Malaria, anaemia and undernutrition in a drought-affected area of the Rift Valley of Ethiopia:

Experiences from a trial to prevent malaria

Taye Gari Ayana

Thesis for the Degree of Philosophiae Doctor (PhD)
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To

Aynalem,

Ruth, Kena and Abenezer

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Summary

Background: In Ethiopia, malaria, anaemia and undernutrition are common childhood health problems. The country is planning to reduce these conditions to a level where they are not a public health problem. Meanwhile, for the success of this aim, a description of the occurrence and interaction of malaria, anaemia and undernutrition could help contribute to design tailored, efficient and effective control strategies. This study was done in the context of malaria prevention trial, which aimed to measure the effect of combining long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) in reducing the malaria incidence compared to LLINs or IRS alone. The study area was affected by a serious drought and famine during the first year of the study.

Objectives: The overall aim of this thesis is to improve our understanding of the effect of malaria prevention on anaemia, and to assess the association between malaria, anaemia and undernutrition among children in a drought-affected area in south-central, Ethiopia.

Methods: A cohort of 5309 residents was followed-up for 16 weeks to measure the variations in malaria incidence among villages (Paper I), and the results were used as a baseline to calculate the sample size needed for the trial. We followed a cohort of children aged 6 to 59 months for one year to describe anaemia and changes in haemoglobin (Hb) concentration (Paper II). A cohort of 4468 children was followed-up for 89 weeks to measure the relationship between malaria and undernutrition (Paper III). Weekly home visits and patient self-referral were also used to identify malaria cases. We conducted Hb concentration (once a year) and anthropometry (twice a year) surveys.

Results: In Paper I, we observed a variation in malaria incidence among villages. On the other hand, the insecticide-treated nets ownership was low (27%), with the distance from the lake or river and younger age being the main risk factors for malaria. The findings of this study were used as a baseline to calculate the sample size for the trial. In Paper II, despite the malaria prevention effort in the community, we observed an unexpected increase in anaemia prevalence over the period of a year, which could be due to the drought and famine that affected the area. A higher incidence of anaemia was observed among children with stunting, malaria infection, young age and in poor families. However, no significant difference in anaemia prevalence was observed among the different trial arms (LLIN+IRS, LLINs alone, IRS alone and routine arm). In Paper III, malaria infection was a risk factor for stunting and wasting, although undernutrition was not a risk for malaria infection. Furthermore, an increase in the prevalence of stunting, but no significant change in a prevalence of wasting was observed over time.

Conclusions: We showed a large variation in malaria incidence among villages. Conducting trials in a drought-prone area may bring an unexpected challenge. We observed an unexpected increase in anaemia prevalence over a year. There was no significant difference in anaemia prevalence among the trial arms. Moreover, a close follow-up of the nutritional status of children with malaria infection may be needed. There could hence be a need to prioritize villages nearer to the main mosquito breeding sites for malaria control.

Trial registration: PACTR 201411000882128 (8 September, 2014)

List of original papers

This thesis is based on the following original research papers, which are referred to in the text by their Roman numerals.

Paper I: Gari T, Kenea O, Loha E, Deressa W, Hailu A, Balkew M, GebreMichael T, Robberstad B, Overgaard HJ, Lindtjørn B. Malaria incidence and entomological findings in an area targeted for a cluster-randomized controlled trial to prevent malaria in Ethiopia: Results from a pilot study. Malaria J (2016) 15:145.

Paper II: Gari T, Loha E, Deressa W, Solomon T, Atsbeha H, Assegid M, Hailu A, Lindtjørn B. Anaemia among children in a drought-affected community in south-central Ethiopia. PLoS ONE (2017) 12 (3): e0170898.

Paper III: Gari T, Loha E, Deressa W, Solomon T, Lindtjørn B. Malaria increased the risk of stunting and wasting among young children in Ethiopia: Results of a cohort study. PLoS ONE (2018) 13 (1): e0190983.

Abbreviations

AHR	Adjusted Hazard Ratio
AIDS	Acquired Immune Deficiency Syndrome
AOR	Adjusted Odds Ratio
CBN	Community-Based Nutrition
CI	Confidence Interval
DDT	Dichloro Diphenyl Trichloroethane
GEE	Generalized Estimating Equation
GLOBVAC	Global Health-and Vaccination Research
Hb	Haemoglobin
HEWs	Health Extension Workers
HIV	Human Immunodeficiency Virus
ICC	Intra-cluster Correlation Coefficient
IMCI	Integrated Management of Childhood Illnesses
IPT	Intermittent Preventive Therapy
IRS	Indoor Residual Spraying
IYCF	Infant and Young Child Feeding
LLINs	Long-Lasting Insecticidal Nets
MOH	Ministry of Health
OR	Odds Ratio
RBM	Roll Back Malaria
RDT	Rapid Diagnostic Test
SD	Standard Deviation
SGD	Sustainable Development Goal
SP	Sulfadoxine-Pyrimethamine
TEM	Technical Error of Measurement
UNICEF	United Nations Children's Fund
US-PMI	United States President's Malaria Initiative
WHO	World Health Organization

Table of contents

Introduction

What is this thesis about?

In 2014, researchers from Hawassa University, Addis Ababa University, the University of Bergen and the Norwegian University of Life Sciences started the trial, "Combining long-lasting insecticidal nets and indoor residual spraying for malaria prevention in Ethiopia: Study protocol for a cluster randomized controlled trial" in the Adami Tullu area in the Ethiopian Rift Valley (1). The aims of this trial were: "to provide evidence on the combined use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) for malaria prevention by answering the following research questions: Can the combined use of LLINs and IRS significantly reduce the incidence of malaria compared with the use of either LLINs or IRS alone? Will the combined use of LLINs and IRS reduce vector density, infection, longevity and the entomological inoculation rate? Will it be determined whether LLINs + IRS improve the haemoglobin (Hb) concentration and reduce anaemia among children under 5 years of age compared with children in LLINs or IRS alone" (1). (The study protocol of the trial, called the MalTrials project, is attached to this thesis in Appendix III).

I worked in this study by taking part in the data collection from the early pilot study to the end of the trial. Some of the results from the trial have already been published (2-5), and the remaining, and primary results will be published separately.

One of the main reasons for doing Paper I was to obtain information about the micro-epidemiology of malaria, variation in malaria occurrence between households in the same village or between villages in the study area. More specifically, we needed information about the

variations in malaria incidence and vector populations among villages so that we could use such information to calculate the sample size for our trial (1). The entomological study of Paper I was conducted by another PhD student, Oljira Kenea, at Addis Ababa University in Ethiopia. In addition, this thesis analyses the effect of LLINs + IRS compared to LLINs or IRS alone on anaemia reduction among children under the age of 5 years (the first one-year data of the trial) (Paper II). In the meantime, we analyze the interaction between malaria and undernutrition (Paper III). Therefore, the thesis addresses some important aspects of the MalTrials project, and topics related to important child health problems in Ethiopia such as malaria, anaemia and undernutrition. A particular emphasis is given on how malaria is associated with anaemia and malnutrition.

Globally, an estimated 5.9 million children under the age of 5 years died in 2015 (6). The risk of dying before the age of 5 years is 14 times higher among children residing in sub-Saharan Africa compared to those in developed countries (6). Infectious diseases, including malaria, are the cause for over 50% of child deaths (6-8), and malnutrition is an underlying cause for 45% of the deaths in young children (9, 10).

Malnutrition is a condition resulting from either an insufficient or excessive intake of various nutrients. It is a broad term that includes both undernutrition and overnutrition (11). This thesis focuses on the undernutrition aspect of malnutrition such as wasting (weight-for-height less than 2 Standard deviation (SD) of the World Health Organization (WHO) Child Growth Standards Median) and stunting (height-for-age less than 2 SD of the WHO Child Growth Standards Median) (12).

Malaria, anaemia and malnutrition are interconnected, and frequently co-exist in highly prevalent areas (10, 13). Although individuals in all ages of life are affected by these conditions, children under the age of 5 represent the most vulnerable group. The largest disease burden of malaria, malnutrition and anaemia are among children living in sub-Saharan Africa, including Ethiopia (14, 15).

Studies have shown that both malaria infection (10, 16, 17) and undernutrition (18, 19) are risk factors for anaemia in children. Furthermore, malnutrition may suppresses the immune system, and could increase the risk of infections for diarrheal diseases, pneumonia and measles (20), all major causes of severe illness, and death in sub-Saharan Africa. On the other hand, infections could compromise the nutritional status of young children and result in undernutrition (20).

A scientific controversy exists about the possible link between malnutrition and malaria infections. For example, some reported an increased risk of malaria infection (21, 22), whereas others observed a lower risk of malaria (23, 24) among undernourished children. In contrast, studies have also shown an increased risk of malnutrition among children with malaria infection (21, 25-27).

So, in this thesis I present data obtained during our malaria prevention trial to discuss the co-existence, and interaction of malaria, anaemia and malnutrition.

Research Environment

The south Ethiopian Malaria Research Group (see https://malaria.w.uib.no/malaria-research-group/) was established in 2007. The three main malaria research projects are the Ethiopian Malaria Prediction System, MalTrials project and the "Medical Entomology and Vector Control" training project.

The focus areas of the malaria research in south Ethiopia has been to improve our understanding of malaria prevention, and assess the possible links between climate change and malaria in south Ethiopia. It was a collaborative research between Hawassa University, Addis Ababa University, Arba Minch University, Norwegian University of Life Sciences and the University of Bergen in Norway.

The Ethiopian Malaria Prediction System project (2007 – 2012), strengthened the PhD and Masters programmes in Ethiopia, and 7 PhD candidates successfully completed their PhD work. Based on the lessons learned and evidence gaps identified during Ethiopian Malaria Prediction System research, MalTrials started in 2013. The MalTrials project, short name for "Combining LLINs + IRS for malaria in Ethiopia: A cluster randomized controlled trial" is a Norwegian Research Council (GLOBVAC) supported research project. The trial includes seven senior researchers, five PhD students and 41 technical staffs. The main focus of MalTrials is to provide evidence on the combined use of LLINs + IRS on malaria prevention by assessing malaria epidemiology, entomology, vector control interventions and costs of the interventions.

As a result of the MalTrials project, two additional PhD students started their research in 2017 on "Malaria in pregnancy and intrauterine growth pattern" and on "Molecular diagnosis of asymptomatic malaria and malaria drug resistance in south Ethiopia". The latter project included the establishment of a molecular laboratory at Hawassa University in 2017.

The Ethiopian Ministry of Health, Regional and Local vectors control units require personnel with the knowledge and expertise to deal with the different vector-borne diseases, operational decision-making, and monitoring, evaluation and surveillance of vector control programmes. Thus, in 2014, a Masters' degree research based programme in "Medical Entomology and Vector Control" was launched at Arba Minch University with the aim of training candidates to lead malaria control and elimination efforts in Ethiopia.

Malaria

Burden of malaria

Global

Malaria is an infectious disease caused by the *Plasmodium* species (28). The five species of *Plasmodium* that can infect human beings are: *P. falciparum, P. vivax, P. malariae*, *P. ovale* and *P.knowlesi* (14, 29). The word "malaria" originates from two Italian words *mal'aria*, meaning bad air. In this view, malaria was understood in terms of "miasmatism." The interpretation was fever caused by a "miasma" or poisoning of the air (30, 31). Despite being an ancient disease, the basic symptoms, aetiology and transmission of malaria were established at the turn of the 20th century by Italian (Golji), British (Ross) and French (Laveran) scientists (32, 33).

Globally, it is estimated that there were 216 million cases of malaria, leading to 445,000 deaths in 2016. The largest proportion (70%) of all malaria deaths were among children under the age of 5 years, with most of them occurring in sub-Saharan Africa (14, 34). A meta-analysis that covers 115 years from 1900 to 2015 of malaria history in sub-Saharan Africa has shown a long-term decline in the prevalence of *P. falciparum*. However, the occurrence of malaria has been interrupted by periods of rapidly increasing or decreasing transmission (35). In Africa, despite the decline in malaria incidence by 20% between 2010 and 2016, an increase in malaria case incidence was reported in 2016 compared to the 2015 (14).

Malaria in Ethiopia

In Ethiopia, nearly 68% of the land mass of the country have favourable condition for malaria transmission, with 60% of the population at risk of the disease (36, 37). Malaria transmission in the country is seasonal and unstable (38). In most parts of Ethiopia, the major malaria transmission season is from September to December, and the minor transmission season is from April to May (37). *Plasmodium falciparum* (60%) and *P.vivax* (40%) are the two common causes of malaria (36, 39). According to the 2017 World Malaria Report, Ethiopia is one of the five countries (Afghanistan, Ethiopia, India, Indonesia and Pakistan) contributing to over an estimated 85% of the global *P.vivax* cases (14).

The primary vector responsible for malaria transmission is *Anopheles arabiensis,* and the secondary vector is *Anopheles pharoensis* (39, 40). In Ethiopia, malaria incidence was substantially reduced by over 50% between 2000 and 2015 (14). Even if the prevalence was low, malaria remains among the five most prevalent reasons for treatment at outpatient departments in

2015 (41). According to the last two national malaria indicator surveys reports, the prevalence of malaria among children was 1.4% in 2011 (42) and 0.6% in 2015 (37). A large geographic variation in the prevalence of malaria in children was reported among the regions (0% in the Somali Region versus 6.0% in the Gambella Region) (37). Although the Somali region represents dry and arid lowlands, and malaria most often occurs near bodies of water, or the transmission is seasonal following rainfall, it is unlikely that there were no malaria cases in this region. However, the region was affected by severe drought and a shortage of rain during the years 2015 and 2016. Thus, a malaria prevalence of zero for this region might not mean a malaria-free area, but could be related to the low rainfall that occurred during the national malaria indicator survey period (37).

Micro-epidemiology of malaria

Malaria transmission can vary much between- and within villages in the same district (36, 43-48). This occurrence of malaria in a small scale area (micro-geographic) is a challenge for sustaining malaria control efforts (49). For example, a failure to focus on malaria hotspots, including a small area with an increased risk of malaria transmission, could reduce the efficiency of control interventions (50). However, the risk factors for malaria hotspots are not completely understood, and some studies observed the distance from local mosquito breeding sites (51-53), housing conditions (49, 54), poverty and individuals' bed net use (51) as risk factors for the occurrence of malaria in micro-geographic areas. These risk factors could differ from region to region, between villages and even from households to households. Hence, it is important to obtain more information about the variability in malaria incidence within small geographical areas, as such information could also help to plan for targeted and efficient interventions to

reduce malaria (Paper I). Furthermore, the risk of developing malaria for individuals within the same village or household may be correlated (46). This correlation within villages or clusters results in the statistical efficiency of cluster randomized designs being lower than that of individually randomized designs, so thus a larger sample size is necessary for equivalent power (55). Therefore, the knowledge of variation in malaria incidence within- and between villages, the intra-cluster correlation coefficient (ICC), was needed to calculate the sample size for malaria prevention trial (MalTrials).

Residual malaria transmission

According to the WHO definition, residual malaria transmission refers to the remaining low-level transmission of malaria after a high coverage has been achieved with vector control interventions to which local vectors are fully susceptible (56). The residual transmission could be due to *Anopheles* mosquito behavioural avoidance to enter houses, or people stay out-door during the early night (56). An entomological study from our malaria prevention trial has shown a high mosquito human-biting activities outdoors compared to indoors during the early part of the night (3). Furthermore, a study from the Arba Minch area in south Ethiopia has shown a residual malaria transmission in an area of a high indoor vector control interventions (57). Hence, targeting residual malaria transmission with new startegies in addition to the existing indoor acting interventions can be important for local malaria control and elimination (58).

Risk factors for malaria

Immunity

Human populations could develop an immunity to malaria following continued exposure to malaria infection (59-62). In areas with a high malaria transmission, newborn babies are protected through the immunity they acquire from their mothers for the first few months after birth (63). This immunity will gradually decrease, and children are at an increased risk of malaria as they become older. A study from Ethiopia has shown a high malaria incidence among children between the age of 1 to 9 years in lowland areas (areas below 1,500 metres above sea level), although the malaria incidence was similar for all age-groups in the highland area (48). This could indicate that people living in highland areas are less exposed to malaria infection, and hence all age groups lack immunity to malaria. On the other hand, in lowland areas where the risk of malaria infection is higher, adults, but not young children, could develop some protection to malaria infections through previous exposure to malaria.

Socio-demographic factors

Studies have reported that people from poorer families (64, 65), housing conditions such as the presence of open eaves (66) and having less educated parents (67) are at increased risk of malaria infection. Moreover, evidence also shows that improved housing (68) and knowledge on the proper use of LLINs (69) could be effective intervention to prevent malaria.

Climate

Temperature (70, 71), rainfall and altitude (72) and relative humidity (73) are reported to be predictors of malaria transmission. The development and survival of malaria parasite in the

Anopheles mosquito is mostly determined by the mean annual temperature (28, 40, 74). For example, climate change influences the El Niño cycle; and during the El Niño season the rainfall decreases and the temperature increase, which could result in a low malaria incidence (75). On the other hand, following the El Niño season, heavy rainfall could create many water bodies that favour mosquito breeding, and result in increased malaria transmission. As a result of climate change or global warming, previously malaria-free highland (with lower temperatures) areas are now becoming at risk of malaria transmission (76). A recent study from the Oromia Region in Ethiopia showed an association between malaria and an increase in sea surface temperature (77). Consequently, wormer highland areas favour malaria transmission (76). This poses a challenge for the future malaria control and prevention programme.

Human activities

In Africa, a high population growth rate has led to an increased demand for food and energy. In order to meet the population demand, many countries have been forced to initiate a large-scale dam and irrigation projects to help cultivate crops (78). In low malaria transmission areas like Ethiopia, such irrigation scheme creates favourable environments for malaria vector breeding (65). Studies have shown that people living close to irrigation dams are at increased risk of malaria infection (71, 78).

The various reasons for the movement of people include a high population pressure in highland areas, environmental deterioration, natural disasters and conflicts (79). The movement of a non-immune population to a malaria endemic area, inaccessibility to basic health services and the absence of housing in such conditions could result in the spread of malaria (80-82).

Economic and social impacts of malaria

Malaria causes illness, suffering and premature death, and has a major impact on the socio-economic status of human beings (83). In sub-Saharan African countries, malaria is related to a decreased productivity through the absenteeism of the labour force from work (84). Malaria can also seriously affect the education system through an absenteeism of teachers and students (85). In summary, countries with a high malaria burden have historically lower annual growth than those without malaria transmission in their country. This is primarily related to direct costs, such as personal and public expenditures on prevention and treatment, as well as indirect costs such as lost productivity or income due to illness or death (86).

History of malaria prevention

In 1955, the WHO launched the Global Malaria Eradication Programme with the aim of eradicating malaria in 10-15 years. The two core strategies to achieve the goal were indoor residual spraying with dichlorodiphenyltrichloroethane (DDT), and treating all malaria cases using Chloroquine (87). The WHO eradication programme achieved malaria elimination from 37 countries in Europe and North America (88). In addition, the malaria eradication effort substantially reduced the burden of malaria illness and deaths in many other countries including Ethiopia (89). However, some countries, including the sub-Saharan African countries, failed to sustain the programme, and after some years it resulted in a resurgence of malaria. Between the 1970s and 1990s, the malaria situation was worsened by the development of parasite resistance to Chloroquine (90, 91) and mosquito resistance to DDT (92). Furthermore, the civil war and political unrest in Ethiopia had a serious and negative impact on the social and economic development of the country between 1974 and 1991. The crisis resulted in a failure of the health

system to respond effectively to the occurrence of malaria which led to a resurgence of the disease in Ethiopia (93).

In 1992, a new Global Malaria Control Strategy was endorsed by the Ministerial Conference on Malaria Control, and confirmed by the World Health Assembly in 1993. This new strategy was mainly implemented using the primary health-care approach through a decentralized programme-based disease control adapted to a local context (94, 95). A growing concern by many governments, particularly in Africa, about the increasing illnesses and deaths due to malaria, resulting in the establishment of the Roll Back Malaria (RBM) initiative, which was launched in 1998, with the aim to half the malaria burden by 2010 (96, 97). The initiative was strengthened through financing from the Global Fund to fight AIDS, Tuberculosis and Malaria. Moreover, as of 2005, the United States Government President's Malaria Initiative (US-PMI), and financial commitments to malaria by the Bill and Melinda Gates Foundation significantly contributed to achieving the formulated goals of RBM in many countries (96). By the end of 2010, the evaluation of these initiatives has shown an increase in funding from international sources. Furthermore, vector control tools such as LLINs and IRS were scaled-up, and the availability of parasitological tests for malaria diagnosis and treatment with artemisinin-based combination therapy were expanded (98). As a result, the overall cases of malaria declined by 23%, with malaria deaths declining by 38% from the 2000 baseline (96). However, the rate of decline in malaria burden has stalled since 2014, and an increase in malaria cases was reported between 2014 and 2016 (14).

Existing strategies to combat malaria

The WHO Global Malaria Programme recommends three malaria prevention and control strategies: 1) early diagnosis and treatment of cases, 2) the use of intermittent preventive therapy for pregnant women and children, and 3) vector control measures, such as IRS, LLINs and larval control (99). IRS and LLINs are the two main vector control interventions (100). IRS is the application of long-acting chemical insecticides on the wall and roof of residential house in order to kill adult malaria mosquitoes, and has a long history in malaria prevention and control programmes (101). Today, IRS continues to be one of the main malaria vector control tools (100). The challenge with IRS is the rapid development of mosquito resistance to chemical insecticides (101).

The insecticide-treated net, or long-lasting insecticidal net, is a mosquito net that serves as a physical barrier against mosquitoes (99). The mosquito nets could also repel, disable and/or kill mosquitoes (99), and is recommended as a key vector control tool to protect at-risk populations from malaria (102). The implementation of insecticide-treated nets on a large scale as part of an integrated approach to control malaria started in the 1980s (103), but was launched in Ethiopia in 2004 (104).

Two types of mosquito nets have been used in malaria vector control: the conventionally treated net is the one treated by dipping it in a WHO-recommended insecticide, and then impregnated after three washes. The other category (currently widely) applied is the LLINs, which have an insecticide incorporated within the fibres (99). The WHO Global Malaria Programme recommends the universal coverage of LLINs to control malaria vectors (105).

Intermittent Preventive Therapy (IPT) involves the administration of an antimalarial drug (Sulfadoxine-pyrimethamine) at intervals to children or pregnant women to prevent malaria infection (106, 107). In areas with seasonal malaria transmission, IPT provides considerable protection from malaria among children sleeping under LLINs (108). In Ethiopia, due to *P. falciparum* resistant to Sulfadoxine-pyrimethamine, IPT is not included in the guidelines to treat malaria (104).

Environmental management for larvae source reduction can reduce vector abundance, and the application of larvicides can reduce malaria transmission (109, 110). Thus, the WHO recommends larval source management as a supplementary strategy to control malaria vectors (111).

On the other hand, among others (climate change, political instability, parasite drug resistance, and vector resistance to insecticides), the persistence of residual transmission poses a major challenge for malaria elimination (58). Thus, new intervention strategies are needed to achieve the post-2015 malaria elimination goals (105).

Malaria diagnosis and treatment

Early accurate diagnosis with Rapid Diagnostic Test (RDT) or microscopy blood film examination (112), and treatment with effective medicines (113) within 24 hours of malaria onset, are essential components of malaria control and elimination strategies. Malaria diagnosis and treatment based on clinical signs and symptoms alone lead to the misdiagnosis and misuse of antimalarial drugs because of a non-specific clinical manifestation (112). Since 2010, the WHO

recommends parasitological confirmation of malaria by microscopy or RDT before treatment is started (113). The gold standard for over a century, Giemsa staining and light microscopy, is useful to identify the *Plasmodium* parasite presence, species and density, whereas RDT based on lateral flow immune-chromatography, which can be done with limited training, have made malaria tests accessible to the larger community (112). Moreover, polymerase chain reaction based assays, highly sensitive and specific even at lower levels of parasitemia, are expensive to do and require highly skilled laboratory personnel, and are not used for diagnosing malaria in resource-poor settings (113).

In Ethiopia, the current malaria prevention and control activities are integrated into the basic health services. In the health centres, malaria diagnosis is primarily performed using microscopic examination, while in the health post, multi-species RDT is used to diagnose malaria (104). Chloroquine has been used to treat all forms of malaria for over 50 years in Ethiopia (40); in the meantime, chloroquine resistant *P. falciparum* was reported in 1986 (114), and Sulfadoxine-Pyrimethamine (SP) was endorsed for the treatment of uncomplicated *P. falciparum* in 1998 (90). In 2003, a national survey observed that *P. falciparum* was resistant to SP, and Artemether-Lumefantrine became the first line of treatment for uncomplicated *P. falciparum,* whereas Chloroquine remains as the first line of treatment of *P. vivax* (104). The LLINs are distributed free of charge for the community, using mass distribution to the community every three years. The LLINs distribution and IRS operations are mainly supervised by Health Extension Workers (HEWs), and supported by a health centre and district health office (115).

Combination of IRS and LLINs

Both IRS and LLINs are effective in preventing malaria when applied independently (53, 100, 116). In Ethiopia, IRS and LLINs are used separately, or in combination in the same geographic area (104). However, there is a paucity and even conflicting evidence on the benefits of combining IRS and LLINs over individual intervention alone to prevent malaria. A malaria prevention trial from The Gambia observed no difference in the reduction of malaria in the LLINs + IRS arm compared to the LLINs alone arm (117), while a study from Tanzania has shown a decreased risk of malaria among the combination arm, LLINs and IRS compared to LLINs alone (118). To provide evidence that could be used either for a modification or designing of the new policy, a community-based trial called "Combining long-lasting insecticidal nets and indoor residual spraying for malaria prevention in Ethiopia: A cluster randomised controlled trial" was conducted in the Ethiopia Rift Valley area. The overall aims of the trial were to evaluate the benefit of combining LLINs and IRS in reducing malaria incidence, vector population, improving Hb concentration, and the cost effectiveness of the intervention over LLINs or IRS alone (1). In order to effectively implement such a trial, measuring baseline data related to the existing malaria incidence and vector population through a pilot study was of importance.

Future plan to control malaria

Since the launch of RBM between 2000 and 2015, eight countries eliminated malaria, and many countries reduced the burden of malaria to low levels (119). Based on the lessons learned, the WHO launched a 15-year post-2015 Global Technical Strategy in line with the sustainable development goals (SDGs) to reduce malaria cases and death by 90% in 2030 compared to the

2015 malaria case and death (105, 120). To achieve this goal WHO recommends maximizing the impact of existing malaria interventions and strategies, while investigating new strategies (105).

Ethiopia is currently shifting to programming (integrated within the overall health system) for a sustainable long-term impact (104) for malaria prevention and control. In line with the Post-2015 WHO Global Technical Strategy and SDGs (105, 120), the Ethiopia National Malaria Strategic Plan for 2014-2020 aims to reduce malaria cases by 75% from the baseline of 2013 and achieve near zero malaria deaths, and to eliminate malaria in selected low transmission areas by the year 2020 (115). However, the success of this goal could be threatened by various factors that could be taken into consideration. For example, despite the formulated targets, to achieve and maintain a universal coverage of vector control interventions between 2011 and 2015 (121), nearly one-third of households in malarious areas were not protected by either LLINs or IRS, or both LLINs + IRS, in 2015 (37). Moreover, the observed high prevalence of asymptomatic malaria (122), complex dynamics in malaria transmission such as the spatial expansion of malaria to highlands related to climate change (48), residual malaria transmission (57), as well as vector resistance to most of the available insecticides (123), could be major challenges to eliminate malaria in Ethiopia.

Anaemia

Burden of anaemia

Global

Anaemia is a condition in which the number and size of red blood cell, or Hb concentration, is below the limit of normal, subsequently impairing the ability of the red blood cell to transport sufficient oxygen around the body (124). Anaemia is not a disease, but the manifestation of underlying condition. These conditions could be a dietary deficiency, such as iron (main cause), folate, riboflavin, Vitamin A and B deficiencies, acute and chronic infections like malaria, helminths, tuberculosis and HIV and cancer (125-127). In addition, anaemia could be due to inherited or acquired disorders that affect Hb synthesis and red blood cell production, such as haemoglobinopathies (128). Even though it is not a primary disease process, anaemia can cause severe illness, and even death (129).

Globally, an estimated 273 million children suffered from anaemia in 2011. The burden of anaemia varied across countries, and the largest proportion of the world's anaemia cases were reported from South-East Asia, the Eastern Mediterranean and African regions (130, 131).

Anaemia in Ethiopia

According to the 2015 WHO report, half (50%) of the children in Ethiopia were anaemic, with the same report classifying anaemia in Ethiopia as a severe public health problem (128). The trend in a national prevalence of anaemia shows a reduction of 10%, from 54% in 2005 to 44% in 2011 (132). The main strategies to control childhood anaemia include promoting the availability and access to iron-rich foods, iron supplementation, prevention of malaria,

deworming for hookworm and trichuris and treating anaemia cases (133). Despite the effort to control childhood anaemia, the condition remains a severe public health problem in the country, and increased from 44% in 2011 to 56% in 2016 (132, 134). The underlying cause for an increase in the prevalence of anaemia remains unknown, though the country was facing a serious drought and famine in 2015 (135). This drought caused a serious shortage of food, which could increase the risk of iron deficiency anaemia (135).

Risk factors for anaemia

The risk factors of anaemia are multiple, and vary across wide geographic areas. Iron deficiency anaemia is the leading (50%) cause of childhood anaemia in developing countries (127, 136). In such countries, protein energy malnutrition (137) and infections such as malaria, diarrhoea and intestinal helminths (hookworm, trichuris and schistosomiasis) are the common risk factors for childhood anaemia (126, 138-140). In addition, poverty (126, 141), illiteracy (142) and poor hygiene and sanitation (18, 143) are among the contributing factors for the occurrence of anaemia.

Economic and social impacts of anaemia

Severe anaemia in children is associated with an increased risk of child deaths (144), and moderate childhood anaemia can negatively affect cognitive performance and physical growth (145-147). In addition, anaemia reduces the work capacity among adolescents and adults, which in turn can lead to economic loss (148, 149).

Strategies to prevent and control anaemia

The current recommendations to prevent and control anaemia are:

Food-based approaches to control anaemia

This approach is considered as a long-term strategy, and a sustainable means of preventing anaemia that can be implemented through community-based nutrition programmes (150). The food-based approaches include dietary improvement through promoting the availability and access to iron-rich foods (151). The other approach includes the addition of iron to foods (food fortification) commonly consumed by the general public such as maize flour, wheat flour, rice and so on (124).

Iron supplementation

The WHO recommends iron supplementation as a public health interventions for children aged 6 months and above, living in an area where anaemia is highly prevalent (152). However, in malaria-endemic settings, the safety of iron supplementation is not clearly understood (153). For example, a community-based randomised placebo-controlled trial on a routine prophylactic supplementation with iron and folic acid in a high malaria transmission area in Zanzibar showed an increased risk of severe illness and death among those who received iron and folic acid supplementation (154). Meanwhile, evidence from different trials have shown that in the presence of regular malaria prevention or management services, iron treatment does not increase the risk of malaria or deaths (155). Therefore, the WHO's current recommendation for malaria endemic area is that iron supplementation should be done concurrently with interventions to prevent, diagnose and treat malaria (152).

Helminth control in order to prevent anaemia

In developing countries like Ethiopia, the high burden of poor sanitation and human waste disposal increases the risk for intestinal parasitic infections such as hookworm and trichuris (153, 156-158). In such settings, periodic deworming with antihelminthic medicines for all preschool children is public health intervention to prevent anaemia (153).

Malaria prevention and treatment in order to prevent anaemia

Malaria can cause anaemia by either suppressing red blood cell production (159, 160), or invading and destroying red blood cells (159, 161). Therefore, malaria prevention could reduce childhood anaemia in malaria endemic areas; hence, malaria prevention is recommended as one strategy to control anaemia (124, 162).

The effect of combining LLINs and IRS to prevent anaemia

The independent applications of LLINs (163) and IRS (164) have shown to be effective in reducing childhood anaemia in malaria-endemic settings. Even so, the added benefit of the combination of IRS and LLINs over LLINs alone on changes in mean Hb concentration is not fully known. For example, in a cluster randomized controlled trial from The Gambia, evaluating the effect of combining IRS and LLINs on anaemia reduction, a secondary endpoint observed no significant difference in the risk of anaemia between IRS + LLINs and LLINs alone arms (117), whereas a malaria prevention trial from a Tanzanian study showed a higher mean Hb concentration among children in the LLINs + IRS arm compared to those children in the LLINs alone arm (118). Recognizing an evidence gap, we did a trial to measure the added benefit of combining LLINs and IRS on anaemia reduction (1).

Future plan to prevent and control anaemia

Globally, anaemia prevalence among children decreased from 47% to 43% between 1995 and 2011 (130), and anaemia declined at an average of 0.2-0.3 percentage points per year in the past two decades. The success of anaemia control varied considerably across the geographic regions, and the decline in the prevalence of anaemia was slow in countries having a high prevalence of anaemia such as Africa and Asia (128, 130). If the present trends are maintained, it is unlikely to achieve the desired anaemia control goals, and therefore the current scaling up and sustaining of existing interventions, such as iron supplementation, deworming and malaria prevention, are recommended to reduce the burden of anaemia (130).

Malnutrition

Burden of malnutrition

Global

Malnutrition is defined as "a state in which the physical function of an individual is impaired to the point where he or she can no longer maintain adequate bodily performance process such as growth, pregnancy, lactation, physical work and resisting and recovering from disease" (165). Malnutrition could refer both to under- and overnutrition (11). This thesis focuses on the undernutrition aspects of malnutrition such as wasting and stunting.

Worldwide, 50 million children were wasted (acute malnutrition) and 159 million children were stunted (chronic malnutrition) in 2014 (15). Asia and Africa account for over 90% of all stunted children under the age of 5 years (15).

Undernutrition in Ethiopia

In the last 50 years, large-scale drought and famine have affected Ethiopia, with the increase in the frequency of droughts in the past few decades primarily due to low and infrequent rainfall (166). Drought has a serious impact in countries like Ethiopia, where the economy and livelihood are mainly dependent on subsistence rain-fed agriculture (167). Evidence from the past also show that the African continent, including Ethiopia, is likely to face severe and extensive droughts in the future (167). Hence, climate change is a major challenge to control undernutrition.

In Ethiopia, malnutrition is a common childhood health problem (168, 169). According to the 2016 Demographic and Health Survey report, 38% of children are stunted and over 10% of the children are wasted (134). Despite a decline in the proportion of stunted children over the last 15 years from 58% in 2000 to 38% in 2016, studies have shown a large spatial and temporal variation in the prevalence of undernutrition related to climate changes (170), and undernutrition remains a common health problem in Ethiopia.

Risk factors for undernutrition

The UNICEF conceptual framework for the causes and consequences of undernutrition identifies the multifactorial causality of undernutrition, which could be categorized as immediate, underlying and basic causes (Figure 1) (131, 171). In Paper III of this thesis, we attempt to assess the association between malaria infection (malaria) as immediate cause for undernutrition. Furthermore, we measured other factors that can affect the child caring and inadequate feeding practice such as wealth index and educational status of the head of household.

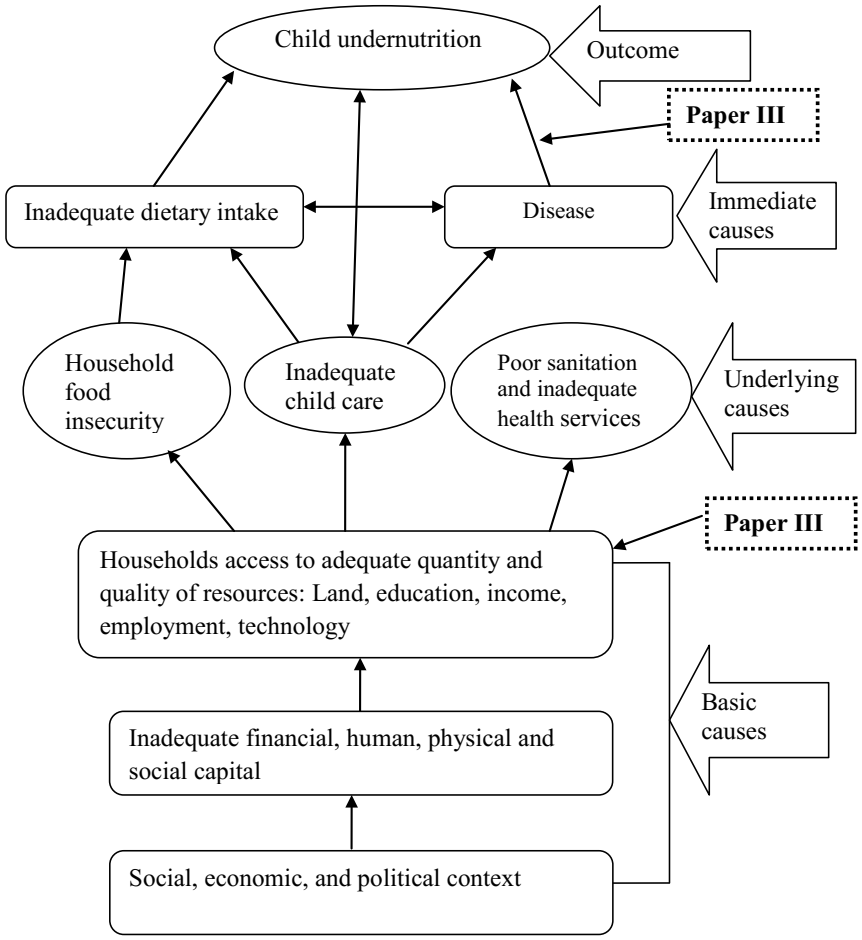

Figure 1: Conceptual framework for the causes of undernutrition in children (adapted from UNICEF nutritional strategy (172) and The Lancet Series (173)).

Immediate causes

The immediate causes work at the individual level, and include inadequate dietary intake and disease (171). Inadequate dietary intake related to suboptimal breastfeeding and poor complementary feeding practices are the most important immediate cause of undernutrition (173). The relationship between infection and malnutrition is like a vicious cycle: malnutrition

compromises the immune system of the children and contributes to an increased risk of infection (174). Meanwhile, infection increases the body's requirement for nutrients through a loss of appetite and poor absorption, and increases body metabolism (172, 175). Studies report that diarrhoeal disease (176), acute respiratory infection (177), parasitic infections (178) and HIV/AIDS (174) are among the main contributing factors of undernutrition in children. However, there is no conclusive finding on the relationship between malaria and undernutrition.

Underlying causes

The underlying factors are those that include household food insecurity (169), inadequate care and feeding practices (179). It is well documented that low household food security is associated with undernutrition in children (180). The presence of food in the household alone does not guarantee optimal child nutrition, and thus, adequate child care and feeding practices are crucial for optimal nutrition. Evidence shows that improving the educational status of the mother is an important determinant in improving child care and feeding practices (181, 182).

Basic causes

The basic causes include the potential resources available to a community, which are limited by human resources, the natural environment and access to technology (183). The utilization of these potential resources for the food security and healthcare of a society are determined by social, economic, cultural and political factors (171, 173). These factors can also differ from country to country.

Occurrence and interaction between malaria, anaemia and undernutrition

In areas where malaria, anaemia and undernutrition are prevalent, the three conditions often exist together (13). Studies show that malnutrition is an underlying cause of many deaths related to infectious diseases such as diarrhoea, pneumonia and measles (9, 10). Malaria infection has shown to be one of the leading causes of anaemia in children in sub-Saharan Africa (16).

However, scientific controversies exist concerning the association between malaria and malnutrition. A randomized controlled trial from Ghana that aimed to measure the impact of malnutrition on malaria and the efficacy of intermittent preventive therapy for malaria observed a higher risk of stunting among children with malaria, but they did not observe malnutrition as a risk factor for malaria incidence (26). A cohort study among children less than 10 years old from Vanuatu showed an increased risk of developing wasting among children with clinical malaria compared to those without malaria, but did not show acute malnutrition as a predictor for clinical malaria (25). Another cohort study that involved children aged 28 to 60 months in Kenya observed an increased risk of stunting among children with malaria compared to those without the malaria illness (21). On the other hand, a cross-sectional study from Kenya observed an increased risk of clinical malaria among stunted children compared to non-stunted children (22). Furthermore, a case-control study from Ethiopia has shown an increased risk of malaria among children with undernutrition (184). Nonetheless, a cross-sectional study from the Democratic Republic of Congo (24), and cohort studies from India (23) observed a lower risk of malaria infection among malnourished children. There are also cross-sectional studies that did not show an association between malaria and malnutrition (137, 185). In conclusion, most of the studies measuring the relationship between malnutrition and malaria were community-based cross-

sectional studies (22, 24, 137, 185), which could have less strength of evidence to support a causal association. Therefore, we did a community-based cohort study with a longer follow-up period on the relationship between malaria and malnutrition (Paper III).

Economic and social impacts of undernutrition

Malnutrition during pregnancy and the first two years of childhood could lead to an intergenerational effects, meaning undernutrition during childhood could affect the growth of the next generation of girls and their future children (131). Undernutrition during childhood could also result in short-term consequences such as illnesses and deaths (173), whereas the long-term consequences of undernutrition are irreversible physical and cognitive damage, and an increased risk of chronic disease such as diabetes mellitus and hypertension as adults (131). Thus, undernutrition during childhood could have a serious impact on future economic well-being and welfare.

History of child healthcare and nutrition intervention

A systematic review by Lindsay H. Allen has shown that nutrition programmes have shifted their primary emphasis from the control of protein deficiency in the 1930s, to energy deficiency in the 1960s to micronutrient deficiencies in the 1990s (186). In 1992, the global intergovernmental conference on nutrition advocated integrating nutritional objectives into general development programming, and to address those at an increased risk of malnutrition (187). Cognizant of the challenges of specific case diagnoses and treatment in poor resource settings, the WHO and UNICEF developed an integrated management for a childhood illness (IMCI) strategy to address the common child health problems, including malaria and malnutrition, in 1995 (188). Following

the 1996 World Food Summit call for food and nutrition security for all, the Millennium Development Goals were endorsed in 2000 to reduce child underweight by 50% (189, 190). This was strengthened in 2002 by the global strategy for infant and young child feeding (191), with the aim of improving nutritional status, health, growth and development, and hence improve the survival of infants and young children. Among others, ending any form of malnutrition (including stunting and wasting) by 2030 is among the SDGs adopted by the United Nations General Assembly (120).

Strategies to prevent and control undernutrition

To control undernutrition, a comprehensive approach with a targeted strategy at the community, health system and national level is recommended (171). In Ethiopia, IMCI, the Community Based Nutrition (CBN) programme, Health Facility Nutrition Services, Micronutrient Interventions and Essential Nutrition Actions/Integrated infant and young child feeding counselling services are the existing guidelines that could assist in tackling the common causes of childhood illnesses and death (133, 192).

The IMCI strategy includes both preventive and curative elements to be implemented by the families, communities and health facilities (193). Furthermore, the Infant and Young Child Feeding Strategy sets the standards for global action in support of optimal breastfeeding and complementary feeding based on the key lessons learned from the past (194). Globally, in using the concerted effort, the trend in prevalence of stunting declined from an estimated 40% in 1992 to 26% in 2011, with an average reduction of 2.1% per year. However, the progress in reducing stunting was less in sub-Saharan Africa, including Ethiopia, with a decline from 47% in 1992 to

40% in 2011 (171). Unfortunately, the prevalence of stunting remains high in sub-Saharan countries. The major challenges related to the slow achievements in sub-Saharan Africa were poverty and repeated droughts, resulting in a lack of food, and political instability (167, 183). In addition, the region is facing the double burden of malnutrition and the co-existence of overweight and stunting (171).

A comprehensive implementation plan on maternal, infant and young child nutrition endorsed in 2012 by the World Health Assembly specified global nutrition targets in 2025 from the 2010 baseline. Among others, reducing stunting by 40%, and wasting to less than 5% among children under 5 years of age are the formulated targets (195).

Malaria, anaemia and malnutrition prevention services in Ethiopia

Ethiopia: The country

Ethiopia, one of the oldest civilisations in the world, covers an area of 1.1 million square kilometres. Based on topography and climate, Ethiopia has three broad agro-ecological zones such as the hot zone (less than 1,500 metres above sea level), areas of average (mid) climatic conditions (1,500-2,400 metres above sea level) and the cool temperature highlands (above 2,400 metres above sea level). The general annual rainfall distribution is seasonal, and varies across geographic locations in the country (132).

The total population of the country was estimated at over 101 million in 2016. Nearly half (49.5%) of the population is female, and 80% of the population lives in rural communities (196), with children under the age of 5 years accounting for 14.5% of the population. Rain-fed

agriculture is the backbone of Ethiopia's economy, which accounts for approximately 50% of the gross domestic product. Even if Ethiopia had achieved a rapid economic growth in the past few years, the 2016 Human Development Index shows that the country is still among the poorest nations in the world (197).

Health services in Ethiopia

Ethiopia has a poor health status compared to other low-income countries (198). The main determinants of poor health are infectious diseases (60-80% of the health problem) and malnutrition (198). Cognizant of the prevailing health problem, the existing health policy of the country gives priority for infectious diseases that affect the vast majority of the population (199). The policy also gives an emphasis to maternal, neonatal and child health services in order to improve the health status of women and children.

The current health service delivery is organized as a three-tier system, and includes Tertiary level health care (Specialized Hospital), Secondary level health care (General Hospital) and the Primary level health care (Primary Hospital, Health Centre and Health Post) (200). On average, a health post serves a *kebele* (the lowest government administrative unit that comprises approximately 1,000 households). The rapid expansion of the health posts, and HEW deployment between 2004 and 2010 have increased the access to, and utilization of primary care by promoting a community-based maternal and child health services (201). The initiative of the health extension programme played a substantial role in the improvement of the coverage of interventions to prevent and treat common causes of child mortality, in particular immunizations, malaria prevention and treatment, and exclusively breastfeeding.

In Ethiopia, under-5 mortality rate has decreased from 204/1,000 live births in 1990 to 67/1,000 live births in 2016. Few preventable and treatable conditions such as infections, including malaria, neonatal conditions and malnutrition, account for over two-thirds of child deaths (134). Malaria, anaemia and malnutrition prevention and control services are integrated into maternal new-born and child health services, and mainly delivered by the primary level health care, particularly the health post, health centre and district hospital (200).

Context of the study

Ethiopia is one of the most drought-prone countries in Africa, and has been affected by repeated droughts in the past (202, 203). In 2015 and early 2016, the country was again affected by a severe drought and famine triggered by the El Niño effect (135), as the occurrence of malaria and malnutrition can be affected by climate change (70, 170). During the drought season, the decrease in rainfall and increase in temperature could decrease the vector density, and result in reduced malaria occurrence (204, 205). On the other hand, the household food shortage related to drought could be associated with an increased prevalence of undernutrition and micronutrient deficiencies, including anaemia (206, 207).

The study setting of this thesis, the Adami Tullu District, is situated in the Rift Valley area. The capital of the district, Zeway (Batu), is located at a latitude of $7^{o}56'$ N and longitude of $38^{o}42'$ E, with an elevation of 1,640 m above sea level. Rain-fed agriculture and cattle rearing are the primary livelihood of the study population. The main crops growing in the district are maize, wheat and sorghum. Like many districts in the country, the study area was affected by repeated droughts and famines over the past few decades (202), and was also affected by the 2015 and

early 2016 El Niño, which triggered a serious drought and food shortage (135). The drought caused an increase in the mean annual temperature of 2^{o}C, while the total rainfall decreased by 60% of that expected in 2015 (208). The Government of Ethiopia categorized the district as one of the most severely affected districts in the country (Figure 2), and mass food distribution as emergency relief was done in the area.

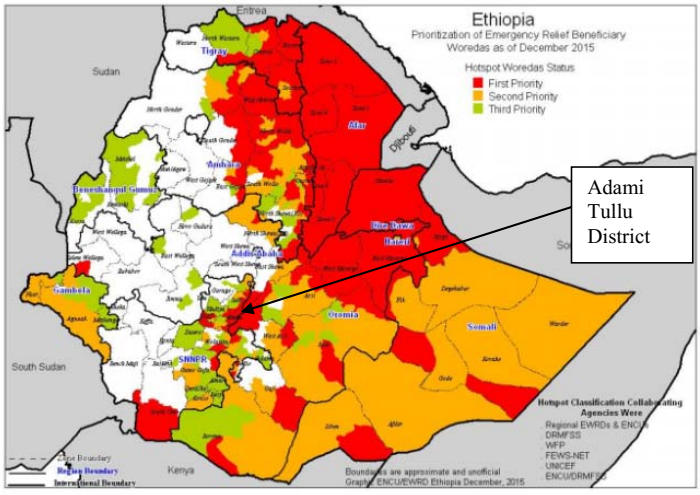

Figure 2: Districts prioritized according to severity of drought in December 2015 (Adapted from International Federation of Red Cross and Red Crescent Societies(135))

Rationale for this thesis

Malaria, anaemia and undernutrition are common childhood health problems in Ethiopia (37, 41, 134). Malaria transmission varies much between villages, and also between households in the same district. The occurrence of malaria in a small-scale area (micro-geographic) is a challenge for malaria control; hence, targeting malaria hotspots, the areas with the greatest risk of malaria transmission, could increase the efficiency of control interventions (49, 51). The risk factors for the micro-geographic variations are not fully understood. Thus, knowledge of the variability in malaria episodes among- and within villages could help to design targeted interventions to help more efficiently reduce malaria. In Paper I, we assessed the variation in malaria incidence and *Anopheles* population between villages in the same *kebeles*, which might provide relevant information for the local health service managers to plan targeted malaria control interventions.

Moreover, our research team conducted a cluster randomized controlled trial with the aim of measuring the effect of combining IRS and LLINs on malaria incidence, Hb concentration and entomological parameters over the individual interventions alone. The knowledge of variation in malaria incidence among- and within the villages from Paper I was used to calculate the sample size for our trials, and to locate the study area for the trial (1). The trial protocol is annexed (Appendix III).

Malaria infection can be a risk factor for anaemia (209), and studies have shown that malaria prevention can help reduce the prevalence of anaemia (210). In addition, it has also been documented that both LLINs and IRS are effective in reducing anaemia when applied

independently (164, 210). Nevertheless, scientific controversies exist on the combined use of the two interventions over individual intervention alone on changes in Hb concentration.
As part of a cluster randomized controlled trial to provide evidence on the combined use of LLINs and IRS for malaria prevention, we assessed Hb concentration among children aged 6 to 59 months (Paper II). The results of this paper, measuring Hb concentrations in the same population through repeated surveys, could be important in describing the occurrence and risk factors of anaemia over a period of one year. Consequently, the findings of Paper II provide important information for the malaria prevention trial on the effect of combining LLINs and IRS compared to individual interventions on anaemia reduction among children during the first year of the trial.

Moreover, malaria, malnutrition and anaemia prevention strategies could be affected by unexpected events such as drought and famine. The study area was affected by serious drought and famine in 2015 and early 2016 (135), and the findings of Paper II could also provide additional information regarding anaemia occurrence during serious drought and famine situations in the presence of mass food distribution, as well as the challenges of conducting malaria prevention trials in drought-prone settings.

Furthermore, additional intervention strategies such as improving nutritional status could be a supplementary option to help malaria elimination goals if undernutrition is a risk factor for malaria. However, the existing evidence on the association between malaria and malnutrition are inconsistent (125). A cohort study follows a group of people forward in time from exposure to outcome; the temporal sequence between exposure and outcome is clear (211), and could yield

valid information in assessing possible causal relationship. Therefore, Paper III may contribute with information for the growing body of scientific knowledge on the interaction between malaria and undernutrition.

Objectives

General objectives

The overall aim of this thesis is to improve our understanding of the effect of malaria prevention on anaemia, and to assess the association between malaria, anaemia and undernutrition among children in a drought-affected area in south-central, Ethiopia

Specific objectives

1. To estimate variations in malaria incidence and vector populations between *kebeles*, and within- and between villages in the same *kebele* (Paper I).

   - To provide baseline data on variations in malaria incidence within- and between villages for the sample size calculations for the trial, "Combining long-lasting insecticidal nets and indoor residual spraying for malaria prevention in Ethiopia: Study protocol for a cluster randomized controlled trial."

2. To estimate the prevalence and risk factors of anaemia among children followed over a period of one year (Paper II).

3. To assess the bi-directional association between malaria and undernutrition among a cohort of children aged 6 to 59 months old (Paper III).

Methods

Study locations

The Adami Tullu District is located in the Oromia Regional State, 160 km south of Addis Ababa in the Rift Valley of Ethiopia (Figure 3). The district is sub-divided into 47 *kebeles* (the lowest government administrative unit), which in turn are divided into smaller villages called "*Gare.*" The Adami Tullu District shares a border with the Abijitta and Langano lakes in the south, and with Zeway lake in the northeast. In 2014, the estimated population of the district was approximately 173,000 people, and 12% of the population were children under the age of 5 years (212).

Each *Kebele* has one health post staffed by two community HEWs. The HEWs are providing basic curative, disease prevention and health promotion services, in addition to malaria diagnosis (with RDT) and curative (with Artemether-Lumefantrine) services (28), deworming for intestinal parasites such as hookworm and trichuris, and nutritional interventions such as nutritional screening and treatment of moderate and severe acute malnutrition without medical complications (213, 214).

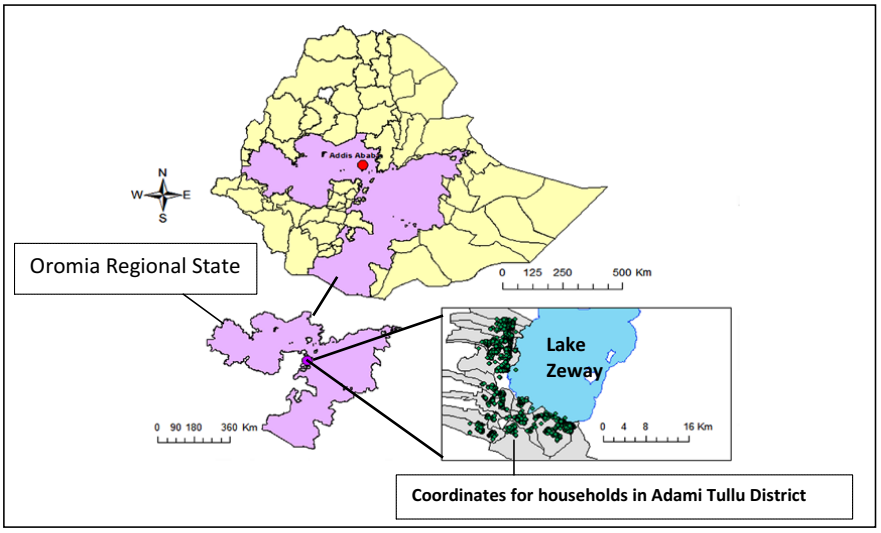

Figure 3: Geographic location of study area, the Adami Tullu District, Ethiopia, 2016

Study design and data

For this thesis, the summary of the study design, population and data sources are presented in Table 1. This thesis work was a part of malaria prevention trial called, "Combining long-lasting insecticidal nets and indoor residual spraying for malaria prevention in Ethiopia," the short name being the MalTrials Project (1).

MalTrials Project

Briefly, the trial was conducted between August 2014 and January 2017. The aims of the trial were to investigate whether the combination of LLINs with IRS would enhance the protective benefits of malaria infection, and improve Hb concentrations and the cost-effectiveness of the interventions against malaria and its effect on mosquito behaviour, as compared to each intervention alone. The calculated sample size was 44 cluster or villages per four intervention

arms, a total of 176 villages. We randomly selected these 176 villages in 13 *kebeles* within 5 km from Lake Zeway and the Bulbula River. The villages were randomly assigned to the four intervention arms: LLINs + IRS, LLIN alone, IRS alone and Routine arm. The study involved 34,548 people living in 6,071 houses. We gave a specific identification number for each household using a metal plate, as well as a unique personal identification number for each inhabitant in the household. The geographic coordinates of the household were taken using a handheld Global Positioning System device. The baseline census on socio-demographic and economic characteristics, malaria prevention and treatment practices, deaths, any illness history and health-seeking behaviours was conducted using an interviewer-administered structured and pre-tested questionnaires between June and July 2014. Following randomization of the villages to either of the four malaria prevention trial arms, LLINs + IRS and IRS alone arms were sprayed with carbamate propoxur once a year, and LLINs were provided free of charge to those in the LLINs + IRS and LLINs alone arms. Next, we carried out weekly home visits to identify a person with fever or having a history of fever within the past 48 hours, and referred the person to the health post with a referral card. The residents were also encouraged to visit health posts if they developed a fever between the once a week visit days. To include residents joining the cohort through birth or newcomers, a census was updated in August 2015 and August 2016.

Study design and data for pilot study (Paper I)

Three PhD students participated in the pilot study, epidemiology (Taye Gari), entomology (Oljira Kenea) and health economics (Alemayehu Desalegne). I worked on the epidemiology part of the pilot study (Paper I). A pilot study involving epidemiological and entomological studies was conducted to gather baseline information that we could use for the trial. This included studies on

the micro-epidemiology of clinical malaria, and a description of the malaria vectors and description of any association between malaria incidence and malaria vector density. We used the variation in the malaria incidence to calculate the intra-cluster correlation coefficient (the coefficient of variation of the malaria incidence rates among the villages) (215), which was used to estimate the needed sample size for the malaria prevention trial (1).

Briefly, the entomological study part of Paper I, conducted by PhD student Oljira Kenea, was conducted from June to October 2013, and a total of 36 *gares* or villages (12 per *kebele*) were randomly selected for adult mosquito collection. The mosquitoes were collected using the Centers for Disease Control light traps set indoors close to a sleeping person, pyrethrum spray sheet catches and artificial outdoor pit shelters. Enzyme-linked immunosorbent assays were also used to detect the sources of mosquito blood meals, while mosquito longevity was estimated based on parity.

For the epidemiological study of Paper I, a cohort study was conducted before the start of the trial in villages from four *kebeles* in the Adami Tullu District. This epidemiological study was conducted between August and December 2013, whereas the entomological study was conducted between June and October of the same year. The *kebeles* were selected based on their distance from Lake Zeway and the Bulbula River, and used as proxy for the main mosquito breeding sites. We included all residents living in the villages within 3 km of the community health posts. Those who refused to participate in the study, and temporary visitors were excluded. The houses were given a number tag, and a household census was conducted to collect socio-demographic and malaria-related data. The residents were followed for 16 weeks, and active (weekly home

visits) and passive (self-referral) surveillance were used to search the malaria cases. In the health posts, the blood samples were collected from the patients for RDT and blood film preparation. The RDT result was used to treat the patients, while blood smears were prepared using the WHO blood film preparation guidelines for microscopic blood film examination for malaria (216). The prepared blood film slides were read by two different senior microscopy readers blinded to the results of each other. In Paper I, we considered a malaria infection if the presence of malaria parasites in the blood sample was confirmed by two microscopic slide readers.

Study design and data for Paper II

For Paper II, a cohort study and cross-sectional study involving all children aged 6 to 59 months old enrolled in the malaria prevention trial were conducted between December 2014 and December 2015. The cross-sectional surveys involved all children enrolled in the main trial, 2,984 children in December 2014 and 3,128 children in December 2015.

Two cohort groups, a dynamic and a closed cohort, were identified from the first Hb concentration survey (December 2014), and we followed the non-anaemic children for one year. Malaria infection was a main exposure, and anaemia was the outcome variable. The dynamic cohort, in which newborn children and newcomers joined the cohort and older children (age greater than 59 months) left the cohort, involving 2,141 children recruited from the December 2014 survey and 1266 children joining the cohort during the follow-up period, making a total of 3,407 children. In contrast, a total of 1,244 children participated in the closed cohort (children aged over 59 months, those who left the area, newcomers and newborns were excluded).

The main exposure (malaria) was assessed through weekly home visits and passive patient self-referral to health posts. A year later, in December 2015, we measured the anaemia incidence (outcome) among these same children.

Study design and data for Paper III

For Paper III, we assessed the association between malaria and undernutrition using two cohort studies followed-up for 89 weeks. The cohort of children was identified through four anthropometry surveys. During the surveys, we included all children aged 6-59 months enrolled in the main trial, with 2,945 children in December 2014, 2,528 children in August 2015, 3,044 children in December 2015 and 2,790 children in August 2016.

In the first approach (malaria/malnutrition), a cohort of 2,328 non-stunted and 4,204 non-wasted children were included to assess the incidence of undernutrition (outcome) among children based on their previous malaria status (exposure). In the second approach (malnutrition/malaria), a cohort of 4,468 children was followed-up to measure malaria (outcome), taking undernutrition as an exposure variable. Both weekly home visits and passive malaria case detection were carried out. Malaria was diagnosed using RDT, and anthropometry surveys were conducted twice a year for two consecutive years.

Table 1: Study design, participants, and data collection method

Papers	Design	Participants	Data
Malaria incidence and entomological findings in an area targeted for a cluster-randomized controlled trial to prevent malaria in Ethiopia: Results from a pilot study (Paper I)	Cohort	Residents from four *kebeles*	Interview with head of household. Microscopic blood film examination for malaria parasite.
Anaemia among children in a drought-affected community in south-central Ethiopia (Paper II)	Cohort and cross-sectional	Children aged 6-59 months	Interview with caregivers, anthropometric and Hb concentration measurements, and RDT for malaria.
Malaria increased the risk of stunting and wasting among young children in Ethiopia: Results of a cohort study (Paper III)	Cohort	Children aged 6-59 months	Interview with caregivers, RDT for malaria and anthropometric measurements.

Assessment of exposure and outcome variables

Malaria status was determined using microscopic blood film examination in Paper I, and RDT results in Papers II and III. Anthropometric indices were calculated based on the WHO 2006

growth reference standard (12), and Hb concentration was assessed using HemoCue HB 301 (217). The details of exposure and outcome variables are presented in Tables 2 and 3.

Table 2: Definitions of outcome variables used in this thesis

Variable	**Definition/measurement**	**Paper**
Malaria	A child or person was considered as having malaria if malaria parasite was seen under microscopic blood film examination (Paper I) or if RDT was positive (Paper III).	I, III
Anaemia	A child was considered anaemic if the Hb concentration was less than 11 g/dl.	II
Stunting	A child with a height-for-age Z-score of less than 2SD (Standard deviation) of the WHO 2006 median growth reference was considered as stunted.	III
Wasting	A child with a weight-for-height Z-score of less than 2SD of the WHO 2006 median growth reference was considered as wasted.	III
Mean mosquito host-seeking abundance	The average host-seeking abundance of *Anopheles* collected per night per house.	I
Mean mosquito resting abundance	The average resting abundance of *Anopheles* obtained per house per day.	I

Table 3: Definitions of exposure variables used in this thesis

Variable	**Level**	**Definition**	Paper
Distance from mosquito breeding site	*Kebeles*	The *Kebeles* distance from the lake or river was calculated from the geographic coordinates of households taken using the Geographic positioning system.	I
Malaria	Individual	A child or person was considered as having malaria if RDT was positive.	II, III
Stunting	Individual	A child with a height-for-age Z-score of less than 2SD of the WHO 2006 median growth reference was considered as stunted.	II, III
Wasting	Individual	A child with a weight-for-height Z-score of less than 2SD of the WHO 2006 median growth reference was considered as wasted.	II, III
Underweight	Individual	A child with a weight-for-age Z-score of less than 2SD of the WHO 2006 median growth reference was considered as underweight.	II, III
Wealth index	Household	Wealth index was constructed using principal component analysis from household assets-related variables. The households were then ranked into three categories such as poor, medium and rich.	I, II, III
Educational status	Individual	Assessed from the question that asked the highest education level attained by the parent or caregiver. Then, it was classified as not attended formal education, primary and secondary and above.	I, II, III
LLINs use	Individual	LLINs use is defined as a self-report of sleeping under an LLINs last night before an interview.	I
Intervention	Household	The type of intervention given to the household could be LLIN + IRS, LLIN alone, IRS alone or routine arm.	II, III

Measurements and standardization

Following the intensive training and skill practice, we did a standardization of anthropometric measurements of 10 children in a neighbouring community not participating in the study. A standard wooden board was used to measure height or length, and a calibrated Salter spring scale was used to measure the weight of the children. The weight measuring scale was adjusted, and the weight was read to the nearest 0.1 kg. The length of children less than 24 months old was taken in the recumbent position, while for the older children the height was measured standing on a vertical measuring wooden board, which was read to the nearest 0.1 cm. There were 12 teams of observers measuring all the children twice. First, each observer measured all the 10 children, and then took a break before taking the second measurement with a separate format.

The inter- or intra-observer technical error measurement (TEM) and reliability coefficient for weight and height were calculated. Technical error measurement is a measure of error variability (12), and could be intra-observer or inter-observer TEM. Intra-observer TEM is estimated from the difference between a repeated measurement taken by one observer. Inter-observer TEM is estimated from single measurements taken by more than one observer, with the reliability coefficient estimating the proportion of inter-subject variance (total measurement variance) that is not due to measurement error (12). The intra- or inter-observer TEM and reliability coefficient were within a range of a suggested cut-off point for the acceptability of the measurements (Table 5) (12).

Table 5: Inter- and intra-observer technical error of measurement (TEM) for 10 children in the standardization session

	Weight in Kg	Height in cm
Mean	10.9	85.1
Intra-observer TEM	0.08	0.14
Inter-observer TEM	0.1	0.2
Reliability coefficient	0.97	0.98

Statistical analysis

Data were analysed using Statistical Package for the Social Sciences 21 (SPSS Inc., Chicago) and STATA 13 (STATA Corp, College Station, Texas). Descriptive statistics were used to present the data. A principal component analysis (218) was further used to construct a household wealth index from 14 variables related to household assets and type of materials from which the houses were made (Papers I, II and III). A multilevel mixed effect and Cox regression models were fitted to measure the risk factors of malaria (Paper I) and anaemia (Paper II), whereas a generalized estimating equation (GEE) model was fitted to measure the risk factors of stunting, wasting and malaria (Paper III). The major statistical methods used in this thesis are summarized in Table 4.

Table 4: Major statistical methods used for data analysis

Statistical methods	Papers
Descriptive statistics	Paper I Paper II Paper III
Anthropometric analysis (Z-scores)	Paper II Paper III
Principal Component Analysis	Paper I Paper II Paper III
Multilevel mixed effect model	Paper I Paper II
Logistic regression model	Paper II Paper III
Cox regression model	Paper II
Generalized estimating equation	Paper III

Ethical considerations

Ethical clearance was obtained from the Institutional Review Board of the College of Health Sciences in Addis Ababa University, from the National Ethics Committee of the Ethiopian Ministry of Science and Technology (ref: 3.10/446/06) and from the Regional Committee for Medical and Health Research Ethics, Western Norway (ref: 2013/986/REK Vest). The trial protocol was registered at the Pan African Clinical Trials Registry under the number PACTR 201411000882128 in 2014.

Written permission was obtained from the Oromia Regional Health Bureau, East Shewa Zone Health Department and the Adami Tullu District Health Office. Informed verbal consent was obtained from the parents or caretakers of the children. Children with malaria and malnutrition were treated according to the malaria and malnutrition treatment guidelines of the Ethiopian

Ministry of Health. Patients with *P. vivax* were treated with Chloroquine, and those with *P. falciparum* or mixed infection were treated with Artemether-Lumefantrine (104). Children diagnosed with uncomplicated severe acute malnutrition were treated with Ready-to-Use Therapeutic Food called Plumpy'nut® (214).

Results

Paper I: Malaria incidence and entomological findings

We aimed to evaluate variations in malaria incidence and the vector population in a small-scale area (micro-epidemiology) such as within- and between villages in the Adami Tullu District. Furthermore, we aimed to provide baseline data on variations in malaria incidence within- and between villages for the sample size calculations for the Trial "Combining long-lasting insecticidal nets and indoor residual spraying for malaria prevention in Ethiopia: Study protocol for a cluster randomized controlled trial."

A cohort of 5,309 residents from four *kebeles* in 996 households was followed from August to December 2013. We collected blood samples from 349 patients with fever (body temperature ≥37.5 ^{o}C and/or history of fever in the last 48 hours), and 39 (11.2%) were microscopically confirmed positive for malaria infection, and *P. vivax* was the dominant species (85%). The overall incidence of malaria was 4.6 cases per 10,000 person-weeks of observation. We showed a large difference in malaria incidence between *kebeles*, 0.5 cases/10,000 person-weeks in *kebeles* beyond 5 km from Lake Zeway and the Bulbula River, and 7.9 cases/10,000 person-weeks of observation in those *kebeles* within 5 km of the lake and river. Moreover, we observed a large variation in malaria incidence among villages within 5 km of the lake and river, a range between zero to 23 cases per 10,000 person-weeks of observation. We estimated an intra-cluster correlation coefficient (ICC) of 0.27, using malaria incidence among villages within 5 km of the lake and river. A high malaria incidence rate was observed among children under the age of 5 years old (6.8 cases per 10,000 person weeks) and among children aged 5 to 14 years (6.4 cases per 10,000 person-weeks).

Only 271 (27.3%) of the households owned insecticide-treated nets. The percentage of LLINs use among households that owned an LLINs was 49%. The risk of malaria was higher among children in the age group of 5 to 14 years, with an adjusted hazard ratio (AHR) =2.7; 95% confidence interval (CI); 1.1–6.6. Residents living in the *kebeles* within 5 km of the lake and river were 14.2 (95% CI 3.1–64.7) times more likely to be malaria-positive than those living further away from the lake and river. However, wealth status, household education, sex and LLINs use were not risk factors for malaria in this study.

Although the entomological part of this study was done by PhD student Oljira Kenea, some of the results of the epidemiological and entomological findings overlap. The relative abundance of mosquitoes increased in September and the beginning of October, and this coincided with an increase in malaria incidence in the same and subsequent months. In villages where entomological and epidemiological studies overlapped, a high mosquito host-seeking density of *Anopheles* was associated with a high malaria incidence. The dominant species was *Anopheles arabiensis,* and the mosquitoes were susceptible to carbamates insecticides, but showed a high resistance to deltamethrin (used in LLINs). Based on these findings, Propoxur (carbamate) was selected as the insecticide for the IRS during the trial.

Paper II: Anaemia among children

We aimed to measure the occurrence and risk factors of anaemia over a period of one year. We also aimed to evaluate the effect of combining LLINs + IRS on anaemia reduction over LLINs or IRS alone during the first one year of the malaria prevention trial among children.

Of the 2,984 children involved in the 2014 survey, 1,851 (62%) children were also examined in the 2015 survey. The main reason for the lost to follow-up were the exclusion of children older than 59 months (64.1%) and left the study area (19.5%). Most of the children who left the study area were from poor families (45.2%), and the prevalence of anaemia in those children who left the area was higher (45.7%) than among those children who stayed in the area (32.9%).

The prevalence of anaemia was 28.2% (95% CI, 26.6-29.8) in 2014, and increased to 36.8% (95% CI,35.1-38.5) in 2015 (P <0.001). The prevalence of anaemia among the different arms of malaria prevention trial was 38% (LLINs + IRS arm), 35% (LLINs alone arm), 39% (IRS alone arm) and 36% (routine arm) in 2015.

On the other hand, the overall mean Hb concentration decreased from 11.6 g/dl in 2014 to 11.2 g/dl in 2015 (P<0.001). The mean Hb concentration was similar among the trial arms, 11.1 g/dl (LLINs + IRS), 11.2 g/dl (LLINs alone), 11.2 g/dl (IRS alone) and 11.4 g/dl (Routine) in 2015. The incidence of anaemia in the dynamic cohort was 30 cases (95% CI, 28-32) per 100 children-years of observation, while in the closed cohort it was 28 cases (95% CI,25-31) per 100 children-years of observation.

The multivariate multilevel logistic regression analysis of the cross-sectional studies showed that children living in poor families, children who were stunted and children aged less than 36 months were at an increased risk of anaemia in both the 2014 and 2015 surveys. However, no statistically significant difference in anaemia was observed among the different malaria prevention trial arms.

In the Cox regression analysis of the dynamic cohort study, the risk of anaemia was high among children with malaria, with an adjusted Hazard Ratio (AHR) =10, (95% CI 3.8-28.8) compared to those without malaria infection. Children from poor families (AHR; 1.2; 95% CI,1.04-1.4), stunted children (AHR 1.2; 95% CI;1.04-1.4) and children aged less than 36 months (AHR; 3.2; 95% CI, 2.8-3.7) were more likely to be anaemic compared to their counterparts. There was no significant difference in risk of anaemia among the trial arms.

Paper III: Malaria associated with an increased risk of undernutrition

We aimed to assess the bi-directional association between malaria and undernutrition among a cohort of children aged 6 to 59 months old (Paper III).

We observed a stunting prevalence of 44.9% in December 2014, 51.5% in August 2015, 50.7% in December 2015 and 48.1% in August 2016, whereas the prevalence of wasting was 7.2% in December 2014, 5.7% in August 2015, 4% in December 2015 and 6.7% in August 2016.

In the malaria/malnutrition cohort, we observed 685 new stunting and 239 new wasting cases. The incidence rate per 10,000 person-weeks of observation was 50.4 cases (95% CI, 46.7-54.3) for stunting and 8.2 cases (95% CI, 7.2-9.4) for wasting. The fitted GEE model showed malaria illness in the six months preceding the nutritional survey (AOR = 1.9; 95% CI, 1.2-2.9) and younger age (AOR= 1.3; 95% CI, 1.1-1.5) were the risk factors for stunting. Furthermore, malaria illness in the six months preceding the nutritional survey (AOR=8.5, 95% CI, 5.0-14.5) and younger age (AOR=1.6, 95% CI, 1.2-2.1) were the risk factors for wasting. Nonetheless, intervention arms, wealth index, educational status of caregiver and gender were not the risk factors for wasting and stunting.

In the malnutrition/malaria cohort, 103 malaria cases with 118 episodes of malaria were diagnosed, and only 10 children had more than one malaria episode. *P. falciparum* accounted for 52% of all malaria cases. The overall malaria incidence was 3.8 cases (95% CI, 3.1-4.6)/10,000 person-weeks of observation. In the GEE model, undernutrition (stunting or wasting) was not a risk factor for malaria after controlling for child age, gender, trial arms and educational status of

head of the household. However, children living in a household in the lowest wealth tertile (AOR= 3.3; 95% CI, 1.7-6.3) had increased odds of malaria compared to children in rich families.

Discussion

Methodological discussion

Study design

This thesis builds on results from the Maltrials Project, "Combining long-lasting insecticidal nets and indoor residual spraying for malaria prevention in Ethiopia: Study protocol for a cluster randomized controlled trial" (1). In Paper I of this thesis, we used the data from a pilot study conducted to provide information needed to calculate the sample size for the trial. The other two studies, Paper II and III, were based on a representative sample selected for the trial. In this thesis, we used a cohort study design (Paper I and III), and both cohort and cross-sectional study designs in Paper II.

Cluster randomized controlled trials are experiments in which the entire clusters or groups of individuals are randomly allocated to intervention groups (219). The strength of a cluster randomized controlled trial is that it minimizes the likelihood of bias in the assignment to alternative interventions, and also minimizes confounding, both known and unknown (215).

A cohort is a group of individuals, and a cohort study is the follow-up of people forward in time from exposure to outcome (211, 219). A cohort study is useful in establishing a temporal relationship between exposure and outcome variable, and less susceptibility to bias compared to other observational studies such as case-control, cross-sectional and ecological studies (220, 221). Some of the limitations of cohort study are it is inefficient for outcomes with very low incidence rate, has less control over confounding variables compared to randomized controlled trials and could be expensive and time consuming (221-223).

A cohort study can directly measure disease incidence or risk; however, the frequency of measuring the outcome could affect the incidence rate. For example, in the anaemia survey (Paper II) that we conducted once a year, it could not have captured some of the children who developed and recovered from anaemia between the surveys, which could have resulted in an underestimation of anaemia incidence. Similarly, we did an anthropometry survey twice a year, and some of the children could have developed and recovered from an acute undernutrition (wasting) between the surveys, and thus could not be captured. This might also have resulted in an underestimation of wasting.

In addition, we also employed a cross-sectional study design in Paper II. In cross-sectional studies, exposure and outcome status are measured at the same point in time (219). The estimation of the prevalence of disease using repeated surveys in the same population could be used to measure changes over time (222). One of the limitations of a cross-sectional study is that it is less able to prove causality, as it provides no direct evidence of the time sequence of events (224). For example, in Paper II, for the cross-sectional study we conducted anthropometry and Hb concentration measurement surveys at the same time, and we observed stunting as a risk factor for anaemia. But we did not know whether stunting occurred before anaemia or not, as we could not capture the exact onset of anaemia and stunting. On the other hand, we examined the relationship between stunting and anaemia among a cohort of non-anaemic children, and we observed stunting as a risk factor for anaemia.

Sample size

A study with an adequate sample size will have sufficient power to detect the difference considered to be important (224). The calculated sample size for the trial for the primary end point, malaria incidence, was 44 clusters or villages per four arms (LLINs +IRS, LLINs alone, IRS alone and routine arm) of the trial. The study involved 34,548 from 6,071 households, and was followed for 121 weeks. Therefore, many clusters (176 clusters or villages) of adequate size (a village having an average 35 houses) followed for a relatively longer time period (121 weeks) could provide valid information on the effect difference of the intervention arms.

We calculated the sample size for Paper II, and the estimated sample size was based on the following assumptions: We assumed that the combination of LLINs + IRS could increase the mean Hb concentration by 0.5 compared to either LLINs or IRS alone. We used an anticipated population mean (standard deviation) Hb concentration of 11.5 (1.66) g/dl reported from a study conducted in a similar rural community in Ethiopia (137), and considering a 95% confidence level, a power of 90% and a design effect of 2, the estimated sample size was 464 children per arm of the trial, resulting in a total of 1,856 children for the entire study. Although the estimated sample size sufficiently addresses the research question in this study, we included and followed all children (n=2,984) aged 6 to 59 months participating in all arms of the main trail. Similarly, in Paper III, all children aged 6 to 59 months old enrolled in the main trial were followed to measure the association between malaria and undernutrition (Paper III).

In the meantime, we did not calculate the sample size for the pilot study (Paper I), in which we included all the population in the selected villages. We assumed that the sample size (Paper I)

was large enough to detect the difference in malaria. We also calculated a post-hoc power calculation in Paper I, considering the distance from the lake and river as exposure (≤5 km = 47,080 person-weeks of observation versus >5 km = 37,808 person-weeks of observation) and malaria as an outcome (37 malaria cases among those within 5 km of the lake and 2 malaria cases among those residents living beyond 5 km from the lake), and the power was 95%.

Internal validity

Internal validity refers to the degree to which a study is free from bias (225), or the extent to which the results of an observation are true for the subjects being studied (224, 226). This implies a validity of inference to the source population, and is considered as a prerequisite for external validity (219). The internal validity could be violated by selection bias, information bias and confounding.

Selection bias

This is a bias that arises from procedures used to select study subjects (219) or the absence of comparability among the studied groups, and can occur from loss to follow-up, refusal, non-response or an agreement to participate in the study (227). Non-response bias occurs when the selected participant is not willing to participate in the study. In Paper I, we studied all residents within 3 km of the health post, and the response rate was 99%. The presence of a dedicated health post that gives a malaria diagnosis and treatment free of charge could have motivated the participants to complete the study. Thus, the result of Paper I is less likely to have been affected by a non-response bias.

In a cohort study, selection bias could result from a loss to follow-up (for example, the study subject can no longer be located or no longer wants to participate in the study) (221). The loss to follow-up can be differential when the loss is more or less likely to occur among exposed participants who develop the outcome than among unexposed participants who develop the outcome, and non-differential when the loss of a person with an outcome that happens equally among those exposed and unexposed (219, 221). We attempted to minimize the loss to follow-up by including all permanent residents and those who intended to stay in the study area for the entire study period. In Paper II, selection bias could have been introduced as a result of a considerable number of children lost to follow-up, which could be related to an unexpected serious drought and famine that affects the study area in 2015 and early 2016 (135). We observed a differential loss to follow-up by wealth status, as most of those who left the area were poor families. Moreover, a significant difference ($P<0.001$) in a prevalence of anaemia was observed among those who left the area compared to those children who completed the study. Despite the differential loss to follow-up that could have lowered the hazard ratio in anaemia even further (anaemia incidence among children in poor families in relation to rich families), we observed a significant association between wealth status and anaemia.

Information bias

This is a systematic bias that could arise during measuring the exposure or outcome variables (228). In Papers I, II and III, the census and weekly data were collected using interviewer-administered structured questionnaires. We tried to minimize the information bias during the interviews by training the data collectors, pre-testing the questionnaires and supervising the data collectors. In Paper I, the self-reporting of sleeping under LLINs, which could be difficult to

observe, could have introduced a social desirability bias. Such a bias occurs when the respondents are systematically more likely to provide a socially acceptable response that is an over reporting of sleeping under LLINs because of the presence of data collectors (220).

The other information bias could be related to recording the age of the child, which was obtained through parents or caregivers recall for some of the children. To minimize age-related recall bias and digit preferences, we used a local calendar to record the age of the child if the child did not have any immunization card or birth certificate. Measurement error could also occur during taking the height or length and weight of the children. We attempted to minimize this error through close supervision, and a standardization of the anthropometry instruments. We present the findings of standardization in Table 5, which measured that the inter- and intra-technical error of measurement was within an acceptable range. We used two microscopic slide readers blinded to each other for malaria blood film examination (Paper I), and discordant results were read by a third microscopic reader. Moreover, to minimize a bias in measuring the Hb concentration and malaria diagnosis using RDT (Paper II and III), we used the recommended standard procedures of the manufacturer. The malaria RDT could lose its sensitivity through exposure to high temperatures and moisture for a long time period (229). However, we obtained new RDT kits from the Oromia Regional Health Bureau, and we thought the RDT kits were stored properly in accordance with the standards.

Confounding

The origin of the word confounding is from a Latin word called *confundere*, meaning to mix together (224). Confounding is defined as the confusion, or mixing, that the effect of the

exposure is mixed together with the effect of a third variable, which can result in a bias (219). Confounding can be controlled during the design or analysis of the study. The methods of controlling confounding in the design phase include randomization, restriction and matching. Randomization is a random assignment of subjects to intervention arms (221). The other method of controlling confounding is restriction, as the admissibility criteria for study subjects are limited for a variable that might be a confounder (219). Restriction could limit the generalizability of the study; thus, we did not use restriction to control confounding. The third control method for confounding is matching, which is the process of making a study group and a comparison comparable with respect to extraneous variables (221).

During the analysis phase, confounding can be controlled using standardization, stratification and a multivariate analysis to adjust for a variable considered to be a confounder (219, 230). We used a multivariate analysis to adjust for the effect of confounders. In Paper I, the socio-demographic versus distance from the malaria breeding site; in Paper II, the effect of age, intervention group and sex was controlled to measure the risk of malaria on anaemia. In Paper III, the effect of socio-demographic variables such as sex, age, educational status of the parent, wealth status and intervention arms were adjusted to measure the association between malaria and undernutrition.

Iron deficiency and intestinal helminths are among the main factors contributing to the occurrence of anaemia (Paper II). However, we did not adjust for these factors, since we did not collect such data. While measuring the association between malaria and undernutrition (Paper

III), we could not control for household food insecurity and intestinal helminths because we did not have data on these variables.

Chance

The observed association between outcome and exposure could be due to chance. Therefore, the researcher must explain the results on the basis of chance (227). One can explain the role of chance using P-values or a 95% confidence interval. Unlike P-values that address only chance, the measure of association with confidence interval is useful to reveal the likelihood of the occurrence of chance, as well as the strength, direction and range of an effect (226). We evaluated the role of chance using different models; for example, to measure the association between malaria as exposure and undernutrition as an outcome in Paper III, we applied both logistic regression and generalized estimating equation models, and a consistent statistically significant association was observed. We used a $P < 0.05$ and a 95% confidence interval to evaluate statistical significance.

External validity

External validity is defined as the degree to which the results of a study may apply, be relevant or be generalized to a population (220). For the pilot study (Paper I), four *kebeles* were purposely selected based on their distance from the main mosquito breeding sites, and the study subjects were all residents within 3 km of the community health post in their respective *kebeles*. The study population are typical rural residents selected from different geographic areas (2 *kebeles* close to the primary mosquito breeding site and 2 *kebeles* far from the primary mosquito breeding site) in the Rift Valley of Ethiopia. Consequently, the findings of Paper I could reflect

the malaria transmission dynamics in a small-scale area, the micro-epidemiology of clinical malaria, in a similar setting.

The study area (Paper II and Paper III), the Adami Tullu District, is located in the Rift Valley area of Ethiopia. Our study was based on a random selection of villages for malaria prevention trials in the district, with the population being a typical rural community. Moreover, we included a large sample with adequate power and an adequate follow-up period. Despite the fact that the population is representative of the rural population living in similar ecological settings in Ethiopia, the generalizability of our findings might be affected by the context of our study period. In the years 2015 and 2016, the study area was affected by an unexpected severe drought and food shortages. Therefore, one should take this scenario into consideration while interpreting our findings.

Discussion of the main findings

In this thesis, the overall aim was to improve our understanding of the effect of malaria prevention on anaemia, and to assess the association between malaria, anaemia and undernutrition among children in a drought-affected area in south-central, Ethiopia.
We showed a large variation in malaria incidence among *kebeles*, and within and between villages in the same *kebele.* We observed that a younger age and *kebele* within 5 km of the lake or river were more at risk for malaria infection. Despite malaria prevention efforts, we observed an unexpected increase in anaemia prevalence over a period of one year. Conducting trials in areas prone to drought can bring unexpected challenges. We did not show a significant difference in anaemia among the various arms of the malaria prevention trial. Furthermore, the data showed that malaria was a risk factor for stunting and wasting in children.

Malaria is an infectious disease that can occur over a small geographic area, with some areas having a higher proportion of malaria cases (hotspot) than others (47). The occurrence of malaria transmission in a small-scale area, the micro-epidemiology, could pose a challenge for malaria prevention and control (43). Although the study area of Paper I was categorized as a malaria endemic area or less than 2,000 metre above sea level, we observed a large variation in malaria incidence among the *kebeles*, and residents living in a *kebele* within 5 km of the lake or river had a higher risk of malaria infection. This could be explained by the persistent presence of water bodies that favour the mosquito existence for most of the months in a year close to the lake (231), and hence, malaria transmission could occur throughout the year in those areas. Studies have shown that a households' proximity to the main vector breeding sites were the main risk factor for malaria infections (45, 232). Moreover, we observed a higher variation in malaria

incidence among villages close to the lake and river. Similar studies have also shown variations in malaria incidence between villages, even between the households' (46). The possible reason for a variation of malaria in the micro-geographic could be due to poor housing (66) and living close to a mosquito breeding site (52, 53).

The abundance and host-seeking density of the primary vector responsible for malaria transmission in Paper I, the female *Anopheles* mosquito, is related to an increased risk of malaria transmission in the community (233). We also observed a higher malaria incidence in the villages with a high mosquito host-seeking density, which could also be a cause for the variation of malaria incidence between villages.

In malaria-endemic areas, children have a lower acquired immunity to malaria infection (234). On the other hand, an immunity to malaria could result from the frequent exposure to malaria infections and thus, adults have a higher immunity to malaria (48). Consistent with other studies (45), we observed a higher malaria incidence among children compared to those of an older age (Paper I).

The calculated ICC (0.27) from Paper I is in a range of what is suggested by others (118, 235). The sample size efficiency to detect a significant difference between groups will be lowered when the unit of study is a cluster (215). Therefore, this ICC value was considered to account for a cluster effect when the sample size for the trial was calculated (1).

In Paper I, we observed a higher incidence of malaria compared to a study from Arba Minch in southern Ethiopia (45). This could be related to differences in length of follow-up time. Unlike the Arba Minch study, which involved all months of the years, we followed the study subjects for 16 weeks from August to December. In Ethiopia, studies have shown that the months between September to December are the major malaria transmission season (36).

In the anaemia study, Paper II, a decrease in anaemia prevalence was expected to be related to the community-wide malaria intervention in the study area. However, we observed an unexpected increase in the prevalence of anaemia (28% in 2014 versus 36% in 2015) over a period of a year. This could be due to the serious drought and food shortage affecting the study area (135), and nutritional factors and not malaria were probably the main reasons for the increase in anaemia prevalence.

Similar to a cluster randomized controlled trial from The Gambia (117), in Paper II we observed no significant difference in anaemia prevalence among the different malaria prevention trial arms (LLINs + IRS, LLINs alone, IRS alone and Routine arm) during the first year of the trial. The Gambian study had a high coverage of LLINs throughout the study period. Whereas in our study, despite a high coverage of LLINs (100%) at the beginning of the trial, the observed LLINs loss was much higher than expected. A preliminary finding of a study looking at the survival-ship of the LLINs distributed by the trial project showed only 35% of LLINs survived to one year (Solomon T. et al, unpublished). The lost of LLINs was much higher during the drought season.

Unlike our Paper II study, a cluster randomized controlled trial from Tanzania has shown a higher Hb concentration among children in the combination arm, IRS + LLINs compared to LLINs alone (118) after 6 months of the intervention. This difference could be partially due to the low malaria incidence in our study related to the serious drought that affected the study area. According to the data from the National Meteorology Agency, the main proxy measure for mosquito survival, the mean annual temperature increased by 2^{o}C (from 26 ^{o}C to 28 ^{o}C), while the annual rainfall decreased by 60% of that expected compared to the year preceding the trial (208). A study comparing different mathematical modelling also shows that the most efficient malaria transmission could occur at a temperature of 25 ^{o}C (74). Furthermore, the lower malaria incidence could be due to malaria residual transmission, remaining low malaria transmission in areas of high coverage of highly effective vector control interventions (56).

The malaria parasite invades red blood cells or suppresses red blood cell production, and therefore could cause anaemia (40). We observed a higher anaemia incidence among children with malaria compared to those without the disease (Paper II), and studies have also reported malaria as a risk factor for anaemia (142, 236). However, we did not find any protective effect of an intervention in reducing malaria. In the study area, the main causes of anaemia could be related to malnutrition. Undernutrition could also result in a lower immunity, ultimately predisposing children to infections that might deplete their iron reserve (17). Supporting the other studies (19), we observed chronic malnutrition (stunting) as a risk factor for anaemia. In line with other studies (13), we also showed that children living in poor families had a higher risk of anaemia compared to those in wealthy families. This could be due to poor families being less likely to afford an adequate and varied diet.

In Paper III, our study focused on the role of malaria as exposure and undernutrition as an outcome, and also undernutrition as exposure and malaria as an outcome in a large community-based cohort study followed for a relatively long time. The risk factors for mild-to-moderate undernutrition could be multifactorial, with an inadequate dietary intake and infection being immediate causes. If undernutrition is the risk factor for malaria infection, improving the nutritional status could be used as an additional strategy to control malaria. On the other hand, if malaria is a risk factor for undernutrition, an effective malaria prevention and control could be used as an additional tool to control undernutrition in children. Unlike our study, community-based cross-sectional studies have shown malnutrition as a risk factor for malaria infection (22, 237), but in line with our study, a cohort study from Ghana (26) did not observe an increased risk of malaria as an outcome among children with undernutrition (exposure). This inconsistent finding could be due to differences in the study design, in which the cross-sectional studies could have less strength to establish a temporal relationship between the two conditions. However, some studies have shown a lower risk of malaria parasiteamia among children with severe malnutrition (23). In this study, we employed RDT to diagnose malaria, which could not be used for malaria parasite count. As a result, we did not assess the association between the risk of malaria parasiteamia or severe malaria among children with malnutrition.

On the other hand, through a decreasing appetite and food intake and increasing metabolism related to infection, malaria could lead to malnutrition (238, 239). We showed that malaria in the 6 months preceding the nutritional survey was a risk factor for stunting and wasting (Paper III). Comparable trials and cohort studies also reported an association between malaria as exposure and malnutrition as an outcome (21, 25, 240).

The prevalence of stunting during the last three anthropometric surveys (range between 48% and 52%) in our study was higher than a study from eastern Ethiopia (45.8%) (241) and that of the 2015 Demographic and Health Survey report (38%) (134). This could be related to the drought and famine that affected the study area. In this study, we reported an increased prevalence of stunting (chronic malnutrition), but not wasting (acute malnutrition) among children over a year. As an emergency relief, the Ethiopian government provided a mass food distribution in 2015 and early 2016 for all residents. We thought the mass food distribution could have helped manage the acute malnutrition, but failed to address the chronic malnutrition.

Conclusion and recommendations

Conclusions

The conclusions for this thesis are presented in relation to each of the main objectives as follows:

Objective 1: We aimed to estimate the variation in malaria incidence and vector populations between *kebeles*, and both within and between villages in the same *kebele* (Paper I). Here, the main conclusions related to epidemiological findings are presented as follows:

- We observed a high variation in malaria incidence between villages (hotspot villages) in the same *kebele.*
- This study showed that children and *kebeles* closer to the lake or river had a higher incidence of malaria.
- The calculated intra-cluster correlation coefficient (ICC) was 0.27.
- A high malaria incidence was observed among villages with a high mosquito host-seeking density

Objective 2: We aimed to estimate the prevalence and risk factors of anaemia among children followed over a period of one year, and we reached the following conclusions:

- Despite the malaria prevention efforts in the community, an unexpected increase in anaemia prevalence was observed over a period of one year.
- Conducting trials in a drought-prone area may bring unexpected challenges.
- There was no significant difference in anaemia prevalence among the different trial arms.
- Malaria was a risk factor of anaemia. Also young age, stunting and poverty were predictors for anaemia.

Objective 3: We aimed to assess the bi-directional association between malaria and undernutrition among a cohort of children aged 6 to 59 months (Paper III), and our conclusions are:

- Malaria infection was a risk factor for stunting and wasting.
- Neither stunting nor wasting were risk factors for subsequent malaria infection.
- An increase in a prevalence of chronic malnutrition (stunting) was observed over the period of a year. However, we did not observe an increase in the acute form of malnutrition (wasting).

Recommendations

Operational and Policy

We aimed to estimate the variation in malaria incidence and vector populations between *kebeles*, and within- and between villages in the same *kebele* (Paper I), and we recommend as follows:

- The malaria prevention and control programme should identify and target villages with a high clinical malaria incidence (hotspots) for efficiently and effectively controlling malaria.
- Malaria prevention and control interventions should give a priority to those more at risk of malaria infection, such as those of a young age and those living near the potential mosquito breeding site (near a lake or river).

We aimed to estimate the prevalence and risk factors of anaemia among children followed over a period of one year, and we recommend the following:

- Children with stunting and malaria illness should be assessed and treated for anaemia.
- The existing anaemia prevention and control programme should be strengthened to control the high prevalence of anaemia among children.
- Long-term and sustainable intervention strategies, such as routine screening and treatment of children with anaemia, should be designed to control anaemia during droughts and food shortages.

We aimed to assess the bi-directional association between malaria and undernutrition among a cohort of children aged 6 to 59 months (Paper III), and we recommend as follows:

- An emphasis should be given to strengthen the existing nutrition intervention in order to prevent the high incidence of stunting in the community.
- A close follow-up of the nutritional status of children with malaria infection may be needed by community health extension workers.

For research

- An alternative means of identifying malaria hotspots such as mapping should be assessed.
- To control for an important confounder in measuring the risk factors of anaemia and undernutrition, detailed data on iron deficiency, intestinal parasitic infections and household food insecurity should be collected in future studies.

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Original articles I-III and Appendices

Paper I

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Malaria Journal

RESEARCH

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Malaria incidence and entomological findings in an area targeted for a cluster-randomized controlled trial to prevent malaria in Ethiopia: results from a pilot study

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Abstract

Background: This study was part of the work to prepare for a cluster-randomized controlled trial to evaluate the effect of combining indoor residual spraying and long-lasting insecticidal nets on malaria incidence. A pilot study was done to estimate the variations of malaria incidence among villages, combined with entomological collections and an assessment of susceptibility to insecticides in malaria vectors.

Methods: A cohort of 5309 residents from four *kebeles* (the lowest government administrative unit) in 996 households was followed from August to December 2013 in south-central Ethiopia. Blood samples were collected by a finger prick for a microscopic examination of malaria infections. A multilevel mixed effect model was applied to measure the predictors of malaria episode. Adult mosquitoes were collected using light traps set indoors close to a sleeping person, pyrethrum spray sheet catches and artificial outdoor pit shelters. Enzyme-linked immunosorbent assays were used to detect the sources of mosquito blood meals, while mosquito longevity was estimated based on parity. The World Health Organization's tube bioassay test was used to assess the insecticide susceptibility status of malaria vectors to pyrethroids and carbamates.

Results: The average incidence of malaria episode was 4.6 per 10,000 person weeks of observation. The age group from 5 to 14 years (IRR = 2.7; 95 % CI 1.1–6.6) and *kebeles* near a lake or river (IRR = 14.2, 95 % CI 3.1–64) were significantly associated with malaria episode. Only 271 (27.3 %) of the households owned insecticide-treated nets. Of 232 adult *Anopheles* mosquitoes collected, *Anopheles arabiensis* (71.1 %) was the predominant species. The average longevity of *An. arabiensis* was 14 days (range: 7–25 human blood index days). The overall human blood index (0.69) for *An. arabiensis* was higher than the bovine blood index (0.38). Statistically significant differences in *Anopheline* mosquitoes abundance were observed between the *kebeles* (P = 0.001). *Anopheles arabiensis* was susceptible to propoxur, but resistant to pyrethroids. However, *An. pharoensis* was susceptible to all pyrethroids and carbamates tested.

Conclusions: This study showed a high variation in malaria incidence and *Anopheles* between *kebeles*. The observed susceptibility of the malaria vectors to propoxur warrants using this insecticide for indoor residual spraying, and the results from this study will be used as a baseline for the trial.

Keywords: Malaria, Incidence, Indoor residual spraying, Long-lasting insecticidal nets, *Anopheles arabiensis*, Ethiopia

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Background

Long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are the two main malaria vector control tools available today [1]. A worldwide coordinated effort achieved a 47 % reduction of deaths from malaria since 2000 by using the existing interventions. However, in its 2014 report, the World Health Organization (WHO) indicated that malaria continues to be a major cause of morbidity with 198 million cases globally, of which 82 % were from Africa [2].

In Ethiopia, malaria is one of the major public health problems. The dominant malaria parasites are *Plasmodium falciparum* (77 % of all reported malaria cases) and *P. vivax* (33 %) [3], with *Anopheles arabiensis* being the main vector [4]. The transmission is seasonal, and determined by altitude, rainfall and temperature [5, 6], in addition to local epidemiology. The first malaria prevention and control strategies in Ethiopia began as pilot projects in the 1950s [7], and are now integrated into the national basic health services [8]. Early diagnosis and treatment of cases, LLINs and IRS are currently the main malaria prevention and control tools [9]. Despite the successes [2] and efforts made thus far, malaria in Ethiopia remains the main cause of morbidity (3,331,599 confirmed cases) and hospital admissions (59,370 cases) in 2012/13 [10].

In Ethiopia, IRS and LLINs are commonly used, both separately or in combination, in the same households [9]. The individual effect of both IRS and LLINs on malaria incidence is quite well documented, but evidence is scarce and contradictory as to the effect of combining them [11, 12]. A cluster-randomized controlled trial examining the utility of each intervention, as well as their combined potential to prevent malaria transmission, will be carried out in Ethiopia to help provide useful information for policymakers and health service managers [13]. In order to effectively implement the trial, measuring baseline data related to the existing vector species composition, density, infectivity, insecticide susceptibility status and malaria incidence were of paramount importance. Therefore, a pilot study was carried out in 2013 to collect epidemiological and entomological data from the trial area to assess variation in malaria episodes and local vector populations within and among study *gares* (a "*gare*" is a local name for village), and to determine insecticide susceptibility status.

Methods

Study area

This pilot study was conducted in the Adami Tullu part of the Adami Tullu-Judo-Kombolcha district (hereafter referred to as the Adami Tullu district) located 160 km south of Addis Ababa, the capital of Ethiopia, and described in detail elsewhere [13]. Briefly, the size of the district is 1403 square kilometres [14] and administratively divided into 48 *kebeles* (the lowest government administrative unit). Each *kebele* is further divided into *gares*, with each *gare* having an average of 35 households. The geographic location of the district and the selected *kebeles* are shown in Fig. 1. The major rainy season is from June to August, whereas the minor rainy season is from February to March. Malaria is a major health problem, and the shores of Zeway Lake and the Bulbula River provide the main mosquito breeding sites [15]. Malaria transmission is seasonal, with the majority of cases occurring from September to December each year. There is one health post in each *kebele* staffed by two health extension workers, who provide comprehensive preventive services to the community, including malaria diagnosis and treatment.

Study design and participants

A cohort of 5309 residents from 996 households in 29 *gares* selected from four *kebeles* was followed from August to December 2013. The study participants were selected through the following procedure: A preliminary mapping and census of 24 *kebeles* (sampling frame) in the Adami Tullu district was done in March and April 2013, with four *kebeles* selected from the enumerated 24 *kebeles* based on their distance from the main mosquito breeding sites (the Zeway lakeshore and the Bulbula River) and malaria burden (district health office malaria report). Two of the *kebeles*, Bochesa and Anano-Shisho, were located within 5 km and the other two, Elka-Chelemo and Gallo Raphe, which were beyond 5 km from the Zeway lake-shore and Bulbula River. To make access easy for patients with clinical sign of malaria, 29 *gares* within a 3-km radius from the community health posts were included. All the residents (5309) in these villages were invited to participate in the baseline survey in August 2013, and were followed-up weekly for 16 weeks.

Weekly home visits and identification of malaria infection

Households were visited weekly (active surveillance), and household members with fever (body temperature $\geq$37.5 °C and/or history of fever in the last 48 h) were identified and referred to health posts. In addition, residents were asked to visit the health posts if they developed a fever when the project staff was not available (passive surveillance). Blood samples were collected by a finger prick of all febrile cases reported to the health posts, and rapid diagnostic tests (RDTs) and microscopic slide examination were performed. Patients with positive RDT were treated at the health posts with anti-malarial drugs (CoArtem® for *P. falciparum* and chloroquine for *P. vivax*) according to the national malaria treatment guidelines [9]. Microscopic slides were prepared

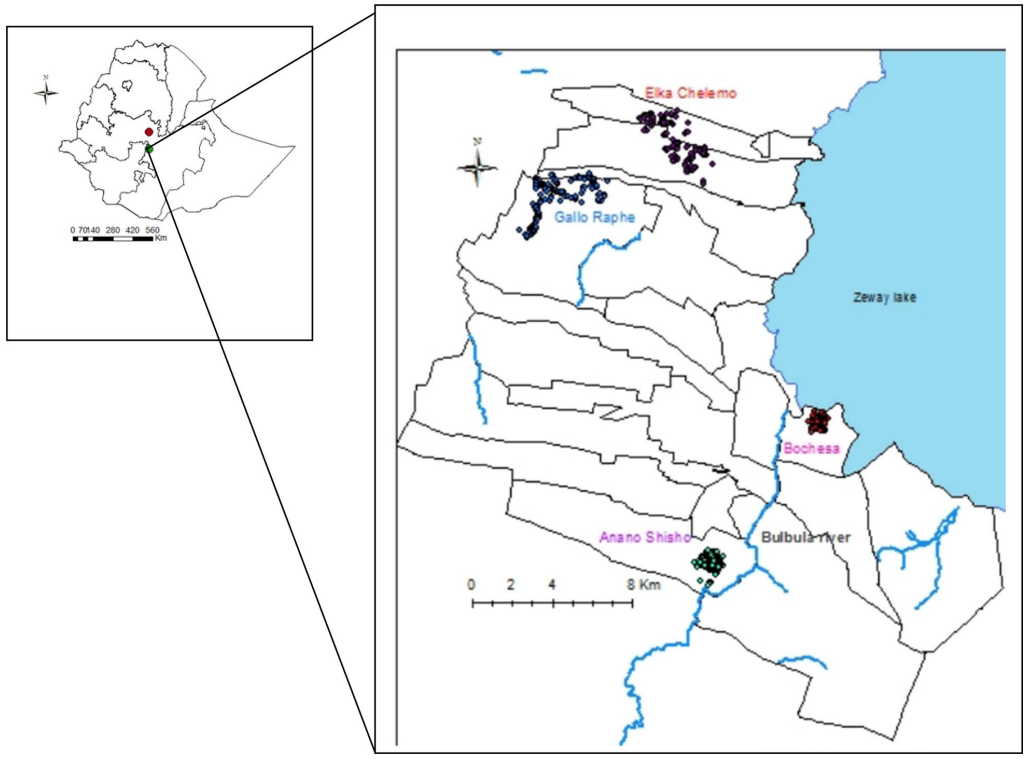

Fig. 1 Geographic location of selected *kebeles*, Adami Tullu district, Ethiopia

according to WHO guidelines [16], and the slides were read by two expert microscopists elsewhere (one in Adama City, the capital of the East Shewa Zone and the other in the Bulbula Health Centre, Adami Tullu district), and the agreement of the two was reported. Discordant slides were read for a third time by a senior microscopist. Data on exposure variables such as socio-economic and demographic variables, ownership and use of insecticidal treated nets (ITNs), whether IRS had been carried out during the past year, history of fever and malaria treatment in householders were all collected using interviewer-administered questionnaires. Information on ITN use was obtained from household heads' response to a question whether anyone in the households had slept under a mosquito net the night before the survey. Data were collected by diploma graduates, while the questionnaires were modified from a malaria longitudinal study tool used in southern Ethiopia [5].

Entomological data collection

The entomological study was conducted from June to October 2013, and a total of 36 *gares* (12 per *kebele*) from each of the three *kebeles* (Bochesa, Elka Chelemo and Gallo Raphe) were randomly selected for adult mosquito collections. Households were selected at random from the 36 *gares*, and indoor and outdoor mosquito collections were undertaken every month. For indoor host-seeking mosquito collections, the Center for Disease Control and Prevention light traps (CDC-LTs) were set in one house per *gare* and allowed to operate from 6:00 pm to 6:00 am. The traps were hung approximately 45 cm above the floor near the feet of occupants protected by LLINs. Indoor and outdoor resting mosquitoes were collected using standard pyrethrum spray catches (PSC) and artificial pit shelters from 6:00 am to 9:00 am, respectively. The PSC was carried out from one randomly selected house per *gare*, while pit shelter collections were performed from six pits per *kebele* from a total of 18 *gares*.

Processing of mosquito samples

Mosquitoes were identified to species by morphological characteristics using an identification key [17]. *Anopheles gambiae* sibling species identification was carried out through the use of the polymerase chain reaction (PCR) method [18]. The head and thorax of each mosquito was separated from the abdomen and tested for the presence of *P. falciparum* and *P. vivax* circumsporozoite protein (CSP) by direct enzyme-linked immunosorbent assay (ELISA) [19].

The sources of mosquito blood meals were determined by the direct ELISA procedure using human and bovine antibodies [20]. The abdomens of unfed *Anopheles* were dissected for parity using Detinova's ovary tracheation method [21]. The parity rate (PR) was measured as the ratio of parous mosquitoes to the total mosquitoes dissected, whereas *Anopheles* mosquito longevity and PR were used to estimate mosquito life expectancy based on the formula proposed by Davidson [22]. Since there was no direct observation of the gonotrophic cycle (gc), the age estimation of *An. arabiensis* and *An. pharoensis* was made on a gc value of 3 days [23]. Because of the small number of the other *Anopheles* species, longevity was only estimated for *An. arabiensis* and *An. pharoensis*.

Insecticide susceptibility

The larvae and pupae of mosquitoes were collected from different breeding sites, and were reared to adults in big cages. Insecticide susceptibility tests were conducted on two species, *An. arabiensis* and *An. pharoensis*. The test for the former species was done in August 2013, a time when the aquatic forms are abundant. In October and November of the same year the tests for the latter species were undertaken. The WHO tube test [24] was employed, and the insecticides included four pyrethroids (0.05 % deltamethrin, 0.75 % permethrin, 0.05 % alphacypermethrin and 0.05 % lambdacyhalothrin) and two carbamates (0.1 % bendiocarb and 0.1 % propoxur). The pyrethroids were impregnated in June/July 2013 and expired in June/July 2014, while bendiocarb was impregnated in May 2012 and expired in May 2015. The dates of impregnation and the expiry of propoxur were July 2013 and July 2016.

After the morphological identification of females [17], 2-3-day-old, non-blood fed mosquitoes were exposed to either insecticide-impregnated filter papers or oil-impregnated papers as controls for 1 h. Each replicate contained 20 mosquitoes, and for each insecticide and control, five to six replicates varying from 100 to 120 mosquitoes were tested. After transferring to holding tubes, mosquitoes were kept for 24 h in a ventilated and humid box free of insecticides. A favourable temperature and humidity were created by placing a damp towel on top of the box. Mortality counts were taken at the end of 24 h, and when control mortality was between 5 and 20 %, the percentage mortality of the experimental was corrected using Abbott's formula. The susceptibility and resistance status of the two vector populations was determined based on the WHO criteria of percentage mortality rates [24].

Outcome variable

Malaria episodes measured by the microscopic slide examination of blood samples for the presence or absence of the malaria parasite was the response variable. Adult mosquito host-seeking and resting density were the response variable for entomological study.

Explanatory variables

The multilevel mixed effect model included covariates associated with malaria episode (grouped into individual, household and *kebele* factors). The individual factors were ITN use, age group (<5, 5–14, 15–24, and ≥25 years) and gender, whereas the educational status of the head of the household and the wealth index were household factors. The *kebele* factors included the distance from lakeshore or river (within or beyond 5 km).

Statistical analyses

Data were entered, cleaned and analysed using IBM SPSS version 21(SPSS Inc., Chicago) and Stata 13 (STATA Corp, College Station, Texas). Descriptive statistics including percentages, mean and standard deviations, were used to summarize the data. A multilevel mixed effects Poisson regression model was fitted to measure associations between response and predictor variables. Malaria incidence (count data) was assumed to follow a Poisson distribution based on random and independent occurrence. Hence, a three-level mixed effects Poisson model with log link was considered to account for malaria episodes, with clustering according to individuals, households and *kebeles*. Wald Chi square test was used to check the fitted model against an intercept-only model. After bivariate analysis, those variables with $P < 0.25$ [25] and main factors were included in the multivariate analysis. The significance level was set at 0.05. The proportion of people using an ITN was calculated by dividing the number of household residents sleeping under an ITN the night before the visit divided by all individuals in the household.

A household wealth index was constructed using a principal component analysis (PCA) [26]. Fourteen variables were included: electricity, watch, radio, television, mobile telephone, separate kitchen, bike, animal cart, bank account or credit association, water source, latrine, window, materials used for a wall and the roof of the house. The first principal component represented 22.8 % of the variance in the sample with an eigenvalue of 3.2.

Households were then ranked into three wealth categories (poor, medium, rich). ArcGIS 10.2 was used for an analysis of village distance from the lakeshore, as well as Stata version13 (StataCorp, Texas) software to calculate the incidence rate ratio (IRR) and to fit the multilevel mixed effects Poisson model.

Variations in adult mosquito host-seeking and resting density, both within and among the *gare*s and the study months, were analysed using a non-parametric Kruskal–Wallis test. The human blood index (HBI) and bovine blood index (BBI) were calculated based on WHO guidelines [27], and all statistical results were considered significant at $P < 0.05$.

Ethical approval

The study protocol and informed consent forms were reviewed and approved by the Institutional Review Board of the College of Health Sciences at Addis Ababa University, Ethiopia and by the Regional Committee for Medical and Health Research (ref: 2013/986/REK Vest), Norway. The national ethical clearance was obtained from the Ethiopian Ministry of Science and Technology (ref: 3.10/446/06). Written permission to undertake the study was obtained from the Oromia Regional Health Bureau, the East Shewa Zone Health Department and the Adami Tullu District Health Offices. Local leaders, village leaders and community elders were also informed about the purposes of the study. Participation in the study was voluntary, and informed consent was obtained from each participant above the age of 18 years. For participants less than 18 years old, consent was obtained from parents or caretakers, while verbal consent was obtained from household heads before routine mosquito collections. Patients who were positive for malaria by RDT were treated at the health posts according to the national guidelines for malaria treatment.

Results

Epidemiological findings

Socio-demographics variables

A total of 5309 individuals in 996 households from four *kebeles* were registered at baseline. The number of households per *kebele* was 325 (32.6 %) in Anano Shisho, 263 (26.4 %) in Bochesa, 210 (21.1 %) in Gallo Raphe and 198 (19.9 %) in Elka Chelemo. Three households (eight individuals) were lost to follow up, with an average household size of 5.3 persons. Nearly half, or 49.9 % (2651) of them were women, and the median age was 15 (IQR = 7–28) years.

Malaria incidence and prevention practices

Of 349 blood samples taken from febrile patients, 39 (11.2 %) slides were microscopically confirmed positive for malaria infection, and 12 (30 %) of these cases were identified through weekly home visits. *Plasmodium vivax* accounted for 33 (84.6 %) of the positive slides (Fig. 2), and patients with repeated malaria episodes were not observed. The overall malaria incidence was 4.6 cases per 10,000 person-weeks of observation (varied from 0 to 23.4 cases per 10,000 person-weeks of observation). However, the average malaria incidence for *gare*s close to the Zeway lakeshore and the Bulbula River was 7.8 per 10,000 person-weeks of observation. A high malaria incidence rate was observed in the under 5 years age group (6.8 episodes per 10,000 person-weeks) and from 5 to 14 years age group (6.4 episodes per 10,000 person-weeks) (Table 1).

Less than one-third of the households (n = 271, 27.3 %) owned insecticide-treated nets. Data on ITN use (self-report of sleeping under ITNs last night) was collected during a census at the beginning of the study and once per week during the home visit. The percentage of ITN use among households that own an ITN was 49 % (n = 657), but the percentage for all participants was low (11 %). The median days individuals slept under an ITN for the 16 weekly visits was 2 days.

Determinants of malaria

The multivariate multilevel mixed effects model results (Table 2) showed that the age group from 5 to 14 years of age (IRR = 2.7; 95 % CI 1.1–6.6) was significantly associated with malaria episode. A high malaria episode (IRR = 2.82; 95 % CI 1.0–7.9) was also observed among children under 5 years of age, though due to a greater variance this was only borderline significant. Residents living in *kebeles* close to Zeway Lake and the Bulbula River had almost a 14.2 (95 % CI 3.1–64.7) times higher risk of malaria infection than those further away from the lake and river. The other variables, including gender, educational status of the head of the household, ITN use and wealth index, were not found to be predictors of malaria in the study area.

Entomological findings

Anopheles species composition and prevalence

Overall, 232 adult *Anopheles* mosquitoes were collected over the 5 months (Table 3). The species composition was 71.1 % *An. gambiae s.l.,* 21.1 % *An. pharoensis,* 5.2 % *An. ziemanni* and 2.6 % *An. funestus s.l.* All *An. gambiae* were confirmed to be *An. arabiensis* by PCR. The malaria vectors *An. arabiensis* and *An. pharoensis* occurred in all *kebeles,* with the largest proportion in Elka Chelemo (48.7 %) and the least in Gallo Raphe (18.5 %). The *Anopheles* abundance varied over the study months with a peak in September after the rainy season (Fig. 3). The average

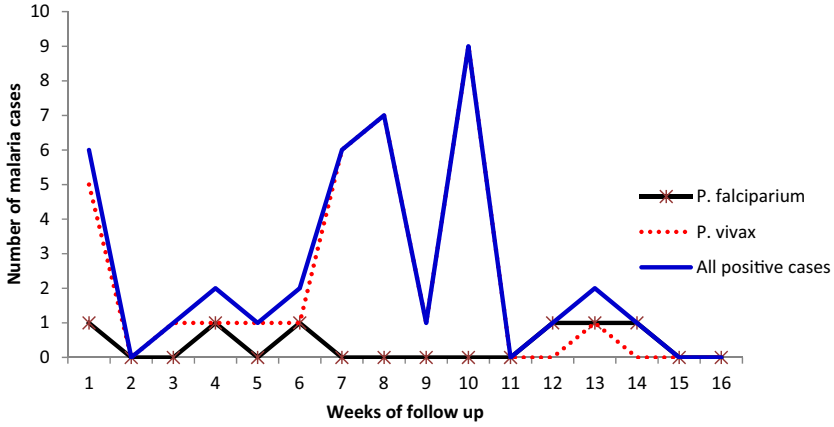

Fig. 2 Malaria cases by *Plasmodium* species, Adami Tullu district, Ethiopia

Table 1 Socio-demographic variables and incidence rate of malaria among study participants, Adami Tullu district

Variables	Person-weeks of observation	Malaria cases	Per 10,000 person weeks
Age (years)			
Under 5	11,888	8	6.8
5–14	28,420	18	6.4
15–24	17,697	6	3.4
25 and above	26,819	7	2.6
Sex			
Male	42,523	22	5.2
Female	42,365	17	4.1
Distance from Lake /river			
≤5 km[a]	47,080	37	7.9
>5 km[b]	37,808	2	0.5
Wealth index			
Poor	27,024	6	2.2
Medium	28,778	11	3.8
Rich	28,910	22	7.7
HH head education			
No formal education	51,634	17	3.3
Primary	18,632	15	8.1
Secondary	8160	5	6.1
Above secondary	6462	2	3.1
ITN use			
No	82,569	38	4.6
Yes	2319	1	4.1

HH household

[a] *kebeles* within 5 km (Bochesa and Anano Shisho)

[b] *kebeles* beyond 5 km (Elika Chelemo and Gallo Rephe) from Zeway Lake shore and river (with potential mosquito breeding sites)

Table 2 Predictors of malaria episodes, Adami Tullu district

Variables	Fixed effects coefficients (IRR)	Standard error	95 % CI	P value
Sex				
Male	1.3	0.41	0.67–2.41	0.46
Female	1			
Age in years				
under 5	2.82	1.48	1.00–7.90	0.049*
5–14	2.7	1.23	1.1–6.60	0.028*
15–24	1.5	0.83	0.48–4.42	0.49
>24	1			
Distance from lake/river				
≤5 km	14.2	11	3.1–64.7	0.0001*
> 5 km	1			
Wealth status				
Poor	0.94	0.47	0.35–2.45	0.9
Medium	0.7	0.27	0.33–1.50	0.34
Rich	1			
HH head education				
No education	1.56	1.2	0.35–7.0	0.56
Primary	1.97	1.5	0.43–8.96	0.38
Secondary	1.67	1.43	0.31–9.0	0.55
Above secondary	1			
ITN use				
No	0.35	0.37	0.04–2.81	0.32
Yes	1			

IRR incidence rate ratio, *CI* 95 % confidence interval

* Significant at $p < 0.05$

Table 3 Species composition of adult *Anopheles* mosquitoes collected in Adami Tullu district

Village	Species	Collection method			Total
		CDC-LT	PSC	Pit shelter	
Bochesa	*An. arabiensis*	19 (14.7)	6 (9.2)	19 (50.0)	44 (19.0)
	An. pharoensis	12 (9.3)	2 (3.1)	0	14 (6.0)
Elka Chelemo	*An. arabiensis*	38 (29.5)	33 (50.8)	9 (23.7)	80 (34.5)
	An. pharoensis	30 (23.3)	3 (4.6)	0	33 (14.2)
	An. funestus	5 (3.9)	0	1 (2.6)	6 (2.6)
	An. zeimanni	9 (7.0)	0	3 (7.9)	12 (5.2)
Gallo Raphe	*An. arabiensis*	14 (10.9)	21 (32.3)	6 (15.8)	41 (17.7)
	An. pharoensis	2 (1.6)	0	0	2 (0.9)
Total	*Anopheles*	129 (55.6)	65 (28.0)	38 (16.4)	232

CDC-LT Center for Disease Control and Prevention light Trap, *PSC* Pyrethrum Spray Collection, figures in parentheses indicate percentage

monthly precipitation peaked in July, and declined with a low precipitation from August to October.

Host-seeking and resting behaviour

The mean host-seeking density of *Anopheles* collected by CDC-LT indoors was 0.7 *Anopheles* per CDC-LT/night/house. The mean indoor resting density of *Anopheles* obtained by PSC was 0.4 *Anopheles* per house per day, whereas the mean outdoor resting density collected from pit shelters was 0.4 *Anopheles* per pit shelter per day over the 5 months. The highest mosquito density was found in Elka Chelemo, where there were significant differences between collection methods (Fig. 4). The average indoor host-seeking density, indoor resting density and outdoor resting density of *An. arabiensis* generally peaked in September, and almost declined to zero in October (Fig. 5).

Anopheles abundance varied significantly between *kebeles* and *gares*. There were significant differences in the abundance of anopheline mosquitoes between the three *kebeles* (Kruskal–Wallis test = 11.25, df = 2, P = 0.004) and between the 36 *gares* (Kruskal–Wallis test = 68.93, df = 35, P = 0.001). The same statistical test revealed that there were significant differences in host-seeking abundances (light trap catches) of *An. arabiensis* (P = 0.025), *An. pharoensis* (P = 0.001) and *An. zeimanni* (P = 0.015) between *kebeles*. However, the indoor host-seeking abundance (light traps) of *An. funestus* s.l. was not significantly different between *kebeles* (P = 0.458).

Moreover, no significant differences were detected between *kebeles* and *gares* in the abundance of indoor resting anophelines (PSC) (P > 0.05) and outdoor resting anophelines (pit shelter) (P > 0.05).

Blood meal sources of Anopheles mosquitoes

Of 107 freshly fed *Anopheles* tested, the overall HBI and BBI was 0.70 and 0.38, respectively (Table 4), with the overall HBI and BBI for *An. arabiensis* 0.69 and 0.39, respectively. *Anopheles arabiensis* preferred to feed more on humans (0.59) than bovines (0.29). The HBI was higher for *An. arabiensis* collected indoors (0.79) than for those collected outdoors (0.37). Inversely, the BBI was higher for *An. arabiensis* caught outdoors (0.68) compared to those collected indoors (0.27). All *An. pharoensis* females that had fed on humans were captured indoors, though none of the indoor- and outdoor-collected *An. pharoensis* females had taken blood from bovines alone. *Anopheles ziemanni* fed more on bovine (BBI = 0.67) than human (HBI = 0.50).

Sporozoite rate, parity rate and longevity of the malaria vectors

All collected mosquitoes (n = 232) were negative for *P. falciparum* and *P. vivax* circumsporozoite proteins. Table 5 shows the parity rate and average longevity of *An. arabiensis* and *An. pharoensis*. The overall average age of *An. arabiensis* and *An. pharoensis* females was 14 days (range: 7–25 days) and 1.6 days (range: 0–6.3 days), respectively.

Indoor host-seeking density of Anopheles and malaria episodes

Both epidemiological and entomological collection was done in 13 *gares* from three *kebeles* (Bochesa, Elka Chelemo and Gallo Raphe). A higher mean (four) indoor host-seeking density of *Anopheles* mosquito was observed in *kebele* (Bochesa) near a lake. In the same *kebele*, a higher (five episodes per 10,000 person week) malaria incidence was observed (Fig. 6).

Status of insecticide susceptibility of An. arabiensis and An. pharoensis

Anopheles arabiensis was highly resistant to deltamethrin, lambdacyhalothrin, permethrin and alphacypermethrin (mortality 0.8–16.8 %), but susceptible to bendiocarb and propoxur (Figs. 7, 8). All tested *An. pharoensis* were found susceptible to all insecticides, as mortality in all cases was 100 %. Control mortality varied from 0–16 % (higher mortality was encountered during the permethrin tests).

Discussion

This study represents one of the few descriptions from Ethiopia on simultaneous epidemiological and entomological information. The overall malaria incidence rate was 4.6 cases per 10,000 person-weeks of observation. However, the incidence varied between the *gares*, and in *gares* closer to the lake or river, the incidence rate was close to eight cases per 10,000 person-weeks of observation. *Plasmodium vivax* was the dominant species, and a higher malaria incidence was observed in a

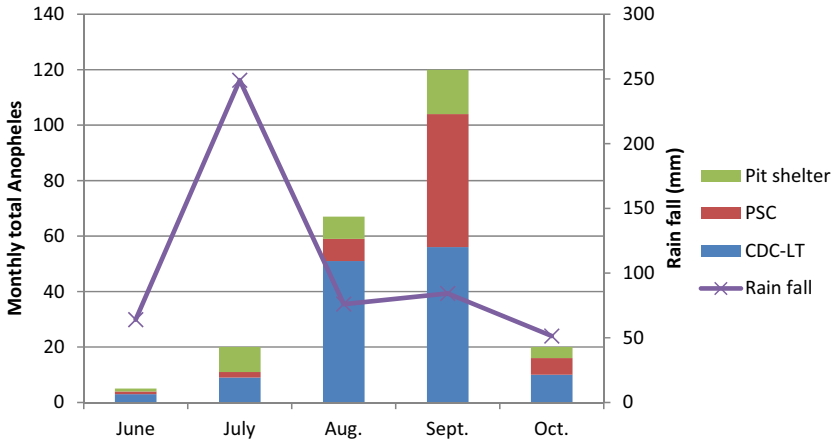

Fig. 3 Monthly *Anopheles* abundance and average precipitation, Adami Tullu district, Ethiopia

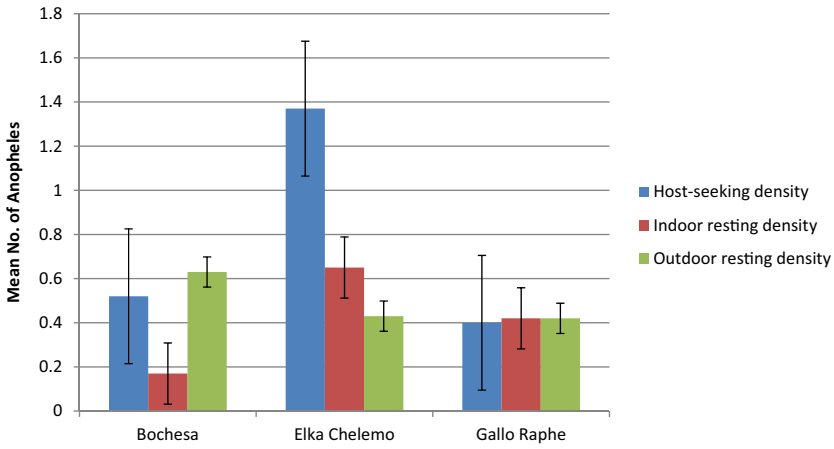

Fig. 4 Mean indoor and outdoor density of *Anopheles,* Adami Tullu district, EthiopiaHost-seeking density (CDC-LT) = Mean no. Anopheles/light trap/night/house, indoor resting density (*PSC* pyrethrum spray catch) = mean no. *Anopheles*/house/45 min in a day, outdoor resting density (Pit shelter) = mean no. *Anopheles*/pit/30 min in a day

kebele with overall higher mean host-seeking density of *Anopheles*.

The observed malaria incidence in this study was higher (4.6 versus 3.6 episodes per person weeks) than what has been reported from southern Ethiopia [5], which could be due to the short (16 versus 101 weeks) follow-up period in the current study.

In line with previous studies from other parts of the country [28, 29], *P. vivax* was the dominant species in the study area, representing about 85 % of the positive cases. The 2011 malaria indicator survey also reported *P. vivax* as the main (60 %) causative agent in the Oromia region [3].

Although all of the study *kebeles* were located below 2000 metres above sea level, which is defined as the threshold for being a malarious area, differences in malaria distribution were observed among the *kebeles* and *gares*. Those *Kebeles* near to the lake or river had

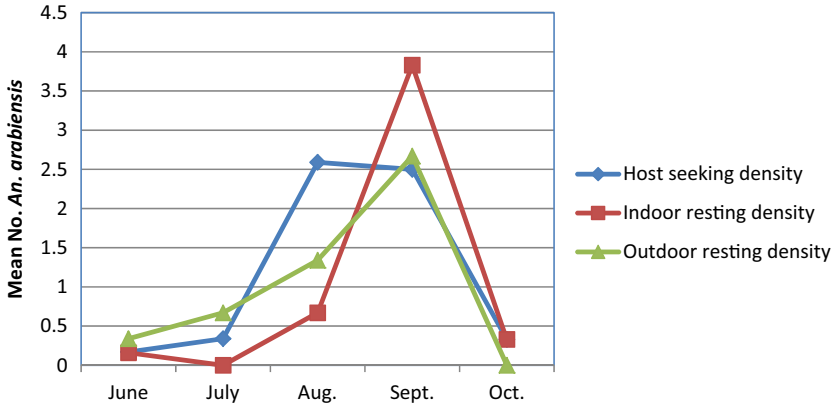

Fig. 5 Overall average monthly host-seeking and resting densities of *An. arabiensis*, Adami Tullu district, Ethiopia. Host-seeking density (CDC-LT), indoor resting density (*PSC* pyrethrum spray catch), outdoor resting density (Pit shelter)

Table 4 Blood meal sources of the *Anopheles* species, Adami Tullu district

Anopheles species	Collection venues	No. analyzed N (HBI)	Blood meals sources			
			Human N (HBI)	Bovine N (BBI)	Mixed N (MBI)	Unknown N
An. arabiensis	CDC-LT	24 (0.75)	15 (0.63)	6 (0.25)	3 (0.13)	0
	PSC	48 (0.79)	35 (0.73)	10 (0.21)	3 (0.06)	0
	Pit shelter	19 (0.37)	4 (0.21)	10 (0.53)	3 (0.16)	2 (0.11)
	Total	91 (0.69)	54 (0.59)	26 (0.29)	9 (0.09)	2 (0.02)
An. pharoensis	CDC-LT	7 (1.00)	6 (0.86)	0	1 (0.14)	0
	PSC	2 (1.00)	2 (1.00)	0	0	0
	Total	9 (1.00)	8 (0.89)	0	1 (0.11)	0
An. ziemanni	CDC-LT	3 (0.67)	2 (0.67)	1 (0.33)	0	0
	Pit shelter	3 (0.33)	0	2 (0.67)	1 (0.33)	0
	Total	6 (0.50)	2 (0.33)	3 (0.50)	1 (0.17)	0
An. funestus	Pit shelter	1	0	1	0	0
Overall *Anopheles*		107 (0.7)	64 (0.60)	30 (0.30)	11 (0.10)	2 (0.20)

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

more incidences of malaria and a higher mean indoor host-seeking density of *Anopheles* mosquito, with similar observations done in other parts of Ethiopia [30]. The persistent presence of infectious *Anopheles* mosquitoes and of water bodies [31] could have resulted in high number of malaria cases in the lakeshore and river areas.

Comparable to other studies [5], children in the age group of under 5s and from 5 to 14-year were more likely to develop malaria than older people. It has been reported that children are more at risk of developing malaria than adults in lowland areas (area <2000 metres above sea level) or malaria-endemic areas [32]. It is a well-known fact that children have a lower immunity to malaria than adults [33].

Families' welfare, as measured by the wealth index, was not associated with the probability of malaria episodes in this study. The inclusion of villages close to a health post (within 3 km), as well as weekly home visits to identify residents with clinical symptoms of malaria, could have motivated febrile cases to seek early treatment and reduce the risk of transmission to other family members in both poor and rich families. The ownership of ITNs in this study (27.3 %) was much lower than the WHO

Table 5 Parity rates and longevity of *Anopheles* species, Adami Tullu district

Kebeles	Species	Number of mosquitoes					
		Collected	Dissected	Parous	PR	P	Age (Days)
Bochesa	*An. arabiensis*	44	3	2	0.67	0.87	7
	An. pharoensis	14	5	1	0.20	0.58	1.8
Elka Chelemo	*An. arabiensis*	80	9	8	0.89	0.96	25
	An. pharoensis	33	18	11	0.61	0.85	6.3
Gallo Raphe	*An. arabiensis*	41	3	2	0.67	0.87	7
	An. pharoensis	2	2	0	0.00	0.00	0
Average for *An. arabiensis*		55	5	4	0.8	0.93	14
Average for *An. pharoensis*		16.33	25	4	0.16	0.54	1.6

PR parity rate, *P* probability of surviving 1 day

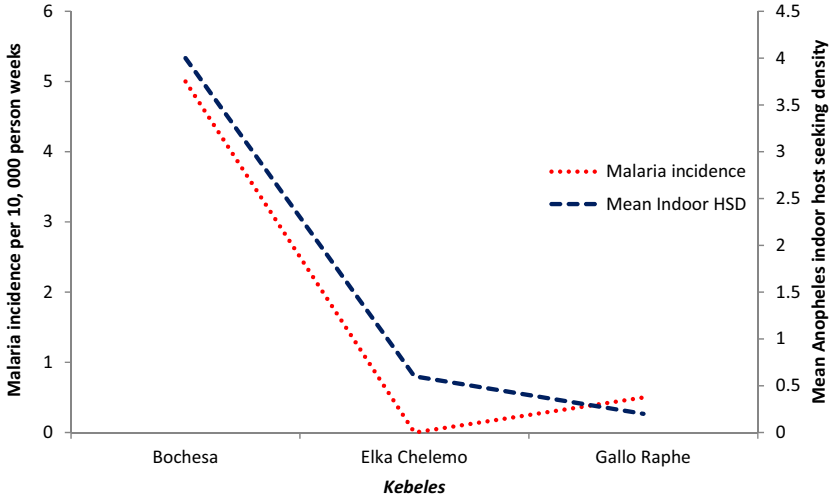

Fig. 6 Malaria incidence and mean indoor host seeking density of *Anopheles*, Adami Tullu district, Ethiopia

recommendations and the national goal (100 % coverage) [1, 8, 34], and also lower than what is reported from other parts of the country [35].

Anopheles arabiensis and *An. pharoensis* were the predominant species in Adami Tullu *kebeles.* These findings were consistent with Abose et al. [36], who reported *An. arabiensis* as the primary- and *An. pharoensis* as secondary vectors in the area. The results also showed that the monthly average precipitation peaked in July and sharply declined from August to October, whereas *Anopheles* abundance rose in September and sharply dropped in October. This was expected since *Anopheles* population dynamics and malaria transmissions are driven by seasonal precipitation in Ethiopia [37]. *Anopheles arabiensis* proliferates in rain-fed residual pools after months of heavy rain in the country [7] and the populations expand during this time; however, excessive rainfall may flush out breeding pools [38]. Therefore, the peak *Anopheles* abundance may not coincide with peak precipitation months.

Results indicate that the overall mosquito density captured by the different mosquito sampling methods was low compared to previous studies in the area [36]. The reason for the low *Anopheles* density could be the rapid scale-up and intensive use of vector intervention measures, particularly ITNs and IRS in the country [28] and elsewhere in eastern Africa [39]. Besides, global climatic changes, particularly changes in hydrologic and climatic factors such as precipitation, humidity, temperature and

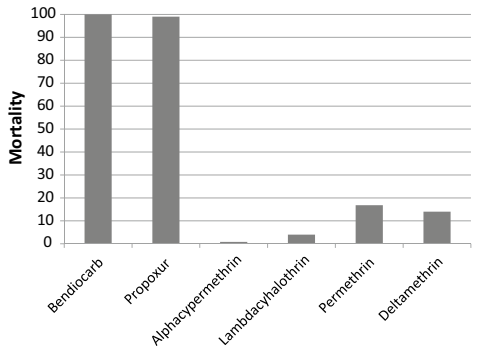

Fig. 7 Insecticide susceptibility of *An. arabiensis,* Adami Tullu district, Ethiopia

wind [40], may have adversely impacted the *Anopheles* population controlling breeding and survival.

The other key potential reason for the low mosquito catches could be the lack of efficient mosquito sampling tools [39]. Efficient indoor and outdoor collection tools are required, especially for vectors such as *An. arabiensis,* which have behavioural plasticity in host preferences and shifts in peak biting time [41]; hence, there is a need to address the inefficient catching techniques. Because adult mosquitoes occur at a certain radius from their breeding sites, a district-wide random sampling of adult mosquitoes without referring to any mosquito breeding sites could also have a potential impact on the occurrence and abundance of mosquitoes, and needs to be revisited.

The overall HBI (0.69) for *An. arabiensis* was higher than the BBI (0.38) for the same species. This finding contrasts prior studies that found a higher BBI for *An. arabiensis* than the HBI in the country [4]. However, the present finding is in line with [42], which found a higher HBI for *An. arabiensis* compared to the BBI in the country. It should be noted that the present study used similar mosquito sampling methods than the previous study [4], thus the potential influence of mosquito trapping on HBI is not expected. But the present finding was similar to the other study [42], that relied on the CDC light trap alone for mosquito collection, which is evidence that the trapping methods used did not impact the HBI. The HBI for *An. arabiensis* was higher indoors (0.73) than outdoors (0.21), but the BBI was higher when collected outdoors (0.53) than indoors (0.21). These results are generally in agreement with prior studies, which observed the opportunistic feeding behaviour of *An. arabiensis* [43]. *Anopheles pharoensis* showed anthropophilic and endophilic behaviour in the area, but more blood-fed females should be tested to reach such conclusions. Furthermore, the average longevity of *An. arabiensis* ranged from 7 to 25 days in the villages, thereby implying that the vector

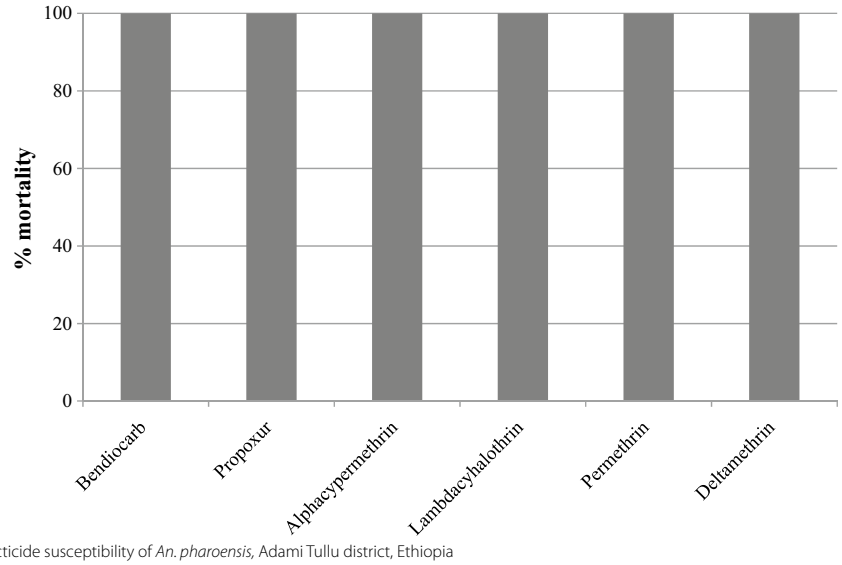

Fig. 8 Insecticide susceptibility of *An. pharoensis,* Adami Tullu district, Ethiopia

had a sufficient longevity for malaria transmission during the study period.

Overall, the relative increase in the abundance of mosquitoes in September and the beginning of October, compared to the other study months, coincides with an increased incidence of malaria episodes in the same and subsequent months. In the entomological study, an overall high mosquito density was observed in Elka Chelemo kebele, although the malaria incidence for the *kebele* was low. This could be due to some of the *gares* being located near the lakeshore in this *kebele,* where a higher mosquito abundance was not followed for malaria episodes. In *kebeles* where the two studies overlapped, high malaria episodes were observed in those with a higher indoor host-seeking density of *Anopheles.*

Anopheles arabiensis was highly resistant to all the tested pyrethroids, including deltamethrin, but susceptible to bendiocarb and propoxur. The insecticide susceptibility study showed a resistance of *An. arabiensis* to the pyrethroids, which is the current insecticide of choice for the treatment of LLINs. This resistance may compromise the efficacy and effectiveness of ITNs. On the other hand, *An. arabiensis* is currently susceptible to the carbamates, which is useful and will be beneficial for the main trial.

The use of weekly active case detection, which was supplemented with passive surveillance, would have maximized the number of cases captured by the study. In addition, the active monitoring of the occurrence and the abundance of adult mosquitoes through house-to-house surveys during the major malaria transmission season in the area is also another strength. Weaknesses of the study include a lack of efficient mosquito sampling methods for the local vectors, the overestimation of malaria incidence due to the inclusion of the major malaria transmission season and the self-report of sleeping under ITNs (difficult to observe), which may have affected the reported ITN usage.

Conclusions

This pilot study showed a high variation of malaria incidence and *Anopheles* among *gares*. Younger age groups and households located near lakes and rivers were significantly associated with malaria infection. *Anopheles arabiensis* had a high HBI (high human contact) and a sufficient longevity for malaria transmission. The observed susceptibility of the malaria vectors to propoxur warrants using this insecticide for indoor residual spraying, and results from this study will be used as a baseline for the trial.

Authors' contributions

BL, WD, EL, MB, HJO, TG, OK and BR and TGM conceived the study, and were involved in the proposal writing and design of the study. OK, TG, and EL participated in the field coordination, data collection, supervision and overall implementation of the pilot study. TG and OK contributed equally to this paper. AH and TG were involved in the initial census. OK and TG analysed the data and drafted the manuscript. All the authors participated in all stages of the study and revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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Paper II

PLOS ONE

RESEARCH ARTICLE

Anaemia among children in a drought affected community in south-central Ethiopia

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Abstract

Introduction

As part of a field trial (PACTR201411000882128) to provide evidence on the combined use of long-lasting insecticidal nets and indoor residual spray for malaria prevention, we measured haemoglobin values among children aged 6 to 59 months. The aim of this study was to estimate the prevalence of anaemia, and to determine the risk factors of anaemia and change in haemoglobin value in Adami Tullu district in south-central Ethiopia.

Methods

Repeated cross-sectional surveys among 2984 children in 2014 and 3128 children in 2015; and a cohort study (malaria as exposure and anaemia as outcome variable) were conducted. The study area faced severe drought and food shortages in 2015. Anaemia was diagnosed using HemoCue Hb 301, and children with haemoglobin <11 g/dl were classified as anaemic. Multilevel and Cox regression models were applied to assess predictors of anaemia.

Results

The prevalence of anaemia was 28.2% [95% Confidence Interval (CI), 26.6–29.8] in 2014 and increased to 36.8% (95% CI, 35.1–38.5) in 2015 (P<0.001). The incidence of anaemia was 30; (95% CI, 28–32) cases per 100 children years of observation. The risk of anaemia was high (adjusted Hazard Ratio = 10) among children with malaria. Children from poor families [Adjusted Odds Ratio (AOR); 1.3; 95% CI, 1.1–1.6)], stunted children (AOR 1.5; 95% CI; 1.2–1.8), and children aged less than 36 months (AOR; 2.0; 95% CI, 1.6–2.4) were at risk of anaemia compared to their counterparts. There was no significant difference in risk of anaemia among the trial arms.

Conclusions

Young age, stunting, malaria and poverty were the main predictors of anaemia. An increase in the prevalence of anaemia was observed over a year, despite malaria prevention effort,

which could be related to the drought and food shortage. Therefore, conducting trials in settings prone to drought and famine may bring unexpected challenges.

Introduction

Anaemia is a common childhood health problem in Africa, and its prevalence among children under the age of five years is estimated at 62%, which is above the cut off points (40%) of the World Health Organization (WHO) classification of anaemia as a severe public health problem [1]. Anaemia has a serious effect on child health, and could result in impaired cognitive function, poor school performance, poor growth and development, and threatens the life of children [2–4]. The risk factors of anaemia are multiple, and vary across geographical areas. Iron deficiency anaemia is the leading (50%) cause of childhood anaemia in developing countries [5, 6]. In such countries, increased risks for childhood anaemia are protein energy malnutrition [7] and infections such as malaria, diarrhoea and intestinal helminths [7–12]. In addition, poverty [9, 13], illiteracy [14], and poor hygiene and sanitation [15] are among the contributing factors for the occurrence of anaemia.

In Ethiopia, the estimated national prevalence of anaemia among children aged 6 to 59 months was about 44% in 2011, and classified as a severe public health problem [16, 17]. To control childhood anaemia, the Ethiopian Ministry of Health (MOH) has been implementing anaemia prevention integrated into the routine child health services and community based nutrition intervention programmes. In addition, the MOH has carried out periodic de-worming campaigns of children since 2004 [18]. However, despite the concerted efforts to control childhood anaemia over the last two decades, anaemia still remains a major childhood health problem in the country [16].

Repeated community-based cross-sectional measurement on the same population has been used to study the effects of anaemia control interventions [19–21]. Unfortunately, such repeated measurements of anaemia prevalence have not been done in Ethiopia [3, 7, 22]. As part of a field trial to provide evidence on the combined use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) for malaria prevention, we measured haemoglobin values among children aged 6 to 59 months [23]. The objectives of this study were to estimate the prevalence of anaemia, and to assess the risk factors of anaemia, and to measure changes in the haemoglobin concentration among children followed over a period of one year.

Methods

Study setting and profile

The study profile was presented in Fig 1, and this study was conducted in Adami Tullu district in the Oromia Regional State in south-central Ethiopia. The district is situated in the Great Rift Valley and has 48 "*Kebeles*" (lowest government administrative unit). Each *kebele* is further divided into villages or *gares*, and about 1000 to 5000 people live in a *kebele* [24]. Lake Zeway, a potential breeding site for malaria vectors, borders most of the study *kebeles* (Fig 2). In 2014, the population of the district was projected to be about 173,000 people, and children under the age of five years accounted for 12% of the population [24]. The livelihood of the households is subsistence agriculture and cattle rearing. Most of the families are dependent on rain-fed agriculture.

Like many districts in Ethiopia, our study area was affected by repeated droughts and food shortage during the past decades [25]. The population also faced severe drought triggered by

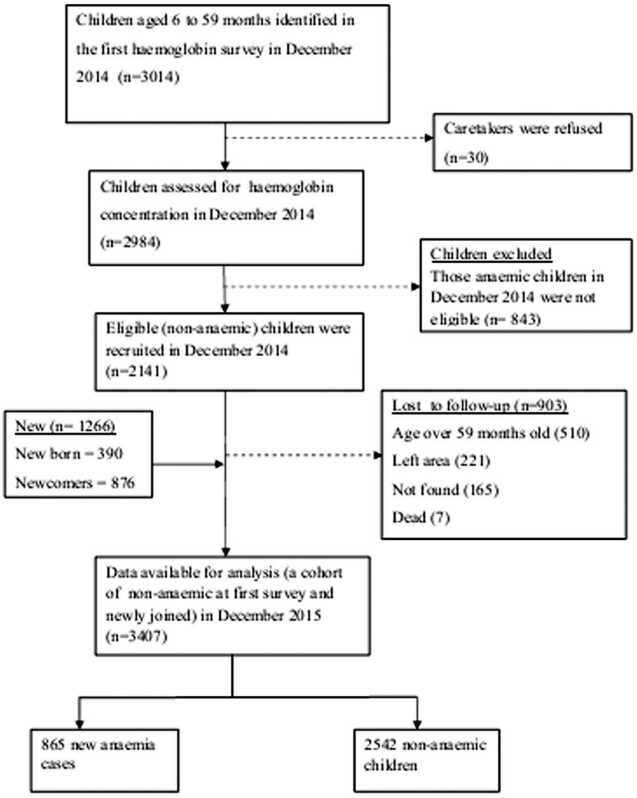

Fig 1. Study profile in Adami Tullu district in south-central Ethiopia, 2014 and 2015.

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El Nino in 2015 and early 2016 [26]. The annual rainfall of the district was 673 mm in 2011, 909 mm in 2012, 745 mm in 2013, 673 mm in 2014 (only 8 months' data available) and decreased to 471 mm in 2015. The average maximum temperature was 27 C in 2014, and 29 C in 2015 [27]. The communities were receiving food aids, and a non-governmental organization was also screening and treating children with acute malnutrition. Malaria is a common health problem in the district [28]. There is one health post staffed by two health extension workers in each *kebele*. Malaria prevention, diagnosis and treatment with anti-malaria drugs, and periodic de-worming of children under five years to control anaemia are among the services given in the health post.

Study design and participants

This study is a part of a cluster randomized controlled trial to measure the combined use of LLINs and IRS over LLINs or IRS alone to prevent malaria [23]. In the anaemia study, repeated cross-sectional surveys, and a cohort study were conducted. In the cross-sectional study, we conducted surveys to estimate the prevalence of anaemia, and anthropometry measurement in December 2014 (n = 2984 children), followed by the second anaemia and anthropometry

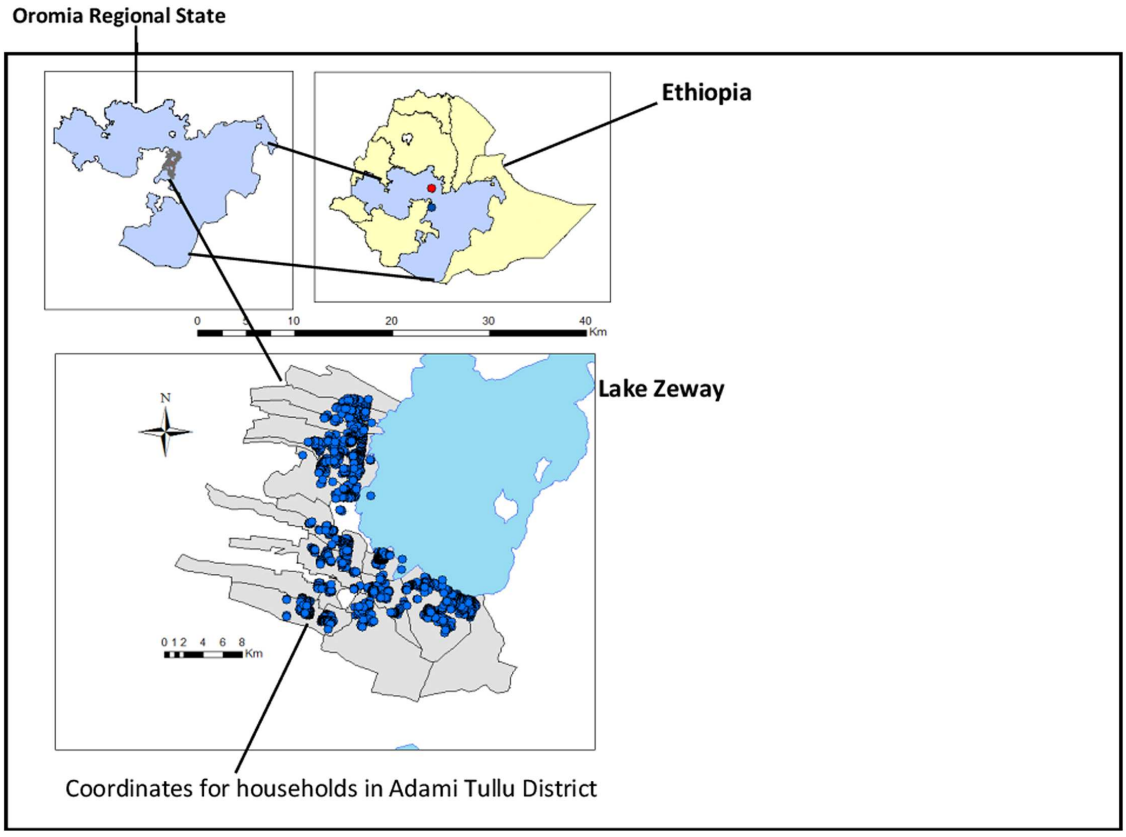

Fig 2. Map of Adami Tullu district in Oromia Regional State in south-central Ethiopia. Re-print under a CC BY license, with permission from Deressa et al Trials (2016).

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survey in December 2015 (n = 3128 children). During the two surveys, we included all children in the age group of 6 to 59 months old in all villages that were enrolled to malaria prevention trials.

Two cohort groups were identified and enrolled in December 2014. In the first cohort, non-anaemic children were recruited in December 2014 and were followed for a year. The main exposure variable was malaria, and the outcome variable was anaemia. A haemoglobin measurement survey was conducted to diagnose anaemia at the end of a year follow-up. This is a dynamic cohort where older children left the study, while children born during the study period and newcomers joined the cohort. The dynamic cohort included 2141 non-anaemic children diagnosed in December 2014, and 1266 children added during the follow-up period, making a total of 3407 children (Fig 1).

The second (closed) cohort was a study in which only children participating in both surveys were included. Among the 1851 children participating in both surveys, 1244 children were non-anaemic in December 2014, and these children were also participated in the second

survey in December 2015. This was a closed cohort, where children who participated in both surveys were included, and children who left the area, children aged over 59 months old and newcomers were excluded.

The study design and the sample size estimation for the malaria prevention trial was described by Deressa et al [23], and included all children aged 6 to 59 months old from 6070 households in 176 villages from 13 *kebeles* living within 5 km from Lake Zeway in the Adami Tullu district. The assumption of sample size determination for haemoglobin concentration was that the combination of IRS and LLINs could increase the mean haemoglobin by 0.5 g/dl compared to either IRS or LLINs alone. A study conducted in south-west Ethiopia showed a mean (standard deviation) haemoglobin of 11.5 (1.66) g/dl [7]. Assuming 90% power, 5% significance level, and a design effect of two, the sample size estimated to be 464 children per arm of the trial, resulting in a total of 1856 children for the whole study. Although the estimated sample size sufficiently addresses the research question in this study, we included and followed-up all (n = 2984) children aged 6 to 59 months participating in all arms of the main trail.

Data collection

Interviewer administered, pre-tested structured questionnaires were used to collect data on socio-demographic and economic variables. Data were collected by diploma graduates, whereas malaria diagnosis and treatment, anthropometric and haemoglobin measurements were conducted by nurses. The nurses were given skill-based training on how to take anthropometric measurements, finger-pricks, blood samples, malaria diagnosis using rapid diagnostic tests (RDTs), haemoglobin measurement, and treatment of malaria cases.

Blood tests for haemoglobin and malaria

Blood samples were obtained from finger-pricks, and haemoglobin concentration was measured using HemoCue Hb 301 (HemoCue AB, Angelholm, Sweden). Based on the manufacturer's instruction, micro-cuvette was filled in with 10μL drops of capillary blood, placed in the HemoCue analyser and the result read after 10 seconds. The WHO criteria were used to define and classify anaemia in children aged 6 to 59 months [29]. Anaemia was defined as haemoglobin <11 g/dl, and classified into mild anaemia if the haemoglobin concentration was in the range of 10–10.9 g/dl, moderate anaemia if haemoglobin concentration was 7–9.9 g/dl and severe if the haemoglobin concentration was <7 g/dl.

Febrile patients were identified through weekly home visit and referred to the nearest health posts for malaria diagnosis and treatment. Capillary blood samples were collected from children presented to health post with clinical sign of malaria for RDTs to diagnose malaria, which was performed using CareStart ^{TM} produced by Premier Medical Corporation Limited in India. Patients with malaria were treated according to the national malaria treatment guideline [30].

Anthropometric measurements

The data collection teams were trained for two days, and the measurement techniques were standardized before each survey. Each observer measured the weight and height or length of 10 children twice. The inter and intra technical errors of measurements (TEM) were within suggested cut-off points for acceptability of measurements [31]. The intra TEM was 0.08 Kg for weight and 0.14 cm for height, whereas the inter TEM was 0.1 Kg for weight and 0.2 cm for height. The weight was taken using Salter scale, which was calibrated daily and adjusted to zero. A standard wooden board was used to measure the height of children older than 2 years,

and recumbent length for those children less than 2 years of age. As most parents did not know their child's date of birth, the parents or caregivers were probed using a local event calendar to obtain an approximate age [32]. The WHO 2006 multi-centre growth reference study [33] was used to calculate nutritional indices such as weight-for- height (WHZ), height- for-age (HAZ) and weight-for-age (WAZ) by using Emergency Nutrition Assessment for SMART software 2011 [34]. Children were classified as wasted (WHZ <-2 Z-scores), stunted (HAZ <-2 Z-scores) and underweight (WAZ <-2 Z-scores).

Statistical analysis

Data were entered into SPSS version 21 (SPSS Inc., Chicago, USA), and also analysed using STATA version 14 (StataCorp, College Station, TX, USA). Descriptive statistics, frequency count, percentage, mean and standard deviation were computed. Principal component analysis (PCA) was used to construct a wealth index [35] from 14 household assets related variables such as electricity, television, radio, mobile telephone, table, chair, bed, separate kitchen from living house, types of roof and walls and ownership of a bicycle, animal cart, animal and land. These variables were dichotomized and coded "1" if the household owned the asset or "0" if not. The Kaiser-Meyer-Olkin (KMO) measure of sample adequacy was 0.79. The first principal component represented 23.6% of the variance in the sample with an Eigen value of 3.3 and categorized into three relative measures of socioeconomic classes (poor, middle and Rich).

To measure risk factors of anaemia among children participating in the cross-sectional surveys, a multilevel model was used to account for clustering within a group at different level [36]. In this study, predictors of anaemia were clustered at two levels; individual child (first level) was nested to households or families with the assumption of differences in risk of anaemia between families but similarity among children within a family. Based on this assumption, the presence of clustering was checked before fitting multilevel model in the following steps: First, a null single level (standard) regression model, and then a null multilevel model with the random household effect were fitted. The calculated likelihood ratio test statistics showed strong evidence of household effect on anaemia status of the children ($P<0.001$). Hence, to account for the clustering, a multilevel model was fitted to estimate crude odds ratios (OR) and adjusted odds ratios (AOR) with 95% CI). We did not adjust for altitude, because the households were located between 1613 and 1758 above sea level, which was assumed to be relatively homogeneous.

We calculated anaemia incidence for the closed cohort, considering the number of children that were diagnosed with anaemia in 2015, among those without these conditions in 2014 survey. Whereas, the incidence rate for the dynamic cohort was calculated considering children that were anaemic in 2015 among those non-anaemic children. These non-anaemic children were those identified in December 2014 survey and those children newly joined the cohort during the follow-up period. Cox regression model was fitted to measure the hazard ratio (HR), malaria was entered as a time varying covariate for anaemia, and socio-demographic factors such as sex, wealth status and educational status were entered as an independent predictor variables for anaemia. Bivariate analysis was computed for all variables. All variables with $P< 0.25$ were fitted to the model and those with $P <0.05$ were maintained and reported.

Ethical issues

Ethical clearance was obtained from the Institutional Review Board of the College of Health Sciences at Addis Ababa University, the National Ethics Committee of the Ministry of Science and Technology in Ethiopia (ref: 3.10/446/06), and from the Regional Committee for Medical and Health Research Ethics, Western Norway (ref: 2013/986/REK Vest). Written permission

was obtained from the Oromia Regional Health Bureau (ORHB), East Shewa Zone Health Department (ESZHD) and Adami Tullu District Health Office. Consultative meeting and discussion was made before the trial implementation with representatives from Adami Tullu District Administration, ESZHD and ORHB. In addition, *Kebele* and village leaders and community elders were also sensitized through meeting before the study. During the meeting and sensitization the objectives, randomization, implementation process and expected outcome of the trial were discussed. As the majority of the study population cannot read and write, we had a challenge to get written consent. Therefore, verbal informed consent was obtained from the head of households, or members of the households older than 18 years in the absence of the head of the households at the beginning of our cluster randomized controlled trial [23]. In addition, the verbal consent was obtained from the parents or caretakers before collecting the blood samples from the children. We used standard information sheet to explain the purpose of the study, and the participants were informed that participation was voluntary and that they had the right to withdraw any time during the study. They were assured that refusal to participate in the study would not affect their health service utilization at the health posts.This information was read to them using the information sheet in their own language, and their consent was recorded using check ($\surd$) mark. We also strictly supervised the data collectors, whether they are following the information sheets or not. Later, this document was stored at our field research station. Children with positive RDT confirmed malaria, and those with uncomplicated severe acute malnutrition were treated in the health post according to the national malaria and malnutrition treatment guidelines [30, 37]. Those children with anaemia were referred to the nearest health centre.

Results

Characteristics of study children

A total of 2984 children in the 2014 survey, and 3128 children in the 2015 survey were included. The mean (SD) age was 33.6 (14.6) months in 2014, and 35.2 (15) months in 2015. In the 2014, the proportion of boys was 51.3%, and 56.6% of the head of households were illiterate (Table 1).

Prevalence of malnutrition

The prevalence of stunting (height-for-age <-2 Z-score) was 44.8% in 2014, and increased to 50.7% in 2015 (P <0.001). The prevalence of underweight was 18.5% in 2014, and 15.4% in 2015 (P<0.001). Whereas, the prevalence of wasting was 7.3% in 2014, and 4.1% in 2015 (P<0.001) (Table 1).

Prevalence of anaemia

The prevalence of anaemia was 28.2% (95% CI, 26.6–29.8) in 2014, and increased to 36.8% (95% CI; 35.1–38.5) in 2015 (P< 0.001). The mean haemoglobin value decreased from 11.6 g/dl in 2014 to 11.2 g/dl in 2015 (P<0.001). The decrease in haemoglobin value (P<0.05) was observed for all factors included in this study (Table 2).

As shown in Fig 3, the prevalence of all types of anaemia, increased over a year period. Mild anaemia prevalence increased from 14.8% in 2014 to 18.6% in 2015 (P<0.001), and moderate anaemia prevalence increased from 11.4% in 2014 to 15.7% in 2015 (P<0.001).

Risk factors associated with anaemia

Bivariate multilevel mixed effect model analysis showed that poor socioeconomic status (OR = 1.4; 95% CI, 1.1–1.7), stunting (OR = 1.9; 95% CI, 1.6–2.2) and age less than 36 months old (OR = 2; 95% CI, 1.6–2.4) were risk factors of anaemia in 2014. Likewise, in 2015,

Table 1. Demographic and anthropometric measurements of children, and household characteristics in Adami Tullu district in south-central Ethiopia, 2014 and 2015.

Variables		2014 (n = 2984)	2015 (n = 3128)
		Number (%)	Number (%)
Sex	Boys	1532 (51.3)	1586 (50.7)
	Girls	1452 (48.7)	1542 (49.3)
Age in months	6–35	1499 (50.2)	1531 (49.0)
	36–59	1485 (49.8)	1597 (51.0)
	Mean age (95% CI)	33.6 (33.0–34.1)	35.2 (34.6–35.7)
Wealth status	Poor	955 (32.0)	966 (32.1)
	Middle	1016 (34.1)	1040 (34.6)
	Rich	1012 (33.9)	1004 (33.3)
Household head education	Illiterate	1689 (56.6)	1856 (59.4)
	Elementary	971 (32.5)	926 (29.6)
	High school and above	324 (10.9)	343 (11.0)
Weight-for height	<-2 Z-score	215 (7.3)	125 (4.1)
	≥-2 Z-score	2737 (92.7)	2953 (95.9)
Height- for- age	<-2 Z-score	1323 (44.8)	1562 (50.7)
	≥ -2 Z-score	1629 (55.2)	1516 (49.3)
Weight-for-age	<-2 Z-score	552 (18.5%)	481 (15.4%)
	≥ -2 Z-score	2432 (81.5%)	2647 (84.6%

CI: Confidence Interval

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educational status of head of household (OR = 1.3; 95% CI, 1.0–1.7) was a risk factor of anaemia in addition to the risk factors identified in 2014.

The multivariate multilevel mixed effect model analysis showed that children living in a poor family (AOR = 1.3; 95% CI, 1.1–1.6), children who were stunted (AOR = 1.5; 95% CI, 1.4–1.9) and children aged less than 36 months (AOR = 2.0; 95% CI, 1.6–2.4) were at risk of anaemia in 2014. Similarly, in 2015, children living in poor families (AOR = 1.3; 95% CI, 1.03–1.6), children who were stunted (AOR = 1.6; 95% CI, 1.3–1.9), children aged less than 36 months (AOR = 2.4;95% CI, 2.0–2.9) and where the head of the household was illiterate (AOR = 1.5; 95% CI, 1.1–2.0) were at increased risk of anaemia. However, wasting was not a risk factor of anaemia during the two surveys, and no statistically significant difference in anaemia was observed among malaria prevention trial arms (Table 3).

Assessment of anaemia using a dynamic cohort

In the dynamic cohort, 2141 non-anaemic children were enrolled in December 2014, and 1266 children newly joined the study, increasing the cohort to a total of 3407 children. In December 2015, after one year of follow-up we observed 865 new anaemia cases. The overall anaemia incidence rate was 30; (95% CI, 28–32) cases per 100 children years of observation. As shown in Table 4, children aged less than 36 months (HR = 3.2), children having malaria (HR = 10.4), and children living in poor families (HR = 1.2) had higher risks of anaemia. However, we did not observe significant differences in risk of anaemia among the trial arms.

Anaemia among children completed the one year follow-up study (closed cohort)

Out of 2984 children included in the 2014 survey, 1851 (62%) were also examined in 2015. The main reasons for lost to follow-up were exclusion of children older than 59 months (64.1%)

Table 2. The mean haemoglobin values, and prevalence of anaemia among children in Adami Tullu district in south-central Ethiopia, 2014 and 2015.

Variables		2014 (n = 2984)		2015 (n = 3128)		P-value†
		HB	Anaemia prevalence	HB	Anaemia prevalence	
		Mean (SD)	Number (%)	Mean (SD)	Number (%)	
Sex	Boys	11.6 (1.7)	441 (28.8)	11.2 (1.7)	592 (37.4)	<0.001
	Girls	11.6 (1.7)	400 (27.5)	11.2 (1.7)	559 (36.2)	<0.001
Wealth status	Poor	11.5 (1.8)	300 (31.4)	11.1 (1.7)	371 (38.4)	<0.001
	Middle	11.6 (1.6)	281 (27.7)	11.2 (1.7)	405 (38.9)	<0.001
	Rich	11.7 (1.7)	259 (25.6)	11.4 (1.7)	333 (33.2)	<0.001
Age in months	6–35	11.2 (1.6)	527 (35.2)	10.7 (1.6)	705 (46.0)	<0.001
	36–59	11.9 (1.7)	314 (21.1)	11.4 (1.7)	446 (28.0)	<0.001
Household's head education	Illiterate	11.5 (1.7)	484 (28.8)	11.2 (1.7)	720 (38.9)	<0.001
	Elementary	11.6 (1.6)	260 (27.4)	11.4 (1.7)	317 (33.7)	0.008
	Secondary and above	11.8 (1.6)	87 (26.9)	11.3 (1.5)	114 (33.2)	<0.001
Weight-for-age	<-2 Z-score	11.2 (2.0)	200 (36.2)	10.9 (1.8)	204 (42.4)	<0.001
	≥-2 Z-score	11.6 (1.6)	641 (26.4)	11.3 (1.7)	947 (35.8)	<0.001
Height-for-age	<-2 Z-score	11.3 (1.8)	468 (34.5)	11.0 (1.7)	642 (41.8)	<0.001
	≥-2 Z-score	11.9(1.5)	372 (22.9)	11.5 (1.7)	509 (32.0)	<0.001
Weight-for-height	<-2 Z-score	11.5 (2.0)	62 (27.1)	11.4 (1.7)	37 (29.6)	0.320
	≥ -2z-score	11.6 (1.6)	777 (28.2)	11.2 (1.7)	1114 (37.1)	<0.001
Intervention arm	IRS + LLINs	11.7 (1.5)	199 (26.8)	11.1 (1.7)	310 (38.1)	<0.001
	LLINs alone	11.5 (1.7)	220 (28.6)	11.2 (1.8)	282 (35.0)	<0.001
	IRS alone	11.5 (1.7)	199 (29.1)	11.2 (1.7)	272 (38.5)	<0.001
	Routine	11.5 (1.7)	223 (28.3)	11.4 (1.7)	287 (35.8)	>0.05

HB: Haemoglobin concentration.

†: t-test was used to compare the mean HB values of the two surveys.

SD: Standard Deviation.

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and out-migration to other districts (19.5%). Among the 221children who migrated to other districts, 100 (45.2%) were from poor, 72 (32.6%) were from the middle and 49 (22.2%) were from more wealthy families. The overall prevalence of anaemia among children who migrated out was 45.7% (101 children), and this prevalence was higher than the prevalence of anaemia among children who took part in both surveys, 32.9% (610 children) (P<0.001).

Two hundred eighty (46.3%) of children with anaemia in 2014 were also having the same condition in 2015. Of 1851 children participating in both surveys, 1244 children were non-anaemic in December 2014. This cohort of non-anaemic children (n = 1244) was followed for one year (making 1223 children years of observation). We observed 336 new anaemia cases after one year of follow-up in December 2015. The overall incidence of anaemia was 28; (95% CI, 25–31) cases per 100 children years of observation.

As shown in Table 5, anaemia incidence was higher among children less than 36 months (HR = 1.5), having malaria (HR = 4.0), were stunted (HR = 1.2) and among children living in poor families (HR = 1.4). We did not observe statistical significant difference in anaemia among malaria intervention arms.

Overall, 63 malaria cases (52% *plasmodium falciparum*, 37% *plasmodium vivax*, and 11% mixed infection) were identified during one year follow-up among children participating in both surveys. Among the 63 malaria cases, only 25 (40%) were registered during the major

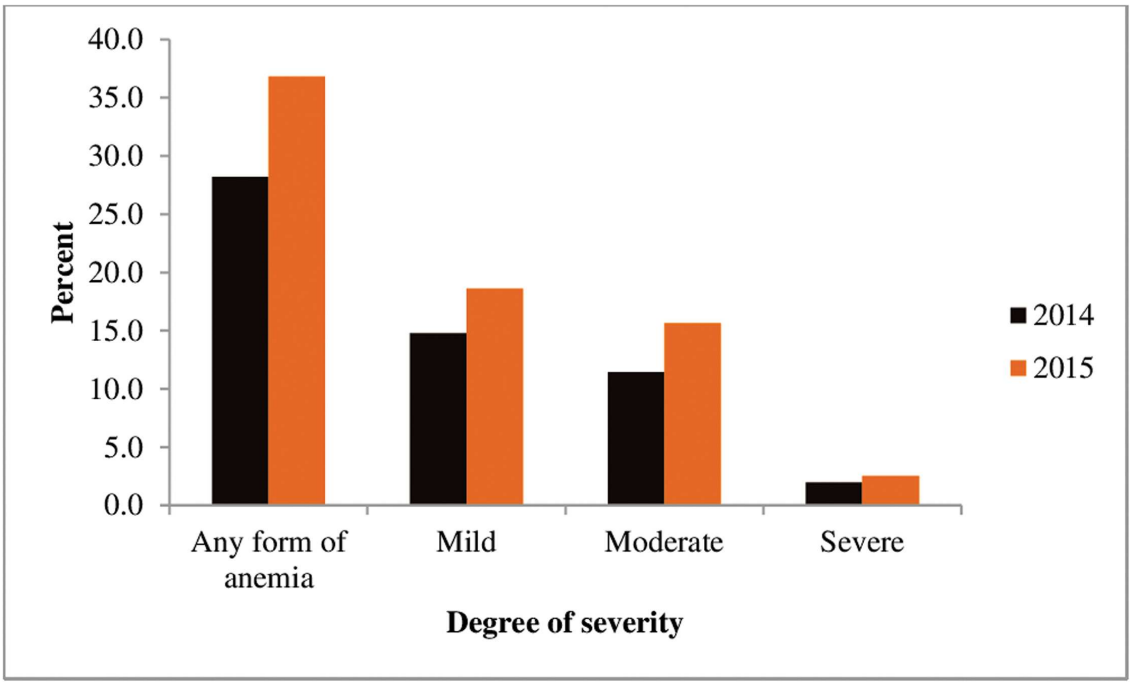

Fig 3. Severity of anaemia among children in Adami Tullu district in south-central Ethiopia, 2014 and 2015.

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malaria transmission (September to December, 2015). The incidence rate of malaria was 6.7 cases per 10,000 person weeks of observation (95% CI, 5.2–8.5).

Discussion

This study was a part of a trial to prevent malaria; and showed that low age, stunting, malaria and poverty were the main predictors of anaemia. Although we had expected a reduction of anaemia due to the effect of the malaria prevention trial, we observed an increase in anaemia prevalence among children aged 6 to 59 months from 28% in 2014 to 37% in 2015. Our study took place during a period when there was 60% less rain. The population experienced severe food shortage followed by an increase in the prevalence of stunting. The prevalence of anaemia and stunting were particularly high among children in the poor families, or among families who moved out of the area, probably because of lack of food. Many (36.5%) of the children who were anaemic in 2014 were also anaemic a year later. This could indicate that children remained chronically anaemic, or that they suffered from recurrent anaemia.

In Ethiopia, most of anaemia studies were done as cross-sectional studies [7, 22]. However, we followed the population for one year to measure changes in anaemia prevalence and to assess the incidence and risk factors for anaemia. As our study population was randomly selected from the base population around Lake Zeway in the Rift Valley, we believe the population is representative of rural population living in similar ecological settings. Thus, our findings can be generalized to similar areas in Ethiopia.

Table 3. Multilevel logistic regression for predictors of anaemia among children in Adami Tullu district in south-central Ethiopia, 2014 and 2015.

Variables		2014 (n = 2984)			2015 (n = 3128)		
		Anaemic Cases N (%)	Unadjusted † OR (95% CI)	Adjusted OR £ (95% CI)	Anaemic Cases N (%)	Unadjusted † OR (95% CI)	Adjusted OR £ (95% CI)
Sex	Boys	440 (28.7)	1.1 (0.6–0.9)	NA	593 (37.4)	1.1 (0.9–1.2)	NA
	Girls	401 (27.6)	1.0	NA	558 (36.2)	1.0	NA
Age in months	6–35	526 (62.5)	2.2 (1.8–2.6)*	2.0 (1.7–2.4)*	705 (46.0)	2.4 (2.0–2.9) *	2.5 (2.1–3.0) *
	36–59	315 (37.5)	1.0	1.0	446 (28.0)	1.0	1.0
Height-for-age	<-2 Z-scores	468 (34.5)	1.9 (1.6–2.2)*	1.6 (1.4–2.0)*	642 (41.8)	1.6 (1.4–1.9) *	1.5 (1.3–1.8) *
	≥ -2 Z-scores	372 (22.9)	1.0	1.0	509 (32.0)	1.0	1.0
Weight-for-height	<-2 Z-scores	62 (27.1)	0.9 (0.7–1.3)	NA	37 (29.6)	0.7 (0.5–1.1)	0.6 (0.4–1.0)
	≥ -2 Z-scores	777 (28.2)	1.0	NA	1114 (37.1)	1.0	1.0
Wealth status	Poor	300 (31.4)	1.4 (1.1–1.7)*	1.3 (1.1–1.6)*	420 (39.0)	1.3 (1.1–1.6) *	1.3 (1.1–1.6) *
	Middle	281 (27.7)	1.1 (0.9–1.4)	1.1 (0.8–1.3)	396 (38.1)	1.3 (1.1–1.6) *	1.3 (1.1–1.7) *
	Rich	259 (25.6)	1.0	1.0	333 (33.0)	1.0	1.0
Education of household head	Illiterate	378 (28.8)	1.1 (0.8–1.5)	NA	554 (39.4)	1.3 (1.0–1.7) *	1.5 (1.1–2.0) *
	Elementary	203 (28.6)	1.1 (0.8–1.5)	NA	273 (37.0)	1.0 (0.8–1.4)	1.2 (0.8–1.6)*
	Secondary and above	260 (27.0)	1.0	NA	322 (32.8)	1.0	1.0
Intervention arm	IRS + LLINs	199 (26.8)	1.0	NA	310 (38.1)	1.0	NA
	LLINs alone	220 (28.6)	1.1 (0.9–1.4)	NA	282 (35.0)	0.9 (0.7–1.1)	NA
	IRS alone	199 (29.1)	1.2 (0.9–1.5)	NA	272 (38.5)	1.0 (0.8–1.3)	NA
	Routine	223 (28.3)	1.1 (0.8–1.4)	NA	287 (35.8)	0.9 (0.7–1.1)	NA

NA: Not Applicable because p >0.25

OR: Odds Ratio

*: Significant at P<0.05

†: bivariate analysis

£: Multivariate analysis

N: Number

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The prevalence of anaemia in our 2015 survey (36.8%) was similar to studies from northern Ethiopia (37%) [22], and western Ethiopia (32%) [7]. In agreement with other studies [16, 38], anaemia was common among all age groups, and the prevalence of anaemia decreased with an increase in age. This could be due to increased requirement of iron during periods of rapid child growth. Inadequate intake of iron rich foods and repeated infections in the low age group could result in an increased risk of anaemia in this age group [39].

Malaria parasites invade and destroy red blood cells, and increase the risk of anaemia in children infected with malaria parasite [8]. In this study, the risk of anaemia was higher among children having malaria compared to children without malaria infection, and this supported previous studies [6, 14, 40].

Children in poor families were more at risk of anaemia, as has been reported by others [38, 41]. The poor the families are the less likely to afford adequate and diversified foods, and they are also less likely to seek early treatment for anaemia [42].The observed increased risk of anaemia among children with stunting was consistent with previous research findings [12, 21].This can be related to deficiency of protein energy that could result in impaired immune function with repeated infection that depletes iron stores [43]. Micronutrient deficiencies, including

Table 4. Incidence rate and hazard ratio of anaemia among dynamic cohort of children in Adami Tullu district in south-central Ethiopia, 2014 to 2015.

Variables		CYO	Anaemia cases	IR/100 CYO (95% CI)	Unadjusted † HR (95% CI)	Adjusted £ HR (95% CI)
Sex	Boys	1473	442	30 (28–33)	1.1 (0.9–1.2)	NA
	Girls	1440	423	29 (27–32)	1.0	NA
Age group in months	6–35	1084	561	52 (48–56)	2.9 (2.6–3.4) *	3.2 (2.8–3.7)*
	36–59	1405	303	22 (19–24)	1.0	1.0
Malaria status¶	Positive	46	19	41 (26–64)	8.5 (3.1–23.4)*	10.4 (3.8–28.8) *
	Negative	2866	846	30 (28–32)	1.0	1.0
Height for age	> = -2 Z-score	1124	406	36 (33–40)	1.0	1.0
	<-2 Z-score	1106	458	41 (38–45)	1.1 (1.0–1.3) *	1.2 (1.04–1.4)*
Weight for height	> = -2 Z-score	1755	314	18 (16–20)	1.0	NA
	<-2 Z-score	134	22	16 (11–25)	0.9 (0.6–1.5)	NA
Wealth status	Poor	945	312	33 (30–37)	1.3 (1.1–1.5) *	1.2 (1.04–1.4)*
	Middle	984	300	30 (27–34)	1.2 (1.0–1.4)	1.2 (1.0–1.4)
	Rich	980	251	26 (23–29)	1.0	1.0
Education of household head	Illiterate	1707	551	32 (30–35)	1.2 (0.9–1.5)	1.2 (0.9–1.5)
	Elementary	868	226	26 (23–33)	1.0 (0.8–1.2)	0.9 (0.7–1.2)
	Secondary and above	322	86	27 (22–33)	1.0	1.0
Intervention arms	LLINs + IRS	765	240	31 (28–36)	1.1 (0.9–1.3)	NA
	LLINs alone	722	207	29 (25–33)	1.0 (0.8–1.2	NA
	IRS alone	661	201	30 (26–35)	1.0 (0.8–1.3)	NA
	Routine	764	217	28 (25–32)	1.0	NA

HR: Hazard Ratio IR: Incidence Rate CYO: Children Years of Observation NA: Not Applicable, because P>0.25 in bivariate analysis

†: bivariate analysis

£: Multivariate analysis

¶: malaria was entered as time varying covariate

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iron, also occur when there is under-nutrition due to food shortage and food lacks diversity [42].

We expected a decrease in prevalence of anaemia among children related to an ongoing malaria prevention trial. However, an increase in the prevalence of anaemia between the two surveys, and high anaemia incidence (30 per 100 person years of observation) could be due to drought and food shortages [26]. In addition, the shortage of water related to decrease in rainfall could also result in poor hygiene and increase occurrence of intestinal parasitic infestation that could contribute to an increase in the prevalence of anaemia [44].

We observed no statistically significant difference in risk of anaemia among the trial arms. This could be related to low malaria incidence. In this study, the observed malaria incidence (6.7 cases per 10,000 children weeks) was lower than our pilot study (8 cases per 10,000 person weeks for the general population, and 11 cases per 10,000 children weeks) as reported elsewhere by Gari et al [28]. The observed low malaria incidence in this study could be due to the short duration and reduced rainfall as a result of the severe drought that affected the area [27].

The present study had some limitations. In the cohort study, children from poor families accounted for 45.2% of all children who left the study area, and the loss to follow-up was most likely related to drought, and to food shortage triggered by El Nino that affected the study area in 2015 and early 2016 [26]. The high prevalence of anaemia among children who left the area (46%) compared to those who completed the two surveys (33%) could have introduced bias in

Table 5. Incidence and hazard ratio of anaemia among closed cohort of children in Adami Tullu district, south-central Ethiopia, 2014 to 2015.

Variables		CYO	Anaemia cases	IR/100 CYO (95% CI)	Unadjusted † HR (95% CI)	Adjusted £ HR (95% CI)
Sex	Boys	612	176	29 (25–34)	1.1 (0.9–1.4)	NA
	Girls	611	160	26 (22–31)	1.0	NA
Age group in months	6–35	415	155	37 (32–44)	1.7 (1.3–2.1) *	1.5 (1.2–1.9)*
	36–59	808	181	22 (19–26)	1.0	1.0
Malaria status¶	Positive	26	9	34(18–65)	3.02 (0.9–10.5)	4.0 (1.1–14.3)*
	Negative	1197	327	27 (25–31)	1.0	1.0
Height for age	> = -2 Z-score	610	143	23 (20–28)	1.0	1.0
	<-2 Z-score	613	193	32 (27–36)	1.3 (1.1–1.7) *	1.3 (1.05–1.63)*
Weight for height	> = -2 Z-score	1178	324	28 (25–31)	1.0	NA
	<-2 Z-score	45	12	27 (15–47)	0.9 (0.5–1.7)	NA
Wealth Status	Poor	367	110	30 (25–36)	1.4 (1.1–1.8) *	1.4 (1.05–1.8)*
	Middle	420	135	32 (27–38)	1.5 (1.1–1.9) *	1.5 (1.2–2.0)*
	Rich	413	91	22 (18–27)	1.0	1.0
Education of household head	Illiterate	688	197	29 (25–33)	1.1 (0.8–1.6)	1.1 (0.8–1.6)
	Elementary	402	105	26 (22–32)	1.0 (0.7–1.5)	1.0 (0.7–1.5)
	Secondary and above	133	34	25 (18–36)	1.0	1.0
Intervention arms	LLINs + IRS	313	98	31 (26–38)	1.1 (0.9–1.5)	NA
	LLINs alone	311	76	24 (20–31)	0.9 (0.7–1.2	NA
	IRS alone	272	71	26 (21–33)	0.9 (0.7–1.3)	NA
	Routine	327	91	28 (23–34)	1.0	NA

HR: Hazard Ratio IR: Incidence Rate CYO: Children Years of Observation NA: Not Applicable, because P>0.25 in bivariate analysis

†: bivariate analysis

£: Multivariate analysis

¶: malaria was entered as time varying covariate

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the estimation of the prevalence and incidence of anaemia. The other limitations were: We included children in the age group of 6 to 59 months old, and those older than 59 months were excluded at the beginning of the study. And by doing so, we unfortunately remained with a smaller sample size. Moreover, the low malaria incidence (smaller sample size than expected) could have under-powered the study to detect the effect difference. The causes of anaemia are multi-factorial, and our study could have benefitted from a more thorough laboratory analysis of the causes of anaemia including stool examination for intestinal helminthes. Unfortunately, we only measured some of the main risk factors of anaemia, such as nutritional status assessed using anthropometry, diagnosed malaria status, and socioeconomic variables. Unlike other studies [6, 15, 21], we did not assess the iron status by measuring serum ferritin, and we did not assess the intestinal parasitic load, which could be potential causes of anaemia. Therefore, future anaemia and malaria prevention studies in drought prone areas should include a more thorough assessment of potential causes of anaemia.

In this study, under-estimation of anaemia incidence rate could resulted from: A year apart anaemia survey may not have captured all the anaemia cases in the year time, particularly anaemia cases that occurred and recovered between the two surveys. In addition, the onset of anaemia was uncertain related to the less frequent, once a year anaemia survey we conducted. Therefore, the time to anaemia was defined as the time from malaria diagnose to anaemia diagnosis. However, the time at onset of anaemia could have occurred earlier than the time at

anaemia diagnosis, and the use of time to anaemia diagnosis as end point could result in under-estimation of anaemia incidence.

Conclusion

In conclusion, young age, stunting, malaria and poverty were the main predictors of anaemia. An unexpected increase in the prevalence of anaemia was observed over a year period, and anaemia prevalence was particularly high among people who moved from the area due to lack of food. We did not observe statistical significant difference in risk of anaemia among malaria intervention arms. Therefore, conducting trials in settings prone to drought and food shortage is a serious challenge. Further study could be needed to measure serum ferritin value to establish the proportion of iron deficiency anaemia among the children.

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Author Contributions

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Formal analysis: TG EL BL.

Funding acquisition: BL EL WD.

Investigation: TG EL WD BL HA MA TS AH.

Methodology: TG EL WD BL.

Project administration: TG EL WD BL HA MA TS AH.

Resources: BL EL WD.

Validation: TG EL WD BL HA MA TS AH.

Visualization: TG.

Writing – original draft: TG EL WD BL.

Writing – review & editing: TG EL WD BL HA MA TS AH.

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Paper III

PLOS ONE

RESEARCH ARTICLE

Malaria increased the risk of stunting and wasting among young children in Ethiopia: Results of a cohort study

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Abstract

Introduction

Given the high prevalence of malnutrition in a malaria-endemic setting, improving nutritional status could serve as a tool to prevent malaria. However, the relationship between the two conditions remains unclear. Therefore, this study assessed the association between under-nutrition and malaria among a cohort of children aged 6 to 59 months old.

Methods

Two cohorts of children were followed for 89 weeks in a rural Rift Valley area of Ethiopia. In the first approach (malaria-malnutrition), a cohort of 2,330 non-stunted and 4,204 non-wasted children were included to assess under-nutrition (outcome) based on their previous malaria status (exposure). In the second approach (malnutrition–malaria), a cohort of 4,468 children were followed-up to measure malaria (outcome), taking under-nutrition as an exposure. A weekly home visit was carried out to identify malaria cases. Four anthropometry surveys were conducted, and generalized estimating equation (GEE) method was used to measure the association between undernutrition and malaria.

Results

The prevalence of stunting was 44.9% in December 2014, 51.5% in August 2015, 50.7% in December 2015 and 48.1% in August 2016. We observed 103 cases with 118 episodes of malaria, 684 new stunting and 239 new wasting cases. The incidence rate per 10,000 weeks of observation was 3.8 for malaria, 50.4 for stunting and 8.2 for wasting. Children with malaria infection, [Adjusted Odds Ratio (AOR) = 1.9; 95% Confidence Interval (CI), 1.2–2.9)] and younger age (AOR = 1.3; 95% CI, 1.1–1.5) were more likely to be stunted. Furthermore, children with malaria infection (AOR = 8.5; 95% CI, 5.0–14.5) and young age group (AOR = 1.6; 95% CI, 1.2–2.1) were more likely to be wasted. However, stunting and wasting were not risk factors of subsequent malaria illness.

Conclusions

Malaria infection was a risk factor for stunting and wasting, but stunting or wasting was not associated with subsequent malaria illness. As our study shows that malaria is a risk factor for stunting and wasting, a close follow-up of the nutritional status of such children may be needed.

Trial registration

PACT R2014 11000 882128 (8 September 2014).

Introduction

Malaria is a public health problem in the developing world; particularly in sub-Saharan African countries where malaria kills a child every two minutes [1]. The disease remains one of the major challenge for people's health and livelihood around the world [1]. On the other hand, malnutrition is an underlying cause of death for approximately 45% of children under the age of five years [2]. Globally, 161 million children under five years old were stunted, and 51 million were wasted in 2012 [3, 4]. One-third of the world's stunted children were living in sub-Saharan Africa [5]. Malnutrition could refer both to under- and over-nutrition [6], but in this study we use the term to refer to under-nutrition (stunting, underweight and wasting).

In Ethiopia, malaria is a common cause of childhood illness [1, 7].Nearly, 68% of the land mass of the country have ecological characteristics favourable for malaria transmission, and about 60% of the population is at risk of malaria infection [8]. The transmission of malaria is seasonal and unstable [8]. *Plasmodium falciparum* (60%) and *P.vivax* (40%) are the two main causes of malaria [9]. According to the recent malaria indicator surveys, the national prevalence of malaria among children was 1.4% in 2011[7] and 0.6% in 2015 [10]. A pilot study conducted for the preparation of a community based malaria prevention trial in Adami Tulu district, Ethiopia (same study area with current study) in 2013, has shown a malaria incidence of 6.8 cases per 10,000 person weeks of observation among children [11]. In addition, under-nutrition is a major public health problem in the country. According to the 2016 Demographic and Health Survey (DHS), 47% of rural children were stunted and 10% were wasted [12–14].

Good nutrition and healthy growth during a period from conception to a child's second birthday—the 1,000 days—have lasting benefits throughout life, and undernourished children reaching this age could suffer from irreversible health problems [15]. Under-nutrition could be the result of poor dietary intake often combined with infectious disease [16]. On the other hand, malnutrition is a well-known underlying cause for many infectious disease-related causes of child deaths [17, 18]. Malaria and malnutrition co-exist in a setting where the two conditions are highly prevalent [19]. However, the relationship between malaria and under-nutrition is complex and remains unclear. Previous research shows mixed findings, e.g.: a community-based survey from Ghana showed underweight as a contributing risk to malaria infection [18], whereas results from a follow-up study and repeated cross-sectional surveys showed stunting as a contributing risk to malaria [20]. A case-control study from Ethiopia reported wasting as a contributing risk to malaria [21]. Some cohort studies showed a lower contributing risk to malaria infection among malnourished children [22–24], although results from other cross-sectional surveys did not report any association between malaria and malnutrition [25, 26]. Meanwhile, others observed malaria infection as a risk factor for under-

nutrition [27–29]. In summary, most of the studies assessing the possible association between malaria and malnutrition were either institutional-based surveys that could not be generalizable [30], or community-based cross-sectional studies, which could have less strength of evidence to support a causal relationship [25]. Moreover, both case control [21] and cohort [31] studies show inconsistent findings.

Long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS) and prompt diagnosis and treatment are the primary tools in reducing malaria-related illness and deaths [1]. In the meantime, evidence on the relationship between under-nutrition and malaria could also be used as an additional means to help the current malaria control activities. Given the high prevalence of malnutrition in a malaria-endemic setting, and if malnutrition is associated with malaria, improving nutritional status could serve as a tool to prevent malaria. However, in Ethiopia, there is a scarcity of follow-up studies measuring the relationship between the two conditions. Therefore, the general objective of this study was to assess the association between malaria and under-nutrition among a cohort of children aged 6 to 59 months old. The specific objectives were: 1) to assess the association between under-nutrition as an exposure and subsequent malaria infection, and 2) to evaluate the association between malaria as an exposure, and under-nutrition as outcome among children aged 6 to 59 months old.

Materials and methods

Study area

The study profile of the participants was presented in Fig 1. The study was conducted in 13 *kebeles* (the lowest government administrative unit) in the Adami Tullu district, located 160 km south of Addis Ababa (Fig 2). According to the 2007 census, children under the age of five accounted for 12% of the total estimated population (147,000) of the district [32]. The total annual rainfall in 2014 (recorded for 8 months) was 673 mm, decreasing to 471 mm in 2015 [33]. The primary livelihood of the study population is based on rain-fed agriculture and livestock rearing, while cereal crops such as maize, wheat and teff are the main crops growing in the district. The study area was affected by repeated droughts and famines in the past few decades [34]. In 2015 and early 2016, the El Nino-triggered drought caused a serious food shortage [35], and the residents were getting food aid. Malaria and malnutrition were the major public health problems in the district [11, 36]. Each *Kebele* has one health post staffed by two community health extension workers. The health post provides basic health services, including the distribution of LLINs, the diagnosis of malaria with RDT and treatment with antimalarial drugs, as well as nutritional intervention, such as treatment for severe acute malnutrition and de-worming.

Study design and participants

This study was a part of a malaria prevention trial, and the details have been presented in an earlier publication [37]. In brief, the trial was a cluster randomized controlled trial, and the unit of randomization was a village. The study villages were selected randomly from those within 5 km from Lake Zeway. The trial was based on four arms: LLINs + IRS, LLINs alone, IRS alone and control arms.

In this study on the association between malaria and under-nutrition, two cohort studies were followed-up from December 2014 to August 2016 with weekly visits. In the first cohort (called the malaria-malnutrition cohort), malaria was the main exposure and under-nutrition was the outcome. In this cohort, 2,330 non-stunted and 4,204 non-wasted children were considered as the study subjects (supplementary "S1 Fig and S2 Fig"). In the second cohort (called

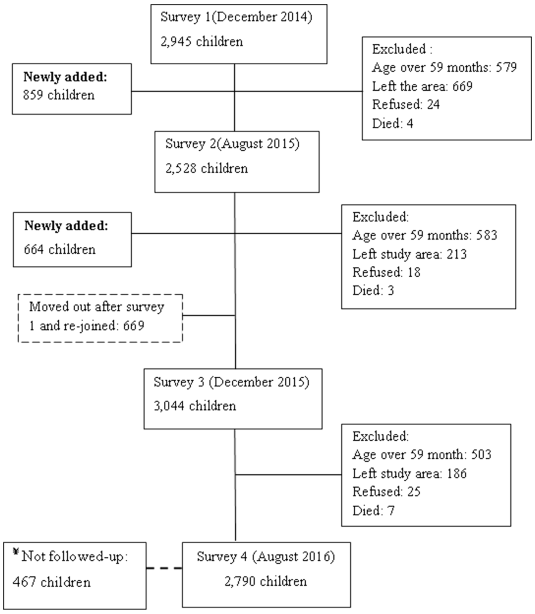

Fig 1. Study profile of children, in Adami Tullu District in south-central Ethiopia 2014–2016. ¥ The broken line to the left of the fourth survey box indicates newly joined children during the last survey (August 2016) and not followed for malnutrition. Thus, they were not included in the cohort study. However, they were included in the calculation of prevalence of undernutrition.

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malnutrition–malaria cohort), a cohort of 4,468 children was enrolled using anthropometry surveys, and followed to determine malaria incidence (outcome) based on nutritional status (exposure).

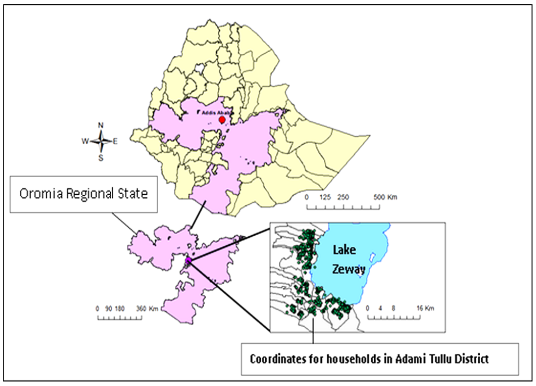

Fig 2. Map of the study area with location of households in Adami Tullu District in south-central Ethiopia. Reprint under a CCBY license, with permission from Deressa et al. Trials (2016).

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Sample size estimation

The sample size calculation for the main trial has been presented elsewhere[37]. In brief, the calculated sample size was 44 villages per four arms (LLINs+ IRS, LLINs alone, IRS alone and control arm), with each village having approximately 35 households. Overall, roughly 31,000 people in 6,072 households were included in the trial. In this study on the association between malaria and malnutrition, we followed all children (4468 children) aged 6 to 59 months who participated in the main trial. The total number of non-stunted children was 2,330, and the number of anthropometric measurements ranged between 1 and 4 for each child. Whereas, the total number of non-wasted children was 4,204, and the number of anthropometric measurements ranged from 1 to 4 for each child.

Data collection

A household census was conducted to collect data on demographic and socioeconomic variables using a pre-tested interviewer-administered structured questionnaire. The data collectors were diploma graduate personnel. We carried out weekly home visits searching for children with a history of fever over the past 48 hours. The identified cases were referred to health posts for a malaria diagnosis. In addition, the families were advised to visit the health post between the visit days if their child developed a fever.

Malaria diagnosis

According to the World Health Organization (WHO) recommendation, the two methods currently considered suitable for routine patient management are light microscopy and RDT [38]. The gold standard for over a century, light microscopy is useful to identify the *Plasmodium* parasite presence, species and accurate parasite counting (identification of high parasite density). However, high quality light microscopy requires well-trained, skilled staff, good staining reagents and electricity to power the microscope [38]. Whereas, rapid diagnostic tests (RDTs) based on lateral flow immune-chromatography, which can be done with little training, and have made malaria diagnostic tests accessible to the larger community [39]. In Ethiopia, light microscopy is used to diagnose malaria at the health centers and hospitals, whereas, at community health posts, the diagnosis of malaria is made using rapid diagnostic testing [40]. For the current study, we used RDT for malaria diagnosis. However, RDT does not measure malaria parasite counts. Therefore, we did not assess the association between malaria parasiteamia or severity and malnutrition.

At the health post, capillary blood samples were collected through a finger prick, and malaria was diagnosed using a multispecies *P.falciparum* and *P.vivax* RDT Care Start ^{TM} produced by Premier Medical Corporation Limited in India.

Anthropometry survey

We conducted four anthropometric surveys in December 2014, August 2015, December 2015 and in August 2016. A standard wooden board was used to measure height or length, and a calibrated Salter spring scale was used to measure the weight of the children. The data collectors were trained, the instrument was standardized in the field work and the inter-rater reliability of the tools were analyzed [41]. The intra-technical error of measurement was 0.08 kg for weight and 0.14 cm for height, whereas the inter-technical error of measurement was 0.1kg for weight and 0.2 cm for height. The weight measuring scale was adjusted, and the weight was read to the nearest 0.1 kg. The length of children less than 24 months old was taken in the recumbent position, while for the older children the height was measured standing on a

vertical measuring wooden board, which was read to the nearest 0.1 cm. Using the 2006 WHO multi-center growth reference study [42], weight for height (WHZ), height for age (HAZ) and weight for age (WAZ) were calculated. The children were classified as wasted with a WHZ < -2 Z score, stunted with a HAZ < -2 Z score and underweight with a WAZ < -2 Z score.

Statistical analysis

Data were entered into IBM SPSS version 21 (SPSS Inc, Chicago, USA), and analyzed using STATA version 14 (StataCorp, Texas, USA). Descriptive statistics were used to summarize the data, and nutritional indices were calculated using the Emergency Nutrition Assessment (ENA) for SMART software 2011 [43]. A household wealth index was constructed using principal component analysis technique [44]. We included 14 variables that were related to household assets and livestock ownership [45], and the constructed index was used to categorize the households into three socio-economic classes, including rich, middle and poor.

The prevalence of under-nutrition was calculated for each survey, whereas the incidence of stunting, wasting and malaria was calculated per person-weeks of observation.

In the malaria-malnutrition cohort, the outcome variables stunting and wasting were assessed using anthropometric measurements. Because we only did anthropometry twice a year, it was difficult to capture the actual start of stunting or wasting, as the time of start of stunting or wasting could have occurred earlier than the time at diagnosis. Therefore, the use of time from malaria diagnosis to stunting or wasting diagnosis as an endpoint could result in underestimation of the incidence rate ratio of stunting or wasting, and could limit the use of Cox regression. On the other hand, the standard logistic regression could not allow for the repeated measures of the outcome variables, and could underestimate the standard error. However, the generalized estimating equations (GEE) procedure extends the generalized linear model to allow for analysis of repeated measurements [46]. The repeated observation within one subject are not independent of each other, and therefore, GEE helps to correct for these within-subject correlations. In this study, stunting or wasting (outcome) was measured four times for a child, and the variable stunting or wasting was dichotomous that follows a binomial distribution. Thus, a logistic generalized estimating equation (GEE) was used to allow the repeated measures for the outcome variables, stunting or wasting. The specified probability distribution was binomial with logit link function and the working correlation matrix structure was exchangeable. The covariance matrix was robust estimator, and the scale parameter was Person chi-square (χ^2). A hybrid with a maximum Fisher scoring iteration of 1 was used as a parameter estimation method. The main effect was the term used to build the reported model, and Kernel was specified for the log quasi-likelihood function. Child age, gender, malaria infection, wealth index, education of the household head and intervention arms were considered as the potential risk factors of stunting and wasting for each child. Lastly, the bivariate and multivariate logistic regression analysis were carried out and odds ratio was reported.

In the undernutrition-malaria cohort, the outcome variable, malaria case was assumed to follow a binomial distribution. The main exposure variables wasting and stunting were measured twice yearly and the outcome malaria variable (malaria) was identified through weekly home visit and patient self-referral between the visit days. Hence to account for within subject measurement correlations, a logistic GEE model was fitted taking into account child age, gender, stunting, wasting, wealth index, education of the household head and intervention arm as the potential risk factors of malaria cases for each child. To construct the model the following were specified: the scale parameter was Pearson χ^2, the scale weight variable was the number of weeks a child had been observed, the covariance matrix was robust estimator and a hybrid with maximum Fisher scoring iteration of 1 as parameter estimation method. The specified

log-likelihood function was Kernel, and the main effect was the term used to build the model. Bivariate and multivariate analysis were done to measure the risk factors of malaria, and odds ratio was reported.

Ethical issues

Ethical clearance was obtained from the Institutional Review Board of the School of Public Health at Addis Ababa University, the National Ethical Committee of the Ministry of Science and Technology in Ethiopia (ref: 3.10/446/06) and the Regional Committee for Medical and Health Research Ethics, Western Norway (ref: 2013/986/REK Vest). A written permission letter was obtained from the Oromia Regional Health Bureau, East Shewa Zonal Health Department and the Adami Tullu District Health Office. As the majority of the study population cannot read and write, we had a challenge to get written consent. Thus, a verbal consent was obtained from the parents or caretakers before collecting the blood samples and anthropometric measurements of the children. A standard information sheet was used to explain the purpose of the study, and the participants were informed that participation was voluntary and that they had the right to withdraw any time during the study. They were assured that refusal to participate in the study would not affect their health service utilization at the health posts. This information was read to them using the information sheet in their own language, and their consent was recorded using check ($\surd$) mark. We also strictly supervised the data collectors, whether they are following the information sheets or not. Later, this document was stored at our field research station. Those children who were positive for malaria, and severely acutely malnourished, were treated according to the national treatment guidelines [40, 47]. Accordingly, those with *P. falciparum* or a mixed infection were given Artemether-Lumefantrin, while children with *P. vivax* were treated with Chloroquine.

Results

Participants and prevalence of under-nutrition

The mean (SD) age of the children in months was 33.6 (14.4) in December 2014, 37.4 (15.0) in August 2015, 35.6 (14.8) in December 2015 and 36 (15.2) in August 2016 surveys. Nearly 49% of them were girls in all the surveys (Table 1).

The overall prevalence of stunting was 44.9% in December 2014, 51.5% in August 2015, 50.7% in December 2015 and 48.1% in August 2016. The prevalence of underweight was 17.2% in December 2014, 21.7% in August 2015, 16.3% in December 2015 and 18.7% in August 2016, while the prevalence of wasting was 7.2% in December 2014, 5.7% in August 2015, 4.0% in December 2015 and 6.9% in August 2016 (Fig 3).

Prevalence of stunting and wasting: Correlation with prevalence of malaria. As shown in Fig 4, stunting increased, but malaria prevalence decreased during the second and third surveys compared to the first survey (December 2014). We observed no significant correlation between prevalence of stunting and malaria (Spearman's correlation coefficient was -0.32, P-value = 0.684), and also no significant correlation between wasting and malaria (Spearman's correlation coefficient was 0.31, P-value = 0.68).

Malaria-malnutrition cohort

Incidence of stunting. We observed 684 new stunting cases during the 89 weeks of follow-up; of which, 285 (41.7%) were severely stunted (HAZ $<$-3 z-score). Stunting was highest in the age group from 23–35 months (46%), followed by the age group 6–23 months (28.4%) and those above 36 months old (26.5%). The incidence rate of stunting was 50.3; (95% CI,

Table 1. Characteristics and nutritional status of children in Adami Tullu District in south-central Ethiopia, 2014–2016.

Variables		December 2014 (n = 2,945)	August 2015 (n = 2,528)	December 2015 (n = 3,044)	August 2016 (n = 2,790)
		Number (%)	Number (%)	Number (%)	Number (%)
Gender					
Boy		1,497 (50.8)	1,302 (51.5)	1,538 (50.5)	1,419 (51.9)
Girl		1,448 (49.2)	1,226 (48.5)	1,506 (49.5)	1,371 (49.1)
Age in months					
6–35		1,461 (49.6)	1,105 (43.7)	1,462 (48.0)	1,274 (45.7)
36–59		1,484 (50.4)	1,423 (56.3)	1,582 (52.0)	1,516 (54.3)
Household Head education					
Illiterate		1,689 (57.4)	1,465 (58.0)	1,794 (58.9)	1,624 (58.2)
Primary		936 (31.8)	775 (30.7)	916 (30.1)	855 (30.6)
Secondary and above		320 (10.8)	288 (11.3)	334 (11.0)	311 (11.2)
Wealth status					
Poor		945 (32.0)	801 (31.7)	1,045 (34.3)	961 (34.4)
Middle		1,000 (34.0)	875 (34.6)	1,015 (33.3)	942 (33.8)
Rich		1,004 (34.0)	852 (33.7)	984 (32.4)	887 (31.8)
Intervention arm					
IRS + LLIN		741 (25.2)	707 (28.0)	797 (26.2)	739 (26.5)
LLIN alone		752 (25.5)	569 (22.5)	784 (25.8)	734 (26.3)
IRS alone		670 (22.8)	541 (21.4)	679 (22.3)	630 (22.6)
Routine		782 (26.5)	711 (28.1)	784 (25.7)	687 (24.6)
Anthropometric indicators					
Median (IQR)	HAZ	-1.8 (-2.8- -0.8)	-2.1 (-3.2- -0.9)	-2.0 (-2.9- -1.1)	-1.9 (-2.8- -1.0)
	WAZ	-1.0 (-1.7- -0.4)	-1.8 (-1.1- -0.3)	-1.05 (-1.7- -0.4)	-1.12 (-1.7- -0.4)
	WHZ	-0.07 (-0.1–0.02)	0.2 (0.1–0.3)	0.1 (0.06–0.2)	-0.01 (-0.8–0.7)

IQR: Interquartile Range; IRS: Indoor Residual Spraying; LLINs: Long Lasting Insecticidal Nets
HAZ: Height-for-Age; WAZ: Weight for Age; WHZ: Weight-for-Height

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46.7–54.3) per 10,000 person-weeks of observation. The stratified analysis of stunting by malaria status and age group showed that age was not an effect-measure modifier of malaria effect (a risk ratio of 1.0 among children aged 6–23 months, 0.8 in the age group above 24 months, an overall crude risk ratio of 0.9 and an adjusted risk ratio of 0.9).

Risk factors of stunting. The fitted GEE model showed that younger age (AOR = 1.3; 95% CI, 1.1–1.5) and malaria infection (AOR = 1.9; 95% CI, 1.2–2.9) were risk factors of stunting after adjusting for the previous height-for-age (6 months preceding anthropometry survey). However, wealth status, education of household head, gender and intervention arms were not found to be the risk factors for stunting (Table 2).

Incidence of wasting. Overall, 239 new wasting cases were registered, with severe wasting (WHZ<-3Z-score) accounting for 31% (74/239). The incidence rate of wasting was 7.2, (95% CI, 6.3–8.1)/10,000 person-weeks of observation.

Risk factors of wasting. In the GEE analysis, children in the younger age group (AOR = 1.6; 95% CI, 1.2–2.1) and children with malaria illness (AOR = 8.5; 95% CI, 5.0–14.5) were more likely to be wasted after adjusting for the previous weight-for-height (6 months preceding anthropometry survey). Nonetheless, education of household head, wealth status of the family, gender and intervention arms were not associated with wasting (Table 3).

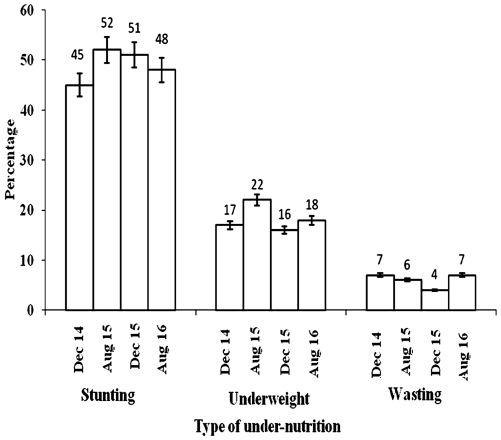

Fig 3. Prevalence of under-nutrition among children in Adami Tullu District in south-central Ethiopia, 2014–2016. I: Bar with 95% confidence level.

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Malnutrition-malaria cohort. Overall, 103 malaria cases with a total of 118 episodes of malaria were observed between December 2014 and August 2016. Of all children diagnosed with malaria, 12 children had more than one malaria episode. Slightly over half (53%) of the malaria cases were due to *P. falciparum*, followed by *P. vivax* (36%) and mixed infections (11%). The incidence of malaria was 3.8 (95% CI, 3.1–4.6)/10,000 person-weeks of observation.

Risk factors of malaria. The logistic GEE model showed that being in the poorest families AOR = 3.3; 95% CI, 1.7–6.3) were more at risk of malaria compared to children in rich

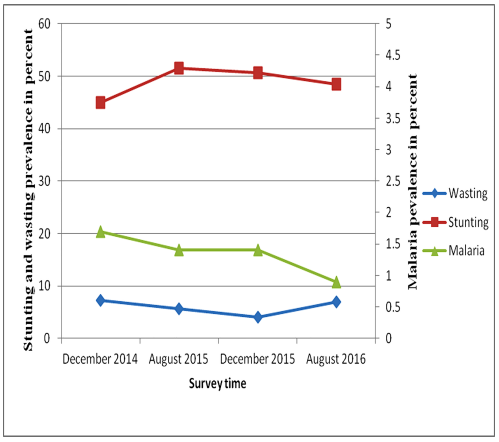

Fig 4. Prevalence of stunting, wasting and period prevalence of malaria among children in Adami Tullu District in south-central Ethiopia, 2014–2016.

https://doi.org/10.1371/journal.pone.0190983.g004

Table 2. GEE model for stunting in children living in Adami Tullu District in south-central Ethiopia, 2014–2016.

Variables (N = 9320)		Unadjusted OR (95% CI)	Adjusted OR (95% CI)	P-value
Gender	Boy	1.0 (0.9–1.2)	1.1 (0.9–1.3)	0.23
	Girl	1	1	
Age in months	6–35	1.3 (1.1–1.4)	1.3 (1.1–1.5)	0.002*
	36–59	1	1	
Malaria infection¥	Positive	1.9 (1.2–2.9)	1.9 (1.2–2.9)	0.01*
	Negative	1	1	
Previous height-for-age†	<-2Z-score	12.0 (9.5–15.3)	12.3 (9.8–15.8)	<0.001*
	≥-2Z-score	1	1	
Wealth Status	Poor	1.2 (1.0–1.5)	1.2 (0.9–1.5)	0.13
	Middle	1.1 (0.9–1.3)	1.1 (0.9–1.3)	0.51
	Rich	1	1	
Education: household head	No formal	1.1 (0.8–1.5)	1.1 (0.9–1.5)	0.42
	Primary	1.4 (1.1–1.8)	1.3 (1.0–1.8)	0.05
	Secondary and above	1	1	
Intervention arm	IRS+ LLINs	1.1 (0.9–1.5)	1.1 (0.8–1.4)	0.4
	IRS alone	1.2 (0.9–1.5)	1.1 (0.9–1.5)	0.39
	LLINs alone	1.0 (0.8–1.3)	1.0 (0.7–1.2)	0.91
	Routine	1	1	

¥: malaria illness in the previous months preceding anthropometry survey

†: Height-for-age 6 months preceding anthropometry survey

OR: Odds Ratio; CI: Confidence Interval

*: P <0.05

IRS: Indoor Residual Spraying; LLINs: Long Lasting Insecticidal Nets

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families. However, stunting, wasting, gender, age, education of head of household and intervention arms were not associated with increased risk for malaria (Table 4).

Discussion

In this study, malaria infection in the six months preceding the anthropometry survey was a risk factor for stunting and wasting. Even so, stunting or wasting did not contribute to an increased risk of malaria infection.

Our study was based on data from a large cohort of children recruited from a rural community, and the follow-up was for a relatively longer period of time (89 weeks). The continuous supply of Artemether-Lumefantrin and Chloroquine, the availability of a dedicated project staff in the health post and weekly active searches for cases motivated the residents to seek early diagnosis and treatment for malaria.

In our cohort studies the outcome variable is repeatedly measured; that means the outcome variable (stunting, wasting and malaria) is measured in the same child on several different occasions (four cross-sectional studies for undernutrition and weekly for malaria). In such studies the observations of one child over time are not independent of each other, and thus it is necessary to apply GEE method to fit logistic regression model, which consider the fact that the repeated measures of each child are corrected [46]. The outcome and main exposure variables (malaria and undernutrition) were measured using objective standard measurement tools.

Table 3. GEE model for wasting in children living in Adami Tullu District in south-central Ethiopia, 2014–2016.

Variable (N = 16,804)		Unadjusted	Adjusted	
		OR (95% CI)	OR (95% CI)	P-value
Gender	Boy	1.2 (0.9–1.5)	1.2 (0.9–1.5)	0.25
	Girl	1	1	
Age in months	6–35	1.5 (1.2–2.0)	1.6 (1.2–2.1)	0.001*
	36–59	1	1	
Malaria infection¥:	Positive	8.0 (4.7–13.3)	8.2 (5.0–14.3)	<0.001*
	Negative	1	1	
Previous weight-for-height†	<-2Z-score	2.4 (0.9–5.9)	2.3 (0.8–5.8)	0.45
	≥-2Z-score	1	1	
Wealth Status	Poor	0.9 (0.7–1.3)	0.9 (0.7–1.3)	0.66
	Middle	0.9 (0.7–1.3)	0.9 (0.6–1.2)	0.51
	Rich	1		
Education: household head	No formal	1.0 (0.7–1.6)	1.1 (0.7–1.6)	0.74
	Primary	0.9 (0.6–1.5)	1.0 (0.6–1.5)	0.83
	Secondary and above	1	1	
Intervention arm	IRS+LLINs	0.9 (0.6–1.3)	0.9 (0.6–1.3)	0.45
	IRS alone	0.9 (0.6–1.3)	0.9 (0.6–1.3)	0.63
	LIINs alone	1.0 (0.7–1.5)	1.0 (0.7–1.4)	0.89
	Routine	1	1	

¥: malaria illness in the previous months preceding anthropometry survey

†: Weight-for-height 6 months preceding anthropometry survey

OR: Odds Ratio; CI: Confidence Interval

*: P <0.05; IRS:

Indoor Residual Spraying; LLINs: Long Lasting Insecticidal Nets

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The observed malaria incidence (3.8/10,000 person-weeks of observation) in this study was lower than that of a previous study from the same area in 2013 (6.8/10,000 child-weeks of observation) [11]. This may be related to reduced rainfall due to the severe drought that affected the study area during our study period [35]. During the drought season, the decrease in rainfall and increase in temperature could decrease the vector density and result in reduced malaria occurrence [48]. The observed stunting prevalence of 51.5% in August 2015, 50.7% in December 2015 and 48% in August 2016 in this study was higher than a study from Eastern Ethiopia (45.8%) [49] and South-West Ethiopia (40.4%) [25]. This could be due to the El Nino triggered severe drought and food shortage that affected large parts of Ethiopia [35].

In this study, malaria infection preceding an anthropometry survey was a risk factor for wasting. This could be explained by the potential effect of malaria to influence the host's nutrition. Malaria could cause an acute weight loss through decreased food intake, and an increased energy requirement related to illness [50, 51]. Furthermore, children with a malaria infection need more protein and calories than uninfected children for rapid recovery, as inadequate food availability could increase the risk factors for under-nutrition. This finding is supported by a comparable cohort study from Vanuatu Island [28].

In addition, our data show that malaria infection preceding the malnutrition survey was a risk factor for stunting, and this finding was consistent with a cohort study from Kenya [27] and Ghana [29], which show a higher risk of stunting among children with malaria. However, a cohort study from Benin [52] did not observe an association between malaria and a

Table 4. GEE model for malaria in children living in Adami Tullu District in south-central Ethiopia, 2014–2016.

Variable (N = 16,720)		Unadjusted OR (95% CI)	Adjusted OR (95% CI)	P-value
Gender	Boy	0.9 (0.5–1.4)	1.0 (0.6–1.6)	0.93
	Girl	1	1	
Age in months	6–35	1.1 (0.7–1.9)	1.1 (0.7–1.9)	0.62
	36–59	1	1	
Height-for-age	<-2Z-score	1.2 (0.8–2.0)	1.0(0.6–1.6)	0.89
	≥-2Z-score	1	1	
Weight-for-height	<-2Z-score	0.9 (0.4–2.0)	0.9 (0.4–1.4)	0.80
	≥-2Z-score	1	1	
Wealth index	Poor	3.5 (1.9–6.6)	3.3 (1.7–6.3)	0.002*
	Middle	1.9 (0.9–3.8)	1.8 (0.9–3.6)	0.12
	Rich	1	1	
Education: household head	No formal	0.7 (0.4–1.5)	0.8 (0.3–1.7)	0.52
	Primary	1.1 (0.5–2.2)	1.0 (0.4–2.4)	0.92
	Secondary and above	1	1	
Intervention arms	IRS+LLINs	1.5 (0.8–2.9)	1.6 (0.8–3.2)	0.18
	IRS alone	1.5 (0.8–2.9)	1.5 (0.7–3.2)	0.26
	LIINs alone	1.1 (0.5–2.3)	1.2 (0.5–2.6)	0.72
	Routine	1	1	

OR: Odds Ratio; IRS: Indoor Residual Spraying; LLINs: Long Lasting Insecticidal Nets

*: P <0.05

CI: Confidence Interval; IRR: Incidence Rate Ratio

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subsequent occurrence of malnutrition. This could be due to a difference in study setting, in which the Benin study was an institutionally based that could be affected by selection bias.

It is well established that under-nutrition weakens the immune system, putting the child at more risk for infectious diseases, such as diarrhea, measles and respiratory infections [53, 54]. However, concerning the relationship between malnutrition and malaria, different studies showed inconsistent findings. Unlike our findings, studies from Kenya and Ghana observed a higher risk of malaria among children with malnutrition compared to those without malnutrition [18, 20]. This could be due to a difference in study design, in which these two studies used a cross-sectional design that could have less strength to establish the temporal relationship between the two conditions. A case-control study from Ethiopia reported a higher odds of malaria among severely wasted children [21], and this could be explained by a difference in the selection of the cases and controls. In such circumstances, the cases were not comparable with the controls, and could result in biased findings. Moreover, the use of institutional records (incomplete or inaccurate) to assess the exposure status could distort the true association in the path of causality. Nevertheless, another community-based cross-sectional survey in Ethiopia did not show malnutrition as a risk marker for malaria [25]. Additionally, a study from Ghana [29] demonstrated a higher impact of malnutrition on malaria-related deaths among children, though they did not observe malnutrition as a risk marker for malaria incidence, thereby supporting our findings. A systematic review of observational studies [55] observed that most of the studies did not show malnutrition as a risk marker of malaria incidence, but it could have a negative impact on malaria severity and death. In the same review, it was observed that malaria was a risk factor for malnutrition, which was in line with our study. On the contrary, studies by Ahmed et al.[23] and Mitangala et al.[24] observed a lower risk of

malaria parasitemia among children with severe malnutrition. In our study, we used the RDT results and we did not assess the relationship between malnutrition and parasitemia or malaria by severity category.

This study had some limitations. The observed malaria incidence in this study (3.8/10,000 person-weeks) was lower than findings from the same area (8/10,000 person-weeks among the general population and 11/10,000 person-weeks among children) prior to our trial [11]. The lower malaria incidence could mainly be due to the decrease in rainfall and an increase in temperature during the trial period. In Ethiopia, a high prevalence of asymptomatic malaria infection was reported [56, 57], and studies also showed a high prevalence of anemia among asymptomatic plasmodium carriage [58]. In the current study, we did not assess asymptomatic malaria infection. In rural Ethiopia, only 57% of the infants were exclusively breast fed [59], nearly 50% of households were food insecure [60] and over half of children were infected with intestinal helminthes such as hookworm and schistosomiasis [61, 62]. Even so, we did not collect data on food security, breast feeding practices, micronutrient deficiencies and the occurrence of intestinal helminths infections such as hookworm and schistosomiasis that could contribute to increased prevalence of under-nutrition. Our previous work from same populations [45] showed a high anemia prevalence, and that stunting and malaria were contributing to the risk of anemia.

In conclusion, the data showed that malaria was a risk factor for stunting and wasting. Meanwhile, neither stunting nor wasting was associated with increased risk of malaria infection. As our study shows that malaria is a risk factor for subsequent stunting, a close follow-up of the nutritional status of such children may be needed.

Supporting information

S1 Fig. Study profile of non-stunted children in Adami Tullu District in south-central Ethiopia 2014–2016. ¥: Newly added include newborn children aged 6 month and above during the survey and newcomers.
(TIF)

S2 Fig. Study profile of non-wasted children in Adami Tullu District in south-central Ethiopia, 2014–2016. ¥: Newly added include newborn children aged 6 month and above during the survey and newcomers.
(TIF)

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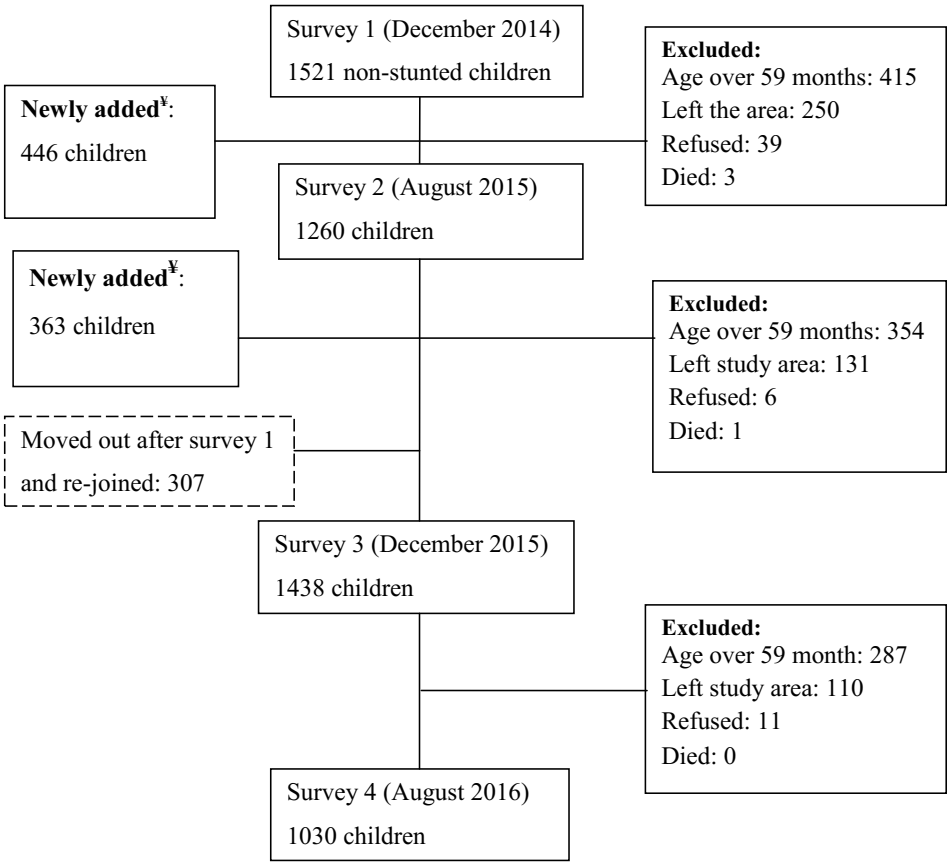
Supplementary Information

Paper III

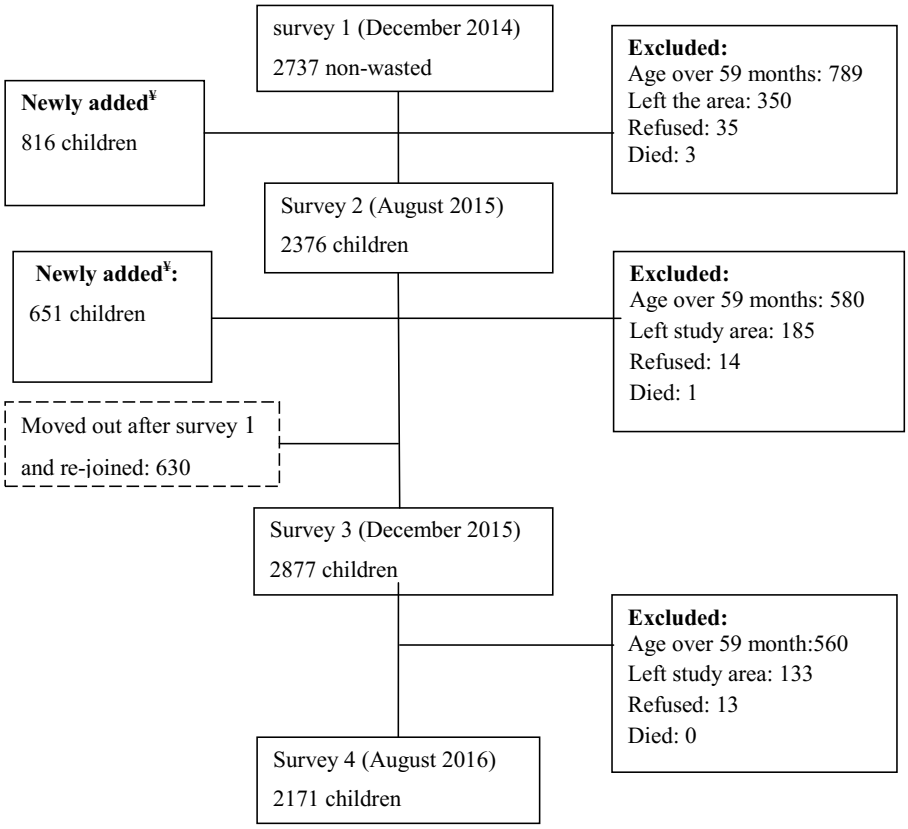
The results presented in the following figure show study profile of non-stunted children in the study area (Paper III)



S1 Fig. Study profile of non-stunted children in Adami Tullu District in south-central Ethiopia 2014 -2016. ¥: Newly added include newborn children aged 6 months and above during the survey and newcomers

Paper III

The results presented in the following figure show study profile of non-wasted children in the study area (Paper III)



S2 Fig. Study profile of non-wasted children in Adami Tullu District in south-central Ethiopia, 2014 -2016. ¥: Newly added include newborn children aged 6 months and above during the survey and newcomers

Appendix I: Questionnaires

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									

MalTrials Project: Weekly malaria data collection format

Kebele_____ Gare_____ House number_____		Date of visit[_____|_____|_____] dd | mm| yyyy	
Data collector		Name _____ Signature _____	
Q01	Did anyone sleep under the bed net last night?	Yes……1 No..……...2 Not sure……3 The household doesn't own net…..4	→ **Skip to Q03** **Skip to Q04**
Q02	Who slept under the bed net last night? (*List the names*)	**1.**_____ **6.**_____ **2.**_____ **7.**_____ **3.**_____ **8.**_____ **4.**_____ **9.**_____ **5.** _____ **10.**_____	
Q03	Presence of fever any time in the last 48 hours	Yes……1 No…….2	If 2 → End

Q04*	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)***	Name_____ Age _____Years/Months Sex Male……1 Female…2 **To be filled in the laboratory** P. falciparum..……..1 P. vivax…….……2 Mixed infection…..…3 Negative ……...4 Other hemoparasite...5 (specify_____) Parasite density _____ Morphology _____	Name_____ Age _____Years/Months Sex Male……1 Female…2 **To be filled in the laboratory** P. falciparum..……..1 P. vivax…….……2 Mixed infection…..…3 Negative ……...4 Other hemoparasite...5 (specify_____) Parasite density _____ Morphology	Name_____ Age _____Years/Months Sex Male……1 Female…2 **To be filled in the laboratory** P. falciparum..……..1 P. vivax…….……2 Mixed infection…..…3 Negative ……...4 Other hemoparasite...5 (specify_____) Parasite density _____ Morphology

* *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***.

Re-enumeration of residents of Adami Tullu district after one year of follow-up

Census conducting week: _____ HH Number: _____

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

(A)
1. yes
2. no

(B)
1. Left the kebele
2. Left the HH

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

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

Sex
1. Male
2. Female

Relationship
1. Father, 2. Mother,
3. Child, 4. Relative,
5. Maid, 6. other

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

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

MalTrials Project:-Data collection format to assess prevalence of anemia among children 6 to 59 months old

kebele _ **Date of collection** _____

Name & sign of data collector_____ **Supervisor sign**_____

HH No.	Name of Gare	Child id	Child Name	sex	Age (month)	Hemoglobin in g/dl	Remark

MalTrials Project:-Data collection format to assess prevalence of under nutrition among children 6 to 59 months old

Date of data collection _____

kebele _____

Supervisor

Name & sign of data collector_____ **sign**_____

HH No.	Name of Gare	Child id	Child Name	Age in months	sex	Wt (Kg)	Ht (cm)

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

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)

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Appendix III: MalTrials Protocol

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Trials

STUDY PROTOCOL Open Access

Combining long-lasting insecticidal nets and indoor residual spraying for malaria prevention in Ethiopia: study protocol for a cluster randomized controlled trial

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Abstract

Background: Long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are the main malaria prevention interventions in Ethiopia. There is conflicting evidence that the combined application of both interventions is better than either LLINs or IRS used alone. This trial aims to investigate whether the combination of LLINs (PermaNet 2.0, Vestergaard Frandsen, Lausanne, Switzerland) with IRS using propoxur will enhance the protective benefits and cost-effectiveness of the interventions against malaria and its effect on mosquito behavior, as compared to each intervention alone.

Methods/Design: This 2 x 2 factorial cluster randomized controlled trial is being carried out in the Adami Tullu district in south-central Ethiopia for about 116 weeks from September 2014 to December 2016. The trial is based on four arms: LLINs + IRS, LLINs alone, IRS alone and control. Villages (or clusters) will be the unit of randomization. The sample size includes 44 clusters per arm, with each cluster comprised of approximately 35 households (about 175 people). Prior to intervention, all households in the LLINs + IRS and LLINs alone arms will be provided with LLINs free of charge. Households in the LLINs + IRS and IRS alone arms will be sprayed with carbamate propoxur once a year just before the main malaria transmission season throughout the investigation. The primary outcome of this trial will be a malaria incidence based on the results of the rapid diagnostic tests in patients with a fever or history of fever attending health posts by passive case detection. Community-based surveys will be conducted each year to assess anemia among children 5–59 months old. In addition, community-based malaria prevalence surveys will be conducted each year on a representative sample of households during the main transmission season. The cost-effectiveness of the interventions and entomological studies will be simultaneously conducted. Analysis will be based on an intention-to-treat principle.

Discussion: This trial aims to provide evidence on the combined use of LLINs and IRS for malaria prevention by answering the following research questions: Can the combined use of LLINs and IRS significantly reduce the incidence of malaria compared with the use of either LLINs or IRS alone? And is the reduced incidence justifiable compared to the added costs? Will the combined use of LLINs and IRS reduce vector density, infection, longevity and the entomological inoculation rate? These data are crucial in order to maximize the impact of vector control interventions on the morbidity and mortality of malaria.

Trial registration: PACTR201411000882128 (8 September 2014).

Keywords: Cluster randomized controlled trial, Cost-effectiveness, Incidence, Indoor residual spraying, Long-lasting insecticidal nets, Malaria, Prevention and control, Ethiopia

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Background

Despite remarkable achievements in the fight against malaria over the last decade, there is still an unacceptably high level of malaria burden worldwide. In 2013, there were an estimated 198 million cases and 584,000 deaths, of which 80 % of the cases and 90 % of the deaths occurred in sub-Saharan Africa (SSA) [1]. The World Health Organization (WHO) recommends the universal coverage of the population at risk with long-lasting insecticidal nets (LLINs) [2] and targeted indoor residual spraying (IRS) with insecticide [3] for the control and ultimate elimination of malaria. In addition, IRS has been recommended to be scaled-up for malaria control across the different malaria endemicities including high transmission settings in SSA [4]. This has brought a significant shift from past practices in which IRS was limited for the prevention and control of malaria epidemics, particularly in unstable and seasonal malaria settings [5, 6].

Both LLINs and IRS have been shown to be effective in reducing malaria transmission when applied independently [7–9]. As a result, both interventions are widely applied for malaria prevention in many countries [10]. Of the 45 countries in Africa with ongoing malaria transmission, 38 adopted both the WHO's policy of universal coverage with LLINs to populations at risk and IRS with insecticide [1]. In an effort to accelerate the control and ultimate elimination of malaria, IRS in combination with LLINs has also been deployed in the same geographical areas in 31 African countries [1]. The available evidence suggests that the joint intervention of the LLINs and IRS should be scaled up and that the combined effect of these interventions should be further evaluated [11–13].

Despite an increasing interest in the simultaneous use of both interventions, there are currently no clear guidelines on how these interventions should be combined [14]. At the same time, there is also a paucity of evidence as to whether their combined use is more effective in reducing the incidence of malaria than using either intervention alone [8, 15–17]. It is poorly understood how the interventions interact to improve malaria control. A few non-randomized observational studies and mathematical modelling exercises suggest a modest effectiveness, or conflicting results, when combining interventions for malaria reduction compared to either intervention alone [11, 12, 15, 17, 18].

Evidence on the effect of the combined use of LLINs and IRS from community-based field trials is conflicting. Consequently, it is difficult to draw any conclusions on whether the combination of IRS and LLINs is beneficial against malaria compared to one of the interventions alone. A recent review indicated that only one of the four published randomized controlled trials showed additional protection against fighting malaria when the use of LLINs was combined with IRS, compared to either method alone [14]. A multi-intervention trial in Benin showed no significant reduction in clinical malaria in children under 5 years of age from houses sprayed with bendiocarb in combination with LLINs, compared to children in houses with LLINs alone [19]. Similarly, in The Gambia a combination of IRS using dichlorodiphenyl-trichloroethane (DDT) and universal coverage of LLINs showed no added protection against malaria among children of 6 months to 14 years compared to the universal coverage of LLINs alone [20]. By contrast, a recent cluster randomized controlled trial in Tanzania, where the usage of LLINs was less than 50 %, reported some evidence of added protection against malaria infection in children 6 months to 14 years from the combining of insecticide-treated nets (ITNs) and IRS with bendiocarb compared to ITNs alone [13]. Lines and Kleinschmidt [21] recently discussed the design issues in conducting studies involving the combination of malaria vector control interventions and recommended further evidence from well-designed trials.

Good evidence on the effectiveness, costs and cost-effectiveness of malaria interventions will provide important information for decision-making in policy formulation, the revision of existing policy and/or the selection of optimal packages of interventions. Since the cost of both LLINs and IRS is greater than the cost of either intervention alone [22], it is important to estimate and evaluate whether the potential extra protection gained by combining both interventions represents good value compared with the added costs. This is particularly important in countries in SSA, where a scarcity of resources is the main impediment to malaria control. A recent review of the evidence of the costs and consequences of large-scale vector control for malaria concluded that both LLINs and IRS are highly cost-effective vector control strategies even though the former method has been identified as more cost-effective than the latter [22].

Malaria is a major public health problem in Ethiopia with approximately 65 % of its 90 million population living in areas at risk of malaria infection [23]. Mainly due to altitudinal and climatic features in most parts of the country, malaria transmission is seasonal and epidemic [24, 25]. The most important malarial parasites in the country are *Plasmodium falciparum* (60 %) and *P. vivax* (40 %) [23]. *Anopheles arabiensis* is considered the main malaria vector in the country, with *An. pharoensis* being a secondary vector.

The National Strategic Plan for malaria prevention and control in Ethiopia aims at scaling up and sustaining both LLINs and IRS interventions in malaria endemic areas [26, 27]. LLINs and IRS are applied either separately or in

combination, and during the period from 2005–2011, more than 43 million LLINs were freely distributed to all households in malarious areas [28]. The Malaria Indicator Surveys (MIS), conducted in 2007 and 2011, revealed that 65 % and 55 % of surveyed households had at least one LLIN, respectively, whereas the reported use by children under 5 years of age, during the night prior to the survey, within households with at least one net ranged from 60 % in 2007 to 65 % in 2011 [29, 30].

In Ethiopia, the increased resistance of *P. falciparum* to chloroquine and sulfadoxine-pyrimethamine necessitated a change as the first-line antimalarial drug for the treatment of *P. falciparum* [31–33]. Consequently, artemether-lumefantrine (AL, Coartem®, Novartis, Basel, Switzerland) has been used as a first-line treatment for uncomplicated *P. falciparum* infection since 2004 [31]. Several studies have shown that AL remains highly efficacious against the treatment of uncomplicated falciparum malaria and with no report of adverse effects [34–37].

Several studies from Ethiopia have shown a high insecticide resistance in malaria mosquitoes, especially in relation to DDT, malathion, permethrin and deltamethrin [38–42]. DDT was the primary insecticide of choice for IRS in the country for a long time until it was replaced by deltamethrin in 2009. Unfortunately, resistance to deltamethrin was reported to be very high [38]. As a result, the National Malaria Control Program has adopted the use of bendiocarb and propoxur insecticides belonging to the carbamate family since 2012 [27, 43]. Currently, bendiocarb and propoxur are the primary insecticides of choice for IRS in Ethiopia. Bendiocarb and propoxur are carbamate insecticides evaluated and approved by the WHO Pesticide Evaluation Scheme, both of which have the potential to control pyrethroid-resistant mosquitoes [44].

This study aims to investigate the effect of combining LLINs and IRS with propoxur for the incidence of clinical malaria and the cost-effectiveness of the interventions against malaria and their effect on mosquito behavior, as compared to each intervention alone. The overall aim is to provide information for national and global policymakers in their pursuit of improving malaria control by evaluating resource demands and the combined effect of both interventions on malaria. This protocol discusses the rationale for the choice of the interventions and describes the designs and methodological approaches being used to determine the effect of each intervention, in addition to evaluating the effect of the interventions.

A 2 x 2 factorial cluster randomized controlled trial was chosen to exploit the robustness of this design to help ascertain the efficacy of the combined interventions compared to either interventions alone and the standard routine practice. This protocol was developed according to the guidelines of the Consolidated Standards of Reporting Trials (CONSORT) statement extension for cluster randomized trials [45]. The scientific value of the inclusion of the control group in this trial was also extensively debated, as it was believed that this arm allows a more reliable comparison between the groups: LLINs + IRS versus LLINs alone or control, LLINs + IRS versus IRS alone and LLINs alone versus IRS alone or control. This is the most robust design in regard to cluster randomized trials to help ascertain the efficacy of the interventions. This study design is also believed to make the results more generalizable and applicable in resource-constrained countries.

Trial objectives

Primary objective

The primary objective of this intervention study is to determine whether the combined use of LLINs and IRS with propoxur provides additional protection against malaria (*P. falciparum* and/or *P. vivax*) among all age groups in the study area compared to LLINs or IRS alone.

Secondary objectives

In the same study population, the secondary objectives of the trial are to:

1. Estimate the costs of LLINs + IRS, LLINs or IRS alone compared to the current routine practice, and to evaluate the incremental costs, effects and cost-effectiveness of interventions
2. Assess whether LLINs + IRS reduce entomological parameters, i.e., human biting rates, mosquito resting density, longevity, sporozoite rates, and the entomological inoculation rate (EIR) inside houses compared with LLINs or IRS alone
3. Determine whether LLINs + IRS improves the hemoglobin (Hb) concentration and reduces anemia among children under 5 years of age compared with children in LLINs or IRS alone

Methods/Design

Study setting

This study is being carried out in the Adami Tullu part of the Adami Tullu-Jiddo-Kombolcha *woreda* (hereafter referred to as the Adami Tullu district) in the East Shewa Zone of the Oromia Regional State in Ethiopia. The *woreda* (or district) is a local administrative unit in the country, followed by *kebeles* (the lowest government administrative unit, which is further divided into *gares*, or villages). The capital of the district, Zeway (or Batu), has a latitude and longitude of 7°56′N 38°42′E with an elevation of 1640 m above sea level. It is located approximately 160 km south of Addis Ababa along the highway

connecting Addis Ababa to Nairobi via Hawassa. The district is set in the Great Rift Valley in south-central Ethiopia, with altitudes ranging from 1500 m to 2300 m. Administratively, the Adami Tullu district has 48 *kebeles*, each with an average population size of approximately 1000 to 5000 people. Figure 1 shows the geographical location of the study district and the description of the study arms in relation to Lake Zeway. The total annual rainfall is approximately 700 mm, with peaks during the main rainy season in July (250 mm) and August (220 mm). The mean minimum and maximum annual temperatures are 14.5 °C and 27.7 °C, respectively.

Based on the 2007 National Census [46], the projected population size of the district for 2014 was about 173,000 people and the population of Zeway town about 60,000. The main ethnic group is the Oromo, and the predominant religion is Islam. The majority of the population live in rural areas in houses made with mud or cement walls and thatched or iron roofs. Local residents primarily depend on farming, livestock rearing, and to a lesser extent on fishing in Lake Zeway for their subsistence. In 2014, there were one public and one non-governmental organization hospital, nine public health centers and 43 health posts in the district. The health centers are primarily staffed by health officers, nurses, midwives, pharmacists and laboratory technicians. Each *kebele* is intended to have at least one health post staffed by two health extension workers (HEWs) reporting to the health center.

Malaria is a leading health problem in the district. Transmission is seasonal and unstable, with several recorded epidemics of varying degrees [47, 48]. The main malaria transmission season occurs between September and December each year following the heavy rainfall between July and August, whereas the smaller peak occurs during May and June each year following small rains during March and April. Lake Zeway, which has many swampy areas, profoundly contributes to mosquito breeding in the study setting. *P. falciparum* and *P. vivax* co-exist in the area in varied proportions [47, 49]. A longitudinal community-based study carried out in 1994 revealed a malaria prevalence of 6.8 % (66 % *P. falciparum*, 31 % *P. vivax* and 3 % *P. malariae*) from July to December, peaking in September at 12.6 % [50]. Community-based cross-sectional surveys conducted in October to November 2006 and April 2007 indicated an overall parasite prevalence of 4.8 %, varying between localities from 1.7 % to 10.4 %, with 88 % *P. vivax* and 12 % *P.*

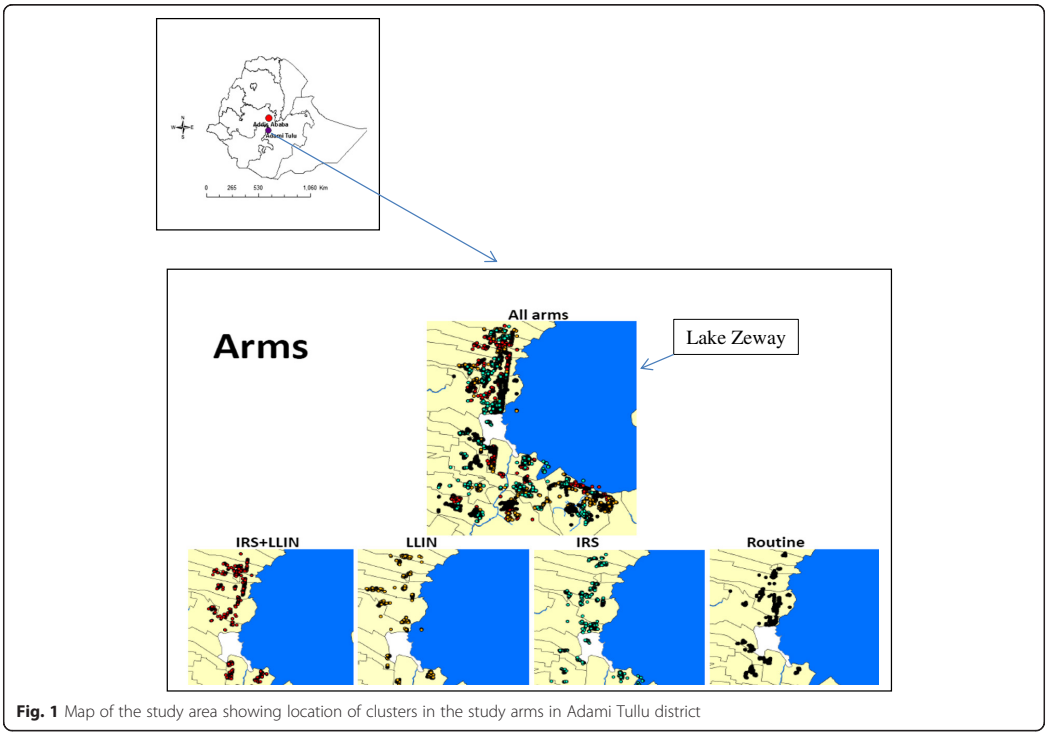

Fig. 1 Map of the study area showing location of clusters in the study arms in Adami Tullu district

falciparum parasite species composition [49]. Artemether-lumefantrine (AL, Coartem®, Novartis, Basel, Switzerland) and chloroquine are the first-line antimalarial drugs for the treatment of uncomplicated falciparum and vivax malaria, respectively. Both LLINs and IRS are the two major malaria preventive interventions implemented by the District Health Office (DHO).

An. arabiensis is the major malaria vector in the district and *An. pharoensis* is considered to have an auxiliary role [50]. An entomological study in the past showed that the former species prevailed from June to October with peak densities from July to September, while the latter species peak months were from September to November [50]. Preliminary insecticide susceptibility tests showed that *An. arabiensis* was greatly resistant to deltamethrin (mortality 14 %), alphacypermethrin (less than 1 % mortality), lambdacyhalothrin (4 % mortality) and permethrin (17 % mortality), but susceptible to bendiocarb and propoxur (mortality 100 %). *An. pharoensis* was fully susceptible to all the aforementioned insecticides with a 100 % mortality (*MalTrials* unpublished pilot data). Adami Tullu district has been one of the sentinel sites for the study of malaria epidemiology and entomology in Ethiopia due to its relatively higher malaria burden [33, 49–52].

Design

This 2 x 2 factorial cluster randomized controlled trial, called *MalTrials*, will be carried out for approximately 116 weeks from September 2014 to December 2016. The village (or cluster) will be the unit of randomization, and an equal number of villages will be randomized to one of the four arms: (1) LLINs + IRS, (2) LLINs alone, (3) IRS alone or (4) control (routine practice). The control arm will receive the routine standard practice of malaria prevention of the Ethiopian Malaria Control Program.

Participants

This trial is only being conducted in the rural communities of the district. The reason for focusing on rural communities is due to the prioritization of IRS for malaria prevention in these areas. Prior to implementing intervention and randomizing villages to arms, a census, mapping, and pilot studies were carried out to estimate an optimum sample size (Fig. 2).

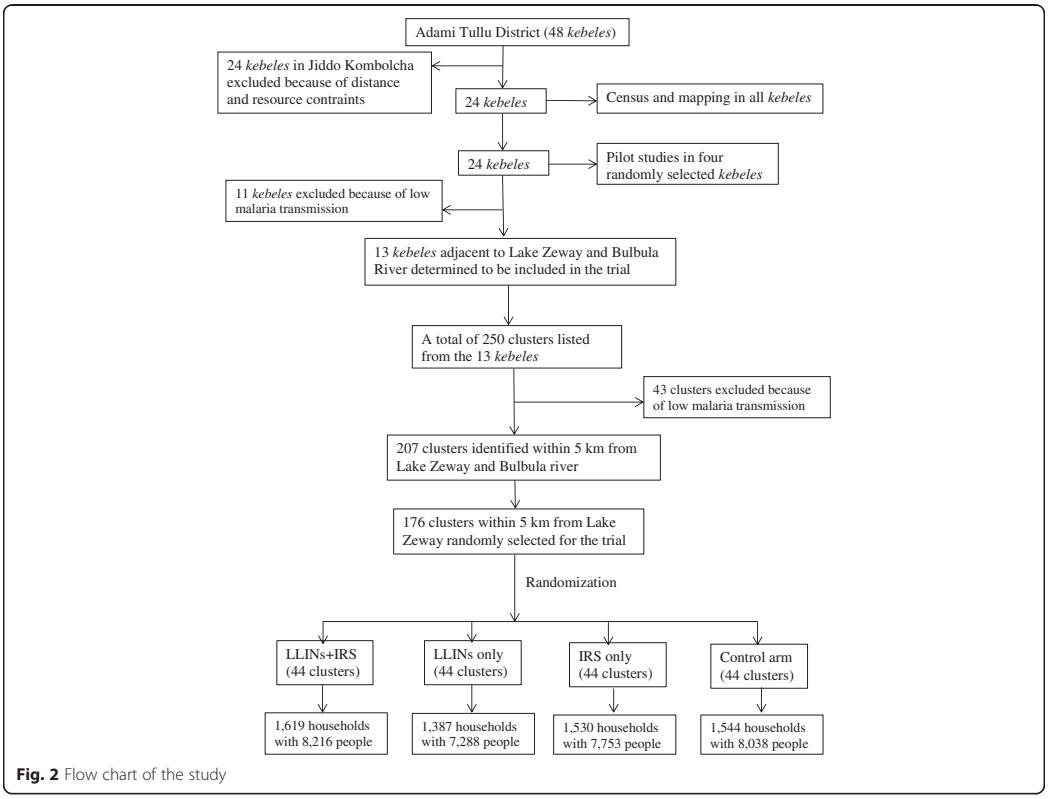

Fig. 2 Flow chart of the study

Village inclusion criteria

Villages with a relatively easy access, relatively higher malaria transmission and located within 5 km from Lake Zeway will be included in the study. The preliminary findings indicated that the incidence of malaria was 8 cases per 10,000 person-weeks of observation for villages within 5 km from Lake Zeway, compared to villages beyond 5 km from the lake where the incidence rate was 0.5 cases per 10,000 person-weeks of observation (*MalTrials* unpublished pilot data).

Village exclusion criteria

Villages with difficult access, a very low malaria transmission, and located beyond 5 km from Lake Zeway will be excluded.

Participant inclusion criteria

All consenting residents of households in all clusters will be recruited for the study.

Participant exclusion criteria

Residents and household heads who are not able to provide informed consent will be ineligible to take part in the trial.

Randomization

From a total of 48 rural *kebeles* in the Adami Tullu district, 13 *kebeles* with a relatively higher malaria transmission adjacent to Lake Zeway were included in the study following a census carried out in 24 *kebeles* and pilot studies in four *kebeles*. From the total list of the clusters in 13 *kebeles*, 207 were located within 5 km of Lake Zeway, had a relatively higher malaria transmission, and were included in the sampling frame, of which 176 were randomly selected. The randomly selected clusters were numbered and equally randomized into the four arms following a computer-generated list using SPSS software, with a flow chart of the study given in Fig. 2. While the study is done in Ethiopia, randomization was done in Bergen in Norway. This was done to prevent selection bias by concealing the allocation sequence from the field researchers assigning villages to the four intervention groups until the moment of assignment. Thus, a researcher not involved in the study randomly allocated a random number from a random number table that was used as the seed for the computer-generated list of villages using SPSS software. The random selection of households for the entomological sampling was done in a similar way.

Due to the nature of the interventions, blinding of the study participants will not be possible, while observer bias will be reduced wherever possible. Microscopists shall read blood films blinded to the identity and intervention status of the subjects. By using standard light traps and exit traps, we shall reduce the mosquito collector bias. Moreover, the entomologists in our research group will examine the trap catches, which are different from the trap collectors.

Interventions

Long-lasting insecticidal nets (LLINs)

The LLINs distributed for this trial were PermaNet 2.0 rectangular, 100 denier, light blue, family size (160 cm width x 180 cm length x 150 cm height) purchased in June 2014 from the Vestergaard Frandsen Group SA (Vestergaard Frandsen, Lausanne, Switzerland). The PermaNet 2.0 net is a factory-treated mosquito net manufactured with deltamethrin, which is expected to retain its biological efficacy for a minimum of 20 standard WHO washes or approximately 3 years under field conditions [53].

The *MalTrials* project bought a total of 10,000 PermaNet 2.0 LLINs for this trial. The nets were imported and arrived in the study site on 16 September 2014, and distributed to study households from 1 to 5 October 2014. All households in the LLINs + IRS and LLINs alone arms received new LLINs free of charge at the beginning of the intervention regardless of the previous ownership, with householders maintaining their existing nets at the time of distribution. The number of new LLINs distributed to each household was based on the household size recommended by the national malaria guidelines [43], i.e., one net for a family of 1–2, two nets for a family of 3–5, three nets for a family of 6–7 and four nets for a family of 8 or more people.

In advance of the LLINs distribution, all village residents were made aware of the distribution of the nets through house-to-house visits, village leaders and community elders. The net distribution was done in the center of the village based on a pre-determined registration list of households in each village by the *MalTrials* project personnel in collaboration with the district and village health workers. Households who did not receive the nets during the distribution schedule were identified and received their nets later. Education about, and a demonstration of how to use, LLINs were given to the recipients by trained field staff and selected village residents.

With an average of 2.57 nets per household, a total of 3006 households (1599 households in LLINs + IRS and 1407 households in LLINs only) in both arms of the trial received 7740 LLINs (4157 nets in LLINs + IRS and 3583 nets in LLINs only). The remaining nets were stored at an ambient temperature under safe storage conditions to be used for net replacement before the peak malaria transmission season each year (in August). Net use and retention at the household level are being monitored during the weekly household visit.

Indoor residual spraying (IRS)

Indoor residual spraying with propoxur will be carried out at three times during the study period in the LLINs + IRS and IRS alone arms. Spraying will be done once a year prior to the peak transmission season at the beginning, the middle and before the end of the trial, following the national spraying operation guidelines [43] and WHO operation manual guidelines [54]. Propoxur (isopropoxy-phenyl methylcarbamate) is highly effective against mosquito vectors for more than 3 months at a dosage of 2 g/m^2 in the form of a water-dispersible powder (WP) [54], and will be acquired from the state-owned Adami Tullu Pesticide Processing Share Company located in the study district. Propoxur 50 % WP contains 2 g of active ingredient and is packaged in 400 g sachets, and two sachets will be mixed with 8 L of water. Preliminary findings using the WHO susceptibility tube tests in 2013 showed a 100 % susceptibility of *An. arabiensis* and *An. pharoensis* to propoxur (*MalTrials* unpublished pilot data).

We will use the average surface area measurement per unit structure (110 m^2) to be sprayed by a spray man per day to calculate the correct amount of insecticide required for the spraying. All IRS operations will be carried out in collaboration with the DHO as per the recommendations of the national guidelines [43]. A 6-day training on spraying operation will also be given for locally recruited spray men and supervisors. The spraying teams will be organized by squads of four spray personnel and a porter, and supervised by a squad leader. The DHO malaria focal persons and HEWs will be used to organize, follow-up, and supervise the daily activity of spray teams, and a spray equipment and personnel protective clothing will be obtained from the DHO.

Approximately 12 houses will be sprayed by each spray operator per day using an 8-liter Hudson X-pert (HD Hudson Manufacturing Company, Chicago, IL, USA). Prior to spraying, a community sensitization will be performed to inform residents in regard to the safety, purpose and time of spraying. On the day of the IRS operation, all targeted households will be informed about the schedule, purpose and requirements of the spraying. The householders will be requested to prepare and vacate the house before spraying, and household items such as water, food and cooking utensils will be removed from the house. Household members will be allowed to enter the sprayed house after 30 minutes, and be requested to clean the floor and bury or burn the dirt. The householders will also be requested not to wash, paint or re-plaster the sprayed walls until at least the end of the main transmission season.

Study endpoints

The primary health outcome measure is malaria incidence determined by the detection of *P. falciparum* or *P. vivax* by rapid diagnostic tests (RDTs) in patients with a fever or having a history of fever within the previous 48 hours upon arrival at health posts by passive case detection (PCD). The sensitivity and specificity of the RDTs compared with the results of the light microscopy will be determined. The secondary health outcome measure is mean Hb concentration in children under the age of 5 years, which is measured using a portable photometer (HaemoCue®, Ångelholm, Sweden) at the end of each transmission season through community-based house-to-house visits.

The primary outcome measure of the economic evaluation is the total cost of the interventions. The secondary outcome measures are the direct and indirect costs of the intervention from both the provider and societal perspectives, which includes Disability- adjusted Life Years (DALYs) and the number of malaria cases averted by each intervention.

The primary entomological outcome measure is malaria transmission expressed as EIR, estimated as the mean number of sporozoite infective bites/person/year. The secondary entomological outcome measures are the mean number of *An. arabiensis*/light trap/night, and mean number of *An. arabiensis* in spray catches/night inside houses.

Sample size

Malaria incidence and anemia prevalence

Sample sizes were calculated based on unpublished epidemiological data collected in a baseline pilot study in villages adjacent to Lake Zeway during September to December 2013. The sample size for the primary endpoint, the incidence of malaria, was calculated using methods for cluster randomized trials [55] that take into account the intra-cluster correlation coefficient (ICC), incidence rate, the expected effect and the power of the study. Using a baseline malaria incidence rate of 7.85 per 10,000 person-weeks and the coefficient of variation between clusters within each group, we used $k = 0.27$ in the sample size estimation (*MalTrials* unpublished pilot data). Thirty-five households (approximately 175 people) per cluster will be followed up for 116 weeks, with 44 clusters achieving a 90 % power to detect a 25 % reduction in the malaria incidence rate in the LLINs + IRS arm compared to LLINs alone or the IRS only arm, using a two-sided 5 % significance level. We plan to follow approximately 1540 households with an estimated 7800 people in each arm of the trial (Fig. 2). Overall, the trial will cover over 31,000 people from approximately 6100 households. The proposed sample size is also assumed to suffice in terms of

having the power to detect a mean reduction between the study arms of 0.5 mg/ml Hb concentration in children under 5 years of age.

Economic evaluation

The sample size calculated for malaria incidence is also assumed to suffice for the economic evaluation of the cost of interventions. The sample size for the cost of illness study (at the patient level) is calculated using a single population mean formula. Hence, 260 participants will be enrolled into the study from the passively detected malaria patients using a consecutive sampling technique.

Entomological study

We aim to calculate the sample size for the entomological collections based on the pilot entomological studies. In a West African study with a 90 % power and 5 % significance level, it was advised to sample eight houses from each of 15 clusters of the study arms, but the mosquito density was lower [56]. A total of 16 villages (four per arm) will be randomly selected for entomological study, in which indoor host-seeking mosquitoes will be collected by CDC light traps from four houses per arm, indoor resting mosquitoes from 16 houses per arm using pyrethrum spray collection and outdoor resting mosquitoes from four artificial pit shelters per arm of the study.

Data collection methods

Each household will receive a specific identification (ID) number tagged onto a colored metal plate placed on the upper front door of the house. Each inhabitant in the household will also receive a unique personal, three-digit ID number (village number/household number/person number). The latitude and longitude of each household will be recorded using a hand-held Global Positioning System (GPS) device. The GPS data will be downloaded to a computer for the creation of maps of the study villages and for a spatial analysis of epidemiological, entomological and economic data. Guidelines for the data collections will be developed during the pilot studies. All questionnaires and forms will be initially prepared in English and translated into a local language for data collection.

Epidemiological data collections

PCD of malaria cases at the health posts will be carried out throughout the trial using RDTs, and thin and thick blood smears for microscopic examination. We will recruit 24 data collectors (two data collectors per *kebele*) for a weekly household visit, 14 nurses (one nurse per *kebele*) for malaria diagnosis and treatment at the health posts and three field supervisors. Data collectors will be trained on questionnaire specifics, interviewing techniques and household visits. They will visit households weekly to help identify residents with fever, or a history of fever in the last 48 hours, to refer to the health posts and track information on LLINs' use by household members during the night before the survey.

The research team and supervisors will make regular field visits for quality control of the work done at health posts. Each field supervisor will be responsible for 59 clusters, and will meet with data collectors and health workers at the health posts at least once a week. During these visits supervisors will check patient registration books, malaria surveillance forms and the availability of antimalarial commodities, and collect completed forms and blood slides and bring them back to the project's office. Throughout the study period, all health posts that participate in the trial will be provided with multispecies and mixed infection-detecting RDTs, artemether-lumefantrine (AL) and chloroquine obtained from the Oromia Regional Health Bureau (ORHB). The RDT that will be provided is the CareStart® Malaria *Pf/Pv* combo test (Access Bio, Inc., Somerset, NJ, USA), which is individually packaged with an alcohol swab, lancet, capillary tube, buffer and test device.

Through weekly household visits, study participants with a fever or having a history of fever within the past 48 hours, will be encouraged to present to the health posts. Residents will be advised to visit the health posts whenever they develop a fever. They will be asked to show a household card whenever visiting a health post. For individuals without a household card, health workers will use other information that allows them to locate the household and its number. Individuals who are found to be positive for *P. falciparum* by RDT will be given AL twice a day for 3 days based on body weight according to national guidelines [31]. AL is a fixed dose combination of 20 mg artemether plus 120 mg of lumefantrine. *P. vivax* positive individuals will be treated with chloroquine, 25 mg/kg for 3 days (10 mg base per kg on days 1 and 2, and 5 mg base per kg on day 3). Treatment of other conditions will be done in accordance with the national guidelines or a referral to higher level health facilities. Patients with severe illness at the time of visit (from malaria or other causes) will be referred to the nearest health facility. Any person from the study villages treated for malaria at a health center or hospital will also be included in the study.

Thin films of blood slides will be fixed with methanol for 30 seconds. Both thin and thick films will be stained with 3 % Giemsa for 20–30 minutes by experienced laboratory technicians in accordance with the standard malaria laboratory procedures. A thick blood smear will be declared negative with a minimum of 100 high power fields by microscopic examination, while the thin film will be examined to identify the *Plasmodium* species for

positive slides. A second reading for all positive and negative slides will be performed by an experienced laboratory technologist and any discordant results will be resolved by a third reader. All blood slide readers will be blinded to the different arms of the trial and to the diagnosis of preceding readers.

At the end of the main malaria transmission season each year, the Hb concentration will be assessed in children 6–59 months old for assessing the prevalence of anemia using a portable photometer (HaemoCue®, Ångelholm, Sweden) in the field. Through house-to-house visits, a single finger-prick sample will be taken from each child, and children's height and weight will also be measured. A Hb cut-off point of less than 11.0 g/dl will be used to decide whether a child has anemia, and will be further classified into mild, moderate and severe anemia.

Community-based malaria prevalence surveys will be conducted on a representative sample of households from each arm of the trial during the main transmission season (September to November) each year among all age groups. All household members will be eligible to be included in the study. Allowing for ineligible households, absence on the day of the survey and refusals at the household and individual level, approximately 5500 individuals from 1100 households will be estimated to be included in the study. This would provide an average 275 households from each arm of the trial to be included in the study. The households will be randomly selected from the total list of households in all arms of the trial. The heads of the households or their representatives will be interviewed using a pre-tested structured questionnaire. Voluntary individuals will be tested for malaria parasites using RDTs. Moreover, blood slides will also be collected for microscopic examination.

Economic evaluation data collections

Costing will be done based on the cost of the interventions from a health systems perspective, with cost data collected by interviewing individual malaria patients, family members and the DHO personnel. The main cost outcome measure will be the total costs of interventions, including all resources used to deliver the interventions and recurrent costs such as personnel, supplies and materials, the operation and maintenance of buildings, utilities and communication costs, in addition to capital costs such as buildings, equipment, and vehicles. The costing of the intervention will be done from the provider's perspective using a standard malaria costing tool developed by the WHO [57]. The economic evaluation of health outcomes will be evaluated by the number of malaria cases averted and DALYs gained as a result of the interventions using incidence data. The number of deaths averted due to the interventions will be calculated based on the case fatality rates of malaria.

Economic evaluation, cost-effectiveness/utility analysis, presenting cost per malaria case prevented and costs per DALY averted will all be employed. The DALYs will incorporate both morbidity estimates from malaria episodes and anemia among children. We will develop a Markov life cycle model to account for the recurrent nature of malaria disease, with a varying amount of risk for repeated episodes during different seasons of the year. The cost of illness study will be conducted in the same district from non-trial villages to account for saved disease treatment costs. Epidemiological and effectiveness parameters will be based on the findings of this research project, while the clinical course of the diseases will be based on a review of the best available literature. Standard DALY weights will be utilized. Moreover, this trial will incorporate an evaluation of the distributional impact of the interventions using inequality analysis techniques and a decomposition of the inequality into various socioeconomic factors using an extended cost-effectiveness analysis (ECEA). In the ECEA, we will estimate the benefit of the interventions across different levels of income group in terms of the number of deaths prevented, the net financial risk protection provided, the out-of-pocket expenditure averted and the number of poverty cases prevented.

Entomological data collections

The results of trials from countries where *An. gambiae* s.s. is the dominant vector might not be fully applicable in countries such as Ethiopia, where *An. arabiensis* is the main vector. The latter vector is less affected by mosquito nets, and is more exophilic and less anthropophilic [58]. Estimating the human biting rate (HBR) of mosquitoes is important for a risk assessment of malaria transmission. The Centers for Disease Control and Prevention (CDC) light traps, used in many studies, are only a proxy measure to estimate HBR. Human landing catches (HLC) are a more direct way to estimate HBR, and are often considered the "gold standard" [59]. Recent research showed that light trap collections placed close to inhabited mosquito nets may not be a reliable method of assessing human biting rates [60].

The CDC light traps and HLC will be compared before starting the trial. Since HLC cannot be used for community-wide mosquito collections due to ethical and logistical drawbacks, CDC light trap collections will be calibrated with HLC locally in order to estimate an operational conversion factor for calculating the HBR and EIRs. Mosquito collections will be carried out during the malaria transmission season following interventions.

The CDC light traps, pyrethrum spray sheet catches and artificial pit shelters will be employed to collect mosquitoes and assess their biting behavior, indoor and outdoor resting densities. Houses will be randomly selected from a computer-generated list and the person will be blinded to the arms and houses. For CDC light traps and pyrethrum spray catches, the number of houses will be four and 16 from each arm, respectively, while four pit shelters in each arm will be constructed. Mosquitoes will be collected weekly from August to November each year, and will be identified to species using a morphological key [61]. Previous studies indicated the presence of *An. arabiensis* as the only species of the *An. gambiae* complex in the study area [50]; however, for reconfirmation purpose a species-specific polymerase chain reaction (PCR) will be applied [62].

From all collections, blood-engorged female mosquitoes will be analyzed using Enzyme- linked Immunosorbent Assays (ELISAs) for determining the blood meal source of vectors in indoor or outdoor situations [63]. All collections of female anophelines, with the exception of blood-fed mosquitoes, will be subjected to a sporozoite test by employing ELISA [64] and a parity determination by ovary dissection [65].

Insecticide resistance in *An. arabiensis* and *An. pharoensis* will be monitored annually throughout the study period using standard WHO tube tests [66]. The insecticides will be the pyrethroids (deltamethrin, alphacypermethrin, permethrin and lambdacyhalothrin) and the carbamates (bendiocarb and propoxur). In order to assess any change in resistance, the resistance intensity will be quantified. For the insecticide susceptibility tests, larvae and pupae of the two species will be collected from breeding habitats and reared to adults. The status of physiological susceptibility/resistance of females will then be determined. Molecular [67] and biochemical analyses [68] will be used to identify potential insecticide resistance mechanisms. Sporozoite rates will be determined by ELISA and rechecked by real-time PCR [69]. The decay rate of propoxur will also be assessed monthly by conducting cone wall bioassays on eight randomly selected houses from the two arms (IRS and LLINs + IRS) [70] for a period of at least 6 months post spraying. An insectary colony of *An. arabiensis* (Debre Zeit strain, being maintained at the Aklilu Lemma Institute of Pathobiology, Addis Ababa University since 2001), which is susceptible to all insecticides including propoxur, will be used for the bioassay test.

Data management

Data will be collected using standardized paper-based forms and questionnaires according to standardized operating procedures. Data will be entered into a computer by trained data entry clerks, we will verify data by range and consistency checks, and data cleaning will be done weekly. Any discrepancies will be corrected by cross-checking against the corresponding original forms and subsequently amended in the final dataset.

All blood slides will be labelled with the patient's unique ID number and date of collection to help ensure anonymity. To minimize any loss to follow-up, we will keep following up all residents and maintain their database, even if they move out of the trial area or move from one cluster to another cluster with a different intervention. For residents or respondents who are not present at the time of the visit by project staff, basic information about dates and reasons for absence will be sought from other community members such as friends or neighbors. The epidemiological data, economic evaluation data and entomological data will all be kept separately. All databases will be password protected and only accessed by research team and data entry clerks for data entry, cleaning and analysis. Furthermore, data will be stored for at least 5 years and be made publically available.

Each of the principal investigators of the epidemiological, economic evaluation and entomological studies will maintain records in compliance with Good Clinical Practice (GCP) for regulatory or institutional requirements. Authorized representatives from the funding agency, ethical committees or regulatory bodies may inspect all documents and records of the trial. The research team will explain any deviation from the originally approved protocol. Moreover, any deviation from the protocol that will have an impact on the conduct of the study will also be immediately reported to the funding agency and the local Institutional Review Board (IRB) as appropriate.

Analytical plan

The primary health outcome measure is malaria incidence determined by the detection of *P. falciparum* or *P. vivax* using RDTs. All analyses will be conducted on an intention-to-treat basis, regardless of whether the individual household members use LLINs, IRS or not. Malaria cases diagnosed with an infection within 28 days of the first episode with the same *Plasmodium* species will be censored and will not be included in the analysis. An analysis will be performed as a community randomized trial with time-person as the denominator. All analyses will be conducted using Stata version 13 (StataCorp LP, College Station, TX, USA), and primary and secondary outcomes will be compared between the different intervention arms and control groups. The main outcome variable, malaria incidence based on PCD, is assumed to follow a Poisson distribution based

on a random and independent occurrence. Hence, a generalized Poisson log linear model will be fitted to measure for associations between the outcome variable and predictors. The main outcome variable will also be analyzed as a binary variable and will be compared in the intervention and control clusters using multilevel mixed-effects logistic regression models, taking into account the clustering effects. In addition, we will also analyze the data using the population as the denominator (e.g., with a generalized estimating equation (GEE), multilevel analysis or spatial analysis) to help assess the effect of the intervention.

To control for potential confounding factors, the clustering effect of villages, the effect of repeated measurement in the same individual and individual level covariates (such as age, gender, LLINs' use) will be taken into consideration during the analysis. Other potential confounding factors will also be adjusted for in the regression analysis, and all estimates will be presented with 95 % confidence intervals. Time will be included as a fixed effect that will allow any interaction with the interventions to be quantified.

The WHO age-adjusted cut-off for Hb will be used to classify anemia in children [71]. For children between 6–59 months of age, a normal Hb level is defined as Hb of 11.0 g/dl or greater and as mild at 10.0–10.9 g/dl. Moderate anemia is defined as children with an Hb level of 8.0–10.9 g/dl while severe anemia is defined as an Hb level of less than 8.0 g/dl. At a community level, a prevalence of anemia will be stated to be severe if over 40 % of the children are anemic (combining mild, moderate and severe) and moderate if the prevalence is 20–39.9 %. We will measure the weight and height of all children under the age of 5 years, and calculate the anthropometric indices such as weight for height, height for age, and weight for age. Both malaria and anemia prevalence data will be compared in the intervention and control clusters using multilevel mixed-effects logistic regression models, taking clustering effects into account.

Cost-effectiveness, expressed as an incremental cost-effectiveness ratio (ICER), will be calculated for each outcome and arm using standard DALY weights. The interventions will be ranked according to cost-effectiveness, whereas inequality in terms of health outcomes, will be measured by the Gini coefficient and the concentration index [72].

The agreement between the two mosquito collection methods, HLC and light traps, in assessing mosquito sampling efficiency will be calculated by a parametric approach based on an analysis of variance and simple graphical methods. Indoor and outdoor *Anopheles* densities will be compared among the study arms using a one-way analysis of variance (ANOVA) if data are normally distributed or using a Mann-Whitney *U* test if data are non-normally distributed.

Ethical considerations

Ethical approval

The study was approved by the IRB of the College of Health Sciences at Addis Ababa University, the Ministry of Science and Technology in Ethiopia (ref: 3.10/446/06) and the Regional Committee for Medical and Health Research Ethics, Western Norway (ref: 2013/986/REK Vest). The protocol was registered online on 8 September 2014 at the Pan African Clinical Trials Registry under the registration number PACTR201411000882128.

Community consultation and sensitization

Prior to the implementation of interventions, a consultative workshop and several meetings were held to explain the objectives, *kebele* selection and randomization, implementation procedures and expected outcomes of the trial to the communities with representatives from the ORHB, the East Shewa Zone Health Department and the Adami Tulu District Administration. Permission through official letters was obtained from various administrative levels. Study communities were sensitized prior to randomization through meetings and discussions with community leaders, *kebeles* and village leaders and community elders.

Information and informed consent

Verbal informed consent to participate in the study was obtained beforehand from the study participants and from parents/guardians for children under 18 years of age using the local *Afan* Oromo language. Information sheets were provided to inform about the purpose of the study, and the participants were informed that involvement in the study was voluntary and that they had the right to withdraw at any time regardless of reason. At each data collection, the verbal consent of the study participants and verbal assent from the parents/guardians for children were obtained using the local language. Assurance was also given that a refusal to participate in this study would not affect their access to services at the health posts in the study villages in the community.

Adverse events and malaria treatment

We do not anticipate any physical harm or risks to the participants. Blood samples for RDTs, microscopic examination of slides and Hb measurements will be collected using aseptically disposable lancets. A finger-prick for blood sample collection may result in mild pain and bruising at the site where blood is obtained, but will not cause any further harm. The collection of blood samples from finger-pricks is part of the routine procedures in the diagnosis of malaria by health workers. Malaria

treatment will be provided according to the national guidelines using AL and chloroquine, which are the first-line antimalarial treatments for *P. falciparum* and *P. vivax*, respectively [43]. All study participants visiting health posts will be examined and treated for malaria free of charge, while the mass distribution of LLINs and IRS spraying will be carried out by the *MalTrials* project for free in collaboration with the DHO. Mosquito collectors will be trained to collect mosquitoes as soon the mosquitoes land and before they bite. To help minimize risk, data collectors will be provided with an appropriate prophylactic drug (Malarone) before the collections.

Confidentiality of information

To the best of our ability, all information from the study households and participants will be held in confidence and appropriate measures will be taken to ensure the confidentiality of information both during and after data collection. Access to information will be limited to data collectors, including health workers and their supervisors at sites of collection, data entry clerks and to the research team.

Trial oversight

There is no need for a Data Safety and Monitoring Board (DSMB) for this trial since all interventions, blood sample collection and treatments are part of routine malaria control in Ethiopia and will be undertaken in collaboration with the health workers at the health posts and the DHO. We do not foresee any adverse effects from the interventions, so we do not intend to apply any stopping rules for this trial. No participant in the control group will be impeded from obtaining mosquito nets from other sources, and malaria incidence in the control villages will be monitored throughout the study for possible case build-up or an outbreak of malaria. If the control villages encounter any malaria outbreaks, an intervention will be taken by the *MalTrials* project and the DHO in accordance with the national guidelines regardless of the trial.

Deltamethrin-treated LLINs and propoxur IRS are WHO recommended and meet the specifications of the WHO's Pesticide Evaluation Scheme, and our interventions will follow the WHO and national recommendations [44, 73]. The hazard which may be associated with this trial is that the insecticides used for the IRS could leak into the environment. Empty sachets, cartons, plastic bags, used gloves, pricking needles and other contaminated materials will be handled properly until they are finally burned. Lastly, all needle safety procedures will be in line with WHO standard.

Timelines of activities

The trial is being carried out for a period of approximately 116 weeks from September 2014 to December 2016, with Table 1 showing details of the timetable of activities.

Discussion

In an effort to accelerate the reduction and ultimate elimination of malaria, IRS with insecticide and the universal distribution of LLINs have been implemented in recent years in many countries in SSA. It is well-accepted that decisions regarding malaria interventions should be based on robust evidence of the benefits and cost-effectiveness of the interventions. Since the rollout of both interventions requires considerable resources, there is an urgent need to evaluate additional protective benefits and the cost-effectiveness of the combination of the interventions. This study aims to measure whether IRS in combination with LLINs increases protection against malaria incidence compared to the use of LLINs alone, IRS alone or current routine practices. The intervention will consist of four "arms": LLINs + IRS, LLINs alone, IRS alone and control (routine practice).

The main outcome of the trial will be the assessment of the incidence of malaria using PCD at health posts. Our sample size is relatively large compared to other trials, e.g., a study in the Gambia used 35 clusters of 110 children in each of two arms to detect a 50 % reduction in the incidence of malaria [56]. Our preliminary sample size calculations based on malaria incidence rates in southern Ethiopia [60] showed that we might need to include 10 clusters per arm with approximately 50 households (250 persons) per cluster. However, a Cochrane review advocates large-sized clusters [8].

Conducting research to evaluate the impact of community interventions raises a number of practical issues [21]. Both LLINs and IRS interventions are implemented by the project, and we anticipate that the coverage of the interventions will be very high despite issues of net use and the re-plastering of sprayed walls of the houses. We understand that the recent relatively low incidence of malaria in the area will have an impact on the proper and consistent use of LLINs by the community, which may challenge an active participation by the community. We will monitor LLINs ownership and use by household members on a weekly basis, and we will also assess the magnitude of the re-plastering rates of houses and teach the community about its consequences on malaria.

Some of the strengths of our study are that we have carried out extensive pilot studies before starting the trial. This enabled us to estimate the variance in malaria incidence among the villages and thus the sample size. In the previous published trials, the IRS insecticide was bendiocarb which has a relatively short residual duration on the walls, or DDT, against which the insecticide resistance has become widespread [38–42]. The current trial evaluates the added protection of propoxur, which

Table 1 Timetable of activities

Activity by year and month	J	F	M	A	M	J	J	A	S	O	N	D
2013												
Ethical approval		x	x	x	x	x	x	x	x	x		
Development of data collection tools					x	x	x	x	x	x	x	x
Census and mapping			x	x								
Epidemiological pilot study									x	x	x	x
Entomological pilot study						x	x	x	x	x	x	
2014												
Selection of clusters		x	x	x	x							
Randomization of study clusters						x						
Procurement of LLINs			x	x	x	x	x	x				
Procurement of insecticide				x	x	x						
Household numbering and tagging						x	x					
Baseline data collection							x	x				
LLINs distribution									x	x		
Protocol registration								x	x			
IRS spraying									x			
LLINs distribution									x			
PCD and weekly household visit									x	x	x	x
Entomological surveys							x	x	x	x	x	
Cost data collection									x	x	x	x
2015												
Anemia survey	x											
Malaria prevalence survey									x	x	x	
PCD and weekly household visit	x	x	x	x	x	x	x	x	x	x	x	x
Entomology lab assays and field surveys	x	x	x	x	x	x	x	x	x	x	x	x
Cost data collection	x	x	x	x	x	x	x	x	x	x	x	x
IRS spraying and LLINs' replacement								x				
Data entry and cleaning	x	x	x	x	x	x	x	x	x	x	x	x
Sensitization of study population								x	x	x		
2016												
Anemia survey	x											x
Malaria prevalence survey									x	x	x	
PCD and weekly household visit	x	x	x	x	x	x	x	x	x	x	x	x
Entomology lab assays and field surveys	x	x	x	x	x	x	x	x	x	x	x	x
Cost data collection	x	x	x	x	x	x	x	x	x	x	x	x
IRS spraying and LLINs' replacement								x				
Data entry, cleaning and analysis	x	x	x	x	x	x	x	x	x	x	x	x
Entomological lab assays and field surveys	x	x	x	x	x	x	x	x	x	x	x	x
Cost-effectiveness analysis	x	x	x	x	x	x	x	x	x	x	x	x
2017												
Dissemination of findings to the study community	x	x										
Data analysis and report writing	x	x	x	x	x	x						
Scientific writing and publications	x	x	x	x	x	x	x	x	x	x	x	x
Final report to the funding agency				x	x	x						
Dissemination of findings to stakeholders							x					

LLINs long-lasting insecticidal nets, *PCD* passive case detection

has a relatively longer duration on walls compared to DDT. In addition, we also determined that the anopheline vectors in the study area are susceptible to propoxur. One of the limitations of this trial is that the communities will not be masked or blinded to the intervention. This would create a bias towards an increased effect of the combined intervention compared with other arms of the trial. This trial will not use a buffer zone between clusters of the different interventions as it will be carried out in rural areas with scattered households. As a result, the proximity of the clusters may influence the results. However, this applies to only some of the selected villages that are close to each other. As the positions of all households are recorded, we shall also try to adjust for possible close proximity of the villages during analysis.

The community in the "routine practice" with a weekly follow-up is in a better position than the community in the "routine practice" without any follow-up. Therefore, we decided to allow the trial with all four arms and emphasized that every participant will have access to weekly visits by the *MalTrials* project field personnel, early diagnosis and state of the art treatment for malaria. Adami Tullu was one of the 19 IRS targeted districts of the President's Malaria Initiative (PMI) and the Ministry of Health (MOH) between 2008 and 2013 [74]. However, since 2014 the PMI has shifted its target areas to other districts in the Oromia Region. However, we will continuously monitor all groups including the control arm of the study if unforeseen malaria epidemics should arise.

This study is proposed at a time of significant need within the countries of SSA on how to effectively use IRS and LLINs interventions for malaria control and elimination. Consequently, the study is well-timed to assess whether the combination of LLINs and IRS could contribute towards the elimination of malaria. The trial addresses how to promote the uptake of research findings into public health programs by enhancing the knowledge base on interventions that will improve the effectiveness and coverage of anti-malaria interventions.

This study is expected to generate important evidence to inform the malaria control programs and the public regarding the effectiveness and cost-effectiveness of the two major vector control interventions. The costs of our intervention is similar to that of the existing malaria control program so that they can be easily accepted by the MOH. We anticipate that the findings of this study will be used for an effective planning and implementation of vector control interventions.

Trial status

At the time of the submission of this manuscript, ethical approval has been obtained and the trial had completed a pilot study, baseline data collection and randomization, and is ongoing.

Abbreviations

AL: artemether-lumefantrine; ANOVA: analysis of variance; CDC: Center for Disease Prevention and Control; CONSORT: Consolidated Standards of Reporting Trials; DALYs: Disability-adjusted Life Years; DDT: dichloro-diphenyl-trichloroethane; DHO: District Health Office; DSMB: Data Safety and Monitoring Board; ECEA: extended cost-effectiveness analysis; EIR: entomological inoculation rate; ELISA: Enzyme-linked Immunosorbent Assay; GCP: Good Clinical Practice; GEE: generalized estimating equation; GPS: Global Positioning System; Hb: hemoglobin; HBI: Human Blood Index; HBR: human biting rate; HEW: health extension worker; HLC: human landing catches; ICC: intra-cluster correlation coefficient; ICER: incremental cost-effectiveness ratio; ID: identification; IRB: Institutional Review Board; IRS: indoor residual spraying; ITNs: insecticide-treated nets; LLINs: long-lasting insecticidal nets; MOH: Ministry of Health; ORHB: Oromia Regional Health Bureau; PCD: passive case detection; PCR: polymerase chain reaction; PMI: President's Malaria Initiative; RDT: rapid diagnostic test; SPSS: Statistical Package for Social Sciences; SSA: sub-Saharan Africa; WHO: World Health Organization; WP: water-dispersible powder.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

WD, MB, BL and EL conceptualized the idea and drafted the initial protocol. WD, MB, BL, AH, EL, TG, HO, OK, TGM and BR finalized the study protocol. TG, AH and OK led the development of the data collection tools. WD and AH processed the approval of the protocol by the ethical committees, IRB and national ethical clearance in Ethiopia. BL processed the approval of the protocol in Norway. EL, TG, AH and OK were involved in the census and pilot study. EL, WD and BL contributed to the design and management of the trial, including statistical inputs. EL computed the sample size. MB, OK, TGM and HO contributed to the design of the entomological study. BR, AH, BL and WD contributed to the design of the economic evaluation of the trial. TG, AH, OK, EL, WD, MB and BL coordinate, manage and implement the trial. EL and TG manage the laboratory activities and data processing of the epidemiological study. BL, WD and MB processed the purchase of the LLINs and IRS insecticide. BL registered the protocol. WD and BL wrote the first draft of the paper. All authors read and approved the final manuscript.

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Errata

Thesis:

On page 24 Figure 1 …" missing and misplaced arrow in the interaction between disease and poor sanitation" should read as shown in the following figure.

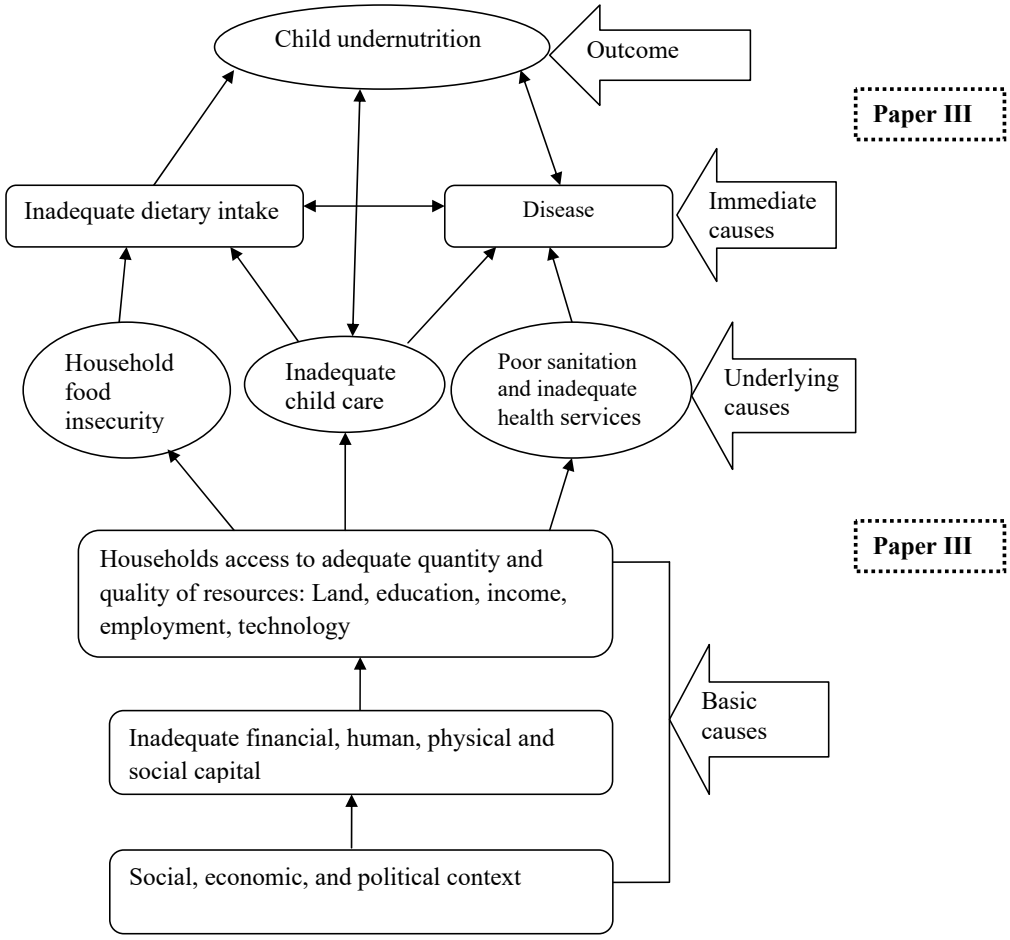

Figure 1: Conceptual framework for the causes of undernutrition in children (adapted from UNICEF nutritional strategy (172) and The Lancet Series (173)).

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