	7.8616 N, 38.7307 E	0.7	1174	40	10.2	14.6	4.39	26.6	<0.001

* *Plasmodium falciparum*, *Plasmodium vivax*, or mixed, RR = Relative risk, LLR = Log likelihood ratio

https://doi.org/10.1371/journal.pone.0222986.t005

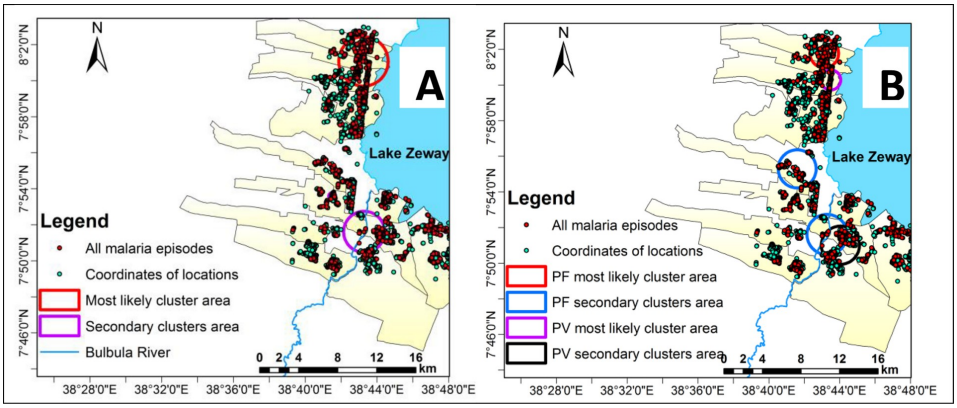

Fig 3. Most likely cluster and secondary clusters of malaria episodes identified using space-time scan statistics, southern-central Ethiopia, October 2014 to January 2017. Panel A shows all malaria episodes. Panel B shows *Plasmodium falciparum* and *Plasmodium vivax* episodes.

https://doi.org/10.1371/journal.pone.0222986.g003

for the *P. vivax* cluster, where people were 10.4 times more likely to contract *P. vivax* than households outside the cluster. This risk was 4.3 for all types of malaria and 8.9 for *P. falciparum*. See the S4 Table for details.

Temporal clustering of malaria

In the study district, most likely purely temporal clusters were observed in all types of malaria, in *P. falciparum*, and in *P. vivax* malaria infections. The most likely purely temporal clusters were observed between September 1, 2015, and November 30, 2015, for all malaria types, when the risk of contraction in the purely temporal cluster was 2.25 times higher than during the rest of the study period. This risk was 2.36 for *P. falciparum* and 2.81 for *P. vivax*. Secondary purely temporal clusters were not observed in all categories of malaria infection in the study period. Table 6 and S3 Fig show the results.

Risk factors for spatial clustering of malaria

In this analysis, we compared the characteristics of malaria cases in the identified spatial clusters (n = 499) with characteristics of cases outside of the clusters (n = 560). In the bivariate, multilevel, logistic regression analysis, we found significant difference in cases within clusters and outside of clusters with regards to distance from a potential vector breeding site. Similarly, in the multivariate analysis, distance from a potential vector breeding site continued as significant predictor of spatial malaria clustering. Living 1 km closer to a potential vector breeding

Table 6. Purely temporal scan statistics of the most likely clusters of malaria, southern-central Ethiopia, October 2014 to January 2017.

Cluster	# locations	Timeframe	# episodes	Expected cases	Annual episodes per 1000	RR	LLR	P-value
All malaria types*	All	2015/9/1 to 2015/11/30	250	126	29.0	2.25	54.8	<0.001
Plasmodium falciparum	All	2015/9/1 to 2015/11/30	143	69.5	16.6	2.36	34.6	<0.001
Plasmodium vivax	All	2015/9/1 to 2015/11/30	75	31.9	8.7	2.81	24.8	<0.001

**Plasmodium falciparum, Plasmodium vivax*, or mixed, RR = Relative risk, LLR = Log likelihood ratio

https://doi.org/10.1371/journal.pone.0222986.t006

Table 7. Multilevel, logistic regression for predictors of spatial clustering of all types of malaria at the household level, southern-central Ethiopia, October 2014 to January 2017.

Variables	Cases within identified spatial cluster		Unadjusted OR (95%CI)	P-value	Adjusted OR (95% CI)	P-value
	Yes n (%)	No n (%)				
Age in years						
<5	118 (48.2)	127 (51.8)	1		NA	
5–14	160 (47.9)	174 (52.1)	0.97 (0.40–2.34)	0.947		
15–24	63 (46.0)	74 (54.0)	0.18 (0.25–2.57)	0.718		
>24	158 (46.1)	185 (53.9)	1.62 (0.61–4.34)	0.332		
Sex						
Male	260 (49.4)	266 (50.6)	1		NA	
Female	239 (44.8)	294 (55.2)	1.17 (0.60–2.27)	0.664		
Family size						
≤5	149 (47.9)	162 (52.1)	1		NA	
>5	350 (46.8)	398 (53.2)	1.22 (0.59–2.51)	0.593		
Educational status of head of household						
No education	228 (41.5)	321 (58.5)	1		1	
Read and write	78 (52.3)	71 (47.7)	0.96 (0.28–3.38)	0.951	0.88 (0.10–7.57)	0.909
Primary	144 (52.9)	128 (47.1)	1.72 (0.77–3.84)	0.188	1.85 (0.76–4.54)	0.176
Secondary and above	47 (58.8)	33 (41.2)	2.88 (0.68–12.22)	0.152	3.45 (0.61–19.59)	0.162
Occupational status of head of household						
Farmer	397 (46.8)	452 (53.2)	1		NA	
Others	95 (47.7)	104 (52.3)	1.02 (0.75–2.33)	0.652		
Wealth index						
Poorest	93 (44.0)	109 (54.0)	1		1	
Poor	106 (46.3)	123 (53.7)	0.67 (0.24–1.85)	0.441	1.70 (0.47–6.15)	0.421
Medium	89 (42.4)	121 (57.6)	0.41 (0.09–1.85)	0.247	0.70 (0.18–2.71)	0.604
Rich	117 (52.9)	104 (47.1)	1.04 (0.19–5.79)	0.966	1.69 (0.14–20.33)	0.680
Richest	94 (47.7)	103 (52.3)	1.18 (0.24–5.86)	0.841	1.67 (0.16–17.59)	0.668
Intervention arm						
LLIN + IRS	136 (47.2)	152 (52.8)	1		1	
LLIN only	112 (44.3)	141 (55.7)	0.35 (0.01–9.22)	0.533	0.56 (0.23–1.38)	0.208
IRS only	123 (47.1)	138 (52.9)	0.33 (0.01–8.58)	0.508	0.45 (0.16–1.26)	0.130
Routine (control)	128 (49.8)	129 (50.2)	0.41 (0.02–8.58)	0.563	1.32 (0.48–3.62)	0.595
Distance from lake or river (km)*						
Mean (SD)	1.30 (1.02)	1.88 (1.38)	33.67 (10.69–106.04)¥	<0.001	41.32 (3.79–138.89)¥	<0.001

n = number of malaria cases, OR = Odds ratio, NA = not applicable (P>0.25 in bivariate analysis), LLIN = long-lasting insecticidal nets, IRS = indoor residual spraying

*At village level: mean (SD) distance from potential breeding site for clusters = 1.40 (0.90), for non-clusters = 2.10 (1.51), unadjusted OR (95%CI) = 1.52 (1.11–2.04).

¥The reciprocal of the OR (95% CI) is presented to show the risk of proximity to a potential vector breeding site.

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site increased the odds of being in a spatial cluster by 41.32 fold (adjusted OR = 41.32, 95% CI = 3.79–138.89). Meanwhile, we found no difference with regard to age, sex, family size, educational status of head of household, occupational status of head of household, wealth index, or study arm between malaria cases found in an identified spatial malaria clusters and cases outside of the clusters (Table 7).

To identify village level risk factor for spatial clustering of malaria, we used logistic regression model. The three independent variables included in the village level analysis were: The

intervention arm, distance from the nearest health facilities and distance from the potential vector breeding site. The only variable that was significantly associated with spatial clustering of malaria was distance from the potential vector breeding site. Villages found in 1 km closer to a potential vector breeding site at increased odds of being in a spatial cluster by 1.5 fold (adjusted OR = 1.5, 95% CI = 1.15–1.93). See the S5 Table for details.

Discussion

We found purely spatial, purely temporal, and spatiotemporal clustering of malaria infection in southern-central Ethiopia. This finding shows that malaria infection was not randomly distributed at the *kebele*, village, or household levels in areas with different malaria control interventions.

As part of a large, cluster-randomized control trial, our study compared the incidence of malaria transmission based on combined interventions (LLINs and IRS) and individual interventions (LLINs alone or IRS alone) [27, 28]. We followed a large cohort of people (n = 34548) in the rural communities of the Adami Tullu district from October 2014 to January 2017 (28 months) to evaluate malaria risk in low-risk and high-risk malaria transmission seasons. The study findings could improve understanding of the micro-geographic heterogeneity of malaria transmission, which can be useful for planning targeted malaria control interventions in small areas. Moreover, the findings can be generalized to many parts of Ethiopia with similar geographic, topographic, and socio-economic conditions.

In the current study, the overall malaria incidence was 16.5 episodes per 1,000 PYOs over the 28 months of follow-up. The incidence was lower than that found in a pilot study that was conducted in the same study area from August 2013 to December 2013, in which the average incidence was 4.6 episodes per 10,000 person-week observations (approximately 24 episodes per 1,000 PYOs) [34]. The difference may be due to the timing of the pilot study, which was conducted during the high malaria transmission season. The incidence also was lower than that of a previous longitudinal study from southern Ethiopia (45.1 per 1,000 PYOs) [19] and the national average incidence between 2011 and 2016 (29.0 cases per 1,000 PYOs) [3]. This lower incidence of malaria observed in the current study area could be related to climate irregularity caused by the 2015 El Nino effect [35] or to differences in coverage of malaria control interventions.

Using spatial scan statistics, we identified locations with high risks of malaria infection. Similar findings have been reported elsewhere in Ethiopia [19, 25, 26, 43]. In the present study, three *kebeles* out of 13 accounted for nearly half (47.6%) of all malaria episodes, and 15.3% of households in the identified clusters accounted for half (50%) of all malaria episodes. Thus, malaria infection was localized and frequent in high-burden clusters in low malaria transmission settings. Targeted interventions in these high-burden clusters can optimize resources and improve effectiveness of malaria elimination programs [10, 11].

Despite variations in size and location of spatial clustering of malaria between study groups, all four study arms (LLINs + IRS, LLIN alone, IRS alone, and routine) showed malaria clustering in separate analyses, with no significant differences in the risk of clustering at individual case or village level (Table 7, S5 Table). The results from the main trials also showed no significant differences in the incidence of malaria across study arms [27]. These results indicate that using LLINs and IRS in combination or alone may not prevent malaria clustering in areas with low rates of malaria transmission. The reason for this lack of difference might be related to the effect of residual transmission, which primarily occurs due to the outdoor and early evening indoor biting behavior of *An. arabiensis* in the study area [27, 44, 45]. In contrast to our study, another cohort study in southern Ethiopia shows that the use of IRS with deltamethrin affected

the spatiotemporal clustering of malaria, but LLINs did not [19]. This difference in findings might be due to the difference in malaria burdens between the study areas (16.5 episodes per 1,000 PYO in our study vs. 45.1 episodes per 1,000 PYO in the other study) [19].

The space-time scan statistics identified high-risk areas for all malaria types over space and time. All the most likely clusters were in locations with identified spatial clusters. Although the overall incidence of malaria was low in the study area and period, there were relatively high malaria infections in 12 of 28 months from November 2014 to November 2015. These spikes in infection rates may be related to warmer temperatures from the El Nino effect in 2015 [29]. As the warmest year on record, 2015 had an average maximum temperature of 29˚C, which was 2˚C warmer than 2014 and 1˚C warmer than 2016 [29]. This warmer temperature may facilitate quick sporogonic development of *Plasmodium* species [46]

The purely temporal cluster analysis aimed to identify high-risk periods for malaria transmission. A significant temporal cluster was observed from September 1, 2015, to November 30, 2015, with peaks in October. This high-risk period is consistent with the high malaria transmission season that occurs in most parts of Ethiopia, following heavy rains in June, July, and August [6, 16]. Thus, malaria interventions before September might further reduce malaria transmission in the study area.

The duration and peaks of infection varied in the study period. For example, in 2015, two major peaks of malaria episodes were observed in January and October. In 2016, two major peaks occurred in June and September. Smaller peaks occurred in between the major peaks each year. In addition to the major risk factors for malaria infection, such as rainfall, temperature, and relative humidity [47–49], local irrigation activity in the study area also may have influenced the observed smaller peaks of malaria infection in dry seasons [23, 32].

We compared cases identified within spatial clusters and those outside of the clusters to further understand the risk factors for malaria clustering. In this analysis, the only factor independently associated with malaria clustering was living close to a potential vector breeding site. The proximity to Lake Zeway or the Bulbula River, which have the most confirmed breeding sites [23, 50], increased the risk of malaria clustering at individual and village level analysis. Previous studies also have reported that close proximity to these sites increases the risk of malaria infection and clustering [19, 48, 51–54]. It is not a surprise to see higher risk of infection in a locality near breeding site of potentially infective Anopheles mosquitoes [13]. Therefore, targeting the households or villages found closer to potential vector breeding site with effective malaria control measures could further decrease the burden of malaria infection. Moreover, there was an indication that clustering of malaria associated with low LLIN use, because the most likely cluster of malaria was imbedded within the cluster of low rate of LLIN use, and also there was increased risk of malaria infection in low LLIN use clusters. Thus, it needs to ensure the utilization of LLINs after distribution by all households to maximize the effect of LLINs on malaria infection.

The Ethiopian Ministry of Health plans to eliminate malaria in 2020 in selected districts with low malaria transmission [55]. To achieve this plan, the Ministry may consider targeted intervention at the *kebele*, village, or individual household level in areas with high-burden malaria clusters. Ideally, such targeted intervention strategies will optimize resources and increase program coverage and effectiveness [11]. To ensure effective implementation of these intervention mechanisms, the Ministry might consider improving identification of malaria clusters.

We believe that our study has some limitations. First, comparing malaria clustering by intervention arm might have been affected by the context of our study period, during which unexpectedly dry and warmer weather conditions occurred following the El Nino effect in 2015. Annual rainfall declined by 60%, and the average temperature increased by 2 ºC above

normal [29]. Severe drought and food shortage also occurred in the study area [35]. Due to the unexpected weather conditions and other behaviors [56], ownership and use of LLINs in the study period dramatically declined after six months of intervention [57, 58]. Our study results may have been different if LLIN ownership and use were higher. Second, a spill-over effect could have occurred between villages of each intervention arm, which may have diluted any difference in the clustering of malaria. Third, we used RDT to confirm the diagnosis of malaria. However, RDT is less sensitive in detecting submicroscopic infection than Polymerase Chain Reaction (PCR) [59, 60]. Compared to all infection, the proportion low density malaria parasite infection is common and have been estimated to be about 20–50% of all malaria episodes in low transmission setting [61, 62]. Therefore, a considerable proportion of submicroscopic infection might be missed in the current study. A study shows that malaria hotspots identified by RDT were not predictive of PCR or microscopy, and long-term stability of hotspots was not observed by RDT in low malaria transmission setting [63]. Moreover, we cannot rule-out the presence of other plasmodium species (such as *Plasmodium ovale* and *Plasmodium malariae)*. However, the prevalence of these infections is less than 1% of malaria cases [64]. Fourth, we opted to use a circular window in the spatial scan statistics to identify the clusters due to its ability to detect other cluster shapes and isotropy with respect to map rotation; however, the true clusters may be elliptic or rectangular. Scan statistics using elliptic or rectangular windows cannot detect these shapes, though, unless all possible angles are considered, which is difficult to compute [36]. Fifth, we did not include all possible risk factors for malaria clustering, such as irrigation-related vector breeding sites and climate (rainfall, temperature, relative humidity). The non-random distribution of these excluded risk factors could be responsible for the observed clustering of malaria.

Conclusion

In conclusion, the risk of malaria infection varied significantly in the study area. We observed high rates of spatial, temporal, and spatiotemporal clustering of malaria episodes at the *kebele*, village, and household levels. Spatial clustering occurred in all four study arms, and the risk of clustering was similar across the arms. Therefore, the results of this study can be used in planning and implementation of malaria control strategies at micro-geographic scale.

Supporting information

S1 Table. Purely spatial scan statistics of the most likely cluster and secondary clusters of malaria episodes at the household level among children and adults, southern-central Ethiopia, October 2014 to January 2017.
(DOCX)

S2 Table. The risk of malaria in the low long-lasting insecticidal net use clusters and non-clusters, southern-central Ethiopia, October 2014 to January 2017.
(DOCX)

S3 Table. Purely spatial scan statistics of the most likely cluster and secondary clusters of all types of malaria episodes by intervention arm at individual level, southern-central Ethiopia, October 2014 to January 2017.
(DOCX)

S4 Table. Space-time scan statistics of the most likely cluster and secondary clusters of malaria at the household level, southern-central Ethiopia, October 2014 to January 2017.
(DOCX)

S5 Table. Predictors of spatial clustering of all types of malaria at the village level, southern-central Ethiopia, October 2014 to January 2017.
(DOCX)

S1 Fig. The most likely cluster and secondary clusters of malaria episodes at the household level among children and adults, southern-central Ethiopia, October 2014 to January 2017.
(TIF)

S2 Fig. Most likely and secondary clusters of all malaria types and areas with low long- lasting insecticidal net use in southern-central Ethiopia, October 2014 to January 2017.
(TIF)

S3 Fig. Monthly malaria incidence showing temporal clusters of all types of malaria (shaded part) and total rainfall (lagged by one month), southern-central Ethiopia, October 2014 to January 2017.
(TIF)

S1 File. Variables used to construct the wealth index and their correlation with the first component.
(DOCX)

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Supplementary information

Paper III

S1 Table. Purely spatial scan statistics of the most likely cluster and secondary clusters of malaria episodes at the household level among children and adults, southern-central Ethiopia, October 2014 to January 2017

Types of malaria	Age (years)	Clusters	# locations	Pop.	# episodes	Expected cases	Annual episodes per 1000	RR	LLR	P-value
All malaria types*	1-15	Most likely	762	2427	236	88.67	41.59	3.7	196.3	<0.001
		Secondary	305	1114	116	40.70	44.53	3.3	51.3	<0.001
		Secondary	23	83	19	3.03	97.90	6.4	19.1	<0.001
	>15	Most likely	342	937	112	30.19	51.12	4.46	72.4	<0.001
		Secondary	58	149	31	4.80	88.98	6.81	32.3	<0.001
		Secondary	92	257	25	8.28	41.60	3.12	11.2	<0.001
Plasmodium falciparum	1-15	Most likely	303	1008	94	19.21	39,88	6.5	84.5	<0.001
		Secondary	393	1477	76	28.15	22.0	3.2	31.7	<0.001
		Secondary	5	22	7	0.42	136.1	17.0	13.2	0.005
	>15	Most likely	146	387	53	7.29	58.57	8.61	63.2	<0.001
		Secondary	58	149	23	2.81	66.01	8.79	28.9	<0.001
		Secondary	126	341	23	6.42	28.84	3.80	13.2	<0.001
Plasmodium vivax	1-15	Most likely	239	748	32	7.35	18.29	5.2	24.4	<0.001
		Secondary	18	65	12	0.64	78.95	20.2	24.2	<0.001
		Secondary	344	1289	38	12.66	12.60	3.6	18.6	<0.001
	>15	Most likely	26	59	7	0.45	50.74	16.6	12.9	0.0046
		Secondary	152	403	15	3.05	15.91	5.5	12.6	0.0062
		Secondary	66	223	11	1.69	21.09	7.07	11.7	0.024

S2 Table. Malaria incidence in the low rate long-lasting insecticidal net use clusters and non-clusters, southern-central Ethiopia, October 2014 to January 2017

Variable	Person years	*Plasmodium falciparum*		*Plasmodium vivax*		Mixed		All types of malaria		Crude HR (95% CI)	Adjusted HR (95% CI)*
		Episodes	IR (95% CI)	Episodes	IR (95% CI)	Episodes	IR (95% CI)	Episodes	IR (95% CI)		
Significant Low rate LLIN use cluster											
No	16706	103	6.2 (5.1-7.4)	50	3.0 (2.2-3.9)	28	1.7 (1.1-2.4)	181	10.8 (9.3-12.5)	1	1
Yes	19250	250	13.0 (11.4-14.6)	105	5.5 (4.5-6.6)	65	3.4 (2.6-4.3)	420	21.8 (19.8-24.0)	1.98 (1.54-2.55)	2.20 (1.80-2.60)

IR=Incidence rate (All IR was calculated per 1,000 person-year observations), LLIN= long-lasting insecticidal nets, HR=hazard ratio,
*adjusted HR calculated for all types of malaria episodes and adjusted for distance from potential mosquitoes breeding site

S3 Table. Purely spatial scan statistics of the most likely cluster and secondary clusters of all types of malaria episodes by intervention arm at household level, southern-central Ethiopia, October 2014 to January 2017

Clusters	# locations	Pop.	# episodes	Expected cases	Annual episodes per 1000	RR	LLR	P-value
Long-lasting insecticidal nets + Indoor residual spraying arm								
Most likely	305	1780	169	63.0	40.6	4.54	86.0	<0.001
Long-lasting insecticidal nets only arm								
Most likely	103	617	88	21.3	61.0	5.58	67.6	<0.001
Secondary	19	118	24	4.1	87.0	6.35	23.4	<0.001
Secondary	9	53	11	1.83	88.8	6.21	10.7	0.011
Indoor residual spraying only arm								
Most likely	50	291	58	9.8	85.2	7.15	59.3	<0.001
Secondary	26	137	38	4.6	118.6	9.32	48.7	<0.001
Secondary	5	26	10	0.9	164.5	11.78	15.4	<0.001
Routine (control) arm								
Most likely	377	2335	147	77.8	26.9	2.78	36.9	<0.001
Secondary	71	384	45	12.8	50.1	3.97	26.3	<0.001
Secondary	16	103	15	3.4	62.3	4.55	10.8	0.009

RR=Relative risk, LLR=Log likelihood ratio

S4 Table. Space-time scan statistics of the most likely cluster and secondary clusters of malaria at the household level, southern-central Ethiopia, October 2014 to January 2017

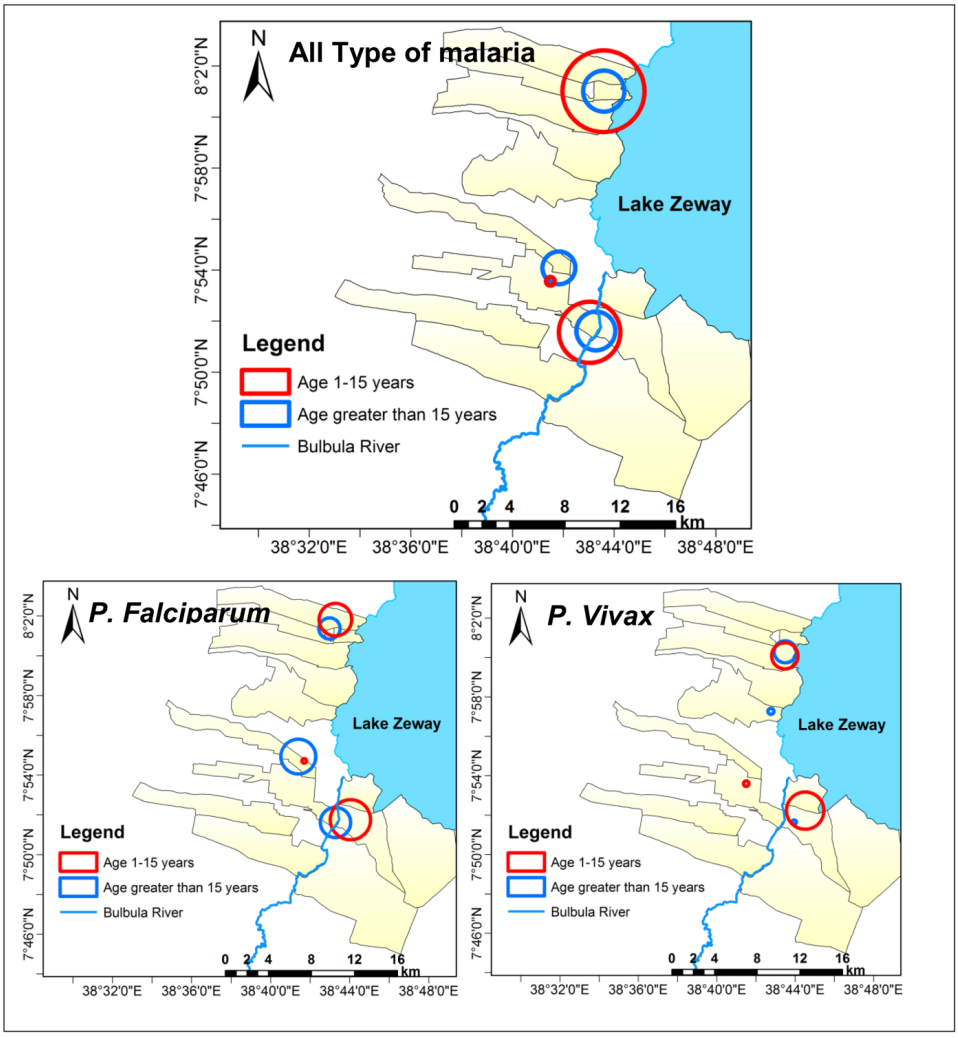
Cluster	# locations	Timeframe	Pop.	# episodes	Expected cases	Annual episodes per 1000	RR	LLR	P-value
All malaria types*									
Most likely	842	Nov. 2014 to Nov. 2015	4706	267	74.5	52.5	4.31	166.0	<0.001
Secondary	403	Sep. 2015 to Sep. 2016	2377	142	37.7	55.1	4.12	88.8	<0.001
Secondary	29	Oct. 2014 to Jan. 2015	183	18	0.9	292.1	20.22	39.9	<0.001
Plasmodium falciparum									
Most likely	355	Dec. 2014 to Dec. 2015	1989	128	17.4	59.4	8.89	155.1	<0.001
Secondary	398	Sep. 2015 to Sep. 2016	2340	77	20.5	30.4	4.07	48.1	<0.001
Secondary	145	May. 2015 to Jun. 2016	899	34	8.5	32.4	4.98	22.2	<0.001
Plasmodium vivax									
Most likely	163	Oct. 2014 to Oct. 2015	847	32	3.4	34.8	10.37	44.6	<0.001
Secondary	651	Oct. 2015 to Nov. 2015	3826	26	2.4	40.7	11.89	39.7	<0.001
Secondary	28	Oct. 2014 to Jan. 2015	174	12	0.2	204.8	57.58	36.6	<0.001

* *Plasmodium falciparum*, *Plasmodium vivax*, or mixed, RR=Relative risk, LLR=Log likelihood ratio

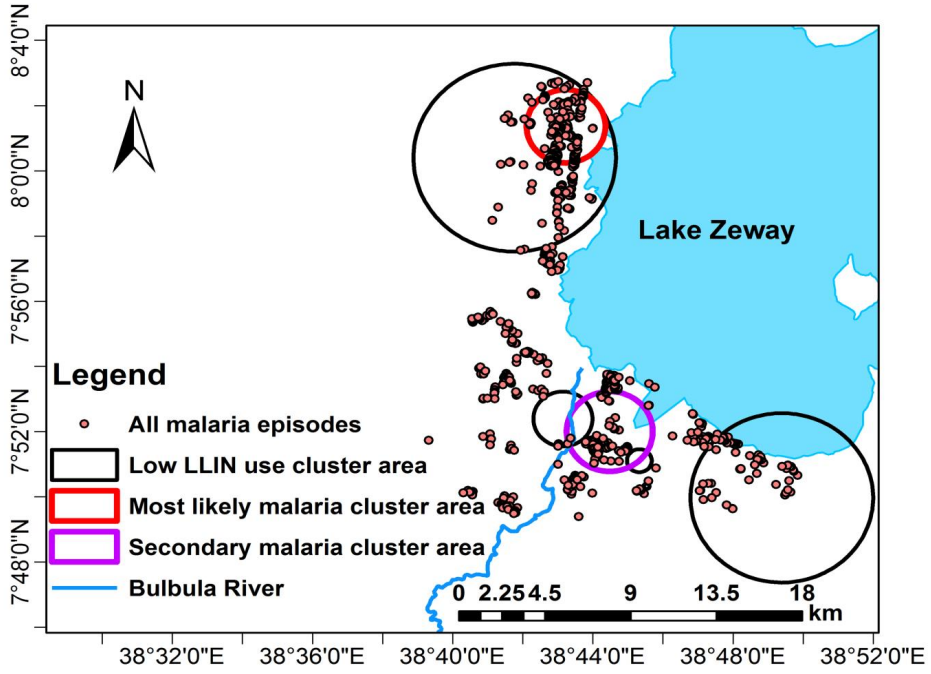
S5 Table. Predictors of spatial clustering of all types of malaria at the village level, southern-central Ethiopia, October 2014 to January 2017

Variables	Village within identified spatial cluster		Unadjusted OR (95%CI)	P-value	Adjusted OR (95% CI)	P-value
	Yes n (%)	No n (%)				
Intervention arm						
LLIN + IRS	13 (29.5)	31 (70.5)	1			
LLIN only	11 (25.0)	33 (75.0)	0.79 (0.31-2.04)	0.633	0.88 (0.33-2.38)	0.802
IRS only	7 (15.9)	37 (84.1)	0.45 (0.16-1.27)	0.133	0.50 (0.17-1.50)	0.218
Routine (control)	8 (18.2)	36 (81.8)	0.53 (0.19-1.45)	0.216	0.67 (0.23-1.93)	0.463
Distance from nearest health facility (km)						
Mean (SD)	1.75 (0.67)	2.01 (0.88)	0.68 (0.45-1.02)	0.062	0.68 (0.42-1.08)	0.102
Distance from lake or river (km)						
Mean (SD)	1.40 (0.90)	2.10 (1.51)	1.51 (1.17-1.93)[¥]	0.001	1.50 (1.15-1.93)[¥]	0.003

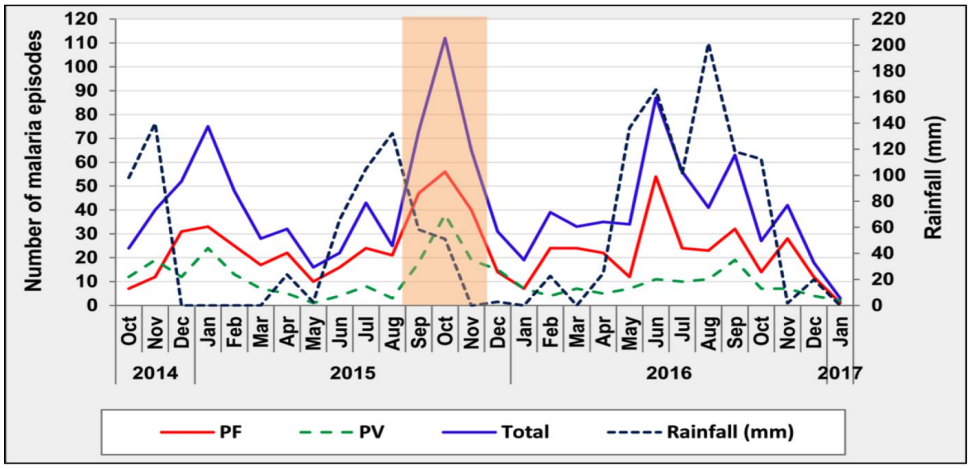
n=number of village, OR=Odds ratio, LLIN= long-lasting insecticidal nets, IRS= indoor residual spraying
[¥]The reciprocal of the OR (95% CI) is presented to show the risk of proximity to a potential vector breeding site.



S1 Figure. The most likely cluster and secondary clusters of malaria episodes at the household level among children and adults, southern-central Ethiopia, October 2014 to January 2017.



S2 Figure. Most likely and secondary clusters of all malaria types and areas with low long-lasting insecticidal net use in southern-central Ethiopia, October 2014 to January 2017.



S3 Figure. Monthly malaria incidence showing temporal clusters of all types of malaria (shaded part) and total rainfall (lagged by one month), southern-central Ethiopia, October 2014 to January 2017.

S1 File. Variables used to construct the wealth index and their correlation with the first component.

Construction of wealth index

Principal component analysis (PCA) was done using 14 variables to construct wealth index. Among these variables twelve variables were binary and the other two variables were dichotomized into meaningful categories (Table 1). The Kaiser-Meyer-Olkin measure of sample adequacy was 0.79. The total variance explained by the first principal component and the corresponding Eigen value was 23.6% and 3.3, respectively. Table 2 shows frequencies, communalities, and correlations.

Table 1. Variables and assigned values

S. No	Variables (n=6071)	Assigned value
1	Electricity	Present = 1, Absent = 0
2	Radio	Present = 1, Absent = 0
3	Television	Present = 1, Absent = 0
4	Mobile telephone	Present = 1, Absent = 0
5	Chair	Present = 1, Absent = 0
6	Table	Present = 1, Absent = 0
7	Bed	Present = 1, Absent = 0
8	Bicycle	Present = 1, Absent = 0
9	Any land used for agriculture	Present = 1, Absent = 0
10	Separate kitchen from living house	Present = 1, Absent = 0
11	Livestock	Present = 1, Absent = 0
12	Animal cart	Present = 1, Absent = 0
13	Main material of the roof	Corrugated iron or cement or concrete = 1, Thatch or leaf = 0
14	Main material of the wall	Wood with mud or wood with mud and cement = 1, No wall or only wood = 0

Table 2. Frequencies of the dichotomized variables, communalities, and correlations with the first component

S.No	Variables (n=6071)	Number (%)	Communalities	Correlations with the first component
1	Electricity	1296 (21.4)	0.652	0.436
2	Radio	1901 (31.3)	0.434	0.477
3	Television	249 (4.1)	0.703	0.427
4	Mobile telephone	3640 (60.0)	0.442	0.530
5	Chair	4293 (70.7)	0.495	0.586
6	Table	2108 (34.7)	0.460	0.659
7	Bed	4257 (70.1)	0.451	0.519
8	Bicycle	1398 (23.0)	0.338	0.487
9	Any land used for agriculture	5606 (92.4)	0.603	0.344
10	Separate kitchen from living house	3128 (51.5)	0.619	0.630
11	Livestock	5000 (82.4)	0.592	0.212
12	Animal cart	1150 (18.9)	0.679	0.616
13	Main material of the roof	3110 (51.2)	0.361	0.339
14	Main material of the wall	1224 (20.2)	0.652	0.436

Appendix I: Questionnaires

HOUSEHOLD QUESTIONNAIRE
The useful life of Bed nets for malaria control in Ethiopia: Durability and Bio-efficacy (Paper I)

Introduction: Hello, my name is "……..." I am from MalTrilas and work on a project investigating how long bed nets last in Ethiopia. You know I am collecting data in weekly base from each household in this village/Gare

To be filled in before the interview

0.1 Household Identification number _____

0.2 Name of interviewer _____

0.3 Date of interview _____/_____/_____ Day/Month/Year)

0.4 Name of Kebele _____

0.5 Name of Zone _____

0.6 Name of village/Gare _____

0.7 Informed Consent Obtained: 1… Yes 2…. No ⟶Stop

Section 1: Household characteristics ***"Now I would like to ask you some general questions about this household."***		
Q #	**Questions and filters**	**Coding category**
1.1	Who is responding to the questions?	1…Head of household 2…Partner of household head 3…Other adult in household, specify_____
1.2	How old is the respondent Age in years?	_____
1.3	Sex of the respondent	1…Male 2…Female
1.4	Is the house has windows? **(Observe)**	1…Yes 2…No, **go to 1.7**
1.5	Are any of the windows screened with netting? **(Observe)**	1…Yes 2…No, **go to 1.7**
1.6	What are the windows screened with? **(Observe)**	1…Wire mesh (metal/plastic) 2…Old bed net 3…Other material, specify _____
1.7	Does the house have an open eave gap? **(Observe)**	1…Yes 2…No

Section 2: *"Now I would like to ask you some questions about your bed nets."*		
2.1	How many people slept in your household last night?	_____
2.2	How many sleeping places are there in your household? ***Include all a net could be hung up, or has ever been hung up, including if there is more than one sleeping space in each room used for sleeping* (Observe)**	Indoors _____ Outdoors _____ Indoors Temporary _____ Outdoors Temporary _____
2.3	How many sleeping places were used last night in your household? **(Observe)**	Indoors _____ Outdoors _____ Indoors Temporary _____ Outdoors Temporary _____
2.4	How many bed nets do you have?(ITN given by Maltrials project)	_____

Now could you please show me the nets in your household? I will need access to the barcode that is attached to the net

Q #	**Questions and filters**	**Net 1**	**Net 2**	**Net 3**	**Net 4**
2.5	Net serial number (Given during data collection for each net)	_____	_____	_____	_____
2.6	Net still in the household ***(those still in package / being used for other purpose)***	1…Yes 2…No → **go to 2.8**	1…Yes 2…No → **go to 2.8**	1…Yes 2…No → **go to 2.8**	1…Yes 2…No → **go to 2.8**
2.7	If yes Q#2.6, Can the net be used for sleeping under? **(Observe)**	1…Yes → **go to 2.18** 2… No	1…Yes → **go to 2.18** 2… No	1…Yes → **go to 2.18** 2… No	1…Yes → **go to 2.18** 2… No
2.8	If "no" Q#2.7, why not?	1…Net thrown away 2…Net used for something else 3…Net was given away 4…Net was sold 5…Net was stolen 99…Don't know	1…Net thrown away 2…Net used for something else 3…Net was given away 4…Net was sold 5…Net was stolen 99…Don't know	1…Net thrown away 2…Net used for something else 3…Net was given away 4…Net was sold 5…Net was stolen 99…Don't know	1…Net thrown away 2…Net used for something else 3…Net was given away 4…Net was sold 5…Net was stolen 99…Don't know
2.9	If "1" Q#2.8 Why was the net thrown away?	1…Too damaged for sleeping under 2…Did not like the net for sleeping under 99…Don't know	1…Too damaged for sleeping under 2…Did not like the net for sleeping under 99…Don't know	1…Too damaged for sleeping under 2…Did not like the net for sleeping under 99…Don't know	1…Too damaged for sleeping under 2…Did not like the net for sleeping under 99…Don't know

2.10	If "1" Q#2.9 How was the net damaged? ***Do not prompt. Record all reasons***	1…By fire 2…Rodents 3…Children 4…Wear and tear 5…Other, specify ______________ 99…Don't know	1…By fire 2…Rodents 3…Children 4…Wear and tear 5…Other, specify ______________ 99…Don't know	1…By fire 2…Rodents 3…Children 4…Wear and tear 5…Other, specify ______________ 99…Don't know	1…By fire 2…Rodents 3…Children 4…Wear and tear 5…Other, specify ______________ 99…Don't know
2.11	If "2" Q#2.9 Why did you not like the net? ***Do not prompt. Record all reasons***	1…Too hot 2…Net too small 3…Net too big 4…Mesh size too big 5…Don't like the feel of the material 6…Don't like the colour 7…Net too dirty 8…Don't like the smell 9…Net makes me sneeze, itch, head ache 10… Infested with bed bugs 11… Doesn't protect against mosquitoes 12…Other, specify____________ 99…Don't know	1…Too hot 2…Net too small 3…Net too big 4…Mesh size too big 5…Don't like the feel of the material 6…Don't like the colour 7…Net too dirty 8…Don't like the smell 9…Net makes me sneeze, itch, head ache 10… Infested with bed bugs 11… Doesn't protect against mosquitoes 12…Other, specify____________ 99…Don't know	1…Too hot 2…Net too small 3…Net too big 4…Mesh size too big 5…Don't like the feel of the material 6…Don't like the colour 7…Net too dirty 8…Don't like the smell 9…Net makes me sneeze, itch, head ache 10… Infested with bed bugs 11… Doesn't protect against mosquitoes 12…Other, specify____________ 99…Don't know	1…Too hot 2…Net too small 3…Net too big 4…Mesh size too big 5…Don't like the feel of the material 6…Don't like the colour 7…Net too dirty 8…Don't like the smell 9…Net makes me sneeze, itch, head ache 10… Infested with bed bugs 11… Doesn't protect against mosquitoes 12…Other, specify____________ 99…Don't know
2.12	If "2" Q#2.8 Why did you use the net for something else?	1…Too damaged for sleeping under 2…Did not like the net for sleeping under 3…More useful things to do with it 99…Don't know	1…Too damaged for sleeping under 2…Did not like the net for sleeping under 3…More useful things to do with it 99…Don't know	1…Too damaged for sleeping under 2…Did not like the net for sleeping under 3…More useful things to do with it 99…Don't know	1…Too damaged for sleeping under 2…Did not like the net for sleeping under 3…More useful things to do with it 99…Don't know
2.13	If used for something else, what was it used for?	1…Screen windows/doors 2…Screen fence/ toilet 3…Protect garden (fence in or cover crops from birds) 4…Protect animals (chickens) 5…Fishing 6…Mattress/pillow 7…Agriculture, e.g. corn collection from field 8…Make rope 9…Stored for visitors 10…Other, specify____________ 99…Don't know	1…Screen windows/doors 2…Screen fence/ toilet 3…Protect garden (fence in or cover crops from birds) 4…Protect animals (chickens) 5…Fishing 6…Mattress/pillow 7…Agriculture, e.g. corn collection from field 8…Make rope 9…Stored for visitors 10…Other, specify____________ 99…Don't know	1…Screen windows/doors 2…Screen fence/ toilet 3…Protect garden (fence in or cover crops from birds) 4…Protect animals (chickens) 5…Fishing 6…Mattress/pillow 7…Agriculture, e.g. corn collection from field 8…Make rope 9…Stored for visitors 10…Other, specify____________ 99…Don't know	1…Screen windows/doors 2…Screen fence/ toilet 3…Protect garden (fence in or cover crops from birds) 4…Protect animals (chickens) 5…Fishing 6…Mattress/pillow 7…Agriculture, e.g. corn collection from field 8…Make rope 9…Stored for visitors 10…Other, specify____________ 99…Don't know

2.14	If "3" Q#2.8 To whom given away?	1…Neighbors 2…Children going to school/college 3…Other relatives 4…Others, specify 99…Don't know	1…Neighbors 2…Children going to school/college 3…Other relatives 4…Others, specify 99…Don't know	1…Neighbors 2…Children going to school/college 3…Other relatives 4…Others, specify 99…Don't know	1…Neighbors 2…Children going to school/college 3…Other relatives 4…Others, specify 99…Don't know
2.15	If "3" Q#2.8 why given away?	1…Too many nets in household 2…Someone else needed net more 3…Replaced it with a better net 4…I do not like to use nets 5…Other, specify____________ 99…Don't know	1…Too many nets in household 2…Someone else needed net more 3…Replaced it with a better net 4…I do not like to use nets 5…Other, specify____________ 99…Don't know	1…Too many nets in household 2…Someone else needed net more 3…Replaced it with a better net 4…I do not like to use nets 5…Other, specify____________ 99…Don't know	1…Too many nets in household 2…Someone else needed net more 3…Replaced it with a better net 4…I do not like to use nets 5…Other, specify____________ 99…Don't know
2.16	If replaced by a better net, why did you like the replacement net more? ***Do not prompt. Record all reasons***	1…Colour, specify which colour is preferred 2…Less damaged 3…Cleaner 4…More suitable size 5…More suitable length 6…Nicer texture / material 7…It was free 8… Other, specify __________ 99…Don't know	1…Colour, specify which colour is preferred 2…Less damaged 3…Cleaner 4…More suitable size 5…More suitable length 6…Nicer texture / material 7…It was free 8… Other, specify __________ 99…Don't know	1…Colour, specify which colour is preferred 2…Less damaged 3…Cleaner 4…More suitable size 5…More suitable length 6…Nicer texture / material 7…It was free 8… Other, specify __________ 99…Don't know	1…Colour, specify which colour is preferred 2…Less damaged 3…Cleaner 4…More suitable size 5…More suitable length 6…Nicer texture / material 7…It was free 8… Other, specify __________ 99…Don't know
2.17	If "5" Q#2.8 When was the net lost from the household?	1…less than 1 month ago 2…between 1 and 3 months ago 3…between 4 and 6 months ago 99…Don't know	1…less than 1 month ago 2…between 1 and 3 months ago 3…between 4 and 6 months ago 99…Don't know	1…less than 1 month ago 2…between 1 and 3 months ago 3…between 4 and 6 months ago 99…Don't know	1…less than 1 month ago 2…between 1 and 3 months ago 3…between 4 and 6 months ago 99…Don't know
2.18	If "yes" Q#2.7 How is the net found? **(Observe)**	1…. Stored away 2….Hanging tied in knot 3…. Hanging loose over sleeping place 4….Visible but not hung up 5….other specify__________	1…. Stored away 2….Hanging tied in knot 3…. Hanging loose over sleeping place 4….Visible but not hung up 5….other specify__________	1…. Stored away 2….Hanging tied in knot 3…. Hanging loose over sleeping place 4….Visible but not hung up 5….other specify__________	1…. Stored away 2….Hanging tied in knot 3…. Hanging loose over sleeping place 4….Visible but not hung up 5….other specify__________
2.19	Why has the net never been used?	1...Save the net for visitors 2…Save the net for future use 3…No place to hang up 4…Currently have enough nets in use	1...Save the net for visitors 2…Save the net for future use 3…No place to hang up 4…Currently have enough nets in use	1...Save the net for visitors 2…Save the net for future use 3…No place to hang up 4…Currently have enough nets in use	1...Save the net for visitors 2…Save the net for future use 3…No place to hang up 4…Currently have

		5…Other, specify ______________	5…Other, specify ______________	5…Other, specify ______________	enough nets in use 5…Other, specify ______________
2.20	What type of sleeping space is the net used for?	1…Wooden bedframe 2…Stick bedframe 3…Iron bedframe 4…Mattress (no frame) 5…Reed mat (no frame) 6…Floor 7…Never used 8…Other, specify_____________	1…Wooden bedframe 2…Stick bedframe 3…Iron bedframe 4…Mattress (no frame) 5…Reed mat (no frame) 6…Floor 07…Never used 08…Other, specify_____________	1…Wooden bedframe 2…Stick bedframe 3…Iron bedframe 4…Mattress (no frame) 5…Reed mat (no frame) 6…Floor 07…Never used 08…Other, specify_____________	1…Wooden bedframe 2…Stick bedframe 3…Iron bedframe 4…Mattress (no frame) 5…Reed mat (no frame) 6…Floor 07…Never used 08…Other, specify_____________
2.21	Was this net used last night?	1…Yes 2…No	1…Yes 2…No	1…Yes 2…No	1…Yes 2…No
2.22	If not, why not? ***Do not prompt. Record all reasons that the person mentions.***	1…User did not sleep here 2…Net was not available to use 3…Used a different net 4…No malaria now 5…No mosquitoes 6…Net too old or too torn 7…Net is dirty 8…Net full of bedbugs 9…Net too hot 10…Net too small 11…Net too big 12…Mesh size too big 13…Don't like the material 14…Don't like the colour 15…Don't like the smell 16…Net made me sneeze, itch, head ache 17…Other, specify______________ 99…Don't know	1…User did not sleep here 2…Net was not available to use 3…Used a different net 4…No malaria now 5…No mosquitoes 6…Net too old or too torn 7…Net is dirty 8…Net full of bedbugs 9…Net too hot 10…Net too small 11…Net too big 12…Mesh size too big 13…Don't like the material 14…Don't like the colour 15…Don't like the smell 16…Net made me sneeze, itch, head ache 17…Other, specify______________ 99…Don't know	1…User did not sleep here 2…Net was not available to use 3…Used a different net 4…No malaria now 5…No mosquitoes 6…Net too old or too torn 7…Net is dirty 8…Net full of bedbugs 9…Net too hot 10…Net too small 11…Net too big 12…Mesh size too big 13…Don't like the material 14…Don't like the colour 15…Don't like the smell 16…Net made me sneeze, itch, head ache 17…Other, specify______________ 99…Don't know	1…User did not sleep here 2…Net was not available to use 3…Used a different net 4…No malaria now 5…No mosquitoes 6…Net too old or too torn 7…Net is dirty 8…Net full of bedbugs 9…Net too hot 10…Net too small 11…Net too big 12…Mesh size too big 13…Don't like the material 14…Don't like the colour 15…Don't like the smell 16…Net made me sneeze, itch, head ache 17…Other, specify______________ 99…Don't know

2.23	Who used this net last night?	_____ _____ _____ _____	_____ _____ _____ _____	_____ _____ _____ _____	_____ _____ _____ _____
2.24	Was this net big enough for the person(s) sleeping under?	1…Yes 2…No 99…Don't know	1…Yes 2…No 99…Don't know	1…Yes 2…No 99…Don't know	1…Yes 2…No 99…Don't know
2.25	During the previous week, how many times has the net been used?	1…Every night 2…5-6 nights 3…1-4 nights 4…None of the nights 99…Don't know	1…Every night 2…5-6 nights 3…1-4 nights 4…None of the nights 99…Don't know	1…Every night 2…5-6 nights 3…1-4 nights 4…None of the nights 99…Don't know	1…Every night 2…5-6 nights 3…1-4 nights 4…None of the nights 99…Don't know
2.26	During which periods of the year is this net used to sleep under?	1…All year 2…Rainy season only 3…Dry season only 99…Don't know	1…All year 2…Rainy season only 3…Dry season only 99…Don't know	1…All year 2…Rainy season only 3…Dry season only 99…Don't know	1…All year 2…Rainy season only 3…Dry season only 99…Don't know
2.27	Is this net ever used for sleeping under away from the main house?	1….Yes 2….No→ **Go to 2.29** 99….Don't know	1….Yes 2….No→ **Go to 2.29** 99….Don't know	1….Yes 2….No→ **Go to 2.29** 99….Don't know	1….Yes 2….No→ **Go to 2.29** 99….Don't know
2.28	If "yes"Where is the net used for sleeping under away from the main house?	1….Taken to school/college 2….Taken to other house 3….Taken to the farm hut/forest 4….Other, specify_____	1….Taken to school/college 2….Taken to other house 3….Taken to the farm hut/forest 4….Other, specify_____	1….Taken to school/college 2….Taken to other house 3….Taken to the farm hut/forest 4….Other, specify_____	1….Taken to school/college 2….Taken to other house 3….Taken to the farm hut/forest 4….Other, specify_____
2.29	Do you tuck the net in at night?	1….Yes 2….No→ **Go to 2.31** 99….Don't know	1….Yes 2….No→ **Go to 2.31** 99….Don't know	1….Yes 2….No→ **Go to 2.31** 99….Don't know	1….Yes 2….No→ **Go to 2.31** 99….Don't know
2.30	Why do you not tuck the net in?	1…Net not long enough 2…Nothing to tuck under 3…Feel too closed in 4…Too much effort 5…Other, specify	1…Net not long enough 2…Nothing to tuck under 3…Feel too closed in 4…Too much effort 5…Other, specify	1…Net not long enough 2…Nothing to tuck under 3…Feel too closed in 4…Too much effort 5…Other, specify	1…Net not long enough 2…Nothing to tuck under 3…Feel too closed in 4…Too much effort 5…Other, specify
2.31	Has the net ever been washed?	1….Yes 2….No → **Go to 2.38** 99….Don't know	1….Yes 2….No → **Go to 2.38** 99….Don't know	1….Yes 2….No → **Go to 2.38** 99….Don't know	1….Yes 2….No → **Go to 2.38** 99….Don't know
2.31.1	If "yes" for Q#2.31 how many times net washed in the past 6 months?	_____	_____	_____	_____

2.32	When was the last time you washed the net?	1…less than 1 month ago 2…between 1-3 months ago 3…between 4-6 months 99…Don't know	1…less than 1 month ago 2…between 1-3 months ago 3…between 4-6 months 99…Don't know	1…less than 1 month ago 2…between 1-3 months ago 3…between 4-6 months 99…Don't know	1…less than 1 month ago 2…between 1-3 months ago 3…between 4-6 months 99…Don't know
2.33	What type of soap was used?	1….None 2….Local soap bar 3….Detergent powder 4….Mix (bar and detergent) 5….Bleach 99….Don't know	1….None 2….Local soap bar 3….Detergent powder 4….Mix (bar and detergent) 5….Bleach 99….Don't know	1….None 2….Local soap bar 3….Detergent powder 4….Mix (bar and detergent) 5….Bleach 99….Don't know	1….None 2….Local soap bar 3….Detergent powder 4….Mix (bar and detergent) 5….Bleach 99….Don't know
2.34	How long did the net soak for?	1….Did not soak the net 2….less than 1 hour 3…..more than 1 hour 99….Don't know	1….Did not soak the net 2….less than 1 hour 3…..more than 1 hour 99….Don't know	1….Did not soak the net 2….less than 1 hour 3…..more than 1 hour 99….Don't know	1….Did not soak the net 2….less than 1 hour 3…..more than 1 hour 99….Don't know
2.35	Was the net scrubbed hard?	1….Yes 2….No 99….Don't know	1….Yes 2….No 99….Don't know	1….Yes 2….No 99….Don't know	1….Yes 2….No 99….Don't know
2.36	Was the net beaten on a hard surface (e.g. rocks, with sticks)?	1….Yes 2….No 99….Don't know	1….Yes 2….No 99….Don't know	1….Yes 2….No 99….Don't know	1….Yes 2….No 99….Don't know
2.37	Where was the net dried?	1….Outside in the direct sun light 2….Outside in the shade 3….Inside 99….Don't know	1….Outside in the direct sun light 2….Outside in the shade 3….Inside 99….Don't know	1….Outside in the direct sun light 2….Outside in the shade 3….Inside 99….Don't know	1….Outside in the direct sun light 2….Outside in the shade 3….Inside 99….Don't know
2.38	In the past month, have you noticed any new holes appear in the net?	1….Yes 2….No → **Go to 2.45** 99….Don't know	1….Yes 2….No → **Go to 2.45** 99….Don't know	1….Yes 2….No → **Go to 2.45** 99….Don't know	1….Yes 2….No → **Go to 2.45** 99….Don't know
2.39	What caused these new holes? ***Allow multiple answers***	1…Tore or split when caught on edge or nail 2…Pulled and torn by a corner 3…Was burned 4…Was caused by rats or mice 5…In another way, specify _____ 99…Don't know	1…Tore or split when caught on edge or nail 2…Pulled and torn by a corner 3…Was burned 4…Was caused by rats or mice 5…In another way, specify _____ 99…Don't know	1…Tore or split when caught on edge or nail 2…Pulled and torn by a corner 3…Was burned 4…Was caused by rats or mice 5…In another way, specify_____ 99…Don't know	1…Tore or split when caught on edge or nail 2…Pulled and torn by a corner 3…Was burned 4…Was caused by rats or mice 5…In another way, specify _____ 99…Don't know
2.40	Have you tried to fix any of these holes?	1….Yes 2….No	1….Yes 2….No	1….Yes 2….No	1….Yes 2….No
2.41	How did you repair the hole?	1…Stitched 2…Knotted/tied 3…Patched 4…Other way, specify_____	1…Stitched 2…Knotted/tied 3…Patched 4…Other way, specify_____	1…Stitched 2…Knotted/tied 3…Patched 4…Other way, specify_____	1…Stitched 2…Knotted/tied 3…Patched 4…Other way, specify_____

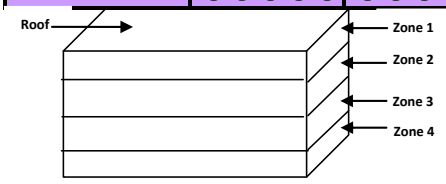
2.42	**If "no"Q#40 ,** what was the main reason?	1…Too busy/no time 2…Not necessary 3…Don't know how to fix 4…Too damaged to fix 5…Other, specify_____	1…Too busy/no time 2…Not necessary 3…Don't know how to fix 4…Too damaged to fix 5…Other, specify_____	1…Too busy/no time 2…Not necessary 3…Don't know how to fix 4…Too damaged to fix 5…Other, specify_____	1…Too busy/no time 2…Not necessary 3…Don't know how to fix 4…Too damaged to fix 5…Other, specify_____
2.43	Has the net been modified?	1…Yes 2…No	1…Yes 2…No	1…Yes 2…No	1…Yes 2…No
2.44	How was the net modified?	1…Shape was changed 2…Material was added to lengthen 3…Material was added to reinforce 4…Other, specify _____	1…Shape was changed 2…Material was added to lengthen 3…Material was added to reinforce 4…Other, specify _____	1…Shape was changed 2…Material was added to lengthen 3…Material was added to reinforce 4…Other, specify _____	1…Shape was changed 2…Material was added to lengthen 3…Material was added to reinforce 4…Other, specify _____
2.45	Do you use any of the following sources for cooking, heating or lighting in the same room as the net?	1…Firewood 2…Charcoal 3…Gas 4…Candle	1…Firewood 2…Charcoal 3…Gas 4…Candle	1…Firewood 2…Charcoal 3…Gas 4…Candle	1…Firewood 2…Charcoal 3…Gas 4…Candle
46	Is there anyone in the same room as the net smoking cigarette	1…Yes 2…No	1…Yes 2…No	1…Yes 2…No	1…Yes 2…No
2.47	Do you ever store food in the room this net is in? (***Observe)***	1…Yes 2…No 99…Don't know	1…Yes 2…No 99…Don't know	1…Yes 2…No 99…Don't know	1…Yes 2…No 99…Don't know
2.48	In the last 6 months, have you seen any rats or mice in this room or their traces (faeces or damage)?	1…Yes 2…No 99…Don't know	1…Yes 2…No 99…Don't know	1…Yes 2…No 99…Don't know	1…Yes 2…No 99…Don't know
2.49	Do cats have access to this room?	1…Yes 2…No 99…Don't know	1…Yes 2…No 99…Don't know	1…Yes 2…No 99…Don't know	1…Yes 2…No 99…Don't know
2.50	What is the main material of the roof in	1…Grass /palm thatch 2…Corrugated iron sheets	1…Grass /palm thatch 2…Corrugated iron sheets	1…Grass /palm thatch 2…Corrugated iron sheets	1…Grass /palm thatch 2…Corrugated iron sheets

	this room? ***Observe***	3… roofed with soil 4… Other, specify_____	3… roofed with soil 4… Other, specify_____	3… roofed with soil 4… Other, specify_____	3… roofed with soil 4… Other, specify_____
2.51	What is the main material of the walls in this room? ***Observe***	1…Mud and sticks 2…Burned bricks 3…Cement bricks 4…Mud bricks 5…Other, specify_____	1…Mud and sticks 2…Burned bricks 3…Cement bricks 4…Mud bricks 5…Other, specify_____	1…Mud and sticks 2…Burned bricks 3…Cement bricks 4…Mud bricks 5…Other, specify_____	1…Mud and sticks 2…Burned bricks 3…Cement bricks 4…Mud bricks 5…Other, specify_____
2.52	What is the main material of the floor in this room? ***Observe***	1…Earth 2…Cement 3…Tiles 4…Carpet 5…Other, specify_____	1…Earth 2…Cement 3…Tiles 4…Carpet 5…Other, specify_____	1…Earth 2…Cement 3…Tiles 4…Carpet 5…Other, specify_____	1…Earth 2…Cement 3…Tiles 4…Carpet 5…Other, specify_____

Section 3: Net inspection ***"Now I will have a look at your nets and count the number of holes. The net will be returned to you and hung up again if you wish."***						
Net serial number (Given Serial no. during data collection for each net)			__________	__________	__________	__________
3.1	The ITN is clean **(Observe)**		1…Yes 2…No	1…Yes 2…No	1…Yes 2…No	1…Yes 2…No
3.2	What types of holes are observed? ***Answer every category***		1…Horizontal tears at bottom 2…Holes at hanging points 3…Open seams 4…Burn holes 5…Holes from rodents 6…Whole section missing 7…No holes	1…Horizontal tears at bottom 2…Holes at hanging points 3…Open seams 4…Burn holes 5…Holes from rodents 6…Whole section missing 7…No holes	1…Horizontal tears at bottom 2…Holes at hanging points 3…Open seams 4…Burn holes 5…Holes from rodents 6…Whole section missing 7…No holes	1…Horizontal tears at bottom 2…Holes at hanging points 3…Open seams 4…Burn holes 5…Holes from rodents 6…Whole section missing 7…No holes
3.3	**Zone 1**	**# of holes size 1** **# of holes size 2** **# of holes size 3** **# of holes size 4**	|__|__| |__|__| |__|__| |__|__|	|__|__| |__|__| |__|__| |__|__|	|__|__| |__|__| |__|__| |__|__|	|__|__| |__|__| |__|__| |__|__|
3.4	Zone 2	# of holes size 1 # of holes size 2 # of holes size 3 # of holes size 4	|__|__| |__|__| |__|__| |__|__|	|__|__| |__|__| |__|__| |__|__|	|__|__| |__|__| |__|__| |__|__|	|__|__| |__|__| |__|__| |__|__|
3.5	**Zone 3**	**# of holes size 1** **# of holes size 2** **# of holes size 3** **# of holes size 4**	|__|__| |__|__| |__|__| |__|__|	|__|__| |__|__| |__|__| |__|__|	|__|__| |__|__| |__|__| |__|__|	|__|__| |__|__| |__|__| |__|__|
3.6	Zone 4	# of holes size 1 # of holes size 2 # of holes size 3 # of holes size 4	|__|__| |__|__| |__|__| |__|__|	|__|__| |__|__| |__|__| |__|__|	|__|__| |__|__| |__|__| |__|__|	|__|__| |__|__| |__|__| |__|__|
3.7	**Roof**	**# of holes size 1** **# of holes size 2** **# of holes size 3** **# of holes size 4**	|__|__| |__|__| |__|__| |__|__|	|__|__| |__|__| |__|__| |__|__|	|__|__| |__|__| |__|__| |__|__|	|__|__| |__|__| |__|__| |__|__|
3.8	Overall holes size for each net	Total size 1 Total size 2 Total size 3 Total size 4	|__|__| |__|__| |__|__| |__|__|	|__|__| |__|__| |__|__| |__|__|	|__|__| |__|__| |__|__| |__|__|	|__|__| |__|__| |__|__| |__|__|

Hole Tally Sheet

					TOTAL			
Zone 1	5	10	15	20	Size	Size	Size	Size
Size 1 (0.5–2 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
Size 2 (2–10 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
Size 3 (10–25 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
Size 4 (> 25 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
Zone 2	5	10	15	20	Size	Size	Size	Size
Size 1 (0.5–2 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
Size 2 (2–10 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
Size 3 (10–25 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
Size 4 (> 25 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
Zone 3	5	10	15	20	Size	Size	Size	Size
Size 1 (0.5–2 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
Size 2 (2–10 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
Size 3 (10–25 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
Size 4 (> 25 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
Zone 4	5	10	15	20	Size	Size	Size	Size
Size 1 (0.5–2 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
Size 2 (2–10 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
Size 3 (10–25 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
Size 4 (> 25 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
ROOF	5	10	15	20	Size	Size	Size	Size
Size 1 (0.5–2 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
Size 2 (2–10 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
Size 3 (10–25 cm)	○○○○○	○○○○○	○○○○○	○○○○○				
Size 4 (> 25 cm)	○○○○○	○○○○○	○○○○○	○○○○○				



TOTAL Size 1				
TOTAL Size 2				
TOTAL Size 3				
TOTAL Size 4				

Appendix II: Ethical Approvals

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Officer:	Telephone:	Our date:	Our reference
REK vest	Øyvind Straume	55978497	01.07.2013	2013/986/REK vest
			Your date:	Deres referanse:
			28.05.2013	

Bernt Lindtjørn
Postboks 7804

2013/986 Vil en kombinasjon av myggnett og innendørs sprøyting med insektmidler bedre forebyggelsen av malaria i Etiopia?
Combining indoor residual spraying and long-lasting insecticidal nets for malaria prevention: a cluster randomized controlled trial in Ethiopia.

Body responsible for the research: University in Bergen
Project Manager: Bernt Lindtjørn

With reference to your application about abovementioned project. The Regional Committee for Medical and Health Research Ethics, Western Norway (REK Vest) reviewed the application in the meeting, 20.06.2013 , pursuant to The Health Research Act § 10.

Description of the project
This study aims to assess whether the combined use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) increases protection against malaria. The proposal is to conduct a cluster randomised controlled trial in Ethiopia to measure malaria incidence and transmission, insecticide resistance, and to assess whether mosquito nets result in an age shift in malaria morbidity before and after trials. The intervention will consist of four "arms": (IRS+LLINs, LLINs alone, IRS alone and control (routine practice)). The study will include up to 10 000 participants.

The Committee`s considerations

Application/Study Protocol
The Committee finds the project to be of great scientific and social importance and relevance for a major health burden in large regions of the world.

Is randomisation justifiable?
The Committee discussed at some length whether or not it was acceptable to include a control group which receives no intervention, other than routine practice, in the project. According to the Health Research Act, research must be based on respect for the research participants' human rights and dignity. The participants' welfare and integrity shall have priority over scientific and social interests. From that perspective; a randomisation where 1/4 of the participants receive a considerable poorer treatment (routine practice) is highly problematic.

Furthermore the Committee debated the scientific value of the inclusion of the control group. The applicant points to Pinder and colleagues which estimates 50% incidence reduction from LLINs and a 75% incidence reduction from IRS+LLINs. Quite clearly IRS and LLIN will have effect, compared to nothing, so how is it justified to include the control group?

Besøksadresse:
Haukeland Universitetssykehus, Sentralblokken, 2. etg, Rom 4617

Telefon: 55975000
E-post: rek-vest@uib.no
Web: http://helseforskning.etikkom.no/

In the end the Committee decided to allow the study with all four arms and emphasises that every participant will have access to weekly visits, early diagnosis, transportation and state of the art treatment for malaria. The paradox the Committee pointed out is that if 1/4 of the participants were forced not to attend the study they would actually be put in a worse situation ("routine practice", with no particular follow-up) than as participants in the control group. The Committee also emphasised that from a scientific point of view, inclusion of the control group allows for a more reliable comparison between groups. The protocol also includes environmental risk factors such as availability of and distance to mosquito breeding sites, temperature and rainfall, which is relevant for comparison between groups, the control group included. The Committee accepts the applicant`s argument that a wide study design would make the results more applicable in countries with resource limitations.

Furthermore the Committee emphasises that a single project were one would follow-up the control group only, in itself probably would have been approved.

Finally the Committee stresses that no participants in the control group in any way can be impeded of getting hold of mosquito protection from other sources.

Consent
The Committee notes that consent might be problematic if one member of the family household does not wish to attend. Any reluctance to attend by any family member must be respected.

Assessment by local ethics Committee
REC Western Norway notes that the project will be submitted to the Institutional Review Board of the College og Health Sciences at Addis Ababa University, Ethiopia. REC Western Norway asks the Review Board to carefully consider and assess the problematic aspects discussed in the chapter above.

Information
The Committee recommends using the Norwegian REC`s template in English. It can be found at helseforskning.etikkom.no under "deadlines and forms" and "Templates for Participation Information and Consent."

Timeframe
The project will end 31.12.2016 and all data will then be anonymised.

Decision
REC Western Norway approves the project in accordance with the submitted application.

Final Report and Amendments
The Project Manager shall submit a final report to the REC Western Norway no later than 01.07.2017., according to Health Research Act § 12. The Project Manager shall submit an application of approval to REC Western Norway if there is significant changes in the project protocol, according to Health Research Act § 11.

Appeal
The Project Manager may appeal the committee's decision, see the Administration Act § 28. The appeal must be sent to the REC Western Norway within three weeks of receiving this letter. If the decision is upheld by REC Western Norway, the appeal will be forwarded to the National Research Ethics Committee for Medical and Health Research for a final assessment.

Med vennlig hilsen

Jon Lekven
Committee chairman

Øyvind Straume
Senior Executive Officer

Kopi til: postmottak@uib.no

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK vest	Øyvind Straume	55978496	19.08.2015	2013/986/REK vest
			Deres dato:	Deres referanse:
			14.08.2015	

Vår referanse må oppgis ved alle henvendelser

Bernt Lindtjørn

2013/986 Vil en kombinasjon av myggnett og innendørs sprøyting med insektmidler bedre forebyggelsen av malaria i Etiopia?

Body responsible for the research: Universitetet i Bergen
Project Manager: Bernt Lindtjørn

With reference to your application dated 14.08.2015 about the abovementioned project. The application has been processed by Committee leader of REC Western Norway in accordance to the Health Research Act § 11.

Assessment

Change to the project
The amendment to the project is to add a new PhD-student which will use already collected data on malaria incidence combined with additional information on the physical integrity of distributed nets and analyse if the nets have adequate concentrations of insecticides.

Committee leader of REC Western Norway reviewed the application.

Review
REC Western Norway has no objections the the amendment of the project.

Decision
REC Western Norway approves the project change in accordance with the submitted application.

Appeal
The Project Manager may appeal the committee's decision, see the Administration Act § 28. The appeal must be sent to the REC Western Norway within three weeks of receiving this letter. If the decision is upheld by REC Western Norway, the appeal will be forwarded to the National Research Ethics Committee for Medical and Health Research for a final assessment.

Med vennlig hilsen

Ansgar Berg
Prof. Dr.med
Head of Committee

Øyvind Straume
Head of Secretariat

Kopi til: *postmottak@uib.no*

በኢትዮጵያ ፌዴራላዊ ዴሞክራሲያዊ ሪፐብሊክ
የሳይንስና ቴክኖሎጂ ሚኒስቴር
The Federal Democratic Republic of Ethiopia
Ministry of Science and Technology

ቁጥር 3:10/837/07
Ref. No.
ቀን 21/5/07
Date

To: Addis Ababa University, College of Health Sciences, and Ethics Committee

Addis Ababa

Re: Combining indoor residual spraying and long lasting insecticidal net for malaria prevention: a cluster randomized controlled trials in Ethiopia.

Dear Sir/Madam /Mr./Mrs./Dr.

We are writing this letter in reference to your renewal request letter dated January 20, 2015.

After having in depth review of your request, the National Research Ethics Review Committee has accepted your renewal request for one year from January 27, 2015 to January 26, 2016. This is, therefore, to notify that the ethical approval is renewed and your group can proceed in accordance to the latest approved document. Please ensure that you submit a biannual report and an annual renewal application 30 days prior to expire date. We are confident that you as PI of the project and your esteemed organization will monitor the ethical implication of the project as it is stipulated in the latest approved document.

With regards,

Yohannes Sitotaw
Secretary of NRERC

CC: _ Chairperson, NRERC

_ **Dr. Wakgari Deressa** (PI)

ማነጋገር ቢያስፈልግዎ
You may Contact

ፖ.ሳ.ቁ. P.O.Box 2490
አዲስ አበባ ኢትዮጵያ Addis Ababa, Ethiopia E-mail most@ethionet.et
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በኢትዮጵያ ፌዴራላዊ ዴሞክራሲያዊ ሪፐብሊክ
የሳይንስና ቴክኖሎጂ ሚኒስቴር
The Federal Democratic Republic of Ethiopia
Ministry of Science and Technology

ቁጥር 3.10/085/2016
Ref. No.

ቀን Jan 5, 2016
Date

To: Addis Ababa University, Collage of Health Science Ethics Committee

Addis Ababa

Re: Combining indoor residual spraying & long-lasting insecticidal nets for malaria prevention: a cluster randomized controlled trail in Ethiopia

Dear Sir/Madam /Mr./Mrs./Dr.

We are writing this letter in reference to your Amendment request letter dated Dec 8, 2015.

After having in depth review of your request, the National Research Ethics Review Committee has accepted your renewal request for one year from **(January 5, 2016- January 4, 2017).**This is, therefore, to notify that the ethical approval is renewed and your group can proceed in accordance to the latest approved document. Please ensure that you submit a biannual report and an annual renewal application 30 days prior to expire date. We are confident that you as PI of the project and your esteemed organization will monitor the ethical implication of the project as it is stipulated in the latest approved document.

With regards,

Yohannes Sitotaw
Secretary of NRERC

CC: _ Chairperson, NRERC

_Dr. Wakgari Deressa (PI)

ማነጋገር ቢያስፈልግዎ
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Errata for
Bed nets for malaria prevention under field conditions in Ethiopia:

Durability, use, and impact on spatial variation of malaria

Tarekegn Solomon Shanka

Thesis for the degree philosophiae doctor (PhD)
at the University of Bergen

05-10-2020
(date and sign. of candidate)

05.10.2020
(date and sign. of faculty)

Errata

Thesis

Page 44 Error in citations: "Reference number 281 cited incorrectly; therefore it should be removed from citation".

Page 82 Error in reference list: "Reference number 281 listed incorrectly; therefore it should be removed from list of references"

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