Malaria vectors in southern Ethiopia

Some challenges and opportunities for vector control

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To

Biniam, Nebiyu and Dibora

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General summary

Background: Malaria is a public health problem in Ethiopia, where more than 60% of the population lives in risky areas. Since 2005, malaria-related sicknesses and deaths have substantially decreased in the country, mainly due to the increasing coverage of vector control interventions and chemotherapy. On the other hand, resistance to most public health insecticides is widely spreading among the populations of the principal malaria vector *Anopheles arabiensis.* Therefore, assessing the susceptibility status of local malaria vectors is an essential activity to improve the effectiveness of the interventions, by introducing the appropriate insecticide resistance management strategies. There are also substantial gaps in knowledge regarding the entomological inoculation rate (EIR), which is an indicator of the intensity of malaria transmission, and are used to assess the impact of vector control interventions. Understanding the species composition, feeding and resting behaviours, parity rate, as well as human biting and sporozoite rates, are all important in evaluating the effectiveness of interventions and planning for supplementary vector control tools. Moreover, improving housing, such as screen doors and windows, and closing openings on walls and eaves, might reduce the entry of malaria vectors and provide protection from infectious bites of malaria vectors.

Objective: The study was carried out to help assess the species composition, age structure, feeding patterns, sporozoite infection rate, entomological inoculation rate and insecticide susceptibility status of *An. arabiensis,* and evaluate the impact of screened houses on its indoor density.

Methods: The study was done in the Chano Mille *Kebele* in southwestern Ethiopia. The longitudinal entomological study was conducted from May 2009-April 2010, whereas the house screening intervention was done between April-November 2011. Thirty houses (10 houses for each collection method) were randomly selected for biweekly *Anopheles* mosquito sampling. The *Anopheles* mosquitoes were collected by the Centers for Disease Control and Prevention (CDC) light traps, pyrethrum spray catches (PSC) and from artificial pit shelters by aspirating. Enzyme-linked-immunosorbent assay (ELISA) was used to analyse the blood meal origins and circumsporozoite proteins. The EIR of *P. falciparum* and *P. vivax* of *An. arabiensis* was calculated by multiplying the sporozoite and human biting rates from CDC light traps and PSC collections.

A randomized control trial was conducted to assess the impact of screening windows and doors with wire mesh, and closing openings on eaves and walls by mud on the indoor density of *An. arabiensis*. Baseline

mosquito data was gathered biweekly from 40 houses by CDC light traps in March and April 2011 to randomize houses into both control and intervention groups. The windows and doors of 20 houses were screened by mosquito-proof wire mesh, and openings on the walls and eaves were closed by mud. The rest of the 20 houses were assigned to the control group. Mosquitoes were collected biweekly in October and November 2011 from both the control and intervention houses.

Results: *Anopheles* species, comprised of *An. arabiensis, An. marshalli, An. garnhami, An. funestus* group*, An. pharoensis, An. tenebrosus, An. rhodensiensis, An. flavicosta, An. longipalpis, An. daniculicus, An. pretoriensis, An. chrysti, An. moucheti, An. distinctus* and *An. zeimanni,* were documented in the area. *Anopheles arabiensis* was by far the most dominant species.

The overall human blood index (HBI) of *An. arabiensis,* including the mixed blood meals, was 44%, whereas the bovine blood index (BBI), including mixed blood meals, was 69%. The majority of *An. arabiensis* (65%) from the indoor-resting collection had bovine blood meal, which was unexpected. The higher proportion (75%) of indoor host-seeking *An. arabiensis* collected by CDC light traps had contact with humans. Only 13% *An. arabiensis* from pit shelters had human blood meal, while 68% had bovine blood meal. *Anopheles arabiensis* showed a consistently higher feeding pattern on cattle than on humans, regardless of collection sites and the high number of the human population. The human and bovine feeding patterns of *An. arabiensis* showed little change due to the number of cattle to human ratio of each household. *Anopheles marshalli* and *An. garnhami* showed similar feeding patterns.

Anopheles arabiensis was highly resistant to four pyrethroid insecticides tested (lambdacyhalothrin, cyfluthrin, alphacypermethrin and deltamethrin) and DDT, with a maximum mortality rate of 56% due to lambdacyhalothrin and a minimum of 10% due to DDT.

The circumsporozoite protein ELISA test revealed 11 *P. falciparum* infections out of 14 sporozoite positive *An. arabiensis* (the other three were *P. vivax*), thereby confirming that this species is the principal vector of *P. falciparum* and *P. vivax* parasites. The *P. falciparum* sporozoite rate of *An. arabiensis* was 0.32% for CDC light traps, 0.28% for pit shelters and 0.23% for PSCs. The overall estimated annual *P. falciparum* EIR of *An. arabiensis* from CDC light traps was 17.1 infectious bites/person/year (ib/p/y), but it varied between houses, from a 0 EIR in 60% of houses to 73.2 in a house close to the major breeding site. Hence, those houses nearest

to the mosquito breeding sites had a higher risk of exposure to infectious bites. The *P. falciparum* EIR of *An. arabiensis* was 2.4 in the dry season and 14.7 in the wet season, indicating 6.1-fold more infectious bites in the wet- than in the dry season. The *P. falciparum* and *P. vivax* EIR of *An. arabiensis* from PSC was 0.1ib/p/y, while the *P. vivax* EIR of *An. arabiensis* from CDC light traps was 2.41ib/p/y.

The screening of doors and windows with wire mesh, and closing the openings on eaves and walls by mud, significantly reduced the indoor density of host-seeking *An. arabiensis* by 40%. The intervention was cheap, and can be incorporated into malaria vector control programmes by local communities.

Conclusion: *Anopheles arabiensis* showed a consistently higher feeding pattern on cattle than on humans regardless of collection sites and the high number of human population. It was the most abundant and the principal vector of *P. falciparum* and *P. vivax*, while *An. marshalli* and *An. garnhami* were the second and third most abundant species, but neither of them was positive for CSPs. The transmission of malaria is heterogeneous; those houses nearest to the mosquito breeding sites (hot spots) had a higher risk of exposure to the infectious bites of *An. arabiensis*. *Anopheles arabiensis* was resistant to pyrethroid insecticides, the only class of insecticides recommended for LLINs treatment; as a result, there should be an action programme to manage insecticide resistance. Finally, supplementary methods of vector control, such as the screening of houses, could be included to help improve malaria control in the area based on the principle of integrated vector management.

List of original published papers

This thesis is based on the following papers listed as Paper I, Paper II, Paper III and Paper IV in the main text.

Paper I: Fekadu Massebo, Meshesha Balkew, Teshome Gebre-Michael and Bernt Lindtjørn. Blood meal origins and insecticide susceptibility of *Anopheles arabiensis* from Chano in South-West Ethiopia. *Parasit Vectors*. 2013, 6:44

Paper II: Fekadu Massebo, Meshesha Balkew, Teshome Gebre-Michael and Bernt Lindtjørn. Entomologic Inoculation Rates of *Anopheles arabiensis* in Southwestern Ethiopia. *Am J Trop Med Hyg* 2013; 89:466-473.

Paper III: Fekadu Massebo and Bernt Lindtjørn. The effect of screening doors and windows on indoor density of *Anopheles arabiensis* in South-West Ethiopia: A randomized trial. *Malar J*. 2013, 12:319

Paper IV: Fekadu Massebo, Meshesha Balkew, Teshome Gebre-Michael and Bernt Lindtjørn. Zoophagic behaviour of anopheline mosquitoes in South-West Ethiopia: Opportunity for malaria vector control. *Parasit Vectors.* 2015, 8:64

Abbreviations

AChE	Acetylcholinesterase
ACTs	Artemisinin-based Combination Therapies
BBI	Bovine Blood Index
CDC	Center for Disease Control and Prevention
CSPs	Circumsporozoite proteins
DDT	Dichlorodiphenyltrichloroethane
DEET	N, N-Diethyl-3-Methylbenzamide
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immuno-Sorbent Assay
GPIRM	Global Plan for Insecticide Resistance Management
GST	Glutathione S-transferases
HBI	Human Blood Index
HBR	Human Biting Rate
HLC	Human Landing Catches
IRS	Indoor Residual Spraying
ITNs	Insecticide Treated Nets
IRM	Insecticide Resistance Management
IVM	Integrated Vector Management
LLINs	Long-Lasting Insecticide Treated Nets
LSM	Larval Source Management
MOH	Ministry of Health
PCR	Polymerase chain reaction
PSC	Pyrethrum Spray Catches
RDT	Rapid Diagnostic Test
SIT	Sterile Insect Technique
SNNRPs	Southern Nations Nationalities and Peoples' Region State
SR	Sporozoite Rate
WHO	World Health Organization

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1. Introduction

1.1. General overview

Human malaria is caused by five *Plasmodium* species: *Plasmodium falciparum, P. vivax, P. ovale*, *P. malariae* and *P. knowlesi* (zoonotic species mainly occurring in Asia).[1, 2] *Plasmodium falciparum* is the most fatal malaria parasite,[2] threatening millions of lives primarily in Africa south of the Sahara. An estimated 187 million clinical cases of *P. falcipa*rum were reported in Africa in 2015.[3] More than 90% deaths and 88% of cases occur in Africa,[4] while some species of *Anopheles* are vectors of malaria.[5] *Anopheles gambiae*, *An. coluzzii, An. arabiensis* and *An. funestus* are the major malaria vectors in Africa.[6]

Globally, the number of cases and deaths due to *P. falciparum* has substantially declined in Africa and elsewhere (Figure 1).[3, 4] Between 2000 and 2012, deaths due to malaria declined by 42% globally, and the reduction was higher (49%) in the World Health Organization's (WHO) African region.[7] In 2013, malaria mortality rates declined by 47% globally and 54% in Africa compared to 2000, with malaria causing 584,000 deaths.[8] Between 2000 and 2015, the malaria mortality rate was reduced by 60% worldwide and 66% in Africa among all age groups.[4] The number of deaths due to malaria declined to 438,000 in 2015, from 839,000 in 2000.[4] The decline in the number of malaria cases and deaths is associated with the widespread use of long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), rapid diagnostic tools and effective anti-malarial drug artemisinin combination therapies (ACTs). It was reported that in 2015, more than 50% of people in Africa slept under LLINs compared to roughly 2% in 2000.[4] The interventions have averted an estimated 663 million clinical malaria cases since 2000, with insecticide treated bed nets being the largest contributors to reducing deaths, followed by ACTs.[3, 4]

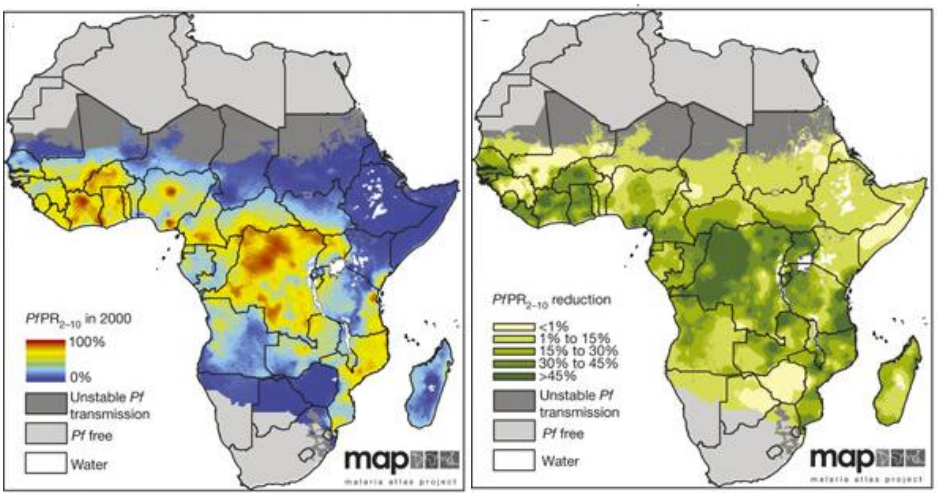

Figure 1: Changes in malaria infection prevalence in 2015 compared to 2000;[3] predicted *P. falciparum*$_{2\text{-}10}$ infections in 2000 (*left*) and absolute reduction in prevalence of *P. falciparum*$_{2\text{-}10}$ infection in 2015 (*right*)

In Ethiopia, the coverage of LLINs and IRS, as well as anti-malaria drugs, has increased since 2005.[4] Since then, malaria cases and mortality rate have significantly declined.[9] For example, the infection prevalence of *P. falciparum* was reduced by 1%-15% in 2015 in children aged 2-10 years, compared with the baseline infection prevalence in 2000 (Figure 1).[3] The transmission pattern of malaria is seasonal, unstable and prone to epidemics,[10] but the number and magnitude of epidemics have been substantially reduced since 2004. The majority of localities in Ethiopia are characterized by *P. falciparum* parasite prevalence ≤ 5% (Figure 2), and only 16% of the population lives in areas with stable malaria transmission.[11] Despite all the achievements and gains, more than 60% of the population in Ethiopia lives in areas at risk for malaria (Figure 2).[11] *Plasmodium falciparum* and *P. vivax* are the two common malaria parasites, and *An. arabiensis* is the principal malaria vector in Ethiopia.[11, 12] Unlike *P. falciparum*, the epidemiology and clinical consequences of *P. vivax* are less known.[11]

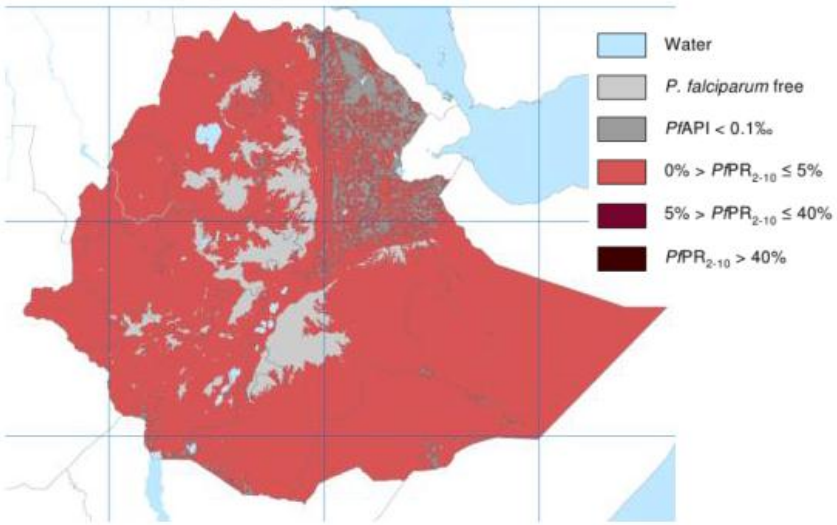

Figure 2: Map of malaria risk in Ethiopia based on *P. falciparum* parasite prevalence (very few localities are characterized by parasite prevalence ≥ 40%)[11]

The impact of LLINs and IRS can be measured by using entomological indicators such as entomological inoculation rates (EIR),[13] parous rates (longevity of vectors), sporozoite rates and human blood index.[14] The EIR is one of the most important indicators in evaluating the impact of interventions and assessing the intensity of malaria transmission in a particular site and time.[13] Very few attempts have been made to estimate EIR in Ethiopia. Krafsur, working in Gambella town and riverside villages, was the first to estimate an annual EIR of *An. gambiae* s.l. (presumably *An. arabiensis*) in Ethiopia.[15] After approximately four decades, an estimation of EIR was reported from a highland in the south-central region of the country.[16] Hence, there is a gap in knowledge of the EIR values of *An. arabiensis* or other malaria vectors.

The feeding patterns and resting behaviours of malaria vectors affect the effectiveness of LLINs and IRS because they primarily target those resting and feeding indoors.[17] The success of LLINs and IRS interventions also depends on the effectiveness of the insecticides, while the development of resistance in malaria vectors might limit their effectiveness. Today, LLINs and IRS are under the constant threat of insecticide-resistant vectors.[18] The principal malaria vectors are resistant to almost all classes of insecticides in many malaria-

endemic countries, including Ethiopia.[19] In 2009 in Ethiopia, DDT was banned from IRS use due to the widespread problem of resistance in populations of *An. arabiensis,* and was substituted for by deltamethrin. A shift to carbamate insecticides was made in 2011 in favour of IRS, as *An. arabiensis* developed a resistance to all the available pyrethroid insecticides in most parts of the country within a relatively short period of their application.[20, 21] Hence, insecticide resistance surveillance is one of the priority areas of research to detect resistance at an early stage and to design appropriate resistance management strategies.

The principal malaria vectors mainly bite humans indoors in many malaria-endemic areas in Africa regardless of the high coverage of LLINs and IRS, thereby making houses the principal site of malaria infection.[22] Improving housing reduces the infectious bites of malaria vectors by blocking house entry.[23] Screening doors, windows and ceiling eaves are known to reduce the number of vectors entering a house.[24] Moreover, modern houses reduced the number of malaria episodes by 47% compared to traditional houses.[25] Housing improvement is a non-chemical approach that can be a component of integrated vector management to help prevent mosquitoes from accessing houses.[23, 25]

In southern Ethiopia, malaria is a public health problem, although the intensity of transmission varies between regions.[26] Chano Mille is one of the malarious *kebeles* near Arab Minch in southwest Ethiopia,[27] where little or no information is available on the entomological indicators of malaria transmission. This gap urged us to conduct a longitudinal entomological study to assess host feeding patterns, sporozoite rates, parity rates and the EIR of local malaria vectors, in addition to the status of insecticide susceptibility of *An. arabiensis* and the impact of housing on its indoor density. The knowledge gap is high, particularly on EIRs. A better understanding of the species composition, feeding patterns, resting behaviour and the age structure of local malaria vectors are also important to plan supplemental vector control tools to support the existing interventions, all of which are essential to help sustain the gains in malaria control and plan towards its elimination.

1.2. Bionomics of anopheline mosquitoes

Understanding the ecology and behaviour of the malaria vectors is relevant in monitoring their response to the existing interventions and deciding on the appropriate control strategies.[28] There are entirely aquatic developmental stages (egg, larva and pupa), as well as the adult stage, which is responsible for malaria

transmission. The feeding preference, resting and biting behaviours of the female *Anopheles* mosquitoes determine the competence of the species.[29]

1.2.1. Life cycle

The development of larval and pupal stages might be influenced by the oviposition site selection of gravid female *Anopheles* mosquitoes. Chemical cues and some physical factors direct the oviposition site selection of adult females.[28, 30] African *Anopheles* mosquitoes also have numerous breeding sites, including shallow and sunlight-exposed temporary water bodies, permanent shaded water bodies, permanent man-made concrete structures, drainage canals and natural swamps.[31] Some species are salt water breeders, whereas others prefer hot springs.[32] After finding the appropriate habitats, adult *Anopheles* females lay their eggs on the surface of the water. The eggs are characterized by the presence of air-filled floating structure called air floats. The eggs hatch to larvae, which are active feeders on decaying organic matters and microorganisms.[28] Larva subsequently molts into the second, third and fourth instar. The final instar develops into the non-feeding stage pupa, with the adults emerging from pupae within a few days (Figure 3). The duration of the life cycle (usually 10-14 days in the tropics) depends on water temperature, type of larval food and species.[28]

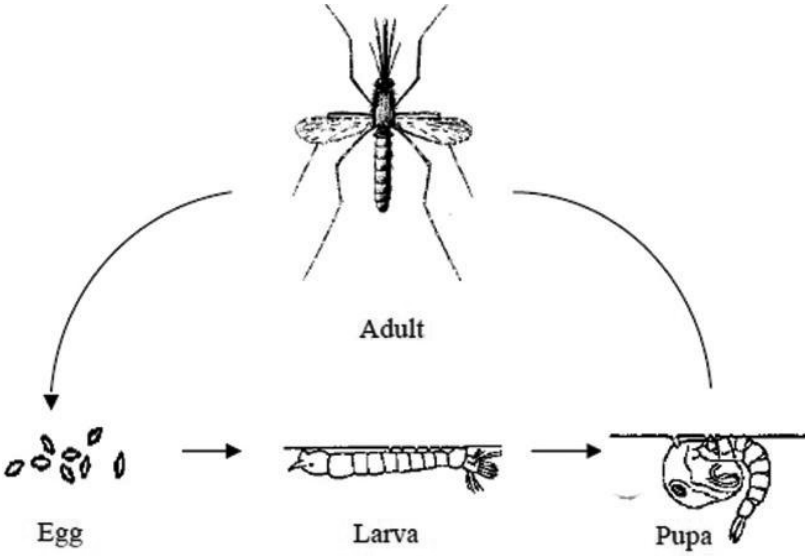

Figure 3: Life cycle of *Anopheles* mosquitoes[33]

1.2.2. Mating, blood feeding and gonotrophic cycle

A female mosquito mates only once after emerging from a pupa, though sperm cells remain viable in the spermatheca throughout her life.[28] Both male and female mosquitoes feed on nectar to obtain energy for flight and dispersal as soon as they emerge from pupae. A female seeks blood meal as a source of protein for egg development and maturation. After blood feeding, it rests for 2-3 days to digest blood meal in the tropics, and even more than a week in a temperate climate.[28] Endophilic mosquitoes prefer to rest indoors during blood meal digestion, while exophilic mosquitoes spend this period outside human dwellings.[28] At the end of blood feeding, the abdomen of a mosquito looks bright red, but in subsequent hours it changes to dark red (Figure 4). As the digestion of blood meal continues, the abdomen becomes whitish due to the development of eggs; the mosquito then lays eggs in appropriate oviposition sites and the cycle continues.

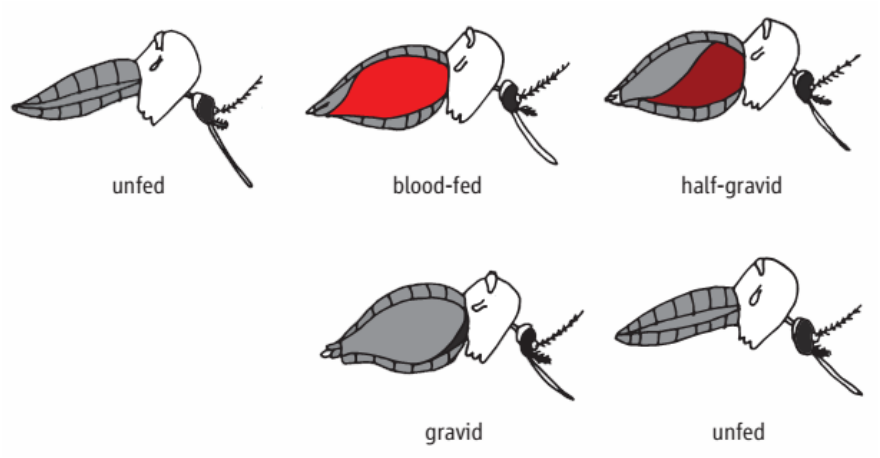

Figure 4: Abdominal condition of a female mosquito[33]

A female mosquito undergoes a repeated cycle of blood feeding on the appropriate blood meal source, resting for blood meal digestion and egg development, and the oviposition of eggs, which is called the gonotrophic cycle.[28] The length of a gonotrophic cycle depends on temperature,[28] availability of blood meal sources and oviposition sites, and is important in determining the vectorial capacity and stability of malaria transmission.[34]

For example, the deprivation of the oviposition sites prolong the gonotrophic cycle of malaria vectors, hence reducing the biting frequency of mosquitoes and malaria transmission.[34] The digestion of blood meal is very fast under warmer temperatures, which in turn shortens the duration of the gonotrophic cycle. As a result, the fast digestion of blood meal increases the frequency of host-seeking, which may intensify the transmission of malaria.[35] In contrast, cooler temperatures extend the gonotrophic cycle, hence reducing the feeding frequencies of malaria vectors.[36]

1.3. Malaria vectors in Africa

Africa is the home for many *Anopheles* species with variable roles in malaria transmission. *Anopheles gambiae, An. coluzzii, An. arabiensis* and *An. funestus* are the most important malaria vectors. There are also locally important vector species, but most *Anopheles* mosquitoes are not incriminated as vectors of malaria.[6]

1.3.1. *Anopheles gambiae* and *An. funestus* complexes

Sibling species in the *An. gambiae* complex are reproductively isolated, but difficult to recognize morphologically. The advancement of molecular techniques has enabled the identification of new species and the incrimination of new malaria vectors.[37, 38] The *An. gambiae* complex comprises *An. gambiae* (molecular S form), *An. coluzzii* (molecular M form), *An. arabiensis, An. merus*, *An. melas*, *An. bwambae, An. quadriannulatus, An. amharicus* and *An. comorensis*.[32, 39] Of these, *An. gambiae, An. coluzzii* and *An. arabiensis* are the most competent malaria vectors in Africa (Figure 5), while the first two species are highly anthropophilic (a preference of feeding on humans) and endophilic. [40, 41] *Anopheles arabiensis* shows anthropophilic- and zoophilic- (a preference of feeding on animals), and exophilic and endophilic behaviours in different regions.[42, 43]

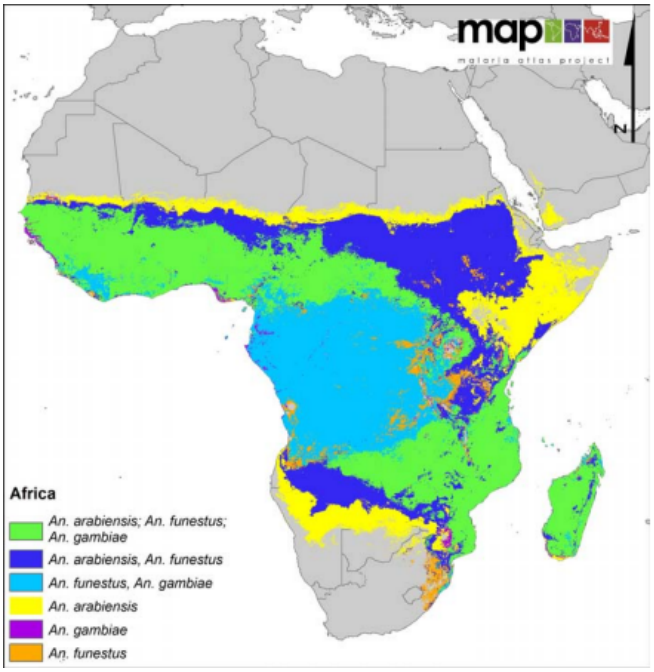

Figure 5: Map showing the distribution of the dominant malaria vectors (*Anopheles gambiae, An. coluzzii, An. arabiensis* and *An. funestus*) in Africa[6]

The role of *An. gambiae* as an important malaria vector is reflected by its sporozoite rate, reaching 10% in Uganda.[44] Nonetheless, the *P. falciparum* sporozoite rate of *An. arabiensis* rarely exceeded 1%, and its occurrence is mostly associated with unstable and epidemic malaria.[45] However, in Tanzania, *An. arabiensis* is replacing *An. gambiae,* and its importance is increasing.[46] *Anopheles gambiae* had higher *P. falciparum* sporozoite rates than *An. coluzzii*.[32, 47] Furthermore, *An. gambiae* is found in most parts of Africa, whereas *An. coluzzii* is limited to western Africa.[48, 49] The hybrids of the two molecular forms are rare, though Nwakanma and colleagues recently documented 5%-42% hybridization frequencies and a high efficiency of gene flow (inbreeding).[50] Thus far, *An. arabiensis* does not show any further speciation.[45]

Both the East African salt water breeder *An. merus* and the West African species *An. melas* are locally important malaria vectors.[32] *Anopheles merus* has been incriminated as a malaria vector in Madagascar,[51] and

along the Kenyan[52] and Tanzanian coasts.[53] Diop and colleagues have reported sporozoite rates of 1%-3% for *An. melas* in Senegalese fishing communities.[54] In Equatorial Guinea, the sporozoite rate of 4.4% was reported for *An. melas*.[55] *Anopheles bwambae* from geothermal hot springs has also been identified as a local malaria vector in Uganda.[56]

The non-malaria vector species *An. quadriannulatus* of South Africa and *An. amharicus* of Ethiopia are primarily zoophilic.[32] *Anopheles comorensis* was first described as a distinct species of *An. gambiae* complex from the Comoros Islands in the Indian Ocean in 1997.[32] The description of the species was based on a single specimen, so hence the biology of the species was not fully understood. Therefore, the information may not be conclusive, so further characterization of the species might be needed.

The *An. funestus* complex comprises 13 morphologically similar sibling species (differentiated by molecular technique PCR), including *An. funestus*, *An. vaneedeni*, *An. parensis*, *An. leesoni*, *An. aruni*, *An. confusus*, *An. rivulorum*, *An. brucei, An. fuscivenosus, An. funestus*-like*, An. longipalpis* type C*, An. longipalpis* type A and *An. rivulorum*-like species.[57-59] Of these, *An. funestus* is the most anthropophagic and endophilic, and is an important vector of malaria in some parts of Africa.[60] A sporozoite rate of 22% was reported in South Africa by salivary gland dissections[61] and 11% by nested PCR in Tanzania.[62] In Tanzania, the role of *An. funestus* is currently increasing.[46] *Anopheles rivulorum* was incriminated as a vector of malaria by salivary gland dissection and ELISA in Tanzania in 1990s.[63] The species has shown a tendency to bite humans outdoors in the early hours where and when people were not protected by bed nets. *Plasmodium falciparum* positive *An. rivulorum*, *An. leesoni* and *An. parensis* were identified by PCR in the same country.[62] PCR confirmed *P. falciparum* positive, and *An. rivulorum* was also documented in Kenya.[64] Other members are regarded as zoophilic, and several of them are outdoor resting.[65] *Anopheles vaneedeni* was experimentally infected in the laboratory, but there is no evidence in the wild population.[66]

1.3.2. Other major malaria vectors in Africa

Anopheles nili complex and *An. moucheti* are the major vectors of malaria in forested and humid areas in Africa.[67, 68] The *An. nili* complex comprises four morphologically similar species, including *An. nili* s. s, *An. somalicus*, *An. carnevalei* and *An. ovengensis*.[57, 69] The first species is a widespread and efficient malaria vector in Cameroon,[70, 71] Senegal,[72] Côte d'Ivoire,[73] Zaire[74] and the lowland regions of Ethiopia.[75] It is highly anthropophagic, but exhibits both endophilic and exophilic behaviours.[71-73] *Anopheles ovengensis* plays a

substantial role in malaria transmission in Cameroon,[70] while *Anopheles moucheti* is a widespread malaria vector in Nigeria,[76] Congo,[57] Uganda,[57] Cameroon,[70, 77] Gabon[78, 79] and Equatorial Guinea.[55] The infection rates of wild females of *An. moucheti* were 1.68% in Cameroon[70] and 1.8% in Nigeria.[76] In these settings, the main blood meal sources of *An. moucheti* were humans, thus suggesting its strong anthropophilic behaviour, and it also tends to rest indoors.

1.3.3. Localized secondary vectors in Africa

Secondary vectors are responsible for a small proportion of malaria transmission, and they can also maintain malaria transmission, though at a lower level. *Anopheles marshalli* was incriminated as a vector of *Plasmodium* in forest zones of southern Cameroon based on the detection of CSPs by ELISA,[77] whereas *An. coustani*, *An. pharoensis* and *An. squamosus* were incriminated by salivary gland dissection in the Muheza district in Tanganyika.[80] A recent study from Kenya has shown the ability of *An. coustani* in transmitting malaria, both indoors and outdoors.[81] In Zambia, *An. coustani* was highly anthropophilic, but negative for CSPs.[82] *Plasmodium malariae* CSP positive *An. coustani* was documented in Cameroon.[70] *Anopheles ziemanni* is a local vector of malaria in Cameroon.[83] *Anopheles pharoensis* is an important vector of malaria in Cameroon,[70] Senegal [84]and Chad.[85] Lastly, the anthropophilic behaviour of *An. squamosus* has been reported from Zambia.[82]

1.4. Malaria vectors in Ethiopia

More than 42 species and subspecies of anopheline mosquitoes were documented in Ethiopia.[86] Few species are incriminated as primary and secondary vectors of malaria, while most species are considered as non-vectors.

1.4.1. The principal malaria vector

In Ethiopia, one of the two species of the *An. gambiae* complex is the major vector of both *P. falciparum* and *P. vivax.* The two sibling species are *An. arabiensis* and *An. amharicus*. The former is the most abundant and relatively anthropophagic species, and is consequently responsible for most malaria transmission,[12] whereas the latter has a limited distribution, and it is mainly zoophilic and therefore not involved in malaria transmission. The predominance and principal role of *An. gambiae* (presumably *An. arabiensis*) in malaria transmission have been documented in the 1930s by Italian malariologists.[11]

Anopheles arabiensis shows variable feeding and resting behaviours with both anthropophagic and zoophagic, and exophilic and endophilic behaviours. For example, Tirados and colleagues have reported its anthropophagic behaviour, both indoors and outdoors, in the Konso district in southern Ethiopia.[42] On the other hand, Habtewold and colleagues documented the zoophagic behaviour of *An. arabiensis* from another locality in the same region.[87] In southwestern Ethiopia, it demonstrated a strong zoophagic behaviour, with only 7.3% being of human blood meal origin.[88] Seventy-eight percent of *An. arabiensis* from CDC light traps had human blood meal origin in Ziway, central Ethiopia.[89]

The *Plasmodium* infection rate of *An. arabiensis* varied from place to place in Ethiopia. In 1977, Krafsur reported a sporozoite rate of 1.87% for *An. arabiensis* from Gambella by microscopic dissection,[15] whereas in 1994 Nigatu and colleagues documented a CSP rate of 0.77% [90] from the same area. The CSP rate (*P. falciparum* and *P. vivax*) of *An. arabiensis* was 0.24 % from indoor-resting collections in four villages in southwestern Ethiopia.[88] From human landing catches (HLCs), *Anopheles arabiensis* had a sporozoite rate of 0.5% for *P. falciparum* and 1.76 % for *P. vivax* in Sille in southern Ethiopia.[91] From Ziway in the Central Rift Valley of Ethiopia, a CSP rate of 1.18% s was reported from CDC light traps.[89] A study from south-central Ethiopia documented a *P. falciparum* CSP rate of 0.3% from CDC light traps.[16]

In Ethiopia, few attempts have been made to estimate the EIRs of *An. arabiensis*. The first attempt was made in 1977 by Krafsur, who estimated an overall EIR of 96.67ib/p/y in the river villages of Gambella based on PSCs.[15] More recently, an EIR of *P. falciparum* was estimated to be 3.66 ib/p/y for *An. arabiensis* in the central highlands of southern Ethiopia from CDC light traps.[16] As a result, there is a substantial gap in knowledge regarding entomological transmission levels.

Anopheles amharicus has not been incriminated as a malaria vector in Ethiopia.[32] Fettene *et al.*[88] and Hunt *et al.*[92] collected a higher proportion of *An. amharicus* from cattle sheds. Only a small proportion (1.1%) of *An. amharicus* had human blood meal origins in the Jimma area of southwestern Ethiopia. A recent study from the Jimma Valley, however, showed the occurrence of *An. amharicus* in houses occupied by humans.[93]

1.4.2. Secondary vectors

Anopheles pharoensis is one of the secondary vectors of malaria in different parts of the country.[12] Several investigations in various localities have shown variable results of sporozoites rates. In 1966, Rishikesh dissected the salivary glands of 2,577 *An. pharoensis* from Awasa and Adamitulu, although none of them were found to be sporozoite positive.[94] In Gambella, *An. pharoensis* was found with a *P. vivax* CSP rate of 0.47% based on PSCs.[90] *Anopheles pharoensis* from Ziway had a CSP rate of 0.59% from CDC light trap collections,[89] while sporozoite rates of 0.47%-0.7% were documented around the Koka reservoir dam.[95] *Anopheles pharoensis* had a *P. vivax* CSP rate of 1.4% from CDC light trap collections in south-central Ethiopia.[16]

With regard to the feeding patterns of *An. pharoensis*, an overall HBI of 22.5% was reported in Gambella from indoor resting collections, but the proportion of human blood fed varied from 0%-75% in different villages.[90] A low HBI of *An. pharoensis* was reported from south-central Ethiopia.[16] Outdoor biting behaviours of *An. pharoensis* have also been documented.[90, 96]

Although *An. funestus* is an important vector in some parts of Africa, it is one of the secondary vectors which has its known distribution in Gambella and around the Rift Valley Lakes in southern Ethiopia.[96, 97] Its distribution was wide from the1930s to the 1960s, and in 1966 Rishikesh attempted to dissect the salivary glands of 339 *An. funestus* from the Zwai and Awasa areas, but all were negative for sporozoites.[94] This was followed by Krafsur in 1977, who reported a sporozoite rate of 1.23% from PSCs.[15] It appears that *An. funestus* has only a local importance in Gambella in Ethiopia, whereas it seems to be of little significance elsewhere since several attempts have produced negative results, as well as a scarcity in its abundance. In Ethiopia, it is probable that DDT spraying during the malaria eradication campaigns might have eliminated the species, as has been reported in other East African countries.[98, 99] After the massive application of DDT, *An. funestus* s.s. was substituted by *An. rivulorum* (with outdoor biting and resting behaviours) in East Africa.[99, 100] In Ethiopia, *An. parensis* was the only member identified by PCR technique.[98] The current status of *An. funestus* is not well known, and hence needs to be investigated.

The other secondary vector with local importance in Ethiopia is *An. nili*. Krafsur was the first to incriminate *An. nili* from Gambella in 1970, where he reported sporozoite rates of 0.84% in 1967 and 1.57% in 1968,

thereby concluding that the species was responsible for malaria transmission, mainly in the wet season.[75] A sporozoite rate of 1.29% was reported in 1977 in the same place.[15] *Anopheles nili* showed a preference to rest outdoors. This species was later shown to be rare in other parts of the country, [96,116] and thus appears to be less important in malaria transmission elsewhere.

1.4.3. Other potential *Anopheles* vector species

In many countries, the dynamics of the vectors is changing and those considered as non-vectors are becoming either potential or proved vectors of malaria.[83, 101] For example, in Kenya an unidentified but highly *Plasmodium* susceptible *Anopheles* species was recently documented.[102] In Ethiopia, there is no such conclusive data on the biodiversity of malaria vectors because most of the studies are biased to the vector species that are dominant and well documented, with the due limitations of entomological skills to identify all species in an area.[103]

The salivary glands of *An. coustani* was found to be positive for the *Plasmodium* parasite in the 1940s in Ethiopia.[104] The human biting behaviour of this species was reported from the central highlands of Ethiopia.[12] In southern Ethiopia, *An. coustani* was the second dominant species, and had a biting peak in the early hours of the evening (18:00-20:00), primarily outdoors.[91] After many years, *An. coustani* from Jima town was found to be positive to CSP using ELISA,[105] but because of morphological misclassification and the false positivity of ELISA, there is a need to conduct further investigations using a more sensitive molecular technique like PCR to consider it as a proven vector of malaria.

Human blood was identified from *An. demeilloni* (a maximum of 11.5%) and *An. christyi* (a maximum of 26.4%) in the south-central highlands of Ethiopia.[16] *Anopheles ziemanni* was mainly biting humans outdoors in Gambella, but was negative for CSPs.[90] *Anopheles marshalli, An. demeilloni, An. squamosus, An. garnhami, An. cinereus, An. tenebrosus, An. rhodensiensis, An. longipalpis* and other anopheline species were documented in Ethiopia.[86] Many of them exhibit human biting behaviour, and are vectors of malaria elsewhere in Africa.[70] Lastly, it is important to monitor those species that have contact with humans, since they may be involved in malaria transmission, thus complicating control and elimination operations.

1.5. Entomological indicators of malaria transmission

The transmission intensity of malaria can be measured by entomological variables, including the EIR, the longevity and feeding preferences of vectors, the susceptibility of the vectors to parasites and the length of the extrinsic incubation period of the parasites.[106] *Anopheles* mosquitoes with a higher EIR, susceptibility to parasites, longevity and higher human-biting behaviours are potentially more important as vectors than others, and also increase the intensity of malaria transmission.

1.5.1. Vectorial capacity

Vectorial capacity, the number of new infections that are induced by a vector population per case per day in a particular place and time to a susceptible human population, is used to determine the intensity of malaria transmission.[107] There are intrinsic and extrinsic factors that affect the vectorial capacity of malaria vectors.[106] Worldwide, there are more than 528 *Anopheles* species, of which approximately 60 are potential vectors of malaria, and yet they are different in their competence.[45] The difference is also obvious, even between individuals of the same species. The factors which determine the vectorial capacity of a malaria vector species include vector longevity, strong human blood preference, susceptibility of the vector to parasite infection and the duration of sporogonic development (the parasite development within a vector).

1.5.1.1. Vector longevity

Age grouping of adult malaria vectors is important to help understand the epidemiology of malaria and for assessing the efficacy of vector control interventions.[108] Most anti-vector interventions, such as LLINs and IRS, are designed to shorten the lifespan of mosquitoes by killing older mosquitoes, thereby consequently reducing the burden of malaria transmission.[28] Malaria parasites undergo development in the vectors (extrinsic incubation) before transmission occurs, which comprises a significant proportion of the expected life expectancy of the vectors.[28, 106] Hence, those malaria vectors that live long enough allow the parasite to complete the extrinsic incubation period, and become infectious to transmit malaria to susceptible hosts.

The physiological age of female mosquitoes is determined by dissecting their ovaries and grouping them into nulliparous (young) and parous (old) using the Detinova method.[109] Nulliparous female mosquitoes have coiled tracheolar skeins, whereas the parous females have stretched tracheolar skeins. Parous mosquitoes are those

that have oviposited one or more batches of eggs, and therefore, they could potentially transmit parasites because of their repeated contacts with hosts for blood meals. A female mosquito usually becomes infectious after three gonotrophic cycles. Nulliparous females have not laid their first batch of eggs, and are thus not yet infective.

A more precise method of age determination is the Polovodova method, which counts the number of dilatations left after each oviposition in the ovary.[108] The number of dilatations shows the number of times a female mosquito had a blood meal and laid eggs, hence showing both age and the number of gonotrophic cycles (number of contacts with hosts). The more the number of gonotrophic cycles, the more likely the mosquito becomes infectious to susceptible hosts, but the method is difficult and labourious.

1.5.1.2. Physiological competence of vectors to parasites

The *Plasmodium* parasite must complete the sexual stage of its life cycle (zygote to viable sporozoite in the salivary glands) in the body of female *Anopheles* mosquitoes before its transmission to humans can cause malaria.[106, 107] The development and transmission of the malaria parasite is dependent on the competence of the species. Non-anopheline mosquitoes may have human-vector contact and ingest malaria parasites along with human blood, but cannot support the development of the malaria parasites.[110] The absence of developmental signals, specific cellular receptors and parasite-specific resources may justify the inability of non-anopheline mosquitoes to support malaria parasite development.[106] Even within *Anopheles* mosquitoes, some species are natural vectors of malaria parasites and more susceptible to human *Plasmodium* than others.[111] Consequently, only a fraction of parasites ingested complete the extrinsic incubation period in a small proportion of female *Anopheles* mosquitoes due to either the innate immune system of vectors against *Plasmodia*,[112] gut wall barriers during parasite development and the innate ability of a species to permit development of the parasite.[113]

1.5.1.3. Blood feeding patterns

The blood feeding behaviour of anopheline is essential for malaria transmission due to the human-vector interactions, and those vectors that show strong anthropophagic behaviour are more efficient because this behaviour increases the risk of parasite transmission.[114] The genetics and physiology of the vectors (intrinsic factors) and the defensive behaviour of hosts, host species, colour, body heat, body mass and other (extrinsic)

factors may influence the feeding patterns of vectors.[29] Nonetheless, in most conditions, blood feeding is highly influenced by the accessibility of the hosts.[114] For example, blood feeding is primarily associated with reproduction, so to safeguard the reproduction the mosquito may feed even on non-preferred hosts if the hosts of choice are not available.[29] *Anopheles gambiae* has shown a tendency to feed on hosts with previous contact (last encountered hosts) than new hosts.[115]

The feeding patterns of malaria vectors may also be manipulated by malaria parasites, mainly to reduce mortality and ensure efficient parasite transmission to the susceptible hosts.[116] Blood feeding frequency, persistence and the number of probing are relatively high in infectious vectors, which in turn may increase the efficiency of parasite transmission.[117] A sporozoite-infected *An. gambiae* could be more likely to have fed on multiple hosts in one feeding cycle compared to an uninfected one.[118] A parasite-induced feeding behavioural change of infectious vectors may change the transmission pattern of malaria and risk of infection. High feeding activities of sporozoite-infected *An. gambiae* might increase mortality, but it depends on the defensive behaviour of hosts.[119]

1.5.1.4. Sporogonic development period (extrinsic incubation period)

Anopheles mosquitoes take gametocytes (male and female) while feeding on infected hosts.[120] Gametocytes transform into female and male gametes inside the gut and fuse to form zygotes, and then transform into the motile ookinetes. Ookinetes pass the mid-gut epithelial cells and form oocysts on the outer surface of the mosquito gut. The nuclei divide and form sporozoites in the oocysts, and yet, these stages are not infective to humans. When mature, the occysts burst and release sporozoites, which spread throughout the haemocoel. Some enter into salivary glands and further develop to become infective to a human host.

The vector-parasite interaction is very complex, and is determined by several factors. It is well-known that temperature plays a role in parasite development inside vectors.[121] The duration of the extrinsic cycle of the malaria parasite is shorter at a higher temperature.[121] If the extrinsic incubation period is short, the vectorial capacity may be high, even if the daily survivorship of the mosquito is relatively low.[45] However, the impact of temperature on the sporogonic cycle varies between *Plasmodium* species. At 25°C, for example, the extrinsic incubation period is faster for *P. vivax* (10 days) than *P. falciparum* malaria (12 days). This means a *P. vivax*-infected vector becomes infective to humans in a shorter time than *P. falciparum.*[122] Hence, intervention tools

targeting on the longevity of vectors should consider this variation for effective control of both types of malaria parasites.

1.5.2. Entomological inoculation rate

The entomological inoculation rate is the estimated number of infective bites a person receives in a given time in a defined place.[123] It is a product of sporozoite rate (SR: the percentage of mosquitoes with sporozoites in their salivary glands) and human biting rate (HBR: the number of vectors attempting to bite an individual over a fixed period of time).[123] Changing the values of any of this variable changes the value of the EIR. The estimated value of the EIR is used to quantify the levels of human exposure to the infectious bites of malaria vectors, and measures the intensity of malaria transmission.[13] Moreover, the EIR is one of the sensitive and important parameters for evaluating the impact of public health interventions against malaria vectors, but the accuracy and precision of HBR and SR may affect the reliability of the EIR.[124]

Accuracy and precision are associated with a lack of standardized collection methods for SR and HBR. Different studies use different collection techniques to estimate SR and HBR, which makes the comparison of studies difficult.[123] The HLC is the most direct and widely used to estimate HBR. The method involves collecting landing mosquitoes from volunteer human baits positioned indoors and outdoors by exposing their legs at night. However, this may not reflect the population level of exposure if the distribution and utilization of anti-vector control interventions are high.[124] Moreover, the HLC may overestimate the HBR if insecticides on LLINs and IRS lose their killing effects since mosquito collectors may be challenged with an increased number of mosquitoes. The sporozoite rate is estimated using salivary gland dissection, ELISA (most common) and PCR (rarely) techniques.[123] The subjectivity of salivary gland dissection and the false positivity of ELISA are some sources of uncertainty for SR. CDC light traps, PSC and exit traps are other methods used to estimate HBR and SR.[123] The uncertainty of SR and HBR make the estimation of the EIR difficult. Hence, EIR data is rarely available in many malaria endemic countries in Africa, including Ethiopia.[123] Also, measuring the impact of intervention using the EIR may be difficult unless the standard vector sampling method for HBR and the accurate assay technique for SR is established.[124]

The EIR of the principal malaria vectors is greatly reduced in many malaria-endemic countries after the intense anti-vector control interventions, such as LLINs and IRS. However, the EIR could not be lower than one

infectious bite/person/year to move from control to the elimination of malaria.[13] Based on the EIR analysis, LLINs and IRS are prefect tools to reduce the EIR, but are not sufficient to interrupt malaria transmission, as none of the interventions resulted much below one infective bite per person per year.[13, 125] The integrated vector control strategy has been advocated to supplement the core intervention tools for the further reduction of EIR.[126] For example, implementing LLINs and improved housing against adults, as well as microbial larviciding against the aquatic stages of malaria vectors, substantially reduced the EIR compared to LLINs alone.[127]

1.6. Malaria vector control

The control of human malaria has relied on anti-vector control strategies since the discovery of the malaria-mosquito association.[5] The use of larval source management (LSM) was the principal malaria vector control method before the DDT era. Housing improvement had a substantial role in both the US and Europe. The IRS of DDT was the cornerstone for the 1950s and 1960s malaria eradication campaign, and some countries achieved eradication, while many others reduced the geographical distribution of malaria.[128] Even after the phase out of the eradication programme of the WHO, IRS continued as the main vector control tool. Conventional ITNs were introduced in the 1970s, and both ITNs and LLINs have been widely scaled up since the 2000s for malaria vector control.[3] In 1980, zooprophylaxis was recommended as a component of vector control interventions by the WHO.[129] The contribution of both IRS and LLINs are immense for the current global malaria reduction effort.[3] In addition to LLINs and IRS, improving housing, LSM and zooprophylaxis are also potential candidates. Moreover, the integrated vector control approach, using the combination of available tools against malaria vectors, is likely the most effective.

1.6.1. Insecticide treated nets and LLINs

Since 2005, the coverage of malaria vector control interventions has tremendously increased in most malaria-endemic countries.[4] Insecticide treated nets and LLINs are fundamental tools mainly used against indoor resting and biting malaria vectors.[130] Unlike LLINs, ITNs need a frequent retreatment of insecticide, which was a challenge for the communities in malaria-endemic areas to accomplish, and it was substituted by LLINs in most places. LLINs significantly reduced malaria incidence and mortality in many malaria-endemic countries in Africa.[3, 4] As part of a global malaria strategic plan, the Roll Back Malaria partnership set goals for the universal coverage of interventions, and to reduce the incidence of malaria by 75% in 2015 compared to

2000.[131] LLINs are the most prioritized interventions to achieve these goals. The cost-effectiveness and acceptance of LLINs make them the most important tools to control malaria vectors. According to the WHO 2015 malaria report, LLINs contributed more than 60% in averting malaria incidence and deaths globally.[4] Bhatt *et al*. identified LLINs as the most valuable contributor in averting an estimated 68% of malaria cases.[3]

The nets are designed to avoid human-vector contact, with the chemicals impregnated to repel and/or kill those mosquitoes entering houses and attempting to feed on humans under insecticide treated nets.[130] The effectiveness of nets is guaranteed when the vectors are susceptible to the insecticides.[132] The wide spread of pyrethroid insecticide resistance in the population of malaria vectors may compromise the effectiveness of nets.[19] However, those people sleeping under nets are still getting protection from the infectious bites of mosquitoes because the nets are acting as physical barriers.[3]

1.6.2. Indoor residual spraying

The insecticidal activity of DDT first took place in early 1940, with DDT-based IRS bringing a radical change in malaria vector control.[133] It was mainly used by military personnel during World War II, and was successful in killing indoor resting malaria vectors and reducing malaria transmission.[134] DDT has a long residual effect on the wall of houses, and was either applied once or twice a year. It was introduced in many national malaria vector control programmes during the late 1940s and early 1950s.[134] In the south of the Sahara region, Monrovia, the capital city of Liberia, was the first site used in implementing large-scale DDT house spraying in 1945.[135] It was planned to assess the feasibility of malaria eradication in tropical Africa.

The success of DDT in the 1940s and early 1950s helped to convince global communities to launch the 1955 malaria eradication programme.[128] Thus, malaria was eliminated from several countries in Europe, the Americas, Asia and Australia.[136] It also played a substantial role in shrinking the geographical distribution of malaria, mostly in Asia.[128] The least amount of success was achieved in Africa, possibly due to political conflicts, a lack of trained personnel, transportation difficulties in rainy seasons and weak health infrastructures.[136] The effectiveness of DDT against agricultural pests and household insects made prices go up (including financial constraints for DDT use in 1951), and its widespread application rapidly led to the appearance of vector resistance in Greece in 1949.[137]

In Ethiopia, an organized malaria control programme was first initiated at the national level in 1959, during which DDT was used in pilot projects.[11] Four pilot projects (the Upper Awash Valley, the Kobo-Chercher plain, the Dembia plain and Gambella) were established to assess the technical feasibility of malaria eradication in both highlands and lowlands. The first national malaria eradication training centre was established in Nazareth in the late 1950s. In the 1960s, a national malaria eradication service was launched based on DDT IRS, and malaria was significantly reduced form parts of the country.[11] However, this campaign was replaced by a malaria control programme in the 1970s aimed at reducing malaria mortality and sickness.[136] The Division for Malaria Control (1979-1985), and the later national organization for Malaria and Other Vector-borne Diseases Prevention and Control (1986-1993), was created under the Ministry of Health to coordinate malaria control in Ethiopia.[138] The benefit of the malaria eradication campaign was substantial in Ethiopia for those people protected by DDT, and only DDT was used for IRS until the 1990s. Malathion was only considered in areas with DDT resistant *Anopheles* mosquitoes.[12] In the 1980s and 1990s, the frequency of malaria epidemics and its burden was increased, as the health infrastructure collapsed due to civil war and an acute shortage of vector control personnel mainly because of retirement.[139] Starting in early 1990, the operation of IRS was decentralized to the regional and district health teams, but a lack of technical personnel at the district level became a bottleneck for the operation.[11]

Both DDT and malathion were continued to be used as spray chemicals based on the status of local vector resistance. DDT use continued until 2009 and was replaced by delthametrin, which was then shortly replaced by carbamate insecticides for IRS,[11] as *An. arabiensis* populations developed a resistance to pyrethroid insecticides in most parts of the country.[20, 21] The extensive use of pyrethroid insecticides, both for IRS and LLINs, might shorten the efficacy of pyrethroid insecticides.[19] The resistance of *An. arabiensis* to DDT may have contributed to the rapid spreading of resistance to pyrethroid insecticides, since the two classes of insecticides have similar mode of actions.[18] The use of insecticides with a similar mode of action for IRS and LLINs was against the WHO recommendation, which encouraged using insecticides with different modes of actions to delay resistance development in public health vectors.[18, 19]

Indoor residual spraying prevents malaria transmission by killing vectors that spread malaria parasites. Those malaria vectors that rest indoors on insecticide-sprayed wall surfaces are the most targeted species. For IRS to be effective, at least 80% of homes need to be sprayed. However, the IRS programme can face resident refusal

and re-plastering, which may influence the effectiveness of the operation.[140] The improper use of IRS against the guidelines on dose and application might also affect the effectiveness of IRS, thus leading to insecticide resistance development.

1.6.3. Larval source management

Before the investigation of the insecticidal property of DDT, malaria vector control mainly relied on LSM, which target mosquitoes at aquatic stages to prevent completion of the development of immature stages.[141] Larval source management includes habitat modification (permanently destructing breeding sites), larviciding of breeding sites (application of chemical or biological insecticides), biological control (using predators) and habitat manipulation (temporarily making the breeding sites unsuitable). Larval source management has been used by the Tennessee Valley Authority in the United States[142] and Panama during the canal construction.[143] In Brazil, *An. gambiae* (recently identified as *An. arabiensis* by PCR)[144] was successfully eliminated mainly by the well-targeted application of Paris green on breeding sites, and was sometimes supplemented by pyrethrum house spraying to target adult mosquitoes.[145] Malaria had declined with the subsequent elimination of *An. gambiae* from Brazil.[145] The same strategy of applying Paris green larviciding supplemented by pyrethrum house spraying was followed in Egypt in 1944 to 1945 to eliminate *An. gambiae* s. l.[146] Historically, Paris green and petroleum oils were the most successful and widely used chemicals for larval control. In some parts of Africa, larval control using bacterial agents has shown promising results.[127] In Ethiopia, LSM, such as the drainage of mosquito breeding sites and larviciding with Temephos (Abate), are thought to be effective in urban areas, resettlement villages and military camps.[12]

The behaviour and ecology of the vector species might determine the efficacy of larval control. For instance, *Anopheles gambiae* often breeds in small, temporary rain pools, which are numerous and difficult to locate.[127] Larval source management would be very effective if many of the mosquito breeding sites were identified and well defined.[147] Chemical or biological larviciding and habitat manipulation can play a substantial role in resistance management by killing resistant-malaria vectors in aquatic stages.[148] Those malaria vectors that tend to bite and rest outdoors (less targeted by IRS and LLINs) can also be killed at the aquatic stages.

1.6.4. Housing and malaria

The link between poor housing and the higher risk of malaria infection has been well documented. Improved housing played substantial roles in malaria vector control and elimination programmes by breaking human-vector contacts.[23] In Missouri in the US, screening houses provided a considerable degree of protection from malaria vectors, and the incidence of malaria was highest in houses without screening.[149] In Tennessee River area in the US, a substantial reduction in the incidence of malaria was obtained by improving rural houses.[150] From the 1910s to the 1930s in Italy, Greece, Panama, India and Malaysia, people were protected from the infectious bites of mosquitoes by modifying their houses.[23, 151] Angelo Celli from Italy conducted house screening interventions in 1900, and has reported a substantial lower number of malaria cases in screened houses than those without screening.[152] In 1900, Patrick Munson conducted a house screening study against malaria in Italy, and reported a huge reduction of malaria vectors.[152] The British army in Pakistan and India benefited from screening their barracks against malaria.[153] Regardless of all its success stories, house screening interventions were ignored due to the development of new and effective insecticides.[23]

In Africa, the majority of human exposure to malaria vectors occurs indoors,[22] so improving housing conditions and screening can break the transmission cycle by preventing the entry of mosquitoes. The modification of houses reduced the entry of *An. gambiae* by 78%-80%, while the closing of the eaves of houses reduced entry by 43% in the Gambia.[154, 155] The indoor density of host-seeking *An. gambiae* was reduced by 59% in fully screened houses in the Gambia.[24] The screening of houses provides equal protection for all occupants, and has no contribution to insecticide resistance.[156] In Uganda, children in modern homes had lower malaria episodes than traditional homes.[157] If the malaria vectors predominantly bite indoors, screening houses reduces house entry and minimizes indoor human-vector contacts.

Currently, improving housing is getting attention insofar as complementing existing core interventions.[158] Despite the successful impact of housing interventions on vectors and incidences of malaria, there are still unanswered questions that need to be addressed before the scaling up of the intervention.[159] The protective efficacy of specific housing structures, the socio-economic and cultural diversity of housing and the cost of building are among major unanswered questions associated with housing interventions. But nevertheless, the rapid economic development in malaria endemic countries might support the implementation of housing interventions.[159]

1.6.5. Zooprophylaxis

Zooprophylaxis is the use of animals to divert malaria vectors away from human hosts.[129] However, the results are controversial; some claim that animals reduce malaria infection, whereas others make claims for zoopotentiation (animals increase mosquito bites and malaria infection). In the1980s, the WHO advocated zooprophylaxis as a supplementary malaria vector control.[129] Services in the1990s reviewed the potential of domestic animals in protecting humans from malaria vector bites and malaria transmission.[160] In most cases, the reduction of domestic animals due to changes in agricultural systems was associated with an increase of human bites.[160] The most anthropophagic malaria vector *An. gambiae* even had animal blood meals when the animal population was higher than that of humans.[160] On the other hand, an additional blood meal source of animals was claimed by increasing the density of vectors and human biting rates. Interestingly, the reduction of the livestock population resulted in a shift of zoophilic malaria vectors to bite humans.[160] Moreover, the proximity of cattle to human houses has been associated with increased bites of malaria vectors and the risk of malaria infection.[161] Hence, the number of animals and the way the animals are deployed may influence the impact of zooprophylaxis.

It is known that a passive zooprophylaxis reduces infectious bites and parasite transmission by diverting vectors to the dead-end hosts, and that this has had an impact on malaria transmission in that the infectious vectors "waste" their sporozoites; the susceptible mosquitoes cannot acquire parasites from animals (dead-end hosts).[162] The impact of zooprophylaxis can further be enhanced by the treatment of animals with insecticides (insecticide zooprophylaxis) to kill those vectors that feed on animals (longevity of vectors is the most important component of vectorial capacity) and reduce malaria transmission.[163] Insecticide zooprophylaxis has been recommended to enhance the role of animals in vector control.[164] Rowland and his colleagues who worked in Pakistan have confirmed the effectiveness of insecticide zooprophylaxis against zoophilic malaria vectors.[165] Spraying cattle with pyrethroid insecticide resulted in a 56% reduction of malaria incidence.[165] In Africa, the treatment of cattle by non-repellent and stronger insecticides is suggested to control *An. Arabiensis,* a species that exhibits both anthropophagic and zoophagic tendencies.[164] Pyrethroid insecticides are recommended for the treatment of cattle, but *An. arabiensis* is highly resistant to these insecticides in most parts of East Africa, including Ethiopia.[18, 19] Moreover, topical applications of pyrethroid insecticides were subject to weathering, easily washing off from animals' bodies and less effective.[166] Hence, the use of systemic insecticides is recommended for the treatment of domestic animals, so the vectors ingest insecticide when

feeding on the various body parts of animals. The inclusion of insecticide zooprophylaxis into an integrated vector management (IVM) package may be used to control residual malaria transmission by killing outdoor feeding malaria vectors. However, it is important to conduct more rigorous research to decide when and how to use zooprophylaxis in IVM.

1.6.6. Genetic control

The genetic control method of insect vectors includes the release of males that are sterile (sterile insect technique) or contain lethal genes, and the replacing of genes of the parasite-susceptible population with refractory genes, thereby preventing the transmission of *Plasmodium* (transgenesis).[167] With the sterile insect technique (SIT), males are sterilized by radiation or chemosterilants, and released into wild populations to compete for wild females so that no progeny are produced. The males that contain lethal genes pass the traits to progeny, and hence the progeny may die before parasites complete their life cycle. Therefore, the former two strategies are to crush vector populations,[168] while transgenesis focuses on population replacement.

The sterile insect technique has been widely used in agricultural pest control, and has brought the elimination of some pests. For example, tsetse fly species *Glossina austeni* have been eradicated by using SIT in Zanzibar.[169] The application of SIT is less suitable for malaria control, as multiple vectors are involved in transmission.[168] It also requires the frequent release of large numbers of sterile males to improve the effectiveness by increasing the chance of mating, though the males are less likely to compete with wild males.[170] For an effective use of SIT, it is quite important to select areas with a low vector density, and a good geographic or biological isolation.

1.6.7. Biological control

Biological control is the use of biological agents to control malaria vectors in adult or larval stages. *Bacillus thuringiensis* and *B. sphaericus* are the two of the most widely used larviciding strains of bacteria which produce toxic crystals that degenerate the gut and kill mosquito larvae when they feed on spores.[171] Attention has been given to entomopathogenic fungi due to their effect on the adults of *An. gambiae*. The mode of action of fungi is different from bacterial, viral and protozoan agents in that mosquitoes take a lethal dose by contact (like insecticides), but not by ingestion.[172] Some strain of fungi (for example, *Metarhizium anisopliae*) caused a 100% mortality in *Anopheles* mosquitoes 7-14 days post-application.[172] Moreover, pyrethroid insecticide-

resistant populations of *An. gambiae* were highly susceptible to pathogenic fungi compared to the susceptible populations, and hence it can be used in insecticide-resistant management, which will selectively remove the resistant genes from the population.[173] Interestingly, the pathogenic fungi kill mosquitoes slowly, which reduces the selection pressure, and thus resistance development is likely low.[172] Fungal infections caused a higher mortality of *Plasmodium* positive- than negative mosquitoes, thereby reducing malaria transmission. Furthermore, the blood feeding success of vectors is affected by a fungal infection, which in turn may affect malaria transmission by reducing human-vector contact.[172] Like many insecticides, pathogenic fungi can be applied on the indoor and outdoor resting surfaces of malaria vectors.

Gambusia affinis is the most widely suggested fish species in controlling the larvae of malaria vectors in different parts of the world since 1905.[174] In Somalia, the tilapia fish has shown a substantial impact on the density of *An. arabiensis* and malaria parasites.[175] In Assab, Eritrea (then in Ethiopia), *Aphanius dispa,* a native larvivorous fish, has significantly declined the density of mosquito larvae in intervention villages compared to the control villages.[176] However, in many cases, the introduction of exotic fish species into new aquatic habitats has been discouraged because of the unintended consequence on native fishes and beneficial organisms.[174] Moreover, most studies did not report on the impact of larvivorous fishes on the density of adult malaria vectors, which are the proxy indicator of malaria transmission than larval stages. Furthermore, a recent Cochrane Database of Systematic Reviews on larvivorous fish has concluded that the evidence generated so far on the impact of larvivorous fish on malaria vectors and parasites is insufficient to promote this method as a malaria vector control tool.[174] Hence, high-quality evidence of the impact of larvivorous fishes on adult mosquito density and infectivity, and malaria transmission, is needed to consider this method.

1.6.8. Repellents

Repellents are products that reduce the bites of malaria vectors, and therefore malaria transmission. Olfactory cues are the principal strategy of biting insects to locate their hosts.[177] Their importance is substantial in targeting those vectors biting in early hours and outdoors, such as *An. arabiensis*. Special attention has been given to *An. Arabiensis,* as its importance is increasing after the intense use of LLINs and IRS.[178] Even so, the mode of action of repellents is very complex and poorly understood. Host-seeking insects detect the odour from hosts when the odour molecule enters through the pores on the sensilla of the antennae, palps or proboscis, and then binds with odourant binding proteins, which in turn bind to odour receptor neurons.[177] The

depolarization of odour receptor neurons occurs to carry the signals to the brain, and the insect responds accordingly. For example, the repellent N, N-Diethyl-3-Methylbenzamide (DEET) blocks the odour receptor neurons so that the insect cannot detect the odour. It prevents humans from receiving the bites of mosquitoes and other biting insects by blinding their senses to certain chemical attractants contained in human odours. Some repellents interfere with the host-attractant signals of vectors (attraction inhibition), so the vectors cannot locate hosts and therefore inhibit blood feeding.[177] Repellents can be synthetic or plant-based. DEET is the oldest and the most widely used synthetic repellent against biting insects.[179]

1.7. Challenges of vector control

The current interventions against malaria vectors are effective, and have brought a substantial change in the epidemiology of malaria.[3] However, the gain is suffering from many challenges. The behavioural plasticity of malaria vectors is among the threats of vector control. Some malaria vectors tend to bite during the early night and morning when no protection is used.[180] Some species also avoid indoor-based interventions, preferring to rest outdoors after feeding. Nonetheless, the most important threats of malaria control could be the development of insecticide resistance by most major malaria vectors.[19]

1.7.1. Insecticide resistance

Insecticide resistance is the ability of a vector population to withstand a standard insecticide dose which is lethal to susceptible populations, thereby most likely leading to the failure of vector control programmes.[107] The extensive use of DDT for the control of agricultural pests and public vectors led to the first emergence of resistance in *An. sacharovi* in 1949 in Greece and *An. quadrimaculatus* in the US.[128] Dieldrin-resistant *An. gambiae* was reported in Nigeria in 1955, while DDT resistance was reported in 1958. In the 1960s, DDT-resistant *An. gambiae* was identified in Togo, Senegal and Sudan.[181] In the 1960s, DDT resistance detrimentally affected the malaria eradication plan.

In the 1970s, resistance to DDT and the incidence of malaria increased in developing countries.[18] In the 1980s, DDT was partially replaced by organophosphate and pyrethroids insecticides. In Ethiopia, DDT-resistant *An. arabiensis* populations were reported in the1990s, and resistance became widespread throughout the country. Pyrethroid insecticide resistance was first detected in East Africa in Sudanese *An. arabiensis* populations in the 1970s and in the *An. gambiae* of West Africa (Côte d'Ivoire) in the 1990s.[182] At present, pyrethroid resistance

is widespread, and a principal challenge of malaria control and elimination efforts in many countries in Africa (Figure 6). The principal malaria vectors are resistant to pyrethroids used for the treatment of LLINs and all classes of insecticides used for IRS in many malaria-endemic countries.[18, 19] Although organophosphate and carbamate insecticides are currently being used for IRS, resistance to these insecticides is also increasing in many African countries (Figure 7).

In Ethiopia, pyrethroid resistance is widely distributed in populations of *An. arabiensis*.[19] The resistance of *An. arabiensis* to malathion has been reported, while the vector is susceptible to pirimiphos-methyl and the carbamate insecticides, bendiocarb and propoxur.

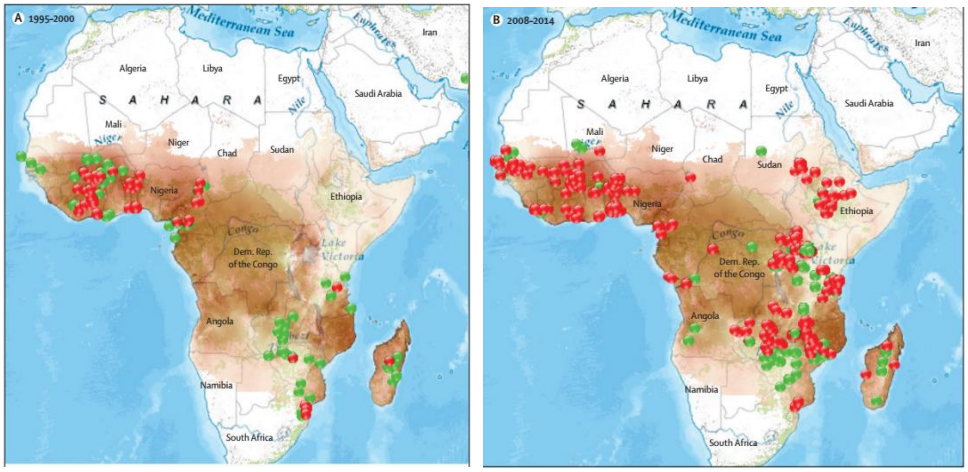

Figure 6: Distribution of pyrethroid resistance in African malaria vectors (A) 1995-2000 (B) 2008-2015. Red dots show resistant populations according to the WHO definition of less than a 90% mortality after exposure to a discriminating dose; green dots show susceptible populations.[19]

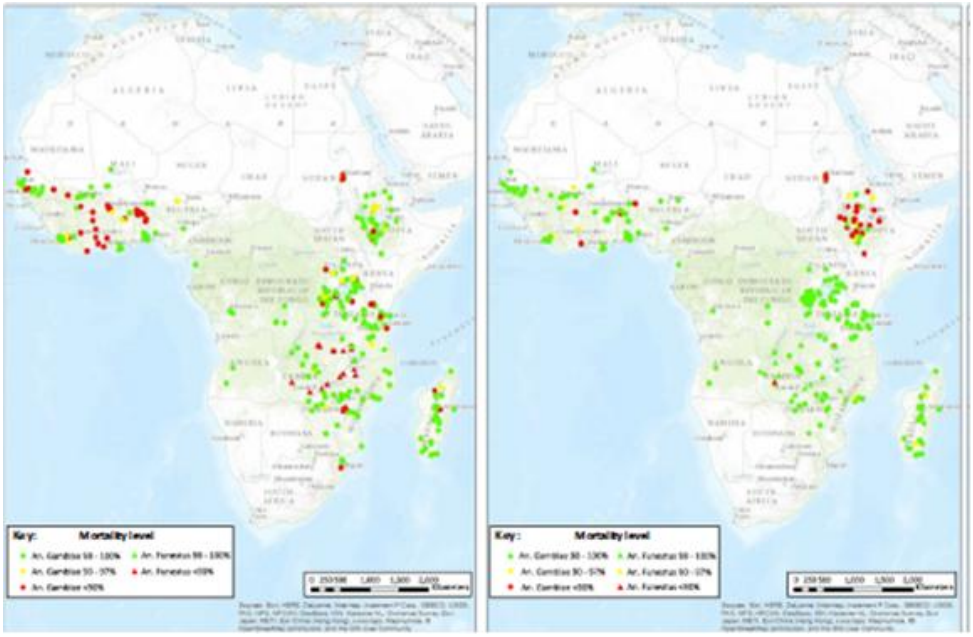

Figure 7: The distribution of carbamate (*left*) and organophosphate (*right*) insecticides resistance in Africa[18]

1.7.1.1. Insecticide resistance mechanisms

Resistance of vectors to insecticides can be due to target-site insensitivity, metabolic resistance, a lower penetration through their cuticle or behavioural responses.[183] Metabolism and target site insensitivity are the most important and common types of resistance mechanisms. Target-site insensitivity is due to the mutations of the proteins targeted by the insecticide, whereas metabolic resistance is a biodegradation of the insecticide due to the over-expression of detoxifying enzymes.[184] Some vector species developed multiple resistance mechanisms to insecticides of different classes, which make the control programmes extremely difficult and expensive. A reduction in the rate of the penetration of the cuticle may contribute to both metabolic and action site-insensitivity mechanisms.[185]

Metabolic resistance: Metabolic resistance is associated with the over-expression of the genes encoding the major enzymes detoxifying insecticides before reaching the target sites (receptors or enzymes in the nervous

system), and makes the insecticides ineffective.[186] Metabolic resistance mechanisms are more challenging compared to other types of mechanisms, and might influence vector control programmes unless appropriate resistance management strategies are implemented.[187] It is known by conferring resistance to all four classes of insecticides.[188] There are three main gene-regulated insecticide detoxifying enzymes. These are oxidases (cytochrome P450), glutathione S-transferases (GST) and esterases.[186] Some enzymes are multifunctional and detoxify insecticides from different classes.[183]

Cytochrome-P450 monooxygenase enzymes (mainly associated with pyrethroid resistance) are the most diversified (more than 100 identified) and are very efficient in detoxifying insecticides,[189] which might make this resistance mechanism the most problematic and difficult for management. Some cytochrome-P450 enzymes, in particular CYP6M2, are capable of detoxifying both pyrethroid and DDT, indicating cross-resistance between the two insecticide classes.[190] The over-expression of P450 alleles conferred resistance to carbamates and DDT in *An. gambiae,* thus indicating that P450 is able to confer resistance to insecticides of different classes.

Glutathione S-transferase is the metabolic enzyme identified in the four classes of public health insecticides. In DDT-resistant *An. gambiae,* the production of GSTs was much higher than the susceptible *An. gambiae*.[191] Glutathione S-transferase primarily metabolizes the organochlorine insecticide DDT and has a secondary role in detoxifying organophosphate insecticides, which indicates the role of GSTs in resistance mostly to DDT, and to a lesser extent to organophosphates.[192] The over-expression of GSTs also played at least a secondary role in conferring resistance to pyrethroid insecticides.[193]

Esterases are also contributing to the resistance of organophosphate insecticides due to the over-production of ester bonds hydrolyzing or sequestering enzymes. In resistant populations of *An. culicifacies* and *An. subpictus,* esterases metabolize the organophosphate insecticide malathion.[194] A recent study from Benin described the role of esterases in resistance development in *An. gambiae* to pyrethroids and carbamates.[195]

In Ethiopia, there is no conclusive evidence on the metabolic resistance mechanisms of *An. Arabiensis,* but the possible involvement was determined by bottle bioassay for some public health insecticides.[196] It is very clear that information on the metabolic resistance mechanisms of *An. arabiensis* is urgent for planning and implementing resistance management strategies to elongate the functional life of available insecticides. There

is the need for new technologies to identify all the potential markers of metabolic resistance for an effective monitoring and management of resistance.

Target site resistance: Target site resistance mechanism is a common type of resistance in malaria vector populations that contributes to resistance for all classes of public health insecticides.[186] Organophosphate and carbamate insecticides inhibit acetylcholinesterase (AChE) (an enzyme which hydrolyzes acetylcholine in nerve cell synapses of susceptible populations), leading to the paralysis and death of susceptible populations.[197] In resistant vector populations, however, the insecticides cannot bind with the enzyme due to the modification of amino acid sequences in AChE, and hence the enzyme continues its normal function.[197]

DDT and pyrethroid insecticides target the voltage-gated sodium channel proteins of the nerve cells membrane.[198] They stimulate the nerve cells to produce repetitive impulses by preventing the voltage-gated sodium channels from closing so that the channels conduct sodium continuously, which might cause paralysis and death in susceptible populations. The channel becomes insensitive to DDT and pyrethroid in resistant vector populations because of the modification of channel proteins (mutation of genes encoding the channel proteins and hence the substitution of some amino acids), which inhibits the binding of insecticides.[186] The most common form of resistance to DDT and pyrethroids is the knockdown resistance (*kdr*).[199] L1014S and L1014F are the common types of *kdr* mutations in African malaria mosquitoes.[18]

A recent study conducted by Alout *et al*.[200] demonstrated the association between the insecticide-resistant strain of *An. gambiae* and the prevalence of the sporozoite stage after the feeding of infectious blood meals by membrane feeding assays. The study concluded that insecticide-resistant strains in general had a higher prevalence of sporozoite stage in their salivary glands than the susceptible strains, but the relative mean sporozoite infection was higher in *kdr*-resistant *An. gambiae* (not statistically significant) than AChE, which might raise a concern for vector control because of the widespread existence of pyrethroid-resistant (*kdr*) malaria vectors. In Ethiopia, the extensive use of pyrethroid insecticides, both for IRS and LLINs, as well as the wide distribution of DDT-resistant *An. Arabiensis,* might result in the wide spread of pyrethroid resistance in the vector populations.[20, 21] *Kdr* resistance is well documented in Ethiopia in *An. arabiensis* populations.[20, 21] The resistance of *An. arabiensis* to malathion has been confirmed by WHO bioassay in Ethiopia,[201] but the target site insensitivity alleles for AChE has not yet been documented.

Cuticular resistance: Cuticular resistance is considered as a minor resistance mechanism, in which the cuticle of insects is modified and become less permeable to insecticides. The modification of cuticle slows down the absorption of wide classes of insecticides (non-specific mechanism of resistance). For malaria vectors in particular, the cuticular resistance is primarily due to the thickening of the tarsal cuticle. The thickness of the cuticle was higher in pyrethroid insecticide-resistant *An. funestus* than the susceptible females, which might justify the association between cuticular thickness and pyrethroid resistance.[185] The production of some proteins increased in pyrethroid-resistant mosquitoes, which may be associated with pyrethroid resistance.[202] The contribution of cuticular resistance for phenotypic resistance is not known, and therefore should be studied.

1.7.1.2. Resistance detection mechanisms

The WHO tube test bioassay using fixed diagnostic doses of insecticides is the most widely used technique to assess resistance in malaria vector populations, and interpreted based on the cut point of the WHO.[203] The method is very simple to perform, is standardized and is used in routine resistance monitoring. But it lacks sensitivity, does not provide information on the type, level and mechanism of resistance, and is preformed using live mosquitoes.[203] The level of resistance can be determined by using dose response bioassays.[203]

CDC bottle bioassay is the method used to determine the time by which the insecticides penetrate the body of insects and reach the site of action, and any delay of these processes contribute to the resistance of vector populations.[203] It is used to monitor insecticide resistance, such as the WHO tube test bioassay. The CDC bottle bioassay is also simple, rapid and economical, and provides information on the presence/absence of resistance in vector populations like the WHO tube test bioassay. Unlike the WHO tube test, the CDC bottle bioassay allows for the evaluation of different concentrations of insecticides (not fixed dose), and simple and multiple resistance mechanisms.[203]

Biochemical and molecular assays are sensitive and specific techniques for detecting resistance in malaria vector populations. Biochemical assays are used to assess the activities of enzymes contributing to resistance, whereas the molecular assays are used to determine the alleles contributing to target site resistance mechanisms, and also to detect enzyme gene families. A substantial number of resistance mechanisms are not identified (diagnostics are not available for all resistance mechanisms), and therefore there is a need for the

development of molecular markers to identify all the potential enzymes and alleles implicated in the resistance in field malaria vector populations.[204] Understanding the genomic changes associated with insecticide resistance is useful to support insecticide resistance management programmes.

1.7.1.3. Insecticide resistance management

In 2012, the WHO issued the Global Plan for Insecticide Resistance Management (GPIRM) for malaria vectors, urging to ensure timely entomological and resistance monitoring, and to develop and implement comprehensive insecticide resistance management (IRM) strategies.[203] IRM implementation needs the understanding of the insecticide resistance mechanisms and the development of new products.[18, 204] It includes rotation, mosaic application and a mixture of insecticides, in addition to a combination of interventions to reduce the operational problem of resistance.[18, 19, 205] The principal assumption of rotation and the mosaic application of insecticides is to revert the vector to the status of susceptibility by removing selection pressures, whereas the purpose of a mixture of insecticides and combining interventions is primarily to kill resistant malaria vectors.[205, 206]

Rotation of insecticides with different modes of action is a strategy to reduce the selection pressure from insecticides and to delay resistance development within the vector population. The susceptibility of vectors to insecticides, and shortly rotating insecticides, is crucial to protect the building up of resistant genes within vector populations.[187] If the vectors develop resistance to the first insecticide, the introduction of a second insecticide is assumed to reduce the resistant genes in the populations.[187] The operational cost of insecticide rotation may limit its implementation.

Combining two interventions is another type of IRM, and is used to reduce the development of resistance to insecticides. If two insecticide-based interventions are combined, the two insecticides should be with different modes of actions.[187] It can easily be implemented if the human and financial resources are sufficiently available. The cost of combining two intervention tools is high compared to rotation and mosaic strategies.[187] Although the combination of IRS and LLINs is the method currently in use, different studies have reported variable and inconclusive results. A number of randomized controlled trials have shown no added impact of combining IRS with LLINs on malaria incidence and vector density over LLINs alone,[132, 207] whereas other studies have documented significantly added protection, including the reduction of EIR.[208, 209]

The mosaic application of insecticides is the application of insecticides with different modes of actions in a separate manner. Different houses in the same village can be sprayed with insecticides with different modes of actions, or the application can be done on a broad scale in adjacent communities. The mosaic application of pyrethroid and organophosphate insecticides was used to monitor insecticide resistance in Mexico, where the resistance to these insecticides was slowly selected compared to pyrethroid alone.[210] The mosaic application of IRS is practically difficult because it requires huge logistics.[211] The mosaic application of two insecticides with different modes of action on a single bed net can be practical, and might slow down resistance development. But, bed nets treated by two insecticides in different sides are currently not available for public health vector control. The experimental evaluation of bed nets impregnated by two insecticides (carbamates and organophosphates) did not improve vector control compared to bed nets impregnated by pyrethroids.[212]

Insecticide resistance can be slowed down if insecticides with different modes of actions are co-formulated and applied for vector control. The most important role of this approach is that the vectors come in contact with all the insecticides at the same time. Bed nets impregnated by the mixture of insect repellent- and low-concentration organophosphate showed a promising role, especially by killing *An. gambiae* carried *kdr* and AChE resistance genes.[213] However, there are many preconditions to formulate the mixture of insecticides. There should be no cross-resistance between partner insecticides and the decay rate of insecticides should be the same (the residual life of the insecticides).[211] Mixing different insecticides might raise the cost of interventions.[205] At present, there is no mixture formulation of insecticides for public use.

1.7.2. Misuse of LLINs

LLINs are the principal contributor for current malaria reduction globally. LLINs have saved the lives of many children and pregnant women. Deaths due to malaria is surprisingly coming down, which is mainly due to the LLINs.[3] They provide protection by killing mosquitoes and reducing human-vector contacts.[130] Unfortunately, the killing effect could be lost due to a high level of resistance to insecticides used for the impregnation of LLINs. The physical protection (prevent human-vector contact) depends on the permanent use of LLINs. Hence, the gain on eradicating malaria can be sustained if the coverage and use rate of interventions is high in the communities. In typical rural communities, where houses are grass thatched and circular, the position to hang bed nets may not be suitable, possibly leading to less of an adherence in the use of bed nets. Moreover, the use of LLINs for an unintended purpose is observed in some malaria-endemic communities. For example, a

substantial number of LLINs were used for fishing in lake areas of Kenya.[214] Some communities used bed nets to protect and store maize, and for weeding veils or room dividers, but there is a claim that the old nets or worn-out nets are re-used for other purposes.[215] In Ethiopia, net misuse has been uncommon, as reported by a qualitative study conducted in Amhara and Oromia regional states.[216] The concept associated with net misuse may require an understanding of the condition of bed nets and the year of provision.[215] It is clear that the misuse of nets, the lifesaving tool, may not be intentional; as a result, there is a need for education of the communities and a clear follow-up after mass distribution campaigns.

1.7.3. Behavioural resistance of malaria vectors

The behavioural resistance of malaria vectors is associated with a shift of biting and resting behaviours of malaria vectors against current interventions. This behavioural shift of malaria vectors is increasing, and it might affect the malaria control and elimination programmes because outdoor biting and resting malaria vectors are less targeted by LLINs and IRS.[180] These behavioural resistances have a selective advantage,[217] which has been reflected by the wide distribution and abundance of *An. arabiensis* after the intense distribution and use of LLINs and IRS (less targeted by LLINs and IRS) in Africa.[178] The avoidance of indoor-based interventions by malaria vectors was one of the causes for the failure of the 1955 global malaria eradication campaign.[100, 218] Moreover, the changing behaviour of malaria vectors have led to a residual malaria transmission, a potential challenge for malaria control and elimination efforts.[219]

Today, early hour and outdoor biting malaria vectors are complicating malaria transmission.[220] In South Africa, *An. funestus* still prefers to feed on humans, but changed its feeding time to daylight hours to avoid LLINs and IRS.[221] The behavioural resistance of vectors in certain conditions can be operationally more challenging than physiological resistance because it may not be reverted by introducing new insecticides with different modes of action.[180] But new insecticides with different modes of action can effectively control physiologically resistant vectors. It is clear that the behavioural resistance of vectors should be monitored to design appropriate interventions such as LSM to target in the aquatic stage.[148]

1.8. Future prospective of malaria vector control

The current vector control interventions have reduced malaria transmission throughout the world. Despite this significant reduction, malaria transmission continues globally.[8] The rapid spread of resistance against all the

classes of insecticides in most principal malaria vectors might compromise the vector control and malaria elimination effort in Africa and elsewhere.[18] New insecticides are not available for public health vector control, which has made the problem more serious, so it is therefore urgent to develop new insecticides.[222] Although some new insecticides are in the pipeline (and some might be available after 2020),[222] the resistance management strategies should be in place to lengthen the useful life of current and future insecticides. Longitudinal surveillance and monitoring systems should be established to identify insecticide resistance at an early stage (low level of resistance) and implement appropriate insecticide resistance management.[19]

Either inherent and/or influenced by behavioural resistance to insecticides, outdoor resting and the biting habits of malaria vectors challenge malaria control and elimination programmes.[219] Those people active outdoors in the early hours of night are at risk of malaria infections, and no intervention other than repellants is currently available to protect them from outdoor infectious bites of malaria vectors.[219] Hence, the sustainability of the gains, and the future elimination plan of malaria, needs the integration of several tools. The existing intervention tools should be supplemented by improving houses, especially in rural areas, to prevent the entry of vectors into houses where the most transmission is occurring.[25] Moreover, the treatment of cattle with appropriate insecticides to control zoophagic vectors and those changing their behaviour in response to IRS and LLINs (leading to residual malaria transmission) is needed.[180, 219]

1.9. Statement of the problem and rationale of the study

Malaria is a common public health problem in Chano Mille *Kebele* ('village'). It is one of the resettlement sites where the population was moved from the highlands to the fertile lowlands. The movement of a non-immune population from the highlands to malarious sites in the lowlands aggravated malaria transmission in the 1980s in Arba Minch and its surrounding areas. A recent cohort study in Chano Mille has reported the incidence of 3.6/10,000 person-weeks,[27] and little or no entomological components of malaria transmission were studied. Hence, understanding the species composition, and the feeding and resting behaviours of local malaria vectors, have a paramount significance to plan evidence-based interventions. For example, indoor feeding and resting malaria vectors can successfully be controlled by currently available interventions. The changing behaviour of malaria vectors, if any, needs planning supplementary interventions.[180] Assessing the susceptibility status of local malaria vectors is quite relevant in planning appropriate resistance management strategies, and looking for supplementary non-chemical interventions. The study investigated the blood feeding patterns of malaria

vectors by collecting outdoor and indoor resting, and indoor host-seeking for one year (Papers I and IV). Furthermore, the susceptibility status of *An. arabiensis* to insecticides was also assessed, which might be useful for planning effective vector control interventions and the design of IRM strategies (Paper I).

There is a substantial gap of entomological transmission levels of malaria in the study area and most parts of Ethiopia. Thus, the entomological malaria transmission indicators, such as the density of malaria vectors, sporozoite rates and EIRs were investigated, and variations between houses in relation to distance from breeding sites were also assessed to identify those at a higher risk of infection in Chano Mille *Kebele* **(**Paper II**)**.

Paper I shows the predominant indoor human-vector contacts, resistance of *An. arabiensis* to pyrethroid insecticides (the insecticide used for both IRS and LLINs) and DDT, and the higher tendency of malaria vectors to feed on cattle outdoors. A higher risk of malaria infections has been identified in those houses near to the main mosquito breeding sites, along the shore of Lake Abaya (Paper II). This shows the need for supplementary vector control interventions to reduce house entry, hence minimizing indoor human-vector contacts by diverting them to cattle (the dead-end host) available outdoors. For this reason, a randomized control trial was conducted to assess the impact of screening windows and doors by wire mesh, in addition to closing openings on eaves and walls by mud in the sub-village nearest to the main mosquito breeding sites (Paper III).

The bovine blood meal origin of *An. arabiensis* has remained high regardless of collection site (indoor and outdoor) and number of cattle-to-human ratios (Paper IV). It shows the zoophagic feeding patterns of *Anopheles* mosquitoes.

2. Study objectives

2.1. General objective

The main objective of the thesis is to determine the anopheline species composition, and to assess the entomological indices of malaria transmission, as well as seek evidence on the prevention of the house entry of mosquitoes by simple modifications of houses toward an integrated vector management of malaria in Chano Mille *Kebele* in southwest Ethiopia.

2.2. Specific objectives

1. To examine blood meal origins and the insecticide susceptibility status of *Anopheles arabiensis* from Chano Mille *Kebele* (Paper I).

2. To assess the anopheline species composition and entomological indices of malaria transmission (sporozoite and entomological inoculation rates) from Chano Mille *Kebele* (Paper II).

3. To evaluate whether the screening of windows and doors, and the closing openings on eaves and walls, reduces the indoor densities of *Anopheles* mosquitoes in Chano Mille *Kebele* (Paper III).

4. To assess the relative feeding preferences of *Anopheles* mosquitoes in relation to cattle and human host abundance in Chano Mille *Kebele* (Paper IV).

3. Methods

3.1. Description of the study area

This study was conducted in the Southern Nation Nationalities and Peoples' Region (SNNPR) in the Gamo Gofa Zone, the Arba Minch Zuria Woreda ('district') and the Chano Mille *Kebele* (a kebele is the lowest administrative level in Ethiopia) (Figure 8). The Chano Mille *Kebele* is found north of Arba Minch town and 492 km southwest of Addis Ababa, and has three sub-villages (01, 02 and 03). The altitude of the *kebele* at the centre is approximately 1,206 m above sea level, and its geographic coordinate is 6°6'.666" N and 37°35'.775" E. Lake Abaya is close to the study *kebele*. Sub-village 03 is situated close to the shore of Lake Abaya (1350 m -1570 m), with the livelihood of the farmers living there based on agriculture. They mainly cultivate mangos, bananas and maize, both during the rainy season and dry season, the latter by irrigation. Animal ranching is also a common practice; during the night, animals are either kept in separate houses, outdoors in the compound or in communal places. Animals and humans live permanently in the area.

The principal source of irrigation water is the Harrae River, which is about 5 km from the *kebele*. Inside the *kebele* (where the households are found), the canals are well-constructed and the water continuously flows. Water inside the canals flows to farmlands at the edges the *kebele,* where the farmers irrigate their crops, primarily maize and bananas. Due to high evapo-transpiration and a wise use of irrigation water (irrigate every

three-four days), no suitable *Anopheles* mosquitoes breeding sites are available in the dry seasons in and along farm lands. In the rainy seasons, however, water pools in ditches along the roads, and farmlands are the potential breeding sites for *Anopheles* mosquitoes. The shore of Lake Abaya is bare land serving as a communal grazing land. It is covered by big indigenous trees and home for many wild animals. The agricultural land, however, is mainly covered by mango and banana trees.

Chano Mille *Kebele* is one of the 11 malarious *kebeles* in the Arba Minch Zuria Woreda. Malaria is a public health problem,[27] and its transmission is bimodal. Many mosquito breeding sites are found around the shore of Lake Abaya. Water pools formed in cattle and hippopotami hoof prints are the most favorable breeding sites of anopheline mosquitoes. The climate is hot and humid, and thus favourable for mosquito breeding. In 2009, the annual rainfall was 645 mm, and in 2010 1,061 mm. The average minimum and maximum annual temperatures in 2009 were 17.8 and 32.2°C, respectively, and in 2010, 17.9 and 30.2°C, respectively. There are two rainy seasons, one in March-May (main rainy season) and the other in October-November (short rainy season).[223] The altitude of the weather station is 1,200 m above sea level, which is about the same as Chano Mille *Kebele*. There is a health post at the centre of the *kebele* to provide primary care for the community.

The human population of the *kebele* is 6,661 people, and the number of cattle 2,217. Goats, sheep, donkeys, and chickens were also present. LLINs and IRS are the primary malaria vector control interventions in the *kebele,* which are implemented by the Ethiopian Federal Ministry of Health (FMoH). Grass thatched and corrugated iron-roofed houses are found in the *kebele*, but corrugated houses are rapidly being substituted for grass thatched houses.

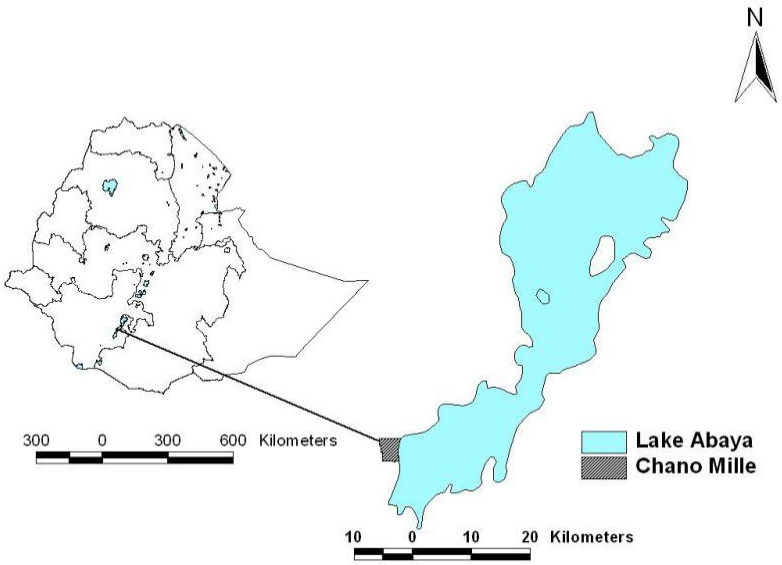

Figure 8: Map of Ethiopia and location of Chano Mille *Kebele*

3.2. Anopheline mosquito sampling

3.2.1. Larval and pupal samplings and insecticides bioassay test (Paper I)

Anopheles larvae and pupae were collected using a standard dipper from natural breeding habitats around the shores of Lake Abaya and the Harrae River, and reared to adults in the entomology laboratory at Arba Minch University under temperatures between 26.2-26.9° C and a relative humidity of 72-84%. The larvae were not provided with any additional food, while the pupae were transferred to 30 cm×30 cm ×30 cm mosquito cages. The adults were provided with a sterilized 10% sucrose solution soaked in cotton pads at the top of mosquito cages.

Anopheles gambiae complex (presumably *An. arabiensis*) were identified using a morphological key and used for susceptibility tests.[57] The susceptibility of two-four-day-old female *An. arabiensis* to insecticides was carried out following the standard World Health Organization (WHO) protocol.[203] Batches of healthy 20 mosquitoes in tubes were exposed to filter papers impregnated with cyfluthrin (0.15%), lambdacyhalothrin

(0.05%), alphacypermethrin (0.05%), deltamethrin (0.05%) and DDT (4%). Four replicates of tests and two replicates of control were also carried out for each insecticide. The knock-down effect of each insecticide was recorded every five minutes during a one-hour exposure period. The mosquitoes were then transferred to holding tubes and kept for a period of 24 h in a ventilated box in an insecticide-free room. All mosquitoes were supplied with a sterilized 10% sucrose solution during the 24-hour recovery period, and a favourable humidity and temperature were maintained by placing a damp towel on the box.

The number of mosquitoes alive and dead, as well as the percentage of mortality was then recorded. When the average mortality of the control mosquitoes was between 5%-20%, the test mortality was corrected by use of Abbott's formula.[224] The susceptibility or resistance of mosquitoes to a given insecticide was determined following the criteria of the WHO basis for the percentage of mortality.[203] The criteria for the interpretation of susceptibility test results are the following: A mortality rate between 98%-100% shows a full susceptibility. When the mortality is in the range between 90%-97%, it is indicative of the presence of resistance, so further confirmation may be needed. A mortality rate of less than 90% confirms resistance in the mosquito populations.

3.2.2. Adult *Anopheles* mosquitoes sampling methods (Papers I, II and IV)

To determine the entomological indices of malaria vectors, a longitudinal entomological study was conducted in Chano Mille *Kebele* for one year (May 2009 to April 2010) (Figure 9). Adult *Anopheles* mosquitoes were collected by CDC light traps, PSC and from artificially constructed pit shelters in all three sub-villages. For each of the methods, mosquito collection was preformed bi-weekly for 12 consecutive months. Moreover, a verbal consent was obtained from each household head before starting collection of mosquitoes.

CDC light traps were used to collect indoor host-seeking *Anopheles* mosquitoes, with 10 houses were randomly selected from the three sub-villages. The traps were hung 45 cm above the feet of sleeping persons protected by insecticide untreated mosquito nets.[225] The collection was done between the hours of 6:30pm to 6:00ams. The light and a person under the nets lure the mosquitoes. All the people in the trapping room were instructed to sleep under the nets. The next morning, the trap bags were tightened to prevent mosquitoes from escaping the bags and transported to the entomology laboratory at Arba Minch University. Mosquitoes that were still alive in the trap bags were transferred to paper cups and placed in a freezer to kill them. All the

female *Anopheles* mosquitoes were morphologically identified into species, and their abdominal stage was determined under a microscope. Unfed *An. gambiae* were dissected for age determination (head and thorax preserved for sporozoite analysis). The specimens were then individually preserved in vials with silica gels inside for further processing (PCR, sporozoite rate and blood meal analysis).

For the PSC, 10 other houses were randomly selected, and indoor resting mosquitoes were collected in the morning from 6:00am to 9:00am. An aerosol (Roach killer, M/S Kafr EI Zayat, Egypt with Registration No. ET/HHP/130) was used to spray houses. Before spraying houses, all the food items were removed. The openings and eaves of windows and doors were closed, with pieces of cloth used to prevent mosquitoes from escaping. The floor and furniture in the rooms were covered with white sheets and a person operated the spraying inside of a house, while another sprayed from the outside by moving round the house. After 10 minutes, knocked-down mosquitoes were collected from the sheets. The morphological speciation of female *Anopheles* mosquitoes was done using a key, and their abdominal stage was determined under a microscope. They were then transferred to vials with silica gels using forceps for PCR, sporozoite rate and blood meal analysis. Unfed *An. gambiae* were further dissected for ageing.

Outdoor resting mosquitoes were collected using hand-held mouth aspirators from 10 artificially constructed pit shelters in the compound of 10 other randomly selected houses. Each shelter was 1.5 m deep with an opening of 1.2 m x 1.2 m. Four cavities, one on each side of the pit shelter, were dug, with each cavity having a horizontal depth of 30 cm.[226] In the act of collecting mosquitoes, the mouth of each pit shelter was covered with an untreated bed net to prevent mosquitoes from flying out of the pit shelter. Mosquitoes were then collected from the pit shelters in the morning from 6:30am to 10:00am, and killed by freezing for morphological identification and abdominal stage determination. Unfed *An. gambiae* were used for age grading. Next, the specimens were preserved in vials with silica gels for PCR, sporozoite rate and blood meal analysis.

3.2.3. Identification of adult *Anopheles* mosquitoes (Papers I and II)

Female *Anopheles* mosquitoes were identified into species using a morphological key,[57] and *An. arabiensis* was identified using the polymerase chain reaction (PCR) technique.[227] A sample of *An. gambiae* from each collection method and month was used for PCR identification. A small portion of a leg of the *An. gambiae*

complex was mixed with a 12.5 µl PCR master mix, which contained deionized water, dNTP, PCR buffer, $MgCl_2$, QD primer, AR primer, UN primer, ME primer, QDA primer, GA primer and Taq DNA polymerase in a PCR tube. The samples were centrifuged and amplified by a PCR apparatus of PTC-100™ Programmable Thermo cycler (MJ Research PTC-100™ Inc., USA). The PCR cycle was run as the following: 95°C/5min x 1 cycle; (95°C/30sec, 50°C/30sec, 72°C/30sec) x 30 cycles; 72°C/5min x 1 cycle; 4°C hold. PCR product (5 µl) was mixed with loading dye (2 µl). A 2% agarose-tris-borate-EDTA containing an ethidium bromide gel was used to run the DNA ladder. The ladder was then visualized under a UV light box (MultiImage™, Pacific Image Electronics Co. Ltd., Taiwan).

3.2.4. Age determination of *Anopheles* mosquitoes (Paper II)

The parity rate of unfed *An. gambiae* (presumably *An. arabiensis*) collected by various means was determined by dissecting their ovaries following the method of Detinova.[109] The ovaries of female mosquitoes were withdrawn from the abdomen into a drop of distilled water on slides and allowed to dry. The ovaries were then observed under a compound microscope to identify nulliparous (tightly coiled tracheolar skeins) and parous (stretched out of the tracheolar skeins). This method is used to identify those mosquitoes that have laid eggs at least once (parous) and or those that have not laid at all (nulliparous).

3.2.5. Blood-meal origin determination (Papers I and IV)

Blood-meal origins of freshly fed *Anopheles* mosquitoes from CDC light traps, PSC and pit shelters were detected by ELISA technique[228] with human and bovine antibodies in different microtitre plate wells. The abdomen of freshly fed *Anopheles* mosquitoes was homogenized in 50 µl phosphate buffered saline (PBS) solution (p^H 7.4), and then further diluted to a volume of 200 µl by PBS. 50 µl of sample was added to each well in a 96-well microtiter plate, and incubated overnight at room temperature. PBS containing a Tween-20 solution was used to wash each well (washed twice). 50 µl host-specific conjugate (anti-human IgG and anti-bovine IgG) was added in each well of separate 96-microtitre plates and incubated for one hr. The wells were washed three times by PBS-Tween-20 solution. Finally, 100 µl of peroxidase was added to each well, and after 30 minutes the absorbance of 405 nm was recorded with in ELISA plate reader (MRX Microplate Reader, Dynex Technologies, 20151-1683, USA). Human and bovine blood meals were used as a positive control, and unfed laboratory-reared *An. arabiensis* were used as a negative control. The results were considered to be

positive when the absorbance value exceeded the mean plus three times the standard deviation of the four negative controls.

3.2.6. The sporozoite rate and EIR (Paper II)

Female *Anopheles* mosquitoes were used for determining the CSPs of *P. falciparum*, *P. vivax* -210 and *P. vivax* -247 by employing ELISA.[229] For this purpose, the head and thorax of each *Anopheles* mosquito was transferred to a vial containing 50 µL of blocking buffer (BB) solution and grinded using a non-absorbent plastic pestle. Next, 200 µL of BB was added. Each well was coated with 50 µL monoclonal antibodies (MAb) of *P. falciparum, P vivax*_210 and *P. vivax*_247, and incubated for 30 minutes at room temperature. The captured MAb was aspirated from the microtiter plate filled by BB and incubated for one hr at room temperature. BB was aspirated from wells of the plate and loaded by 50 µL of mosquito samples. 50 µL of a positive and negative control were loaded on the first and second column wells, respectively. After a two-hr incubation at room temperature, the triturate was aspirated and the wells were washed twice with PBS-Tw20. 50 µL of peroxidase substrate was added to each well, and aspirated after one hr of incubation. The wells were then washed three times with PBS-Tw20. 100 µL of ABTS (2, 2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt) substrate (yields a green end product upon reaction with peroxidase) was added to each well, and incubated for another 30 minutes. The result of each sample was determined visually and by ELISA plate reader. The positive reaction resulted in a green colour. Moreover, the sample was considered to be positive when the optical density was greater than two times the mean of the optical density of negative controls. All positive samples were re-tested.

3.3. House screening intervention (Paper III)

A randomized control trial was conducted to assess whether screening windows and doors with mosquito-proof wire mesh (approximately 64 holes/cm^2), and closing openings on eaves and walls by mud, would reduce the indoor densities of *An. arabiensis* in the sub-village nearest to the main mosquito breeding sites. The study was conducted in two main malaria transmission seasons, April and May, and October and November 2011 (Figure 10). Forty houses were included based on inclusion criteria to collect the baseline data of the indoor density of mosquitoes in April and May 2011. Mosquitoes were collected biweekly for four consecutive nights per week (10 CDC light traps/night). Using the baseline data, the 40 houses were randomized into intervention and control groups. The doors and windows of 20 houses were screened by wire mesh and the holes on eaves and

walls closed by mud. The mosquitoes were then collected biweekly from both intervention and control houses (five from intervention and five from control houses/night).

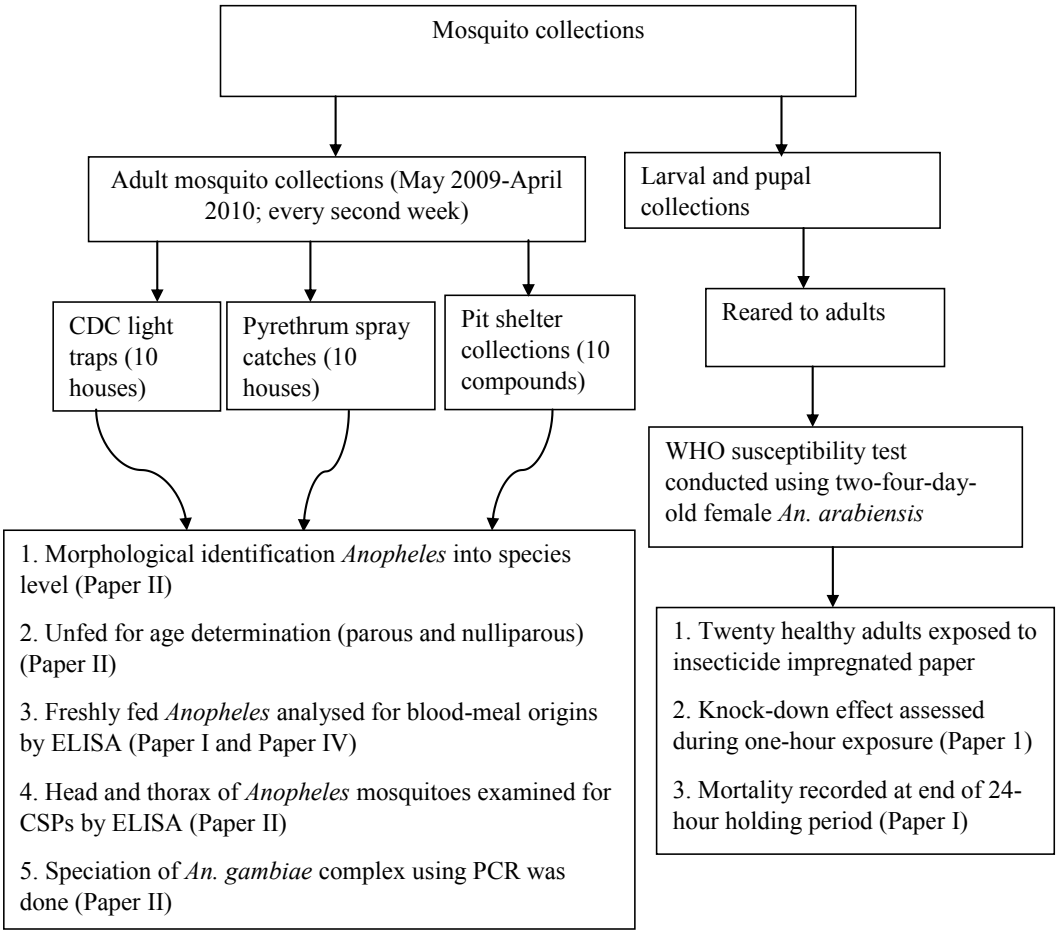

Figure 9: Entomological study design (Papers I, II and IV)

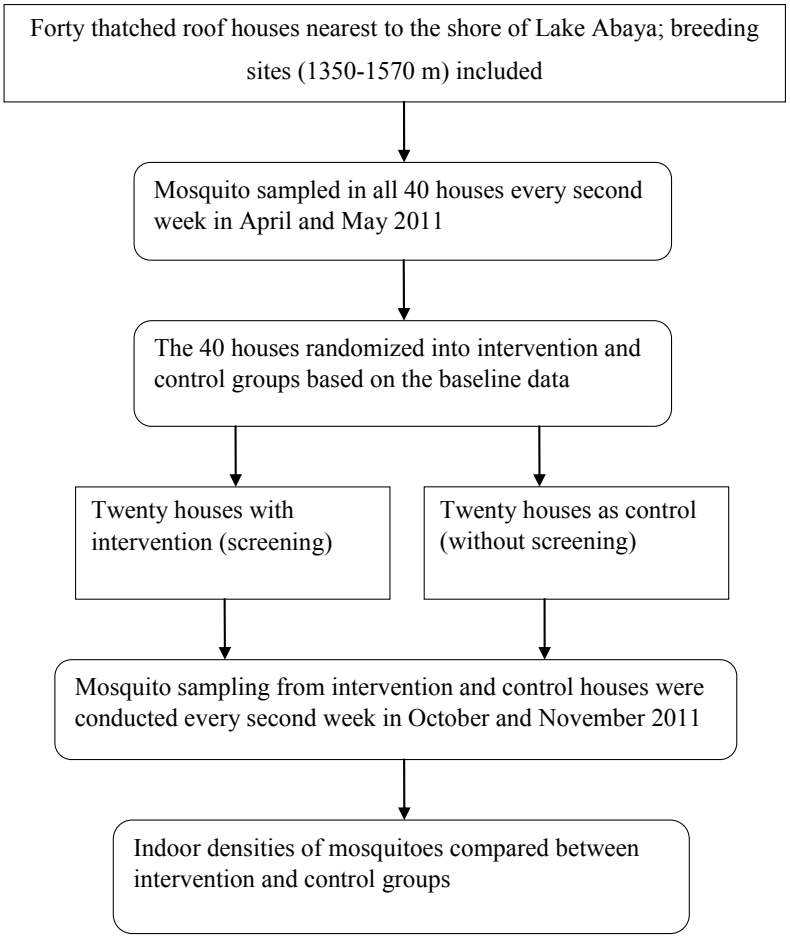

Figure 10: Study design of house screening trial (Paper III)

3.5. Data analysis

The human and bovine blood meal index was calculated as the proportion of the mosquitoes fed on either human or bovine blood meals out of the total blood meals tested (Papers I and IV). A chi-square test was used to compare the human and bovine blood index of *An. arabiensis* collected from different sites. The results of the susceptibility tests were evaluated as recommended by the WHO.[203] A probit analysis was used to calculate the time taken to knock down 50% (KDT_{50}) and 90% (KDT_{90}) of *Anopheles arabiensis* (Paper I). The parity rate was calculated as parous mosquitoes divided by the number of mosquitoes dissected. An analysis of variance was used to compare the mean differences in the number of *An. arabiensis* among months and houses (Paper II). A Tukey Honestly Significant Difference Test was used to distinguish the months and houses with the maximum density of mosquitoes (Paper II). The Spearman's rho correlation was used to test the relationship between mean monthly densities of *An. arabiensis* with rainfall (Paper II). Log-transformed data were also used for parametric statistical analysis. The EIR of *An. arabiensis* was estimated using the standard method, 1.605 × (no. CSP-positive ELISA results from CDC light trap/no. mosquitoes tested) × (no. mosquitoes collected from CDC light traps/no. catches) × 365 days, and the alternative method, 1.605 (no. CSP positive ELISA/no. catches) × 365 days.[230] The EIR from the PSC was estimated by multiplying the human biting rate (HBR) and the CSP rate (Paper II). HBR from PSC was calculated as the number of freshly fed *An. arabiensis* divided by the number of occupants who slept in the houses the night before collection × HBI.[231]

A Generalized Estimating Equation (GEE) with a negative binomial error distribution was used to account for an over-dispersion of *An. arabiensis* and culicine counts (Paper III). A first-order autoregressive correlation structure was used to account for a serial correlation between repeated catches made in the same house. The GEE was fitted separately to counts of different abdominal conditions of *An. arabiensis* and overall culicine to determine the protective effect of screenings against the house entry of the species. The percentage reduction for the house entry of mosquitoes was computed by comparing the mean's ratio of screened and control groups. A non-parametric correlation was used to see the house entry patterns of *An. arabiensis* in pre-intervention and post-intervention months. The statistical significance for the effects of screening on the indoor density of *An. arabiensis* and culicine was tested by the *P*-value obtained from GEEs at the 0.05 level.

The relative feeding preferences of *Anopheles* mosquitoes were calculated according to Hess *et al.* [25] (Paper IV) by taking the percentage of freshly fed *Anopheles* mosquitoes with either humans or bovine blood meals divided by the percentage of either human or cattle in the area. A linear regression analysis was used to assess the impact of cattle-to-human ratios on the human and bovine blood meal index of *Anopheles* mosquitoes. SPSS software (SPSS Inc, Chicago, USA) was used for data entry and analysis.

3.6. Ethical issues

The Regional Health Research Ethics Review Committee of the SNNPR Health Bureau approved the research project. This study was a part of the Ethiopian Malaria Prediction System research project, and ethical permission was obtained from the Regional Ethical Committee of Western Norway. Written permission was obtained from local administrators to conduct the investigation, and the objective of the study was explained for the *kebele* administrators and study participants. Furthermore, informed verbal consent was obtained from all study participants.

4. Results

4.1. Blood-meal origins and insecticide susceptibility of *Anopheles arabiensis* (Paper I)

The blood origins of *Anopheles* mosquitoes and the insecticide susceptibility status of the field populations of *An. arabiensis* were determined in the Chano village in southern Ethiopia. Overall, 3,027 freshly fed anopheline mosquitoes were collected by CDC light traps, PSCs and artificial pit shelters. The blood-meal origins of 2,967 (98%) freshly fed *Anopheles* mosquitoes were determined using the ELISA technique. *Anopheles arabiensis* was the predominant species (75%), and cattle were the main sources of its blood meals. The overall human blood index (HBI) of *An. arabiensis*, including mixed blood meals, was 0.44, and the bovine blood index (BBI), including mixed blood meals, was 0.69. The HBI of *An. arabiensis* from CDC light trap collections was 0.75, which was higher than those for PSC (0.38) and pit shelter collections (0.13), while the BBI was 0.72 for CDC light traps, 0.68 for pit shelters and 0.65 for PSC. More fresh-fed and human blood-fed *An. arabiensis* were sampled from houses close to the shore of Lake Abaya, where the major breeding sites are located.

In this study of susceptibility to insecticides, *An. arabiensis* was resistant to cyfluthrin (0.15%), lambdacyhalothrin (0.05%), alphacypermethrin (0.05%), deltamethrin (0.05%) and DDT (4%), with a mortality rate of 50% for cyfluthrin and alphacypermethrin, 56% for lambdacyhalothrin, 47% for deltamethrin and 10% for DDT.

4.2. Entomologic Inoculation Rates of *Anopheles arabiensis* (Paper II)

The species composition of *Anopheles* mosquitoes, sporozoite, parity and entomological inoculation rates of *An. arabiensis* was assessed in Chano in southern Ethiopia. Overall, 4,708 anopheline mosquitoes (including fresh feds) belonging to 16 species were collected by using CDC light traps, PSCs and from artificial pit shelters. *Anopheles arabiensis* was the predominant species in all collection techniques. The density of *An. arabiensis* was significantly associated with a one-month lag of rainfall (CDC light traps, r = 0.81, $p < 0.001$; PSCs, r = 0.79, $p = 0.002$; and pit shelters, r = 0.63, $p = 0.03$). Those households nearest to the identified major breeding sites of mosquitoes had maximum densities of *An. arabiensis* in all collection techniques. Overall, 4,534 *Anopheles,* including *An. arabiensis* (n = 3,678), *An. marshalli* (n = 763), *An. garnhami* (n = 45), the *An. funestus* group (n = 26), *An. pharoensis* (n = 15) and *An. tenebrosus* (n = 7) were analysed to detect CSPs and for the estimation of EIRs. Of 3,678 *An. arabiensis* tested for CSPs, 11 (0.3%) were positive for *P. falciparum* and three (0.08%) for *P. vivax*. When the collection techniques and number of *P. falciparum* CSPs positive for *An. arabiensis* were considered, most were caught by CDC light traps (seven of 11) followed by artificial pit shelters (three of 11) and PSC (one of 11). Overall, the *Plasmodium* infection rate (*P. falciparum* and *P. vivax*) of *An. arabiensis* was 0.38%, while *P. falciparum* CSPs were 0.3% and the *P. vivax* 210 CSP rate was 0.08%. The *P. falciparum* CSP rate of *An. arabiensis* was 0.32% for CDC light traps, 0.28% for pit shelters and 0.23% for PSCs.

The estimated annual *P. falciparum* EIR of *An. arabiensis* was 17.1 infectious bites per person per year (ib/p/y) [95% confidence interval: 7.03-34.6] based on CDC light traps and 0.1 ib/p/y based on PSCs. The *P. falciparum* EIRs from CDC light traps varied from 0 ib/p/y (in 60% of houses) to 73.2 ib/p/y in a house nearest to the breeding sites. The *P. falciparum* EIR of *An. arabiensis* was 2.4 (95% CI = 0.12-11.7) in the dry season, and 14.7 (95% CI = 5.9–29.4) in the wet season. This finding represented 6.1-fold more infectious bites in the wet than in the dry season. The *P. vivax* EIR of *An. arabiensis* was 2.4 (95% CI = 0.06–13.4) for CDC light traps, and was 0.1 ib/p/y for PSC.

4.3. The effect of screening doors and windows on indoor density of *Anopheles arabiensis* (Paper III)

A randomized control trial was conducted to assess the impact of screening doors and windows with mosquito-proof wire mesh and closing openings on eaves and walls by mud on indoor densities of *An. arabiensis*. Screening doors and windows, and closing openings on eaves and wall by mud, reduced the overall indoor densities of *An. arabiensis* by 40%. The impact was pronounced on unfed *An. Arabiensis,* resulting in a 42% reduction in houses with interventions. The total costs for screening windows and doors, and to close openings on the eaves and walls by mud, was 7.34 USD per house, thereby indicating that it can be incorporated into malaria vector strategies by local communities.

4.4. Zoophagic behaviour of anopheline mosquitoes in southwest Ethiopia (Paper IV)

The relative feeding preference of *Anopheles* mosquitoes in relation to cattle and human host abundance was assessed to understand the feeding patterns of malaria vectors. The blood-meal origins of *Anopheles* mosquitoes from CDC light traps, PSCs and artificial pit shelters were tested using ELISA. The relative feeding preference of *An. arabiensis* to bovine blood meal was 4.7 times higher than that of human blood. *Anopheles marshalli* was six times more likely to feed on bovine blood meal than humans. The majority of *An. arabiensis* (65%) and *An. marshalli* (73%) from indoor-resting collections had a bovine blood meal, which is unexpected in an area practicing IRS and LLINs. The human and bovine feeding pattern of *An. arabiensis* and *An. marshalli* was changed little due to the cattle-to-human ratio of households. The accessibility of cattle outdoors that mosquitoes first encounter may determine the feeding patterns of these mosquitoes.

5. Discussion

5.1. Methodological discussions

Study design

Anopheles mosquitoes were collected bi-weekly for a year to determine the anopheline species present in the study area, human blood index, sporozoite infection rates based on CSPs detections, parity and entomological inoculation rates (Papers I, II and IV). A longitudinal study design enables an understanding of the seasonal variations of *the Anopheles* mosquito density and species composition.[232] It also helps to see the impact of meteorological variables on the density of malaria vectors. *Anopheles* mosquitoes were also collected both indoors and outdoors, as well as host-seeking and resting mosquitoes, using different entomological methods to increase the representativeness of species in the area.[226] The *kebele* was stratified into three sub-villages by considering the patchy distribution of malaria vectors and to measure the average community exposure to the infectious bites of vectors,[147, 233] and houses were randomly selected in each sub-village. This study also has some weaknesses. The entomological data was only collected for a year, so it was therefore not easy to predict the future scenario using one year of data because of the year-to-year variability of meteorological variables and the density of malaria vectors.[234] Moreover, the collection of more data over a longer time period (more than a year) would allow for a better understanding of the changes in density, feeding and resting patterns of malaria vectors.

A randomized control trial was conducted to assess the impact of improving housing on the indoor density of malaria vectors (Paper III). This method is believed to minimize selection biases.[235] The entomological data was collected by CDC light traps to minimize the bias due to the skill of collectors. It is known that assessing the impact of vector control interventions on entomological variables and malaria incidence is valuable. Even so, we only assessed the impact of housing intervention on the indoor density of the malaria vector, which is not a good predictor of malaria transmission. Instead, using the entomological indicators related to malaria transmission, such as the EIR and sporozoite rate, is worthwhile for evaluating the impact of the intervention.[233]

Sample size

The sample size required for entomological sampling varies based on the type of study done. Previously, sample size calculation was uncommon in entomological studies, primarily because these methods are labour-intensive.[226] For example, for monitoring the impact of large interventions, the sampling of 10-30 households per village per month, and analysing 200-500 mosquitoes for sporozoite determination, was recommended in an area with intense malaria transmission.[237] However, to increase the accuracy and precision of the entomological estimates, a sample size determination is advisable.[237]

In our entomological study, no estimate of the sample size was made before we started the studies. Instead, 30 households were randomly selected (10 houses for CDC light traps, 10 for PSC and 10 for pit shelters) to sample *Anopheles* mosquitoes. However, we analysed more than 96% (4534 of 4708) of the sampled *Anopheles* mosquitoes for CSPs, and 98% (2967 of 3027) of those freshly fed to determine blood-meal origins. Hence, the sample sizes were assumed to be sufficient to address the objectives of the study.

To estimate whether our sample size was adequate, we calculated the statistical power of some of the analysis done. For example, in Paper II we wrote that, "The proportion of bovine blood meal of *An. arabiensis* was similar for indoor resting (65%), outdoor pit shelter resting (68%) and CDC light traps (72%) samples." The power of the comparison between CDC light trap and pit shelter was 47%, and for the comparison between CDC light trap and indoor resting, 69%. The power of the comparison between indoor resting and outdoor pit shelter resting was 17%, which is low to detect the true differences. However, our intention was not to detect such small differences, so we therefore think that the sample size for this sub-group analysis was also adequate.

Internal validity

Internal validity is the ability of the study to ensure if the findings are not due to bias or confounding, but are true.[238] A good study design could minimize biases and control confounding. Biases, such as selection and information biases, deviate the results from the truth.[238] On the other hand, confounders are those variables related to outcome and exposure, and could lead to a misinterpretation of the results.[238]

Selection bias

Selection biases mainly occur in the phase of the study subject inclusion. They influence both internal and external validities because they affect the representativeness of the study participants and the procedures used to sample.[239] For Papers I, II and IV, *Anopheles* mosquitoes were collected using CDC light traps, PSC and from pit shelters. All of these collection methods have their limitations. For example, some *Anopheles* mosquitoes may leave houses before PSC is used, and its measurements can hence be less than expected. CDC light traps may attract non-human biting mosquito species, and may overestimate the human-vector contacts. Pit shelter collection is biased toward the outdoor resting mosquito species, and the blood-meal analysis may underestimate the human-vector contacts. Consequently, using all the above-mentioned collection techniques can minimize the bias by collecting indoor host-seeking, indoor and outdoor resting *Anopheles* mosquitoes. Thus, the sampling of *Anopheles* mosquitoes is believed to be representative of all collection sites. The *kebele* was stratified into three sub-villages, and houses were randomly selected in each sub-village for mosquito sampling. More than 90% of the collected mosquitoes were analysed to determine sporozoite rate and blood-meal origins, so the sample selection bias is assumed to be minimized.

The baseline data was used to randomize the participants into control and intervention groups (Paper III). A random allocation of the study participants also helps to minimize selection bias.[238] Using the baseline data also minimizes the baseline difference, which might affect the outcome of the intervention. Mosquitoes were collected from both intervention and control groups on the same night by considering the variability of mosquito density.[240] With regard to methodological selection bias, we know that the human landing collection method is the gold standard to estimate the human biting rate and EIR, but at the time of our study it was considered unethical to do in Ethiopia. Conducting a study for more than one year may enable the catching of the year-to-year variability of vectors density and meteorological variables, which in turn can increase the generalizability of the study.

Information bias

The error in measurement and procedure of classification results in information bias.[238] In our study, the information bias may arise from the morphological misidentification of species, but the morphological identification key we used to identify mosquito species is widely used in Sub-Saharan Africa.[57] The ELISA technique was used to analyse blood-meal origins and sporozoite proteins following the standard protocols and

consumables, and the positive specimens were also reconfirmed. Nonetheless, using more sensitive methods such as PCR can increase the accuracy of the analysis, and help identify the cryptic blood meals (different individuals of the same species).[241] *Anopheles arabiensis,* which is morphologically indistinguishable from the other members of the *An. gambiae* complex, was identified by PCR. The intervention trial data (Paper III) was collected using CDC light trap, which is not dependent on the field worker's skill.[225]

The entomological samples were collected indoors and outdoors prospectively for all analyses (Papers I, II and IV), and both the overall and stratified results were reported. Blood meal and sporozoite analysis were performed following standard techniques. Differentiating parous and nulliparous *An. arabiensis* using tracheolar skeins requires expertise, hence introducing information bias due to misclassification (Paper II). The Polovodova method is more precise to determine the number of human-vector contacts and the potential of the vectors, but is technically more complex.[108]

Confounding

Confounding is a mixing of effects by unexpected factors that attempt to relate to both outcome and exposure.[238] These variables can be controlled during analysis using statistical tools such as stratification and different multivariate techniques. For example, the variability of host feeding behaviour and human-vector contact of mosquitoes (Papers I and II) was managed by stratifying the data and calculating the HBI of mosquitoes from each collection site. Data were stratified in sub-villages to help identify the population at a higher risk of malaria. A Generalized Estimating Equation (GEE) with a negative binomial error distribution was used to account for an over-dispersion of mosquito counts (Paper III). A first-order autoregressive correlation structure was used to account for a serial correlation between repeated catches made in the same house.

Chance

The 95% confidence interval (CI) shows the range of true value and is preferable over *p-value* (usually < 0.05), as it tells the range of possible effects, rather than the cut-off single value.[242] Both the p-value and 95% CI (Paper III) were used to report the impact of intervention on the indoor density of the malaria vector. The EIR of *An. arabiensis* was estimated by using the standard and alternative methods to show the 95% CI (Paper II), as recommended by a study elsewhere.[230] The lack of a significant association between a maximum and

minimum temperature with a density of *An. arabiensis* (Paper II) does not mean that temperature has no effect on the density of the mosquitoes. Biologically, rainfall and temperature link to the vector development and survival.[235] The small size of entomological sampling houses and a one-year data of all variables (short to see the exact lag associations) might limit the power of the association.

External validity

The external validity of the study addresses the audience to which the results of the study are applicable.[243] It is not expected that the findings of this study can be applicable in every setting because of the extreme heterogeneity of malaria and the vectors between regions and villages.[244] The feeding and resting behaviours of malaria vectors vary based on the availability of hosts, human livelihood and climate factors.[245] It is advisable to study the feeding and resting behaviours and species composition of malaria vectors in different settings to help plan the appropriate intervention accordingly. The methodology used (Papers I, II and IV) here and the general findings, such as the higher risk of people close to the breeding sites and the importance of supplementary vector control interventions in hot spots, can be applicable in many similar settings. Our entomological findings agree with the epidemiological study in the same site.[27]

The participants enrolled for the randomized trail (Paper III) are from the hot spot site of malaria against vector *An. arabiensis*. The finding is quite applicable in similar settings, but the frames of doors and windows should be fit for screening to further improve the efficacy of intervention.

5.2. Discussion of the main findings

Sixteen *Anopheles* species were documented in the area. *Anopheles arabiensis* was by far the most dominant, and the only vector of *P. falciparum* and *P. vivax* malaria with the overall CSP rate of 0.38% (14 of 3,678). The *P. falciparum* EIR of *An. arabiensis* from CDC light traps was 17.1 infectious bites/person/year, but it varied between houses and seasons. The infectious bites of *An. arabiensis* was 6.1-fold more in the wet than in the dry season, and those houses close to the breeding sites in the shore of Lake Abaya received much higher infectious bites than those far away. Furthermore, *An. arabiensis* showed a consistently higher feeding pattern on cattle than humans, and it was highly resistant to pyrethroid insecticides (insecticides used for LLINs treatment) and DDT. Screening doors and windows with wire mesh, and closing openings on walls and eaves by mud, significantly reduced the indoor density of host-seeking malaria vector.

Since the1890s[5] (when *Anopheles* mosquitoes were first incriminated as vectors of malaria), many *Anopheles* species have been identified and incriminated.[60] The biting and resting behaviours, in addition to the vectorial capacity of malaria vectors, vary within the same site or region.[123] Understanding this complex interaction of malaria vectors in the field is important for effective malaria vector control. The success of vector control interventions depends on the response of vectors to interventions, their feeding behaviours and interaction with humans and other hosts.[14] Today, understanding the heterogeneity of malaria vectors and their behaviours is important to help control residual malaria transmission (persistence of malaria transmission after a high coverage of quality interventions), which is a substantial challenge to malaria elimination.[219] The challenge of residual malaria transmission further strengthens the need for studying malaria vector behaviour at the local level to plan the appropriate vector control interventions.[219] Assessing the EIR of local malaria vectors is needed because it is an important parameter to evaluate the impact of interventions, and is an indicator of malaria intensity.[13, 123] Insecticide resistance is an urgent global agenda in malaria vector control, and hence the continuous monitoring of the resistance status of local malaria vectors is a top priority to plan resistance management strategies.[18] In addition, the effective implementation of vector control interventions needs evidence. With these and other questions in mind, this entomological study was conducted in southwest Ethiopia.

Several studies have shown that those mosquitoes that tend to feed more frequently on humans are more dangerous than those that feed less frequently.[29, 114] This is because frequent contact between vectors and humans increases the chance of taking malaria parasites from infected humans or inoculating parasites to susceptible human hosts.[29, 114] On the other hand, those mosquitoes that tend to feed on cattle (dead-end hosts) waste their infectious sporozoites or cannot be infected even if they are susceptible for *Plasmodium*.[162] In the current study, *An. arabiensis* exhibited more of a tendency to feed on cattle than humans and rest indoors after feeding on cattle outdoors (Papers I and IV). Many other studies confirmed these behaviours of *An. arabiensis*.[87, 164] Zooprophylaxis, the diversion of mosquitoes to domestic animals, can be considered as a supplementary vector control strategy as *An. arabiesnsis* demonstrated zoophagic behaviour. But the topical application of animals with appropriate insecticides, [165]and increasing indoor coverage interventions to push mosquitoes from houses towards animals outdoors, enhances the impact of zooprophylaxis.[162] However, since *An. arabiensis* has already developed a resistance to pyrethroid insecticides, alternative systemic insecticides such as ivermectin or eprinomectin can be considered for the treatment of animals.[246]

LLINs and IRS are the two most widely implemented malaria vector control interventions, and have resulted in a significant reduction of malaria-related mortality and sickness in Ethiopia.[9] The effectiveness of these interventions could continue as long as the insecticides are effective in killing malaria vectors.[19] Resistance of *An. arabiensis* to pyrethroid insecticides has been reported in many parts of the country,[18] including the study site (Paper I). IRS with deltamethrin and LLINs impregnated by the same insecticide were deployed during the study period.[27] The application of different insecticides with a similar mode of action or the same insecticide for IRS and LLINs may enhance resistance.[205] It is therefore advisable to use the available insecticides by considering their mode of actions to prolong the lifetime of these insecticides, and reduce the threat posed by resistance on the current vector control programme. A high coverage of LLINs is likely to provide community-level protection from the infectious and non-infectious bites of malaria vectors by killing susceptible populations.[19] But in the present study area, the high coverage of LLINs failed to provide community-wide protection, perhaps due to the problem of resistance (Paper I) and also due to a low utilization of LLINs.[27] It seems fair to conclude that the resistance of *An. arabiensis* to pyrethroid insecticides may limit the usefulness of insecticide-based interventions, although some degree of protection may be attained by the net barrier itself provided they are intact and used properly. For this reason, alternative insecticides with different modes of action are required to control the resistant population.

Variations in the risk of malaria due to the location of human habitation has been known for a long period of time.[147] The current study also showed the heterogeneous distribution of the infectious *An. Arabiensis,* which was clustered in those houses located in the sub-village nearest to the mosquitoes breeding sites along the shore of Lake Abaya (Paper II). This study is in agreement with Loha and Lindtjørn,[27] who reported a clustered distribution of malaria cases in those houses near the mosquito breeding sites. Identifying hot spots of infectious malaria vectors (Paper II) and malaria parasite in humans[27] is important to plan targeted interventions[147] because the available interventions are insufficient to protect people at higher risk.[147] Larval source management can be used to control malaria vectors in such clusters if many of the mosquito breeding sites are identified and well-defined.[147] Larval source management plays dual roles by killing insecticide-resistant and outdoor biting malaria vectors in the aquatic stages.[148] Insecticide resistance (Paper I) and residual malaria transmission are among the current global challenges of malaria control.[19, 219] Therefore, the larviciding of the breeding sites may be appropriate strategies to target the aquatic stages along the shore of the lake, and may reduce the infectious bites of malaria vectors in those houses close to the breeding sites.

The current study showed the association between meteorological and entomological variables (Paper II). Most malaria vectors and infectious *An. arabiensis* were found in the wet seasons of the year. The lagged association between the density of malaria vector and meteorological variables is biologically sound and important for the early planning and implementation of vector control interventions in an appropriate time. Some studies identified the density of malaria vectors as a predictor of malaria epidemics in highland areas.[247] The entomological variables, like the EIR and human biting rates, showed a strong association with the incidence of malaria.[125] The higher the EIR of *An. arabiensis* (Paper II), and more malaria cases,[27] have been coincided in those houses close to the malaria vector breeding sites.

House improvement by screening windows and doors and closing openings has been effective against the infectious bites of malaria vectors by reducing indoor human-vector contacts. House improvement was mainly practiced before the DDT era, and contributed to malaria elimination in America and Europe.[23] Today, malaria remains a public health problem despite high LLINs and IRS coverage in some regions of the world, so there is a need for supplementary interventions.[17] House improvement is a fitting intervention to integrate with ITNs and IRS, and it maximizes the existing intervention tools by providing protection to all household members.[23] The screening intervention was conducted in the sub-village with more infectious malaria vectors (Paper II) and malaria parasites.[27] The screening of doors and windows by wire mesh, and closing openings by mud reduced the indoor density of *An. Arabiensis,* and the intervention was relatively cheaper and easy to introduce as a supplementary intervention (Paper III). House screening interventions successfully prevented the home entry of local malaria vectors adapted to biting and resting indoors. Higher indoor human-vector contacts, and even the indoor resting tendency of *An. arabiensis* after feeding on cattle outdoors, were documented (Papers I and IV). Moreover, *An. arabiensis* showed a zoophagic behaviour, which can easily be diverted to animals outdoors by preventing indoor entry by screening doors and windows (Paper IV). When considering screening doors and windows against malaria vectors as a supplementary intervention, the design of the doors should be appropriate for screening; consequently, the efficacy of the screening intervention can be much improved.

5.3. Implications of the study for policy

Malaria vectors are heterogeneous in their biting and resting behaviours. Understanding this behavioural heterogeneity is important to identify the gap between vector behaviour and available interventions, which aids in planning supplementary interventions. Estimating the EIR of local malaria vectors is important because it

shows the extent of local active malaria transmission, and can be used to evaluate the impact of interventions. Monitoring the insecticide resistance of local malaria vectors could be used to plan and implement IRM strategies at an early stage.

The observed clustered distribution of infectious malaria vectors in the village is evidence of the heterogeneity of infectious bites in a small village. Targeting the identified hot spots might enable the use of the available resources more efficiently, and perhaps to control and eliminate malaria. Hence, planning locally applicable interventions like house screening might strengthen the contribution of LLINs and IRS, and therefore reduce the indoor biting density of malaria vectors. The zoophagic behaviour of *An. arabiensis* likely makes house screening an appropriate supplementary intervention to divert them easily to cattle outdoors.

The wide distribution of LLINs and IRS has reduced the number of people at risk of malaria, but are believed to be insufficient to stop malaria transmission for various reasons, including insecticide resistance, a low adherence to LLINs, and the outdoor biting and early biting behaviours of vectors. Considering LSM as part of an integrated vector control approach might be worthwhile because the major cause for the clustering of infections malaria vectors was the location of the breeding sites, but selecting the appropriate LSM strategy is crucial for the effectiveness of the intervention. The principal malaria vector showed zoophagic behaviour, so considering insecticidal zooprophylaxis as a supplementary intervention along with LLINs and IRS can be effective. The main advantage of the insecticide zooprophylaxis is that it kills and reduces the density of malaria vectors. Still, the collaboration between different sectors is vital for the effective use of animal-based intervention to improve both public and animal health.

When planning for the application of certain interventions, considering the lagged patterns of rainfall and the density of malaria vectors might improve the impact of interventions. Using a long time series of data could be needed to understand the lagged patterns and identify the most important predictors of the variables.

6. Conclusions and recommendations

6.1. Conclusions

a) Sixteen *Anopheles* species were identified, and *An. arabiensis* was the dominant species in the area. *Anopheles arabiensis* showed an overall tendency for bovine over human blood meals. The higher bovine blood meal index from indoor resting collections may justify the indoor resting tendency of *Anopheles* mosquitoes after feeding outdoors on cattle.

b) *Anopheles arabiensis* was resistant to pyrethroid insecticides, the only class of insecticides recommended for LLINs treatment; as a result, there should be an action programme to manage insecticide resistance and develop new insecticides for LLINs.

c) *Anopheles arabiensis* was the principal vector of malaria in the region, while *An. marshalli* and *An. garnhami* were the second and third most abundant species. Both *An. marshalli* and *An. garnhami* had contact with humans, but neither of them was positive for CSPs. Most *P. falciparum* CSP was identified from indoor host-seeking *An. arabiensis* collected by CDC light traps, so there is a need to prevent the house entry of malaria vectors to reduce indoor infectious bites.

d) The transmission of malaria is heterogeneous, varying from 0 infectious bites per person per year to 73.2 infectious bites per person per year in the area. Those houses nearest to the mosquito breeding sites (hot spots) had a higher risk of exposure to the infectious bites of *An. arabiensis*. The risk of infection due to *An. arabiensis* was higher in the wet than dry months.

e) The indoor density of *An. arabiensis* was substantially reduced by screening doors and windows using wire mesh and closing holes on eaves and walls by mud. It was also cheap and easy to incorporate into malaria vector control strategies by local communities.

f) *Anopheles arabiensis* showed a consistently higher feeding pattern on cattle than on humans regardless of collection sites and the high number of human population.

6.2. Recommendations

Operational

a) Insecticide resistance monitoring and evaluation should be in place to help detect resistance at an early stage, and to plan insecticide-resistance management strategies.
b) Locally applicable interventions like house screening could be strengthened in order to reduce the indoor density and indoor human-vector contacts.
c) Targeting the malaria hot spots might enable the more efficient use of available resources to control malaria.

For policy

a) Insecticide-resistance management strategies should be implemented to prolong the functional lifespan of insecticides.
b) Supplementary interventions could be implemented in the sub-village nearest to mosquito breeding sites.
c) The local vector behaviours and the lagged patterns of meteorological variables could be considered to maximize the impact of interventions.

For research

a) The indoor human-vector contact and the occurrence of *An. marshalli* and *An. garnhami* suggest a need for further investigations into their potential role as vectors of malaria.
b) The impact of topical application insecticides on cattle or cattle treatment with endocticides (e.g. ivermectin) on malaria incidence and entomological indices could be studied to take advantage of the zoophagic behaviour of *An. arabiensis*.
c) The impact of screening doors and windows on the incidence of malaria should be studied to further strengthen the role of housing improvement.
d) The impact of larval source management, in combination with LLINs and IRS, needs to be evaluated.

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Blood meal origins and insecticide susceptibility of *Anopheles arabiensis* from Chano in South-West Ethiopia

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Abstract

Background: *Anopheles arabiensis*, the main malaria vector in Ethiopia, shows both anthropophilic and zoophilic behaviours. Insecticide resistance is increasing, and alternative methods of vector control are needed. The objectives of this study were to determine the blood meal origins and the susceptibility to insecticides of *An. arabiensis* from Chano village near Arba Minch in South-West Ethiopia.

Methods: Blood meal sources of anopheline mosquitoes collected using Centers for Disease Control and Prevention (CDC) light traps and pyrethrum spray catches (PSC) from human dwellings, and hand-held mouth aspirators from outdoor pit shelters were analysed using a direct enzyme-linked-immunosorbent assay (ELISA). The susceptibility of *An. arabiensis* to pyrethroid insecticides (alphacypermethrin, lambdacyhalothrin, deltamethrin, and cyfluthrin) and DDT was assessed using females reared from larval and pupal collections from natural breeding sites.

Results: The blood meal origins of 2967 freshly fed *Anopheles* mosquitoes were determined. *An. arabiensis* was the predominant species (75%), and it fed mainly on cattle. The densities of both freshly fed *An. arabiensis* and those fed on human blood followed similar seasonal patterns. The overall human blood index (HBI) of *An. arabiensis*, including mixed blood meals, was 44% and the bovine blood index (BBI) was 69%. The HBI of *An. arabiensis* from CDC light trap collections was 75% and this was higher than those for PSC (38%) and outdoor pit shelter collections (13%), while the BBI was 65% for PSC, 68% for outdoor pit shelters and 72% for CDC light traps. More freshly fed and human blood-fed *An. arabiensis* were sampled from houses close to the shore of Lake Abaya (the major breeding site).
A high proportion of *An. arabiensis* was resistant to the pyrethroid insecticides, with a mortality rate of 56% for lambdacyhalothrin, 50% for cyfluthrin and alphacypermethrin, 47% for deltamethrin, and 10% for DDT.

Conclusion: *Anopheles arabiensis* is the predominant species of anopheline mosquito in this region, and cattle are the main source of its blood meals. The greater tendency of this species to feed on cattle justifies the application of insecticides on cattle to control it. However, *An. arabiensis* has already developed resistance to the available pyrethroid insecticides, and alternative insecticides are needed for malaria vector control.

Keywords: *Anopheles arabiensis*, Human blood index, Bovine blood index, Pyrethroid insecticides, DDT, Insecticide resistance, South-West Ethiopia

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Background

Malaria vectors that feed mainly on humans seriously affect human health because this behaviour increases the risk of malaria transmission [1]. The feeding pattern of *An. arabiensis*, the main vector of malaria in Ethiopia, varies among households [2]; it shows both zoophilic [3] and anthropophilic behaviours [4,5]. In Ethiopia, only a few studies have examined the blood meal origins of *An. Arabiensis*, particularly focusing on mosquitoes from animal sheds and human dwellings in the main malaria transmission seasons [3,5,6]. Such studies might have underestimated or overestimated the human–vector contact and the risk of malaria transmission [7].

Pyrethroid insecticides are widely used for bed net treatment, and for indoor residual spraying (IRS) [8] to reduce malaria incidence [9,10]. Long lasting insecticide treated nets (LLINs) and IRS have contributed to a reduction of malaria incidence in many malaria endemic countries by reducing the number of mosquitoes inside houses [11,12]. IRS and LLINs are efficient malaria vector control measures for *An. gambiae* s.s, which mostly feeds and rests indoors [13,14]. In contrast, *An. arabiensis* obtains a large proportion of its blood meals from cattle, apart from humans, and exhibits significant exophilic behaviour [4,6,13]. Thus, treatment of cattle with insecticide may reduce *An. arabiensis* populations in an alternative approach to malaria vector control [15,16]. In southern Ethiopia, Habtewold *et al.* [3] observed normal feeding behaviour of *An. arabiensis* on insecticide treated cattle with no diversion to humans. Moreover, Rowland *et al*. [15] reported a 56% reduction in the incidence of malaria in Pakistan resulting from the application of deltamethrin insecticide to cattle. In Africa, deltamethrin treated cattle provided protection against *An. arabiensis* in experimental huts [16].

Resistance to deltamethrin and permethrin in *An. arabiensis* has been reported from different parts of the country [17,18]. DDT resistant *An. arabiensis* is widespread in the country, including Arba Minch [17-19]. There has not been any information regarding susceptibility/resistance of *An. arabiensis* to pyrethroids from the area. Therefore, it is important to examine the insecticide susceptibility and blood meal origins of *An. arabiensis* from Chano in South-West Ethiopia for planning alternative or additional vector control approach.

Methods

Study area

The study was conducted in Chano, a village 15 km north of Arba Minch town in South-West Ethiopia, from May 2009 to April 2010. The village is located at 6°6.666′ N and 37°35.775′ E and at altitude of 1,206 m above sea level. There are three sub-villages, named sub-villages 1, 2 and 3. The village is close to Lake Abaya and sub-village 3 is found at a distance of 1350 to 1850 m from the lake. Three major irrigation canals pass through the village. The canals are permanent, well-constructed and flow into the agricultural fields outside the village. The inhabitants are subsistence farmers with maize cultivation and cattle ranching as their main source of income. The main cash crops are mangoes and bananas.

Domestic animals are usually kept in compounds in open conditions, but a few households use separate roofed cow shelters. It is not customary to keep animals in human dwellings. The people habitually sleep indoors throughout the year. There is no permanent or seasonal movement of animals out of the village for feeding or watering. The human population size is 6661 while the cattle population is 2217 (approximately three humans per head of cattle) (Table 1).

The climate is hot and humid. Potential mosquito breeding sites are located at the shores of Lake Abaya and Harrae River. Small water bodies created by hoof-prints of cattle and hippopotami are the major breeding sites for *Anopheles* mosquitoes. Harrae River is a potential location for the breeding of anopheline mosquitoes during the dry seasons when many small water pouches are available. However, its influence is much smaller than that of Lake Abaya because it is about 5 km from the village. Monthly rainfall was recorded from the weather station in Arba Minch University, about 6 km from the study area, which is located at an altitude of 1200 m above sea level (the same as Chano village). In 2009, the annual rainfall was 645 mm, and in 2010 it was 1061 mm. The average minimum and maximum annual temperatures in 2009 were 17.8 and 32.2°C, and in 2010 they were 17.9 and 30.2°C.

Study components and vector control activities in the area

This study is a part of the research programme "Ethiopian Malaria Prediction System," which researches malaria and climate. The village was purposely selected, because it is one of the malarious villages in the Arba Minch area, for study of the epidemiological and entomological components of the disease and for the development of

Table 1 Abundance of human and other potential blood meal hosts in the three sub-villages from Chano in Southwest Ethiopia

	Human and other potential hosts					
Sub - villages	Human	Cattle	Goat	Sheep	Donkey	Chicken
01	2289	568	90	112	36	261
02	2154	696	80	161	31	373
03	2218	953	83	166	42	557
Total	6661	2217	253	439	109	1191

mathematical models to predict malaria. A recent publication by Loha and Lindtjørn described the occurrence of falciparum malaria in the village [20]. Antivector interventions, such as the application of IRS with DDT and distribution of insecticide treated nets (ITNs), were carried out by the government in June 2009 and March 2010, respectively. At least two bed nets were provided for each household.

Mosquito collections

Mosquito sampling was conducted biweekly for a total of 12 consecutive months (May 2009 to April 2010) after obtaining verbal consent from the heads of households. Indoor blood-searching *Anopheles* were collected from ten randomly selected houses using Centers for Disease Control and Prevention (CDC) light traps (New Standard Miniature Light Traps 512 6 V 150A; John W. Hock, Gainesville, FL) by positioning the traps 45 cm above the floor at the feet of sleeping persons, who were protected by mosquito nets untreated with insecticide, from 18:30 to 6:00 hours [21]. Indoor resting mosquitoes were sampled in the mornings (6:00 to 9:00) from ten other randomly selected houses by application of the pyrethrum spray catch (PSC) method. Prior to spraying with an aerosol (Roach killer, M/S Kafr EI Zayat, Egypt with Registration No. ET/HHP/130) in each house, all food items and small animals were removed, the openings and eaves of windows and doors were filled with pieces of cloth, and the floor and furniture were covered with white sheets. Two sprayers, one from outside and the other inside the house were engaged, and knocked down mosquitoes were collected after ten minutes [22]. Outdoor resting mosquitoes were collected using a handheld mouth aspirator, paper cup and torch from ten pit shelters constructed under the shade of mango trees in the compound of ten randomly selected houses. Each shelter was 1.5 m deep and had an opening of 1.2 m × 1.2 m. About 0.5 m from the bottom of each pit shelter, a 30 cm horizontally deep cavity was prepared for each of the four sides [23]. The mouth of each pit shelter was covered with untreated bed net during collection periods (6:30–10:00 hours) to prevent mosquitoes from escaping.

Mosquito processing

Live female anopheline mosquitoes were killed by freezing and all females were identified to species level using morphological characteristics [24]. Female anopheline mosquitoes were examined under a dissecting microscope and classified on the basis of their abdominal condition as unfed, freshly fed, half-gravid and gravid [22]. All female mosquitoes were preserved individually in vials with silica gel desiccant for later analysis (blood meal origins, parity rate and sporozoite rate).

Detection of blood meal sources

The blood meal origins of freshly fed anopheline mosquitoes collected outside and inside houses were determined using a direct enzyme-linked immunosorbent assay (ELISA) following the method of Beier *et al.* [25] using human and bovine antibodies. Each mosquito abdomen was crushed in 50 μl phosphate buffered saline (PBS) solution (pH 7.4), which was further diluted by adding 950 μl PBS. Fifty microlitres of sample was added to each well in a 96-well microtitre plate, and incubated overnight at room temperature. Each well was washed twice with PBS containing Tween-20 solution, and 50 μl host specific conjugate (either human or bovine) was added to each well and incubated for one hour. After one hour, each well was washed three times with a PBS–Tween-20 solution. Finally, 100 μl of peroxidase substrate was added to each well and after 30 minutes the absorbance at 405 nm was recorded with an ELISA plate reader. Each blood meal sample was considered positive if the absorbance value exceeded the mean plus three times the standard deviation of the four negative controls (from a laboratory colony of *An. arabiensis* adults not fed with blood). Positive controls contained human and bovine blood.

Species identification

Species specific polymerase chain reaction (PCR) [26] was carried out on 300 morphologically identified individuals from the *An. gambiae* complex obtained by random sampling for each month.

Collection of aquatic forms and rearing to adulthood for susceptibility tests

Anopheles larvae and pupae were collected from natural breeding sites on the shores of Lake Abaya and along the Harrae River. They were reared to adulthood in the entomology laboratory at Arba Minch University in cages and provided with sterilized 10% sucrose solution soaked in cotton pads until testing. Before the test, *Anopheles* mosquitoes were identified using morphological keys [24] and those identified as from the *An. gambiae* complex (presumably *An. arabiensis*) were used for the test.

Insecticide susceptibility tests

Insecticide susceptibility tests were carried out following the standard World Health Organization (WHO) protocol, using insecticide susceptibility test kits and insecticide-impregnated papers [27]. For each replicate, twenty non-blood-fed female *An. Arabiensis*, three to four days old, were exposed to papers impregnated with cyfluthrin (0.15%), lambdacyhalothrin (0.05%), alphacypermethrin (0.05%), deltamethrin (0.05%), and DDT (4%) for an hour. Controls were exposed to insecticide-free papers. The knockdown effect of each insecticide was recorded every

five minutes during the one-hour exposure period [27]. Mosquitoes were then transferred to a recovery tube, supplied with sterilized 10% sucrose solution and kept in an insecticide free box for 24 hours, after which mortality rates were recorded. All susceptibility tests were carried out in a room with temperatures of 26.2–27.4°C and relative humidity of 72–84%. Four replicates of the tests and two replicates of the controls were carried out for each insecticide. For each replicate, new insecticide-impregnated paper was used.

Data analysis

Data were entered and analysed using SPSS version 16 (SPSS Inc., Chicago. IL). The human blood index (HBI) and bovine blood index (BBI) were calculated as the proportion of the mosquitoes fed on either human or bovine blood meals out of the total blood meals determined [7]. Mixed (human + bovine) blood meals were added to the number of human and bovine blood meals when calculating the HBI and BBI [14,28]. Cryptic mixed blood meals were not analysed. The chi-squared test was used to compare the HBI and BBI of indoor and outdoor collected *An. arabiensis*. Analysis of variance (ANOVA) was used to compare the mean differences in the number of freshly fed *An. arabiensis* among months and sub-villages. The Tukey Honestly Significant Difference (HSD) test was used to distinguish the months with the maximum density of mosquitoes.

The results of the susceptibility tests were evaluated as recommended by WHO [27]. Mean mortality was determined across all batches of mosquitoes for a particular insecticide. Probit analysis was used to calculate KDT_{50} and KDT_{90} (the time taken to knock down 50% and 90% of mosquitoes, respectively).

Results

Anopheles species analysed for determination of blood meal origin

Overall, 3027 anopheline mosquitoes engorged with fresh blood were collected from May 2009 to April 2010, and 98% (n = 2967) of these were analysed to identify their blood meal origin. Of the 300 *An. gambiae* complex tested for speciation, 99.3% (n = 298) were *An. arabiensis* and two specimens did not amplify using PCR, and hence, their identity was unknown. Therefore, *An. arabiensis* was regarded as the only member of the complex and the predominant species (75%), followed by *An. marshalli* (22%) and *An. garnhami* (1.7%). *An. funestus*, *An. pharoensis* and *An. tenebrosus* accounted for 0.9%.

Seventy nine per cent of all *Anopheles* species, and 78% of *An. arabiensis*, gave positive reactions against human, bovine or both antibodies. Of all *Anopheles* mosquitoes analysed, 33.5% were found positive for mixed (human and bovine) blood meals. The host blood meals of 21% freshly fed *Anopheles* mosquitoes were not identified, and of these 57% (n = 360) were from outdoor pit shelters (Table 2).

An. arabiensis was the predominant species in outdoor pit shelters (64.8%), in space spray catches (84.6%), and in indoor CDC light traps (84.4%). *An. marshalli* (n = 436, 66.5%), *An. garnhami* (n = 35, 71.4%) and *An. funestus* group (n = 14, 88%) were caught more frequently in outdoor pit shelters, whereas *An. pharoensis* (n = 7) was caught only by indoor CDC light traps.

Feeding behaviour of *Anopheles* mosquitoes

Table 2 shows the blood meal origins of *Anopheles* mosquitoes. *An. arabiensis* showed an overall preference for bovine bloods (33%) above human blood meals. Only 8% of *An. arabiensis* had obtained a blood meal from humans alone. The proportion of mixed blood meals (human–bovine) was high for *An. arabiensis* (36%). A large proportion of *An. arabiensis* had blood meals of unknown origin (22.5%). A high proportion of *An. arabiensis* from CDC light traps (65%) had blood meals of mixed origin, whereas the lowest proportion of mixed blood meals was obtained from outdoor pit shelters (10%). Few *An. arabiensis* from outdoor pit shelters (3%) had human blood meals alone. Similarly, *An. marshalli*, *An. garnhami*, and *An. funestus* group have shown a preference for bovine blood meals above human blood meals, with bovine blood meals alone in 47%, 47%, and 37.5% respectively. No *An. funestus* group had a human blood meal alone.

Blood meal indices of *An. arabiensis*

Table 3 shows the blood meal origins of *An. arabiensis* from different collection sites. The overall human blood index (HBI) of *An. arabiensis*, including mixed blood meals, was 44%, while the bovine blood index (BBI) was 69%. The frequency of human–vector contact was much higher for mosquitoes caught in indoor CDC light traps than for indoor or outdoor resting samples collected by space spraying and from pit shelters. The proportion of human blood meals in *An. arabiensis* from indoor CDC light traps (75%) was significantly higher than for outdoor pit shelters (13%, $\chi^2 = 288.7$, $p < .0001$) and indoor resting space spray catches (38%, $\chi^2 = 36.6$, $p < .0001$). Indoor resting *An. arabiensis* had a HBI of 38% which was significantly higher than the 13% obtained for samples from outdoor pit shelters ($\chi^2 = 58.8$, $p < .0001$). The proportion of bovine blood meals in *An. arabiensis* was similar for indoor resting (65%), outdoor pit shelter resting (68%) and CDC light trap (72%) samples.

Household and seasonal variations in density of blood fed *An. arabiensis*

The densities of freshly fed *An. arabiensis* varied significantly among the three sub-villages ($F = 5.0$; $df = 2$;

Table 2 Sources of blood meal of *Anopheles* mosquitoes collected indoors and outdoors from Chano in Southwest Ethiopia from May 2009-April 2010

Anopheles spp.		Blood meals sources			
	No. analysed (HBI,%)	Human N (%)	Bovine N (%)	Mixed N (%)	Unknown N (%)
An. arabiensis	2234 (44)	180 (8)	745 (33)	807 (36)	502 (22.5)
An. marshalli	656 (37)	68 (10)	308 (47)	175 (27)	105 (16)
An. garnhami	49 (37)	9 (18)	23 (47)	9 (18)	8 (16)
An. funestus group	16 (19)	0 (0.0)	6 (37.5)	3 (19)	7 (44)
An. pharoensis	7 (43)	1 (14)	0 (0.0)	2 (29)	4 (57)
An. tenebrosus	5 (20)	1(20)	1 (20)	0 (0.0)	3 (60)
Total	2967 (42)*	259 (9)	1083 (36.5)	996 (33.5)	629 (21)

Mixed (human + bovine) blood meals were added to the number of human and bovine blood meals when calculating the HBI; numbers in parenthesis. Unknown blood meals are negative for human and bovine antibodies. * Overall HBI of Anopheles mosquitoes.

p = 0.02). Figure 1 shows the variations in freshly fed and human-blood-fed *An. arabiensis* among the three sub-villages. The maximum number of freshly fed *An. arabiensis* was collected in houses in the sub-village nearest to the major breeding site (between 1350 m and 1850 m), with 12.6 per CDC light trap per night, 10.5 per pit shelter and 6 per hut PSC. In contrast, in sub-village 2 (located between 1960 m and 2270 m from the major breeding site), the maximum number of freshly fed *An. arabiensis* was 8.3 per CDC light trap per night, 2.2 per pit shelter per collection time and 0.6 per hut PSC. The maximum number of freshly fed *An. arabiensis* was 1.5 per CDC light trap per night, 4 per pit shelter and 1.5 per hut PSC in sub-village 1 (located between 2350 m and 2600 m from the major breeding site). Similarly, the number of human-blood-fed *An. arabiensis* was highest in sub-village 3, with 11 fed on human blood per CDC trap per night, 1.8 human fed per hut PSC and 1 human fed per pit shelter.

The density of *An. arabiensis* varied with season (F = 3.67; df = 11; p = 0.017) and was associated with rainfall (Figure 2). The density of the total number of freshly fed, human and bovine blood engorged *An. arabiensis* followed a similar seasonal pattern (Figure 3). The highest number of freshly fed *An. arabiensis* was collected in April 2010, comprising 24.7 mosquitoes per CDC light trap, 20.8 mosquitoes per pit shelter and 6.9 mosquitoes per hut in space spray catches. In April 2010, we collected the highest number of *An. arabiensis* with meals of human blood origin from indoor CDC light traps: 16.3 human-blood-fed per CDC light trap per night, 2.5 human-blood fed per pit shelter and 2.4 human-blood fed per PSC. The number of freshly fed *An. arabiensis* declined to zero in August 2009, following the period of lowest rainfall in the preceding two months. The highest densities of *An. arabiensis* were collected during October and November 2009, and in April 2010. However, significantly higher densities of freshly fed *An. arabiensis* were collected in April 2010 than in October and November 2009 (Tukey HSD test, p= 0.004).

Knockdown and mortality of *An. arabiensis*

Table 4 shows the knockdown time for the five insecticides used with *An. arabiensis*. Only deltamethrin resulted in 100% knockdown, with the lowest KDT_{50} (21 minutes) and KDT_{90} (35 minutes) values, whereas DDT resulted in only 10% knockdown within 60 minutes of exposure time. The KDT_{50} values of alphacypermethrin, cyfluthrin and deltamethrin were 27, 25 and 21 minutes, respectively. Only cyfluthrin and deltamethrin resulted in more than 90% knockdown within 60 minutes of exposure time. The KDT_{50} value of lambdacyhalothrin was 1.9 times, and that of alphacypermethrin was 1.3 times, higher than that of deltamethrin.

Table 3 Blood meal origins of *Anopheles arabiensis* collected indoors and outdoors from Chano in Southwest Ethiopia

Collection sites	No. analysed (HBI,%)	Blood meal origins			
		Human N (%)	Bovine N (%)	Mixed N (%)	Unknown N (%)
Indoor CDC light traps	988 (75)	94 (9.5)	70 (7)	644 (65)	180 (18)
Space sprays catches	352 (38)	59 (17)	154 (44)	74 (21)	65 (18.5)
Outdoor pit shelters	894 (13)	27 (3)	521 (58)	89 (10)	257 (29)
Total	2234 (44)*	180 (8)	745 (33)	807 (36)	502 (22.5)

Mixed (human + bovine) blood meals were added to the number of human and bovine blood meals when calculating the HBI; numbers in parenthesis. Unknown blood meals are negative for human and bovine antibodies. * Overall HBI of An. arabiensis.

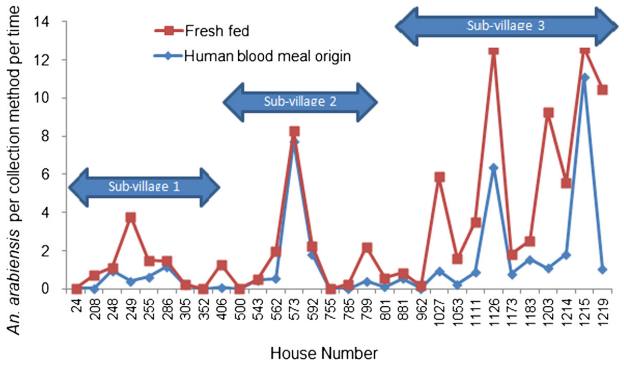

Figure 1 Variation of fresh fed and human blood fed *An. arabiensis* among the three sub-villages from Chano in southwest Ethiopia. (Pyrethrum spray catches: 24, 255, 352, 543, 785, 801, 962, 1111, 1183 & 1214; **CDC light traps:** 248, 286, 305, 573, 592, 755, 881, 1126, 1173 & 1215; **Pit shelter:** 208, 249, 406, 500, 562, 799, 1027, 1053, 1203 & 1219).

The mortality rates of *An. arabiensis* after the 24-hour recovery period was 56% for lambdacyhalothrin, 50% for cyfluthrin and alphacypermethrin, 47% for deltamethrin and only 10% for DDT, much lower than the susceptibility boundary of 80% (Table 4). The mortality rate calculated for the experimental tests was not corrected because mortality in the controls was always less than 5%.

Discussion

The results of this study showed that *Anopheles arabiensis* is the predominant anopheline species in the area, and it feeds mainly on cattle. *An. arabiensis* has already developed resistance to the available pyrethroid insecticides and alternative insecticides may be needed for the treatment of cattle. Houses close to the main mosquito breeding site harboured more freshly fed *An. Arabiensis* and those fed on human blood.

Earlier studies from Ethiopia have examined the blood meal origins of *An. arabiensis* from animal sheds and human dwellings during the main malaria transmission seasons only [3,5,6], neglecting the dry months. A strength of our study is that the blood meal origins of freshly fed *An. arabiensis* were determined by collecting mosquitoes from outdoor pit shelters and inside houses throughout a year, as was recommended by Garrett-Jones [7]. Mosquitoes were sampled from 30 collection sites every two weeks each month and, hence, their blood meals are representative of human contact with the mosquito vector. Our data compare well with those of Loha and Lindtjørn [20], who studied the incidence of malaria in the same village and reported the highest incidence of malaria in the nearest village to Lake Abaya (sub-village 3), where we found the highest densities of freshly fed and human-fed *An. arabiensis*.

One limitation of our study was the inability to determine the cryptic mixed blood meals of malaria vectors that had fed on different individuals of the same species. This might have led to underestimation of human–vector contact and pathogen transmission intensity, as was reported by Norris *et al.* [29] and Scott and Takken [1].

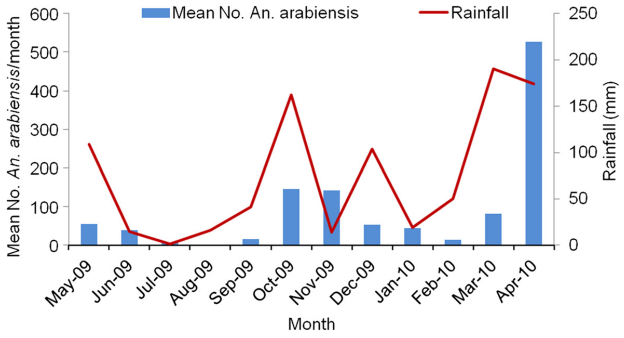

Figure 2 Monthly rainfall (in mm) and the mean density of fresh fed *Anopheles arabiensis* from Chano in southwest Ethiopia.

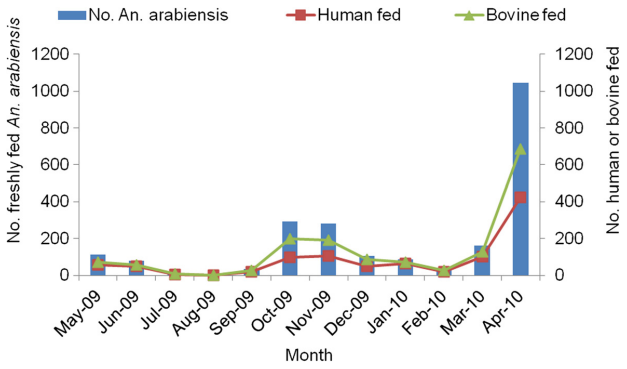

Figure 3 Number of freshly fed, human and bovine blood fed (mixed blood meal included in both human and bovine) *Anopheles arabiensis* from Chano in southwest Ethiopia.

Another limitation is that we could not identify other animal sources of blood meals for malaria vectors in addition to humans and cattle. Such information may be important in the planning of vector control options. The failure to determine the blood meal origins of some freshly fed *An. arabiensis* may have occurred because we lacked antibodies for other hosts, or it could have resulted from enzymatic degradation of the blood.

Many zoophilic *An. arabiensis* were collected indoors using space spray catches after they had fed on cattle outdoors, which provides clear evidence for preference of a bovine blood meal over human. The zoophilic behaviour of *An. arabiensis* observed in this study is consistent with other findings from Ethiopia [3,6]. The HBI (38%) of *An. arabiensis* from space spray catches was lower than the HBI from southern Zambia (92.3%) [30], the Kenyan coast (91%) [31], Konso in southern Ethiopia (55.2%) [3] and the Gambia (82%) [32], but higher than from Eritrea (20%) [33] and western Kenya (23%) [13]. The percentage of mixed blood meals for indoor resting *An. arabiensis* (21.0%) was comparable with that found in other studies [13,34]. No mixed blood meals were identified in resting *An. arabiensis* from inside houses in Kenya [31].

The *An. arabiensis* collected using CDC light traps had higher HBI than those from indoor resting and outdoor pit shelters. Fornadel *et al.* [35] reported an HBI of 94% for *An. arabiensis* from southern Zambia collected using CDC light traps. Interestingly, a high proportion of *An. arabiensis* from indoor CDC light traps had mixed blood meals (65.2%). This suggests that they were interrupted while feeding outdoors on cattle and moved into houses to complete their feeding in a single night or on consecutive nights [29,36]. The lowest HBI was found for *An. arabiensis* from pit shelters located near cattle that are kept outdoors. This reveals that the accessibility of hosts influences the feeding behaviour of this species, as also reported by others [37]. This is the first report of the HBI of *An. marshalli* and *An. garnhami*. Future studies should be conducted to examine the sporozoite rate of these species to determine their possible role in malaria transmission.

The few *An. funestus* collected from outdoor pit shelters was found with cattle blood meal. Unfortunately, we did not identify the species group using molecular method. However, the occurrence of some species from larval identification is known in Ethiopia [38]. Of the members of the group, *An. parensis* and *An. rivulorum* are regarded to be zoophilic elsewhere in Africa [39,40]. *An. funestus* has been incriminated as an anthropophilic and endophilic malaria vector in many

Table 4 Percent knockdown, knockdown time (KDT) (in minutes) and mortality rates of *Anopheles arabiensis* exposed to pyrethroids and DDT from Chano in Southwest Ethiopia

Insecticides tested	Number exposed	% knockdown	KDT_{50} (95% CI)	KDT_{90} (95% CI)	% mortality (±SE)	Status [27] (<80%)
Lambdacyhalothrin (0.05%)	80	80	39 (36–43)	**	56 ± 9.6	resistant
Alphacypermethrin (0.05%)	80	89	27 (20–32)	**	50 ± 5.4	resistant
Cyfluthrin (0.15%)	80	96	25 (19–29)	42 (37–51)	50 ± 9.5	resistant
Deltamethrin (0.05%)	80	100	21 (18–23)	35 (31–39)	47 ± 3.2	resistant
DDT (4%)	80	10	*	**	10 ± 3.5	resistant

* 50% was not knocked down ** 90% was not knocked down, CI= confidence interval, SE = standard error.

countries in Africa [41]. Therefore, the *An. funestus* group identified morphologically in this study could be either *An. rivulorum* or *An. parensis* or both .

In this study area, the distribution of the malaria vector was seasonal. The maximum number of freshly fed and human blood meal-engorged *An. arabiensis* was recorded one month after the peak rainfall. Possible reasons are that the rainfall in the previous month may have provided more breeding sites and increased the relative humidity, which contributes to a high density and longevity of the vectors and consequently increases human–vector contact [42]. In particular, the longevity of the vector is crucial for disease transmission because it increases the chance of an infectious bite occurring [42]. Kristan *et al.* [43] have also shown a one month lag after rainfall as a predictor of vector density in the African highlands. A study from Eritrea also has shown an increase in the *An. arabiensis* population one month after the start of rainfall [33]. Moreover, the distribution of *An. arabiensis* was influenced mainly by the location of breeding sites on the shore of Lake Abaya. A study from the same area [20] and one from Northern Tanzania [44] showed a higher risk of malaria infection in a population living near to mosquito breeding sites. To locate and identify households at greater risk of malaria is, therefore, crucial in the planning and implementation of vector control approaches.

An. arabiensis showed a high level of resistance to knockdown and mortality in response to pyrethroid insecticides (deltamethrin, alphacypermethrin, lambdacyhalothrin and cyfluthrin) and DDT. The knockdown resistance was most likely due to the possession of various detoxifying enzymes. Studies from East and Central Africa [45,46] have reported the occurrence of high levels of mono-oxygenase enzymes in resistant *An. arabiensis*. Elevated levels of mixed function oxidases and β-esterases were also reported in resistant *An. arabiensis* in Tanzania [47]. Moreover, the West African *kdr* mutation (L1014F) detected in high frequencies in South-West and Northern Ethiopian *An. arabiensis* populations [18,48] could be another reason for high knockdown resistance in the study area. The KDT_{50} of lambdacyhalothrin (39 minutes) was higher than that of the other pyrethroid insecticides, but shorter than that reported from Senegal (43.6 minutes) in *An. gambiae* [49]. Compared with studies from Ethiopia, the KDT_{50} values of 25.3 minutes for *An. arabiensis* from Gorgora and 37.6 minutes from Ghibe were higher than that we observed for deltamethrin (21 minutes), but similar to that reported from Sodere (21.9 minutes) [18]. The impact of knockdown resistance is that it can allow the vectors to bite humans even inside the long lasting insecticide treated nets (LLINs) because the vector can withstand a long duration of exposure without being knocked down [50].

The high level of resistance of *An. arabiensis* to deltamethrin and DDT is not surprising because of the long history of the use of DDT for IRS and the widespread use of deltamethrin for LLINs and IRS, and cross-resistance may occur [51]. The high level of DDT (90%) resistance in *An. arabiensis* was expected because 60% resistance was reported from South-Western Ethiopia 14 years ago [19]. The mortality rate (10%) due to DDT was slightly higher than that reported by Yewhalaw and his colleagues [17,48] but lower than that observed by Balkew *et al.* [18,52]. The mortality rate due to deltamethrin (47%) was lower than that observed in other studies in Ethiopia [17,18,48].

The resistance of *An. arabiensis* to alphacypermethrin, lambdacyhalothrin and cyfluthrin was unexpected because they have not been used for vector control. This implies that the use of insecticides with similar modes of action could shorten the duration of efficacy of other insecticides of the same class once resistance has developed in the mosquito population [53]. The most likely explanation is the presence of cross-resistance between insecticides of the same group [51], which might limit the choice of alternative insecticides for vector control. Cross-resistance between DDT and permethrin has been reported in Ethiopia [18] in *An. arabiensis*. No information is available in Ethiopia about the resistance of *An. arabiensis* to alphacypermethrin, lambdacyhalothrin and cyfluthrin. A study from Ghana has shown high survival rates of *An. gambiae* s.s after exposure to cyfluthrin and lambdacyhalothrin [54].

The results obtained in this study have implications for vector control. *An. arabiensis* showed a tendency to feed more frequently on cattle than on humans. In similar settings, Mahande and colleagues [16] and Rowland *et al.* [15] reported the success of treatment of cattle with pyrethroid insecticides in controlling zoophilic malaria vectors. Moreover, the preference of *An. arabiensis* to rest indoors after feeding on cattle outdoors in an area that practises indoor-based vector control activities could explain the low efficacy of LLINs and IRS, owing to the resistance of *An. arabiensis* to pyrethroid insecticides. Previously, N'Guessan *et al.* [55] reported a low efficacy of LLINs and IRS in areas with resistant malaria vectors. On the other hand, the indoor resting preference of *An. arabiensis* is an opportunity to use current indoor based antivector strategies [56] because mosquitoes inside houses are easily targeted [57], but appropriate management of insecticide resistance needs to be implemented.

The possible explanation for the higher HBI and presence of mixed blood meals in *An. arabiensis* from indoor CDC light traps may be that most people are bitten indoors before they go to bed, or that protection from indoor antivector interventions is reduced by the presence of pyrethroid-resistant *An. arabiensis*. In the same setting,

Loha and Lindtjørn [20] described the personal protection role of LLINs, with no impact on community members who did not use the nets. It is the killing capacity that provides protection from the infectious bites of malaria vectors for people in the community who do not use bed nets [58]. In an area with pyrethroid-resistant malaria vectors, even the combination of LLINs and IRS has a low impact on the prevalence of malaria [59], and in other settings an increase in malaria cases has been reported [60]. Asidi *et al.* [61] showed that the treatment of bed nets with pyrethroid insecticides provides additional protection from mosquito bites only if the vectors are susceptible to the chemicals. Our findings also show that the density of freshly fed and human blood-fed *An. arabiensis* increased in April 2010 despite the mass distribution of bed nets in March 2010. Hence, it is advisable to introduce additional vector control strategies that target a reduction in the entry of blood-searching vectors into houses and diversion to alternative hosts available outdoors. However, we should not underestimate the fact that malaria transmission can occur outdoors via human-biting mosquitoes, even if the HBI is low [62].

In addition, the finding of the lowest HBI and percentage of mixed blood meals in *An. arabiensis* from outdoor pit shelters suggests that *An. arabiensis* is less likely to leave houses after feeding indoors on humans [13], or that people are bitten outdoors less frequently in the area. Therefore, IRS and LLINs can provide successful protection from malaria infection if the vectors are susceptible to the available pyrethroid insecticides.

Conclusion

Although a high propensity for *An. arabiensis* to feed on bovine blood was observed in our study area, treatment of cattle with insecticides may not reduce the vector density because *An. arabiensis* has already developed resistance to the available pyrethroid insecticides that are recommended for the treatment of cattle. Thus, alternative insecticides with different modes of action may be needed for treatment of cattle.

Competing interests

The authors have no conflict of interest.

Authors' contributions

FM: Project design, conducted field and laboratory work, data analysis and interpretation, wrote the draft of manuscript, MB: Project design, field and laboratory supervision, and manuscript revision, TG: Project design, supervision and manuscript revision, BL: Project design, field supervision, provided statistical input and manuscript revision. All authors read and approved the final manuscript.

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Entomologic Inoculation Rates of *Anopheles arabiensis* in Southwestern Ethiopia

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Abstract. We collected anophelines every second week for one year from randomly selected houses in southwestern Ethiopia by using Centers for Disease Control (CDC) light traps, pyrethrum spray catches, and artificial pit shelter constructions to detect circumsporozoite proteins and estimate entomologic inoculation rates (EIRs). Of 3,678 *Anopheles arabiensis* tested for circumsporozoite proteins, 11 were positive for *Plasmodium falciparum* and three for *P. vivax*. The estimated annual *P. falciparum* EIR of *An. arabiensis* was 17.1 infectious bites per person per year (95% confidence interval = 7.03–34.6) based on CDC light traps and 0.1 infectious bites per person per year based on pyrethrum spray catches. The *P. falciparum* EIRs from CDC light traps varied from 0 infectious bites per person per year (in 60% of houses) to 73.2 infectious bites per person per year in the house nearest the breeding sites. Risk of exposure to infectious bites was higher in wet months than dry months, with a peak in April (9.6 infectious bites per person per month), the period of highest mosquito density.

INTRODUCTION

Although recent trends show a reduction in the prevalence of malaria in Ethiopia, it is still a challenge to the health of many communities.[1] Long-lasting insecticidal treated nets (LLINs) and indoor residual spraying (IRS) are the two main malaria vector control tools being used to decrease mosquito density and longevity, and human–vector contact of *Anopheles arabiensis,* the species responsible for > 95% of malaria transmission.[2] The impact of LLINs and IRS on indoor populations of vectors is to reduce entomologic transmission indicators, the most common of these is the entomologic inoculation rate (EIR), which is the product of sporozoite rate and human biting rate over a defined time and space,[3] parity (longevity), sporozoite rates, and human blood index.[4] The success of both interventions depends on the response of vectors,[5] their behavior and interaction with humans, and alternative hosts.[6,7]

The EIR measures the intensity of malaria transmission in a particular area.[3] Estimation of the EIR is important for quantifying the potential level of human exposure to infected mosquitoes and for assessing the impact of interventions on malaria transmission in a given area.[4,8] Many studies have reported huge variations in malaria transmission intensity in Africa,[9] not only between urban and rural settings but even within the same locality.[10] It has been reported that the EIR varies between 0.01 and 1000 infectious bites per person per year (ib/p/year) in malaria-endemic countries in Africa.[11]

The human landing catch (HLC) has been the most widely accepted method for estimating the human-biting rate[12] because it measures actual human–vector contact, but it has obvious ethical and technical problems.[13] Other methods such as the Centers for Disease Control (CDC) light trap have been evaluated to replace the HLC by calibrating and determining an index equivalent to the human biting rate. It is believed that CDC light traps when set near sleeping persons protected by nets capture the anthropophilic mosquito species and thus can provide an indirect estimate of the human biting rate.[13] Some investigators have also used pyrethrum spray catches (PSCs) to determine the human biting rate, although this method might underestimate the human–vector contact and consequently the intensity of malaria transmission because many indoor-fed mosquitoes will leave houses before and during spraying.[7] In this study, CDC light traps and PSCs were used to estimate the EIR.

In Ethiopia, few attempts have been made to estimate EIR despite the fact that two-thirds of the country is malarious. However, several reports are available on sporozoite rates of *An. arabiensis* from different localities.[2,14] Based on microscopic dissection, Krafsur[15] in 1977 working in the low-lying and highly malarious town of Gambela and riverside villages in western Ethiopia, was the first to estimate an annual EIR of *An. gambiae* s.l. (presumably *An. arabiensis*). It was not until 36 years later that further estimation of EIR in Ethiopia was published for highland villages with unstable malaria transmission in the south-central region of the country.[16] Thus, there are substantial gaps in knowledge regarding entomologic transmission levels and the impact of the current anti-vector operations (IRS and LLINs).

Malaria is clearly a public health problem in Chano village in southwestern Ethiopia.[17] In the absence of entomologic information on malaria transmission in this village and the surrounding areas, a detailed longitudinal entomologic study was conducted to study host preferences, insecticide susceptibility, anopheline diversity, seasonal variations, and intensity of malaria transmission (EIR). *Anopheles arabiensis* has developed resistance to the pyrethroid insecticides and DDT, and showed a greater tendency to feed on cattle than humans, with an overall human blood index (HBI; the proportion of the *An. arabiensis* fed on human blood meals of the total blood meals determined) of 44% and a bovine blood index of 69%.[18] We report anopheline species diversity, monthly variations of *An. arabiensis* in relation to meteorologic variables, variations between houses in relation to distance from breeding sites, and malaria transmission indices of sporozoite rates and EIR.

MATERIALS AND METHODS

Study area. The study was conducted in Chano (Figure 1), which is north of Arba Minch during May 2009–April 2010.

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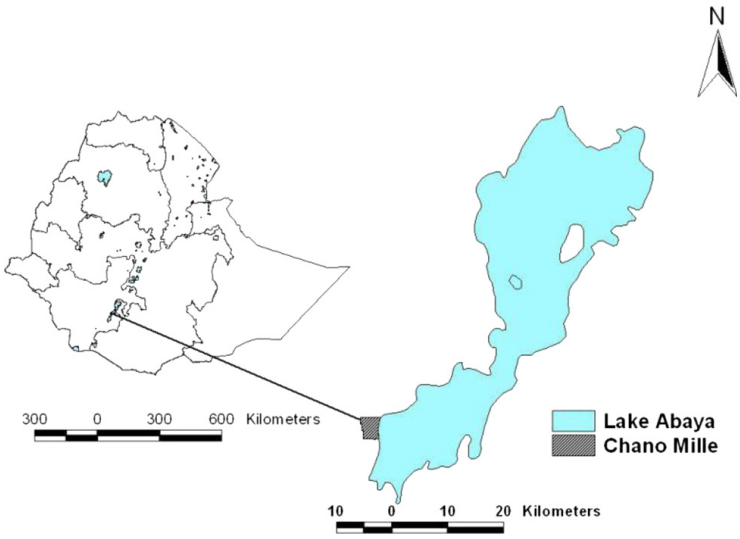

FIGURE 1. Location of the study site in Chano village, Ethiopia.

The health post, which is at the center of the village, is located at 6°6.666′N, 37°35.775′ E and at an altitude of 1,206 meters above sea level. For the purpose of epidemiologic study, the village was divided in to three sub-villages and coded as sub-village 01, 02 and 03. A detailed description of the study area has been reported elsewhere.[18]

Monthly meteorologic data were recorded from the weather station at Arba Minch University, which is 6 km from the study area. In common with most areas in the southern Ethiopian Rift Valley,[19] there were two wet seasons with the main rains falling during March–May and a smaller rainy season during October–December; the dry seasons are during June–September and January–February. Because of high surface water, we classified November 2009 as a wet month despite the low rainfall recorded.

Mosquito sampling. Thirty houses from the three sub-villages were selected for mosquito collections. The selected houses were distributed on the periphery and in the middle of each sub-village and equally divided for sampling by using CDC light traps (10 houses), PSCs (10 houses), or artificial pit shelters (10 houses). The distance from the main larval breeding sites to each house was recorded by using a global positioning system (GPS 60™; Garmon, Olathe, KS).

Anopheles mosquitoes were sampled every second week for 1 year (May 2009–April 2010). Before mosquito collection, verbal consent from the head of each household was obtained. Indoor host-seeking *Anopheles* were collected from 6:30 PM to 6:00 AM in 10 houses by using CDC light traps by hanging them 45 cm above the floor at the feet of sleeping persons, who were protected by untreated mosquito nets.[13] Other occupants in the houses were left to use LLINs provided by the Ministry of Health as part of the routine malaria control. Two trained field assistants from the community turned the light traps on and off at 6:30 PM and 6:00 AM, respectively. In the mornings, collection bags were transported to the entomology laboratory at Arba Minch University for sorting and further processing.

Indoor-resting mosquitoes were sampled in the mornings (6:00 AM to 9:00 AM) from 10 other randomly selected houses by using the PSC technique. All food items and small animals were removed from houses, and all openings and eaves of windows and doors were closed with pieces of cloth. Finally, the floor and furniture were covered with white sheets before spraying houses with a pyrethroid roach killer aerosol (registration no. ET/HHP/130M/S, Kafr; EI Zayat, Egypt). Ten minutes after spraying, anophelines that had been knocked down were collected by using forceps, paper cups, and a torch light. The number of occupants who had slept in each house the previous night was recorded.

Outdoor-resting mosquitoes were collected by using a handheld mouth aspirator, paper cup, and torch from the pit shelters constructed under the shade of mango trees in the compound of each of the 10 houses. Each shelter was 1.5 m deep and had an opening of 1.2 m × 1.2 m. Approximately 0.5 meters from the bottom of each pit shelter, a 30-cm horizontal deep cavity was prepared in each of the four sides.[20] During the collection period (6:30 AM–10:00 AM), the mouth of each pit shelter was covered with an untreated bed net to prevent mosquitoes from escaping.

***Anopheles* mosquito processing.** Female *Anopheles* mosquitoes were killed by freezing, identified to the species level by using a morphologic key,[21] and classified into unfed, freshly fed, half-gravid, and gravid.[12] The abdomens of unfed *An. arabiensis,* the only member of the *An. gambiae* complex in the area,[18] were dissected for parity based on the method of Detinova.[22] The remaining parts of parous and other specimens of *Anopheles* were preserved individually in vials with silica gel for detection of circumsporozoite protein (CSP).

Detection of CSP. The head and thorax of female *Anopheles* were tested for the CSPs of *Plasmodium falciparum*,

TABLE 1

Anopheles spp. mosquitoes collected in Chano in southwestern Ethiopia, by using different collection methods during May 2009–April 2010*

Species	Collection methods			Total no. (%)
	CDC light trap, no. (%)	PSC, no. (%)	Pit shelter no. (%)	
Anopheles arabiensis	2,230 (89)	437 (83.4)	1,057 (63)	3,724 (79.1)
An. marshalli	228 (9.1)	68 (13)	525 (31.3)	821 (17.4)
An. garnhami	7 (0.28)	9 (1.7)	45 (2.7)	61 (1.3)
An. funestus group	2 (0.08)	1 (0.2)	23 (1.3)	26 (0.6)
An. pharoensis	21 (0.84)	0 (0.0)	0 (0.0)	21 (0.4)
Other anophelines†	18 (0.7)	9 (1.7)	28 (1.7)	55 (1.2)
Total	2,506 (53)	524 (11)	1,678 (36)	4,708

*CDC = Centers for Disease Control; PSC = pyrethrum spray catch.
†Others anophelines include *An. tenebrosus*, *An. rhodensiensis*, *An. flavicosta*, *An. longipalpis*, *An. daniculicus*, *An. pretoriensis*, *An. chrysti*, *An. moucheti*, *An. distinctus*, and *An. zeimanni*.

P. vivax 210, and *P. vivax* 247 by enzyme-linked immunoabsorbent assay (ELISA).[23] All positive samples were re-tested for confirmation.

Data and statistical analysis. Data were entered and analyzed by using SPSS version 19 (SPSS Inc., Chicago, IL). The sporozoite rate was calculated as the proportion of mosquitoes positive for *P. falciparum* and *P. vivax* of the total number of mosquitoes tested. Parity rate was determined as the proportion of parous mosquitoes over the number of total mosquitoes dissected.

Analysis of variance was used to compare the monthly total and the house density of *An. arabiensis*. Tukey's honestly significant difference (HSD) test was used to distinguish the months and the houses with the maximum mean density of mosquitoes. Log-transformed data were used for statistical analysis. The Spearman's rho correlation was used to test the relationship between mean monthly density of mosquitoes and EIR with rainfall. All tests were performed at a 0.05 significance level.

The annual *P. falciparum* and *P. vivax* EIR of *An. arabiensis* was calculated from CDC light traps by using the standard method, 1.605 × (no. circumsporozoite-positive ELISA results from CDC light trap/no. mosquitoes tested) × (no. mosquitoes collected from CDC light trap/no. catches) × 365 days, and the alternative method, 1.605 (no. positive ELISA/no. catches) × 365 days.[24] The monthly EIR was determined as a product of the EIRs for each day of the month.

The EIR was also estimated from the PSC as described by the World Health Organization[25] by using the formula: (human-biting rate) × (CSP rate). The human-biting rate was calculated by dividing the total number of freshly fed *An. arabiensis* caught by PSC by the total number of occupants who slept in the houses the night before collection and multiplied by the HBI. The HBI was calculated as the proportion of *Anopheles* mosquitoes that fed on humans (including mixed blood meal origins) of the total *Anopheles* analyzed for blood meal origin.[26] Results of the HBI have been reported elsewhere.[18]

RESULTS

Species composition. Overall, 4,708 anopheline mosquitoes belonging to 16 species were collected and identified. Of the 16 species collected, 14 species were obtained from CDC light traps (n = 2,506), 12 species from pit shelters (n = 1,678), and 9 species from PSCs (n = 524). *Anopheles arabiensis* accounted for 89% of the mosquitoes from CDC light traps, 83.4% from the PSCs, and 63.0% from pit shelter collections. The next most abundant species was *An. marshalli*, which accounted for 9.1% of mosquitoes caught in CDC light traps, 13% in PSCs, and 31.3% in pit shelters (Table 1). Using CDC light traps as a reference, we found that the catch ratio of PSCs to CDC light traps was 0.2 and that of pit shelters to CDC light traps was 0.67 for all anophelines. The figures for *An. arabiensis* were 0.2 for PSC and 0.47 for pit shelters, and the respective values for *An. marshalli* were 0.29 and 2.3. Further analyses were then performed on samples of *An. arabiensis.*

Monthly density of indoor-biting and indoor- and outdoor-resting mosquitoes. The monthly indoor and outdoor density of *An. arabiensis* in relation to rainfall is shown in Figure 2. Monthly density of indoor host-seeking *An. arabiensis* varied significantly (degrees of freedom [df] = 11, F = 6.0, $P < 0.002$). Collections peaked in April 2010 with 53.4/CDC light trap/night. In August 2009, no mosquitoes were found because of extremely low rainfall in June and July 2009. However, over most of the wet months (October, November, March, and April) the variation was not significant ($P > 0.05$, by HSD test).

The density of indoor-resting *An. arabiensis* also varied significantly (df =11, F = 5.5, $P = 0.003$) with a maximum of 7.6/house/day in April 2010. Seasonal trends were also reflected for outdoor-resting density of *An. arabiensis* (df =11, F = 8.1, $P < 0.001$), which had a maximum of 22/pit shelter/day and a minimum of 0/pit shelter/day.

The density of *An. arabiensis* was significantly associated with a one-month lag of rainfall in collections from CDC light traps (r = 0.81, $P < 0.001$), PSCs (r = 0.79, $P = 0.002$), and pit shelters (r = 0.63, $P = 0.03$). However, this association was not significant when *An. arabiensis* density was measured against rainfall in the month of collection or against a two-month lag.

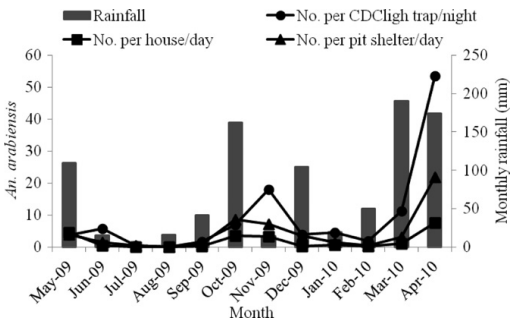

FIGURE 2. Monthly density of *Anopheles arabiensis* collected by three methods in Chano, southwestern Ethiopia. CDC = Centers for Disease Control.

Household variations in indoor-biting and resting mosquitoes. The association between densities of *An. arabiensis* and distance from the major breeding sites is shown in Figure 3. Mosquitoes were more abundant in houses located near main breeding sites on the shore of Lake Abaya than in those further away. Density of indoor-biting *An. arabiensis* differed significantly between houses (df = 9, F = 16.3, $P < 0.001$). Of 2,230 *An. arabiensis* sampled by CDC light traps, 74.3% (1,657) were from the 30% of houses that were closest to larval breeding sites. The distance of the houses closest to the breeding sites was 1350–1570 meters, and the density of indoor-biting *An. arabiensis* in these houses varied from 5.6/CDC light trap/house/night to 46.4/CDC light trap/house/night. In contrast, for houses further from the shore of the lake (2,350–2,600 meters), the density ranged from 0.4/CDC light trap/house/night to 2.8/CDC light trap/night.

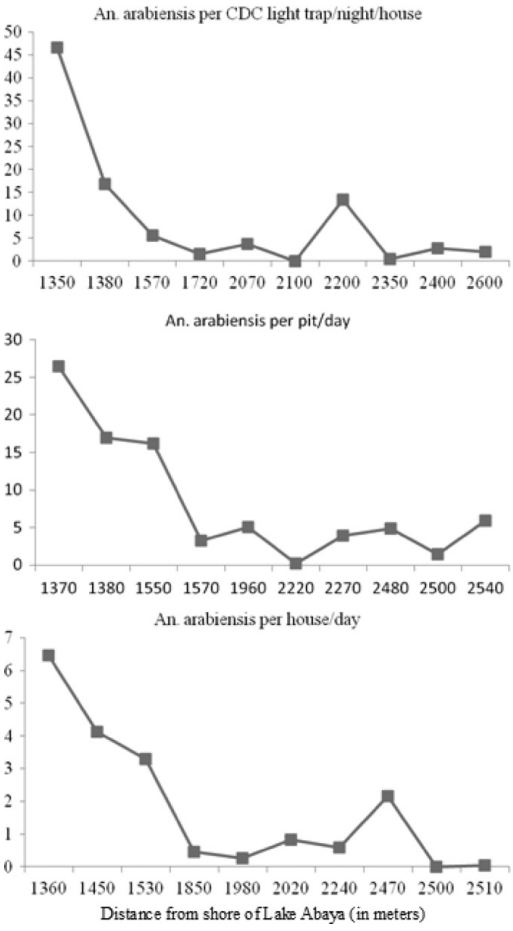

FIGURE 3. Relationship between distance from the identified major breeding site and household *Anopheles arabiensis* density in Chano, southwestern Ethiopia. CDC = Centers for Disease Control.

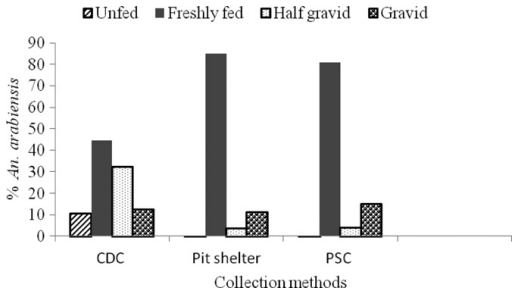

FIGURE 4. Abdominal conditions of *Anopheles arabiensis* from different collection sites in Chano, southwestern Ethiopia. CDC = Centers for Disease Control; PSC = pyrethrum spray catch.

For PSCs, a similar trend in variation of indoor-resting density of *An. arabiensis* (df = 9, F = 8.5, $P < 0.001$) was observed. The density of *An. arabiensis* ranged between 3.3/house/day and 6.5/house/day in those houses closest to the breeding sites (1,360–1,530 meters) but ranged between 0.0 and 2.2/house/day in those houses further from the shore of the lake (1,980–2,510 meters). No significant variation was observed among houses between 1,360 and 1,530 meters from the shore of the lake ($P = 0.83$, by HSD test). The density of *An. arabiensis* in pit shelters also varied (df = 9, F = 9.0, $P < 0.001$).

Abdominal conditions and parity rates. Abdominal conditions of *An. arabiensis* from different collection sites are shown in Figure 4. Freshly fed *An. arabiensis* were dominant (60.3% of 3,724) followed gravid and half- gravid (33.2%), and the percentage of unfed was low (6.4%). The ratio of freshly fed *An. arabiensis* was higher in the PSCs (81% of 437) and pit shelters (85.1% of 1,057) than in CDC light traps (44.5% of 2,230). The proportion of gravid (including half-gravid) to freshly fed *An. arabiensis* was 1.35 times higher in PSCs than in pit shelters.

Unfed *An. arabiensis* were collected almost exclusively in CDC light traps (98.3% of 239). Of the small number of unfed *An. arabiensis* caught and dissected (n = 239) for ovarian ageing, the parous rate was 26.4% (63 of 239). Sixty-two percent (149 of 239) of unfed and 68% (43 of 63) of parous *An. arabiensis* were collected from the house nearest to the shore of the lake.

Sporozoite rates. Overall, 4,534 *Anopheles* were analyzed for CSPs comprising *An. arabiensis* (n = 3,678), *An. marshalli* (n = 763), *An. garnhami* (n = 45), *An. funestus* group (n = 26), *An. pharoensis* (n = 15), and *An. tenebrosus* (n = 7). Of these mosquitoes, 14 *An. arabiensis* were positive for *Plasmodium* CSPs, 11 were positive for *P. falciparum* (78.6%), and 3 were positive for *P. vivax* 210 (21.4%). None of the tested *An. arabiensis* was positive for *P. vivax* 247 CSP, and no other anophelines were found to be positive for CSPs.

Monthly sporozoite rates of *An. arabiensis* from different collection sites are shown in Table 2. The greater numbers of (93% of 14) *Plasmodium*-positive *An. arabiensis* were collected during the wet months (October and November 2009 and March and April 2010). Seven of 11 *P. falciparum* sporozoite-positive *An. arabiensis* were captured by CDC light traps, three were collected in artificial pit shelters (3 of 11), and 1 by PSCs (1 of 11), although there was no statistically

TABLE 2
Monthly circumsporozoite protein–positive *Anopheles arabiensis* collected by three methods from Chano in southwestern Ethiopia*

	CDC light trap			PSC			Pit shelter		
Month and year	No. tested	Pf positive (%, 95% CI)	Pv positive (%, 95% CI)	No. tested	Pf positive (%, 95% CI)	Pv positive (%, 95% CI)	No. tested	Pf positive (%, 95% CI)	Pv positive (%, 95% CI)
May 2009	73	0	0	90	0	0	75	0	0
Jun 2009	105	1 (0.95)	0	8	0	0	32	0	0
Jul 2009	8	0	0	1	0	0	9	0	0
Aug 2009	0	0	0	0	0	0	0	0	0
Sep 2009	28	0	0	6	0	0	16	0	0
Oct 2009	131	0	1 (0.76)	69	0	0	170	0	0
Nov 2009	356	1 (0.28)	0	67	1 (1.5)	1 (1.5)	144	2 (1.4)	0
Dec 2009	76	0	0	4	0	0	70	0	0
Jan 2010	87	0	0	14	0	0	31	0	0
Feb 2010	33	0	0	3	0	0	11	0	0
Mar 2010	224	1(0.44)	0	24	0	0	62	1 (1.6)	0
Apr 2010	1,063	4 (0.38)	0	150	0	0	435	0	1 (0.23)
Total	2,184	7 (0.32, 0.13–0.66)	1 (0.046, 0.001–0.26)	436	1 (0.23, 0.006–1.27)	1 (0.23, 0.006–1.27)	1056	3 (0.28, 0.06–0.83)	1 (0.09, 0.003–0.53)

*CDC = Centers for Disease Control; PSC = pyrethrum spray catches; Pf = *Plasmodium falciparum*; CI = confidence interval; Pv = *P. vivax*.

significant difference between the collection methods. The number of *P. vivax*-positive *An. arabiensis* was similar from all collection sites. The overall *Plasmodium* infection rate (*P. falciparum* and *P. vivax*) of *An. arabiensis* was 0.38%, and the *P. falciparum* sporozoite rate was 0.32% for CDC light traps, 0.28% for pit shelters, and 0.23% for PSCs. When categorized by species, the overall rate was 0.3% for *P. falciparum* and 0.08% for *P. vivax* 210.

Entomologic inoculation rates. The monthly EIRs of *An. arabiensis* estimated from CDC light traps and PSCs are shown in Table 3. The monthly EIRs of *An. arabiensis* were highest in the wet months. A one-month lag of rainfall was significantly associated with the monthly EIR ($r = 0.74$, $P = 0.006$) of *An. arabiensis* from CDC light traps, but there was no significant association with the month of collection or with a two-month lag of rainfall ($P > 0.05$).

The estimated annual EIRs of *Anopheles* from CDC light traps and PSCs are shown in Table 4. Based on the alternative method, the estimated annual *P. falciparum* EIR of *An. arabiensis* from CDC light traps was 17.1 (95% confidence interval [CI] = 7.0–34.6 ib/p/year, whereas that of *P. vivax* was 2.4 (95% CI = 0.06–13.4). The EIR from PSCs was 0.1 ib/p/year for *P. falciparum* and *P. vivax*.

Estimates of the EIRs of *An. arabiensis* were also made individually for the three sub-villages, and for the wet (including main and small rainy months) and dry seasons for CDC light traps (Table 5). The *P. falciparum* EIR was 2.4 (95% CI = 0.12–11.7) in the dry season and 14.7 (95% CI = 5.9–29.4) in the wet season. This finding represented 6.1-fold more infectious bites in the wet than in the dry season. In sub-village 03, *P. falciparum* EIR was 24.4 ib/p/year (95% CI = 6.7–60.3) and *P. vivax* EIR was 5.8 ib/p/year (95% CI = 0.3–29.3). The annual *P. falciparum* EIR of *An. arabiensis* from CDC light traps varied between houses from 0 ib/p/year (in 60% of houses) to 73.2 (95% CI = 15.6–187) ib/p/year in house nearest to the major breeding site.

From the PSCs, two of the *An. arabiensis* collected on the same day from the house nearest the breeding site (with the maximum biting density of mosquitoes) were positive for CSP (1 for *P. falciparum* and 1 for *P. vivax*). The mean biting rate of *An. arabiensis* was 0.33 b/p/n, and the estimated EIR was 0.1 ib/p/year, which was calculated by multiplying the mean

TABLE 3
Monthly EIR of *Anopheles arabiensis* from CDC light traps and pyrethrum spray catches in Chano, southwestern Ethiopia*

	CDC				PSC			
Month and year	No. collected	No. tested for CSP	EIR†	EIR‡ (95% CI)	No. collected	No. tested for CSP	Pf EIR	Pv EIR
May 2009	76	73	0	0	91	90	0	0
June 2009	114	105	2.6	2.4 (0.06–12.0)	8	8	0	0
July 2009	8	8	0	0	1	1	0	0
August 2009	0	0	0	0	0	0	0	0
September 2009	33	28	0	0	6	6	0	0
October 2009	140	131	2.5§	2.4§ (0.06–11.9)	69	69	0	0
November 2009	361	356	2.4	2.4 (0.06–12.0)	67	67	0.1	0.1
December 2009	77	76	0	0	4	4	0	0
January 2010	90	87	0	0	14	14	0	0
February 2010	36	33	0	0	3	3	0	0
March 2010	226	224	2.5	2.5 (0.06–12.4)	24	24	0	0
April 2010	1,069	1,063	9.7	9.6 (2.7–21.2)	151	150	0	0

*EIR = entomologic inoculation rate; CDC = Centers for Disease Control; PSC = pyrethrum spray catch; CSP = circumsporozoite protein; CI = confidence interval; Pf = *Plasmodium falciparum*; Pv = *P. vivax*.
†Standard method: 1.605 (no. enzyme-linked immunosorbent assay [ELISA] positive from CDC light trap/no. ELISA tested) × (no. *An. arabiensis* collected from CDC light trap/no. of catches) × no. days per month.
‡Alternative method: 1.605 (no. ELISA positive/no. catches) × no. days per month.
§*P. vivax* EIR.

TABLE 4
Annual EIR of *Anopheles arabiensis* from CDC light traps and PSCs in Chano, southwestern Ethiopia*

	CDC light trap				PSC		
Variable	No. collected	No. positive/no. tested (%)	EIR†	EIR‡ (95% CI)	No. collected	No. positive/no. tested (%)	EIR
Pf EIR	2,230	7/2,184 (0.32)	17.4	17.1 (7.0–34.6)	437	1/436 (0.23)	0.1
Pv EIR	2,230	1/2,184 (0.046)	2.5	2.4 (0.06–13.4)	437	1/436 (0.23)	0.1

*EIR = entomologic inoculation rate; CDC = Centers for Disease Control; PSCs = pyrethrum spray catches; CI = confidence interval; *Pf = Plasmodium falciparum; Pv = P. vivax.*
†Standard method: 1.605 (no. enzyme-linked immunsorbent assay [ELISA] positive from CDC light trap/no. ELISA tested) × (no. of *Anopheles arabiensis* collected from CDC light trap/no. catches) × 365,
‡Alternative method: 1.605 (no. ELISA positive/no. catches) × 365.

human-biting density by HBI (from a previous report[18]) and the CSP rate.

DISCUSSION

This study showed that the estimated annual *P. falciparum* EIR of *An. arabiensis* in Chano, Ethiopia was 17.1 infectious bites/person/year. *Anopheles arabiensis* was identified as the principal vector of *Plasmodium* in the area, and the risk of exposure to infectious bites was higher in wet seasons than in dry seasons. There was a high variation in EIRs between houses, and a maximum in the house closest to larval-breeding sites.

The estimated EIR from CDC light traps was higher and relatively more representative than that of PSCs because a greater number of *Plasmodium*-positive *An. arabiensis* were collected in CDC light traps. We concluded that the EIR from PSCs cannot be representative of the study area because the two CSP-positive specimens were sampled from one house on the same day. The PSCs also had lower relative sampling efficiency for *Anopheles* mosquitoes than CDC light traps. The most likely explanation is that indoor-resting adults might leave houses immediately after feeding,[7] before spraying, and during spraying.[15] Therefore, using PSCs for estimating EIR can underestimate the human-biting rates.[15] In Senegal, lower EIR was reported for PSCs compared with CDC light traps and HLC for *An. gambiae*,[27] and CDC traps were more efficient than PSCs in collecting more species.[27] Moreover, estimates of human-biting rates of *An. arabiensis* from the CDC light trap were comparable with HLC in an area with low mosquito density and high insecticide-treated mosquito net use.[28] However, CDC light traps failed to determine the human-biting rate of *An. gambiae* s.s. on Bioko Island, Equatorial Guinea.[29] In Tanzania, approximately two-thirds of the human-biting *An. gambiae* complex were collected in a CDC light trap compared with the number caught by HLC.[13]

The HLC is a direct approach for estimating the human-biting rate[12] and is effective in collecting mosquitoes with high sporozoite rates.[27] However, this method could not be applied in our study because it is considered unethical in Ethiopia. It is known that the efficiency of CDC light traps or HLC can vary according to mosquito species.[29] Despite various limitations of CDC light traps as a proxy for HLC, we believe that the EIR values from CDC light traps represented a reasonable estimate of the infective bites of *An. arabiensis* for our study area. However, the use of different methods for estimating EIR makes comparison of EIRs difficult between regions and between countries.[4]

The data from the *P. falciparum* sporozoite rate determination strongly suggest that *An. arabiensis* was the principal vector of malaria in the study area, as reported.[2,14] We identified 16 species of anophelines, of which 5 had been reported from southern Ethiopia.[14] Unlike most studies in this country,[2,14,30] *An. pharoensis* were rarely sampled in our study site. *Anopheles marshalli* was the second most abundant species although in a previous study from Sille in southern Ethiopia, it was found only at low densities.[14] The high proportion of HBI[18] and the moderately frequent occurrence of *An. marshalli* indoors suggests a need for further investigations into its potential role as a vector of malaria in this area and other areas in Ethiopia.

The parous rate of *An. arabiensis* in our study was lower than the 73.2% reported in Sille in 2006[14] and the 58.3% reported in Awash Valley in 1996.[31] This difference could be the result of mass emergence of nulliparous adults from the nearest breeding sites[32] because most unfed *An. arabiensis* were collected from a house near the shore of the lake. The proportion of gravid (including half-gravid) to freshly fed *An. arabiensis* was 1.35 times higher for PSCs than for pit shelters, which suggested a tendency for endophilic rather than exophilic behavior. Exophilic and endophilic behavior of *An. arabiensis* has been reported in southern Ethiopia.[18,33]

We observed a clustered distribution of *Plasmodium* CSPs-positive *An. arabiensis* in a sub-village near the shore of the lake. This finding is consistent with recently reported clustering of malaria cases from the same part of a village[34] and the

TABLE 5
Estimated EIR of *Anopheles arabiensis* from CDC light traps from Chano, southwestern Ethiopia, by sub-village and season*

Variable		No. collected	No. tested	No. catches	No. positive (%)	Pf EIR†	Pf EIR‡ (95% CI)	No. positive (%)	Pv EIR†	Pv EIR‡ (95% CI)
Sub-village	01	124	117	72	0 (0.0)	0.0	0.0 (0.0–24.0)	0	0	0.0 (0–24.0)
	02	411	409	72	3 (0.73)	24.5	24.4 (5.1–68.5)	0	0	0.0 (0–24.0)
	03	1,695	1,658	96	4 (0.24)	25.0	24.4 (6.7–60.3)	1 (0.06)	6.2	5.8 (0.3–29.3)
Season	Wet§	1,949	1,923	120	6 (0.31)	14.9	14.7 (5.9–29.4)	1 (0.05)	2.5	2.4 (0.12–11.7)
	Dry¶	281	261	120	1 (0.38)	2.6	2.4 (0.12–11.7)	0	0	0.0 (0–14.6)

*EIR = entomologic inoculation rate; CDC = Centers for Disease Control; Pf = *Plasmodium falciparum*; CI = confidence interval; Pv = *P. vivax.*
†Standard method: 1.605 (No. enzyme-linked immunosorbent assay [ELISA] positive from CDC light trap/no. ELISA tested) × (no. of *Anopheles arabiensis* collected from CDC light trap/no. catches) × 365.
‡Alternative method: 1.605 (no. positive ELISA/no. catches) × 365.
§EIR calculated by multiplying by 182 days.
¶EIR calculated by multiplying by 183 days.

distribution of malaria vectors in Sille in southern Ethiopia.[35] The *P. falciparum* sporozoite rate of *An. arabiensis* from CDC light traps (0.32%) is comparable with that reported from Sille (0.5%) for HLCs,[14] but the overall sporozoite rate (0.38%) was lower than the 2.26% rate in Sille.[14] A higher *P. falciparum* CSP rate of *An. arabiensis* from CDC light traps was also reported from Ziway in the Central Rift Valley of Ethiopia (1.18%).[36] Krafsur[15] in 1977 reported a higher sporozoite rate (1.87%) for indoor-resting *An. arabiensis*, and a report in 2013 for south-central Ethiopia[16] showed a *P. falciparum* CSP rate of 0.3% for CDC light traps and 0.2% for PSCs for *An. arabiensis*.

This study showed that 93% of sporozoite-positive *An. arabiensis* were found in the wet season and together with EIR were closely associated with rainfall, as has been demonstrated in Gambela in western Ethiopia,[15] in Ifakara, Tanzania,[24] in Eritrea[37] and elsewhere in Africa.[3,9] It is clear that the risk of malaria transmission increases during periods of higher EIR[9,38] and a decrease in density and number of sporozoite-positive mosquitoes results in a decreased EIR.[4] Based on CDC light traps, the estimated annual *P. falciparum* EIR (17.1 ib/p/year) of *An. arabiensis* was higher compared with EIRs of *An. arabiensis* in Gambela (5.36 ib/p/year) and nearby villages (10.47 ib/p/year) estimated from PSCs[15] but much lower than the overall EIR from river villages in Gambela (96.67ib/p/year). Recently, an annual *P. falciparum* EIR of 3.66 ib/p/year for *An. arabiensis* was reported from the central highland of southern Ethiopia.[16] However, in two other studies in neighboring countries, higher EIRs than those recorded in our study have been reported. One study that lasted more than two years and used HLCs[37] reported EIRs of 70.6 ib/p/year from Hiletsidi in the Gash Barka zone and 32.1 ib/p/year in Maiaini in the Debub zone in Eritrea, and another study using the PSC method estimated an annual EIR for *An. arabiensis* of 55.48 ib/p/year in eastern Sudan.[39]

The EIR has implications for monitoring the suitability of vector control interventions.[4,8] Current malaria vector interventions such as LLINs and IRS reduce EIR in many malaria-endemic countries, but none of these interventions has managed to reduce EIR to < 1 ib/p/year,[4] except in the Garki Project (which used propoxur for IRS) and reduced EIR only temporarily.[40] It has been suggested that the annual EIR should be < 1 ib/p/year to reduce and interrupt malaria transmission, based on the finding of a linear relationship between malaria prevalence and EIRs.[41] Our EIR estimate (17.1 ib/p/year) is more than sufficient to continue malaria transmission in the area, where incidence is reported to be 3.6/10,000 person-weeks of observation.[17]

Finally, this study clearly identified *An. arabiensis* as the principal vector of malaria in the Chano area, and the estimated EIR from CDC light traps was higher and more representative than that of PSCs. The risk of exposure to infectious bites was higher in the houses closer to the larval breeding sites, and in wet seasons than in dry seasons. Besides advocating about using the malaria vector control programs for the general population, we advise the vector control programs to focus those households with malaria clustering (hot spots). Such interventions could include IRS during the seasons of the local malaria vector density. Currently, we study if combining screening doors and windows with IRS and LLINs for those houses closer to the breeding sites will reduce malaria transmission. Because the nearby lake was the main mosquito breeding site, it might be worthwhile to assess if aerial spraying of larvicide on the lakeshore would reduce malaria in such populations.

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III

MALARIA JOURNAL

RESEARCH Open Access

The effect of screening doors and windows on indoor density of *Anopheles arabiensis* in south-west Ethiopia: a randomized trial

Fekadu Massebo[1,2*] and Bernt Lindtjørn[2]

Abstract

Background: Screening of houses might have impact on density of indoor host-seeking *Anopheles* mosquitoes. A randomized trial of screening windows and doors with metal mesh, and closing openings on eves and walls by mud was conducted to assess if reduce indoor densities of biting mosquitoes.

Methods: Mosquitoes were collected in forty houses using Centers for Diseases Control and Prevention (CDC) light traps biweekly in March and April 2011. A randomization of houses into control and intervention groups was done based on the baseline data. Windows and doors of 20 houses were screened by metal mesh, and openings on the walls and eves closed by mud and the rest 20 houses were used as control group. Mosquitoes were collected biweekly in October and November 2011 from both control and intervention houses. A Generalized Estimating Equations (GEE) with a negative binomial error distribution was used to account for over dispersion of *Anopheles arabiensis* and culicine counts and repeated catches made in the same house.

Results: Screening doors and windows, and closing openings on eves and wall by mud reduced the overall indoor densities of *An. arabiensis* by 40%. The effect of screenings pronounced on unfed *An. arabiensis* by resulting 42% reduction in houses with interventions. The total costs for screening windows and doors, and to close openings on the eves and walls by mud was 7.34 USD per house.

Conclusion: Screening houses reduced indoor density of *An. arabiensis*, and it was cheap and can easily incorporated into malaria vector strategies by local communities, but improving doors and windows fitness for screening should be considered during house construction to increase the efficacy of screenings.

Keywords: *Anopheles arabiensis*, Screening doors and windows, Indoor density, Metal mesh

Background

Malaria vectors control depends mainly on personal protection, environmental management and use of insecticides for indoor residual spraying (IRS) and mosquito net treatment. The efficacy of long-lasting insecticidal nets (LLITNs) and IRS was reduced in an area where malaria vectors were resistant to insecticide in Benin [1]. In Ethiopia, resistance to pyrethroid insecticides by *Anopheles arabiensis* is increasing [2-4] and, hence, integrated malaria vectors control approach is needed to reduce the challenge from resistance on malaria transmission [5].

Mosquito-proofing houses have a historical success against malaria vectors [6,7]. In Missouri, USA, screened houses afforded a considerable degree of protection against malaria vectors and the incidence of malaria was higher in houses without screening where the population was most accessible for biting mosquitoes [8]. Similarly, in Tennessee River area in USA a substantial reduction of the incidence of malaria was obtained by improving rural houses [7]. Recently, modification of houses reduced houses entry of *Anopheles gambiae* by 78% to 80% in The Gambia [9]. Forty three percent reduction of house entry of *An. gambiae* was reported by closing eves of houses [10]. Screening houses using mosquito proofing materials significantly reduced indoor density of host seeking *An. gambiae* [6,11], and it provides equal protection for all

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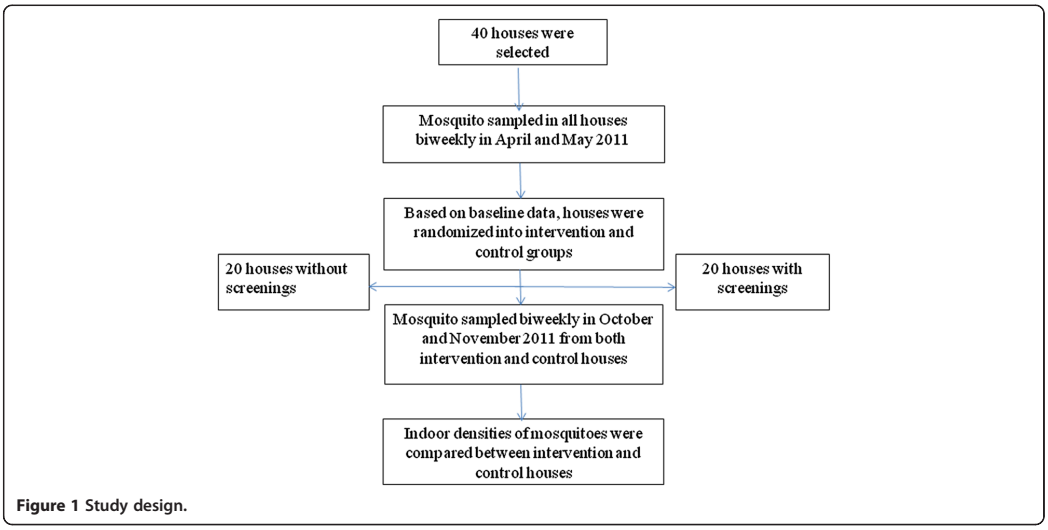

Figure 1 Study design.

occupants in the houses against bites of malaria vectors [12]. *Anopheles arabiensis* predominantly bites humans indoors in study site [4], hence there is a need for additional malaria vector control to reduce house entry and minimize indoors human-vector contact, and divert them to non-human hosts available outdoors. The objective of this study was to assess whether screening windows and doors by metal mesh, and closing openings on eves and walls by mud would reduce indoor densities of *An. arabiensis* in south-west Ethiopia.

Methods

Trial design

A randomized control trial was conducted to assess the efficacy of screening windows and doors with metal mesh, and closing openings on eves and walls by mud on indoor density of *An. arabiensis*. The study was done in Chano, a village 15 km north of Arba Minch town in southwest Ethiopia. The nearest sub-village to Lake Abaya (1,350 to 1,850 m from the shore of Lake Abaya, the major larval breeding sites) was purposely selected for screening trial because both epidemiological [13] and entomological [4,14] findings have shown higher risk malaria exposure in this sub-village than other sub-villages. The detail description of the study area has been reported elsewhere [4,14].

Participants

Forty houses with thatched roof, similar size, found between 1,350 -1,570 m from the main mosquito breeding sites (shore of Lake Abaya), with the number of occupants greater or equal to four and with same number of doors and windows were included for the trial.

Pre-screening mosquito collections

Mosquitoes were collected from all the 40 houses every second week in four consecutive nights per week (10 CDC light traps per night) in April and May 2011. A total of 160 Centers for Diseases Control and Prevention (CDC) light trap nights were conducted to generate the baseline data. Anophelines were identified using a morphological key [15] and classified into unfed, freshly fed,

Table 1 The baseline data of the mean number of *An. arabiensis* per CDC light trap night (April and May 2011)

Abdominal condition	Pre-control houses (n = 20)	# (95%CI)	Pre-intervention houses (n = 20)	# (95%CI)
	N (no. of mosquitoes)		N (no. of mosquitoes)	
Unfed	683	8.5 (2.3, 14.7)	624	7.8 (4.8, 10.8)
Fresh fed	580	7.2 (4.1, 10.4)	639	7.9 (4.4, 11.5)
Half gravid	105	1.3 (0.7, 1.9)	93	1.2 (0.7, 1.7)
Gravid	240	3 (1.8, 4.2)	269	3.3 (2, 4.7)
Overall	1608	20.1(10.9, 29.3)	1625	20.3 (12.8, 27.8)

#Mean number of *An. arabiensis* per CDC light trap per night; Pre-control houses = houses randomized as control group during intervention; Pre-intervention houses = houses randomized for screening during intervention.

Figure 2 External view of screened door.

half gravid and gravid based on abdominal condition. Culicines were counted and discarded.

Randomization

Based on the baseline data, the 40 houses were simply randomized into control and intervention groups using IBM SPSS version 20 (Figure 1). The unit of randomization was an individual house. Table 1 shows the baseline data on number of *An. arabiensis* per CDC light trap per night of the two groups which were similar.

Interventions

Doors and windows of the 20 houses were screened by metal mesh (Figure 2), and openings in the walls and eves were closed with mud (Figure 3) to see if screening the doors and windows reduce house entry and indoor density of host seeking *An. arabiensis*. Any openings in the wall for ventilation purpose were closed by metal mesh only. Timber-frame was used for screening doors. The screened doors were fixed on the frame of the main door externally using hinges, and were removed by rolling to enter or leave the houses. Windows were permanently fixed externally by metal mesh after getting permission from house owners. The costs for metal mesh, timber frame, nails and labour were calculated.

Post-screening mosquito collections

The 40 houses were sampled every second week in October and November 2011 by taking five houses from intervention group and five houses from control group per night for four consecutive nights per week. Anophelines were identified using a morphological key [15] and classified into unfed, freshly fed, half gravid and gravid based on abdominal condition. Culicines were counted and discarded.

Outcome variable

The outcome variable of this study was indoor densities of *An. arabiensis* collected per CDC light trap per night. Mosquito collectors were not masked because CDC light traps are not depending on human skills.

Statistical analysis

Mosquito data within household was described by mean number of *An. arabiensis* per CDC light trap per night. A Generalized Estimating Equations (GEE) with a negative binomial error distribution was used to account for over dispersion of *An. arabiensis* and culicine counts. A first-order autoregressive correlation structure was considered to account a serial correlation between repeated catches made in the same house. The GEE was fitted separately to counts of different abdominal conditions of *An. arabiensis* and overall culicine to determine the protective effect of screenings against house entry of the species. The mean's ratio of mosquitoes between screened and control houses were used to determine the percentage reduction of house entry. Non-parametric correlation was used to see the house entry patterns of *An. arabiensis* in pre-intervention and post-intervention months. All houses were included in analysis because no damaged metal mesh and malfunctioned CDC light traps were observed. The statistical significance of screening effect was tested by P-value obtained from GEEs at 0.05 level. IBM SPSS version 20 (SPSS Inc, Chicago, USA) was used for data entry and analysis.

Figure 3 Openings around eves closed by mud (A) and openings for ventilation closed by metal mesh (B).

Table 2 The efficacy of doors and windows screening on indoor host seeking densities of *An. arabiensis* (October and November 2011)

Abdominal condition	Control N	# (Wald 95%CI)	Intervention N	#(Wald 95%CI)	Means ratio	% reduction	*p*
Unfed	189	2.4 (2.2, 2.7)	115	1.4 (1.1, 1.9)	0.58	42	0.004
Fresh fed	227	2.8 (2.3, 3.6)	143	1.8 (1.5, 2.1)	0.64	36	0.001
Half gravid	13	0.15 (0.1, 0.4)	10	0.13 (0.1, 0.3)	0.87	13	0.83
Gravid	197	2.5 (1.9, 3.5)	122	1.5 (1.2, 1.9)	0.60	40	0.002
Overall	626	7.9 (6.5, 10.1)	390	4.8 (3.9, 6.2)	0.60	40	0.006

Mean number of *An. arabiensis* per CDC light traps per night.

Ethical conditions

A verbal consent was obtained from the household head and they provided with insecticide untreated bed nets.

Results

Mosquito abundance and species composition

A total of 4,778 anophelines and 3,111 culicines were collected during the study period. *Anopheles arabiensis* was the predominant (n = 4249, 89%) species followed by *Anopheles marshalli* (n = 246, 5.1%) and *Anopheles pharoensis* (n = 178, 3.7%). *Anopheles demeilloni, Anopheles dancalicus, Anopheles cinctus, Anopheles culicifacies, Anopheles funestus, Anopheles obscures, Anopheles tenebrosus, Anopheles parensis, Anopheles rufipes, Anopheles ziemanni, Anopheles garnhami* and *Anopheles salbaii* accounted only 2.2% (n = 105).

House entry patterns of *Anopheles arabiensis* at different months

House entry of *An. arabiensis* followed similar patterns before and during intervention. Households with a maximum number of *An. arabiensis* in the months prior to intervention received higher number during intervention both in control houses (r = 0.72, p <0.001) and houses that were subsequently screenings (r = 0.56, p = 0.01).

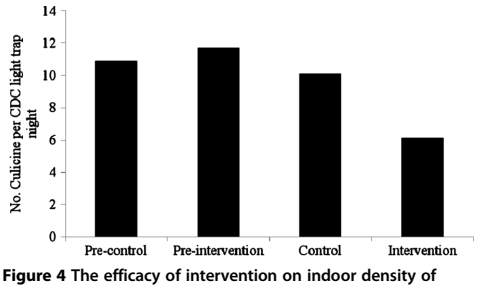

Figure 4 The efficacy of intervention on indoor density of culicine mosquitoes from Chano in southwest Ethiopia.

The efficacy of intervention on indoor density of *An. arabiensis*

The efficacy of screening doors and windows on indoor density of *An. arabiensis* is shown in Table 2. The mean number of *An. arabiensis* was 7.9 (95% Wald Confidence Interval (CI): 6.5, 10.1) per CDC light trap per night in non-screened houses, compared with 4.8 (95% Wald CI: 3.9, 6.2) per CDC light trap per night in houses with screens. There was 40% fewer *An. arabiensis* in houses with interventions than those without interventions (ratio of means 0.6, p = 0.006). The indoor density of hunger *An. arabiensis* was reduced by 42% in intervention group (ratio of means 0.58, p = 0.004). The intervention also had an impact on indoor density of freshly fed *An. arabiensis* by resulting 36% reduction of house entry.

Figure 4 shows the baseline data and the efficacy of intervention against culicine mosquitoes. The mean number of culicine mosquitoes was 10.1 (95% Wald CI: 8.8, 11.9) in houses without interventions and 6.1 (95% Wald CI: 5, 7.8) in screened houses resulting a 40% reduction in door density of biting nuisance culicine mosquitoes. The total costs for screening windows and doors, and to close openings on the eves and walls by mud was 7.34 USD per household (Table 3).

Discussion

The results of this randomized trial show that screening doors and windows, and closing openings on walls and eves by mud reduced the overall indoor densities of *An. arabiensis* by 40%. Although screening intervention reduced indoor density of *An. arabiensis* at all

Table 3 Costs for intervention

Materials	Cost per household
Cost for metal mesh	1.44 USD * 2.5 m = 3.6 USD
Cost for screening including nails and wooden materials	2.3 USD
Closing openings with mud	1.44 USD
Total cost per house	7.34 USD

Total number of houses = 20.

abdominal stages, the reduction was substantially higher against unfed *An. arabiensis*. The intervention was based on locally bought materials, and was affordable.

The houses we assessed were grass thatched, and doors and windows were not well-suited for screenings. The incompatible of doors for screening might reduce the efficacy in such house types. The roofs of grass thatched local houses prevent opening of screened doors outward; consequently, the screened doors were not permanently fixed and people might not used them constantly during the nights before collection.

A house screening study from The Gambia resulted in 43% reduction of house entry of *An. gambiae* which is comparable to the current study [10]. Although the incidence of malaria infection was not assessed, the previous studies have shown less malaria cases in screened houses than in controls [6]. Moreover, the association between the incidence of malaria and the accessibility of a population to mosquitoes was observed with the highest incidence in the population most accessible for mosquito bites [8]. In The Gambia, screening doors, windows and eves resulted in 59% reduction of indoors density of *An. gambiae,* and reduced the prevalence of anaemia [11]. Screening houses by plastic insect-screen resulted 80% protection from indoor bites of *An. gambiae* in The Gambia [9].

The likely explanation for moderate efficacy the current intervention is that people may not use screened doors in the nights before collection because the screened doors were not permanently fixed as windows. Moreover, *An. arabiensis* could enter houses when the people open the doors during earlier hours of the night [16]. The small gaps left in the door and windows could also contribute for the moderate reduction of mosquitoes in the intervention houses. Maximum reduction in number of *An. arabiensis* might be achieved if the screened doors were constantly used by home owner's, and the doors were compatible for screening. The likely reason for the overall lower number of mosquitoes sampled during intervention (October/November 2011) compared to the pre-intervention period (April/May 2011) was presumably due to the seasonal variation of the area. Study from the same area shows the highest density of mosquitoes in April and May; the months with the highest rainfall than the October and November; the months with short and small rains [4,14].

The intervention was cheap, and simple to implement and hence, it can be incorporated into an integrated vector management strategy, and combined with IRS and LLITNs. The cost for screening doors and windows and closing openings on eves and walls (7.3 USD per house) was lower than that was used for fully screening houses (9.98 USD per person) and for screening ceilings (8.69 USD per person) in The Gambia [11]. However, improving doors and windows fitness for screening should be considered during house construction to increase the efficacy of screenings.

Competing interests

The authors declared that they have no competing interests.

Authors' contributions

FM: Project design, conducted field and laboratory work, data analysis and interpretation, wrote the draft manuscript, BL: Project design, field supervision, provided statistical input and manuscript revision. All authors read and approved the final manuscript.

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Parasites & Vectors

RESEARCH Open Access

Zoophagic behaviour of anopheline mosquitoes in southwest Ethiopia: opportunity for malaria vector control

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Abstract

Background: Increased understanding of the feeding behaviours of malaria vectors is important to determine the frequency of human-vector contact and to implement effective vector control interventions. Here we assess the relative feeding preferences of *Anopheles* mosquitoes in relation to cattle and human host abundance in southwest Ethiopia.

Methods: We collected female *Anopheles* mosquitoes bi-weekly using Centers for Disease Control and prevention (CDC) light traps, pyrethrum spray catches (PSCs) and by aspirating from artificial pit shelters, and determined mosquito blood meal origins using a direct enzyme-linked immunosorbent assay (ELISA).

Results: Both *Anopheles arabiensis* Patton and *An. marshalli* (Theobald) showed preference of bovine blood meal over humans regardless of higher human population sizes. The relative feeding preference of *An. arabiensis* on bovine blood meal was 4.7 times higher than that of human blood. *Anopheles marshalli* was 6 times more likely to feed on bovine blood meal than humans. The HBI of *An. arabiensis* and *An. marshalli* significantly varied between the collection methods, whereas the bovine feeding patterns was not substantially influenced by collection methods. Even though the highest HBI of *An. arabiensis* and *An. marshalli* was from indoor CDC traps collections, a substantial number of *An. arabiensis* (65 %) and *An. marshalli* (63 %) had contact with cattle. *Anopheles arabiensis* (44 %) and *An. marshalli* (41 %) had clearly taken bovine blood meals outdoors, but they rested indoors.

Conclusion: *Anopheles* mosquitoes are zoophagic and mainly feed on bovine blood meals than humans. Hence, it is important to consider treatment of cattle with appropriate insecticide to control the zoophagic malaria vectors in southwest Ethiopia. Systemic insecticides like ivermectin and its member eprinomectin could be investigated to control the pyrethroid insecticides resistant vectors.

Keywords: *Anopheles arabiensis*, *Anopheles marshalli*, Bovine blood meal, Feeding preference, Human blood meal, Zoophagic vectors

Background

In Ethiopia, *Anopheles arabiensis* Patton is responsible for malaria transmission [1, 2]. *Anopheles pharoensis* Theobald is the secondary vector [1]. *Anopheles amharicus* Hunt, Wilkerson & Coetzee [3], previously known as *An. quadriannulatus* sp. B, is zoophagic and has no role in malaria transmission [4]. Currently, the roles of *An. funestus* Giles and *An. nili* (Theobald) are uncertain because the species are reported rarely and none of them were positive for *Plasmodium* species [2, 5]. *Anopheles coustani* Laveran, *An. marshalli* (Theobald) and *An. demeilloni* Evans were reported from south Ethiopia [6], but none of them were tested for blood meal origins and circumsporozoite proteins detection. A substantial proportion of *An. christyi* (Newstead & Carter), *An. cinereus* Theobald and *An. demeilloni* had human blood meal origin in south-central highland of Ethiopia [7].

The tendency of malaria vectors to feed on humans (amplifying host of malaria) increases the chance of

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malaria transmission to the susceptible human hosts [8]. On the other hand, those mosquitoes feeding on non-human hosts are likely have a low role in malaria transmission [9]. The current malaria vector control tools such as indoor residual spraying (IRS) and long lasting insecticidal treated nets (LLINs) are targeting endophagic and endophilic malaria vectors [10]. The most anthropophagic and endophagic malaria vectors are can successfully controlled by the LLINs and IRS, whereas LLINs and IRS might have little impact on those species predominantly feed on cattle outdoors [11]. The transmission of malaria continues even in areas with high coverage of indoor-based interventions, due to those vectors feeding on animals and humans outdoors [11, 12], hence there is a need to target all the possible blood meals sources of zoophagic malaria vectors for successful control of the species [10, 12]. Zooprophylaxis is the diversion of vectors to animals or treatment of animals with appropriate insecticides as a supplementary intervention to control the zoophagic vectors [13]. Treatment of animals using toxic chemicals to kill the zoophagic vectors while feeding on animals may decrease the vector population and hence malaria transmission [10]. The impact of zooprophylaxis may however be further maximized by increasing the coverage of indoor-based interventions (LLINs and IRS) to push mosquitoes outdoors where animals are mostly kept, thereby suppressing the human blood meal source and reducing the level of infection in the local vector population [14, 15].

Understanding the blood feeding behaviour of the local *Anopheles* mosquitoes is important to determine the feeding preference of malaria vectors [16, 17], which can inform supplementary vector control interventions [10, 16, 17]. The objective of this study was to assess the relative feeding preferences of *Anopheles* mosquitoes in relation to cattle and human host abundance in Chano, Southwest Ethiopia.

Methods

Study area

This study was conducted in Chano village, 15 km north of Arba Minch town from May 2009 to April 2010. The village is located at 06°6.666' N and 37°35.775' E, at an altitude of 1206 m above sea level. Domestic animals are usually kept in compounds close to the houses at night and people usually sleep indoors throughout the year. There is no permanent or seasonal movement of animals in/out of the village for feeding or watering. Detailed information on the study area and collection methods have previously been published [18].

Host surveys

The total number of human population in the study area was obtained from the epidemiological study conducted in the area during the same period [19]. The total number of cattle and other animals during the study period were obtained from the agricultural office in the village. In addition, during mosquito collections we recorded both the number of people in the houses, and number of cattle in the compounds where collections were made.

Mosquito collections

Freshly fed *Anopheles* mosquitoes were collected bi-weekly for one year from May 2009 to April 2010. We used ten CDC light traps to collect indoor host-seeking *Anopheles* mosquitoes. The CDC light traps were hung 45 cm above the floor at the feet of sleeping persons who were protected by untreated nets. The light traps were turned on at 18:00 and off at 6:00 h by two trained field assistants in the community. On the following morning, the mosquitoes were transported to the entomology laboratory of Arba Minch University for species identification and preservation for blood meal analysis.

Indoor resting mosquitoes were sampled in the mornings (6:00 to 9:00 h) from 10 other randomly selected houses using the pyrethrum knockdown spray collection (PSC) technique following the recommendations of WHO [20]. Outdoor resting mosquitoes were collected in the mornings (6:30-10:30 h) from 10 pit shelters constructed according to the method of Silver [21], under the shade of mango trees in the compound of 10 selected houses. While collecting mosquitoes from pit shelters, the mouth of each pit shelter was covered by untreated bed nets to maximize collection by preventing mosquitoes from escaping.

Mosquito processing

Female *Anopheles* mosquitoes were identified to species using morphological characteristics [22]. Abdomens were examined under a dissecting microscope and females classified into unfed, freshly fed, half-gravid and gravid [20]. Freshly fed *Anopheles* mosquitoes were preserved individually in vials containing dessicating silica gel for later blood meal analysis.

Detection of blood meal sources

The blood meals of freshly fed *Anopheles* mosquitoes were analysed by a direct enzyme-linked immunosorbent assay [23] using human and bovine antibodies. Each blood meal sample was considered positive if the absorbance value exceeded the mean plus three times the standard deviation of four negative controls (laboratory colony of *An. arabiensis* not fed on blood). Positive controls contained human and bovine blood.

Data analysis

The human blood index (HBI) and bovine blood index (BBI) were calculated as the proportion of mosquitoes

fed on either human or bovine blood meals out of the total blood meals tested [17]. Mixed (human + bovine) blood meals were added to the number of human and bovine blood meals when calculating the HBI and BBI [24, 25].

A linear regression analysis was done to see the impact of cattle to human ratio and collection methods on human and bovine blood meal index of *Anopheles* mosquitoes. The relative feeding preference of *Anopheles* mosquitoes were calculated according to Hess et al. [26] by taking the percentage of freshly fed *Anopheles* mosquitoes with either humans or bovine blood meals divided by the percent of either human or cattle in the area.

The following assumptions were made to characterize the host feeding preference: 1) the abundances of people and cattle did not vary throughout the year, 2) there is no seasonal change in sleeping habits of people in the study area, 3) host defensive behaviour did not alter mosquito feeding success, 4) people and cattle available out of doors did not vary at different season. Data were entered and analysed using SPSS version 20 (SPSS Inc., Chicago. IL).

Results

Human and cattle population

The human population size was 6661, some three times higher than number of cattle ($n = 2217$). Goats, sheep, donkeys, and chickens were also present in the village.

Blood meal origins of *Anopheles* mosquitoes

The blood meal origins of *Anopheles* mosquitoes are shown in Table 1. The higher proportion of *An. arabiensis* (58 %; 521 of 894), *An. marshalli* (64 %; 279 of 436) and *An. garnhami* (60 %; 21 of 35) from pit shelters had blood meals of bovine origin. *Anopheles arabiensis* (65 %; 644 of 988) and *An. marshalli* (63 %; 103 of 164) from CDC light traps had mixed blood meals of human and bovine origins. Only a low proportion of *An. arabiensis* (3 %; 27 of 894) and *An. marshalli* (3 %; 14 of 436) from pit shelters contained human blood. Some 44 % *An. arabiensis* and 41 % *An. marshalli* had bovine blood meals, but were found indoors in resting collections.

Relative feeding preference of *Anopheles* mosquitoes

Regardless of the three-fold higher prevalence of humans in the study area, *An. arabiensis* and *An. marshalli* showed a strong preference of bovine blood meal over humans (Table 2 and Fig. 1). The relative feeding preference of *An. arabiensis* on cattle was 4.7 times higher than that on humans and *An. marshalli* was 6 times more likely to feed on cows than humans. The relative bovine blood meal feeding preference of *An. garnhami* was 5.3 times higher than humans. Thus, in this study area, *An. arabiensis, An. marshalli* and *An. garnhami* preferred bovine blood meals over humans.

The HBI of *An. arabiensis* significantly varied between the collection methods ($p = 0.02$), whereas the bovine feeding patterns of the species was not substantially

Table 1 Variation in blood meal origins of *Anopheles* mosquitoes from different collection sites in Chano village in southwest Ethiopia

			Blood meal origins			
Collection methods	Species	No. analysed	Human (%)	Bovine (%)	Mixed (%)	Unknown (%)
CDC light traps	*An. arabiensis*	988	94 (9.5)	70 (7.1)	644 (65.2)	180 (18.2)
	An. marshalli	164	45 (27.4)	6 (3.7)	103 (62.8)	10 (6.1)
	An. garnhami	7	4 (57)	0 (0.0)	2 (29)	1 (14)
	An. pharoensis	7	1 (14)	0 (0.0)	2 (29)	4 (57)
	An. tenebrosus	4	1 (25)	1 (25)	0 (0.0)	2 (50)
	An. funestus group	1	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)
PSC	*An. arabiensis*	352	59 (16.8)	154 (43.8)	74 (21)	65 (18.4)
	An. marshalli	56	9 (16.1)	23 (41.1)	18 (32.1)	6 (10.7)
	An. garnhami	7	3 (42.9)	2 (28.6)	2 (28.6)	0 (0.0)
	An. funestus group	1	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)
Pit shelters	*An. arabiensis*	894	27 (3.0)	521 (58.3)	89 (10)	257 (28.7)
	An. marshalli	436	14 (3.2)	279 (64)	54 (12.4)	89 (20.4)
	An. garnhami	35	2 (5.7)	21 (60)	5 (14.3)	7 (20)
	An. funestus group	14	0 (0.0)	5 (35.7)	3 (21.4)	6 (42.9)
	An. tenebrosus	1	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)
Total		2967	259 (8.7)	1089 (36.7)	996 (33.6)	629 (21)

PSC pyrethrum spray catches, *CDC* centers for disease control and prevention

Table 2 The relative feeding preference of *Anopheles* mosquitoes by considering human and cattle abundance from Chano village in southwest Ethiopia

Species	% HB	% HP	[a]FR	% BB	% BP	[b]FR
An. arabiensis	44	75	0.59	70	25	2.8
An. marshalli	37	75	0.49	74	25	2.9
An. garnhami	37	75	0.49	65	25	2.6
An. funestus	19	75	0.25	38	25	1.5
An. pharoensis	43	75	0.57	29	25	1.2
An. tenebrosus	20	75	0.27	20	25	0.8

% HB percent human blood meals, *% HP* percent human in populations, *% BB* percent bovine blood meals, *% BP* percent bovine, [a]*FR* forage ratios of human (% HB divided by % HP), [b]*FR*, forage ratios of cattle (% BB divided by % BP)

influenced by collection methods ($p = 0.17$). The highest HBI of *An. arabiensis* and *An. marshalli* was from indoors CDC trap collections, while the lowest was from pit shelters (Figs. 2 and 3). *Anopheles arabiensis* showed a higher relative feeding preference on cattle and it remained higher in all collection methods. The feeding patterns of *An. arabiensis* and *An. marshalli* from PSC were inconsistent and showed variation between households (Figs. 2 and 3). Likewise, the human feeding patterns of *An. marshalli* ($p = 0.005$) varied between collection methods whereas the bovine feeding patterns of the species didn't vary much by collection method ($p = 0.86$) and remained higher in all collection methods.

The relative feeding pattern of both *An. arabiensis* and *An. marshalli* on humans decreased as the cattle to human ratio increased, whereas the cattle feeding preference either decreased for *An. arabiensis* or increased for *An. marshalli* as the cattle to human ratio increased (Figs. 4 and 5). The impact of cattle to human ratio of households on HBI ($p = 0.87$) and BBI ($p = 0.86$) of *An. arabiensis* was not significant. Similarly, the HBI ($p = 0.59$) and BBI ($p = 0.18$) of *An. marshalli* was not significantly influenced by the cattle to human ratio of households. This indicates that the human and bovine feeding patterns of *An. arabiensis* and *An. marshalli* slightly changed due to the number of cattle to human ratio of each household which in turn might be due to the accessibility of cattle outdoors in the village throughout the night.

The predicted and observed human and bovine blood meal indexes of *An. arabiensis* and *An. marshalli* were similar (Figs. 6 and 7) but the BBI fitted best for both species than HBI, indicating the bovine feeding pattern of the species is consistent in the area (Figs. 6 and 7).

Discussion

Anopheles mosquitoes are zoophagic; mainly feeding on bovine blood meals than humans. We observed this in spite of the higher human proportion in the area. The relative feeding preferences of *An. arabiensis* and *An. marshalli* on human varied between collection methods with the highest human blood meal indexes from indoor CDC light traps collections. But, many of the human fed *An. arabiensis* and *An. marshalli* had contact with cattle since the higher human blood meal index was because of the mixed (human/bovine) blood meal origins. The bovine blood meal indexes of *An. arabiensis* and *An. marshalli* did not vary, and remained high at all collection methods indicating the consistency of

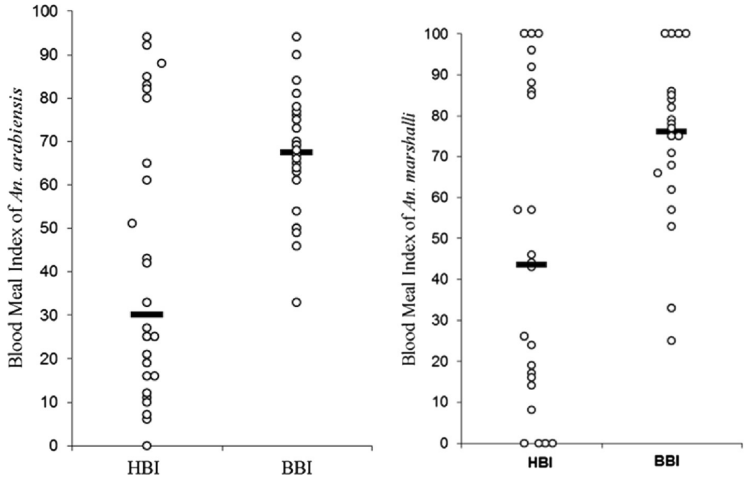

Fig. 1 The human and bovine meal feeding preference of *Anopheles arabiensis* and *An. marshalli* from different sampling houses (Median)

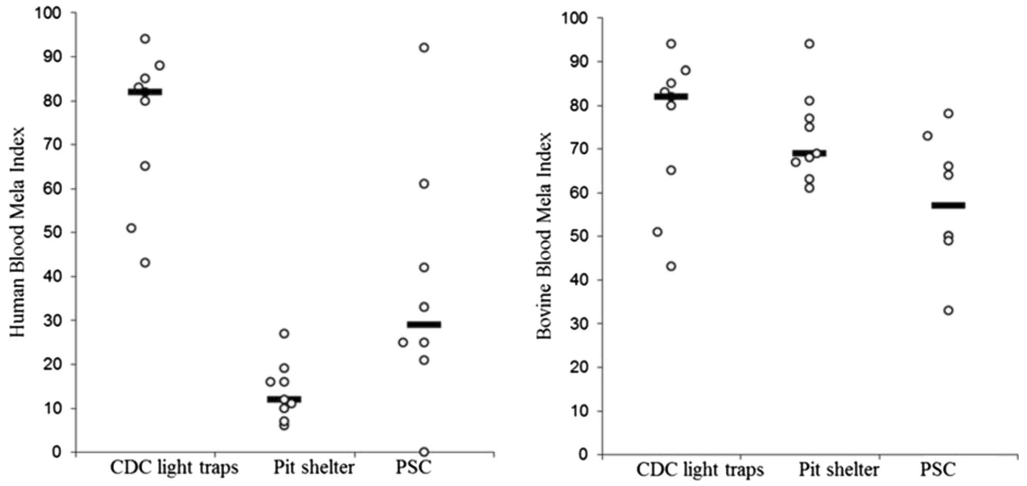

Fig. 2 Human and bovine blood index of *Anopheles arabiensis* from different collection methods (▬ Median)

bovine feeding patterns of the *Anopheles* mosquitoes in the village.

Our results are in agreement with the previous studies that reported the zoophilic feeding preferences of *An. arabiensis* [27–30], *An. marshalli* and *An. demeilloni* [16]. The feeding patterns of mosquitoes might be influenced by proximity, accessibility and defensive behaviours of hosts [18, 31]. In our study area, animals are usually kept outdoors at night where mosquitoes first encounter animals while searching for blood meal sources.

The higher relative feeding preference of *Anopheles* mosquitoes on cattle (zoophagic behaviour) can be considered as an opportunity to introduce supplementary vector control interventions based on zooprophylaxis - the diversion of mosquitoes from humans to animals [13, 14, 28]. Malaria vectors which mostly feed on human indoors can successfully be controlled by the LLINs

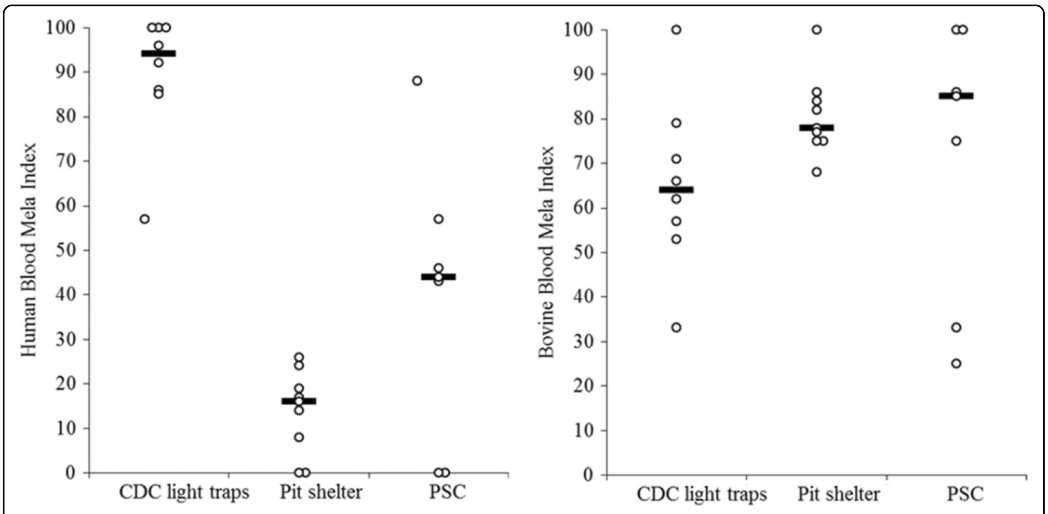

Fig. 3 Human and bovine blood meal index of *Anopheles marshalli* from different collection methods (▬ Median)

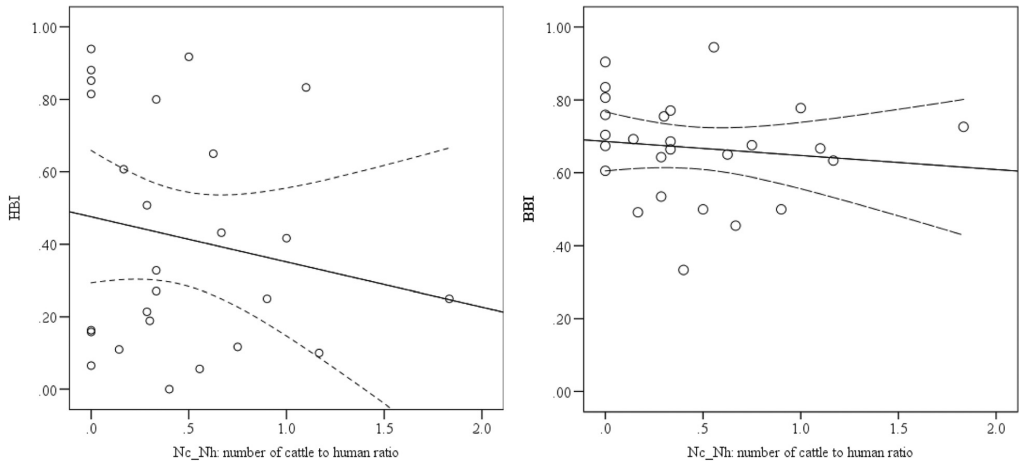

Fig. 4 The relationship between human and bovine blood meal indices of *Anopheles arabiensis,* against the ratio of number of cows to humans in each household. The solid lines represent the linear analysis fit model and dash lines for the 95 % confidence intervals of the mean

and IRS, whereas those species predominantly feeding on cattle outdoors continue to transmit malaria regardless of high coverage of indoor based interventions [11]. Hence, there is a need to target those zoophagic species for control of human malaria [10, 12]. Zooprophylaxis can reduce malaria transmission by pulling mosquitoes toward the dead-end hosts so that the infectious mosquitoes effectively "waste" their sporozoites, and the susceptible mosquitoes cannot acquire parasitaemia from non-human hosts. The impact of zooprophylaxis can be further enhanced by increasing indoor interventions (e.g. bed nets) to protect humans from bites, thus, pushing mosquitoes outdoors towards the alternative mammalian blood sources [14] (dead-end host), effectively reducing infectious bites on humans [14]. In Ethiopia, keeping animals in separate sheds reduced the human biting rates of *An. arabiensis* showing that the animals had the capacity to pull mosquitoes [15]. In the same study, Seyoum

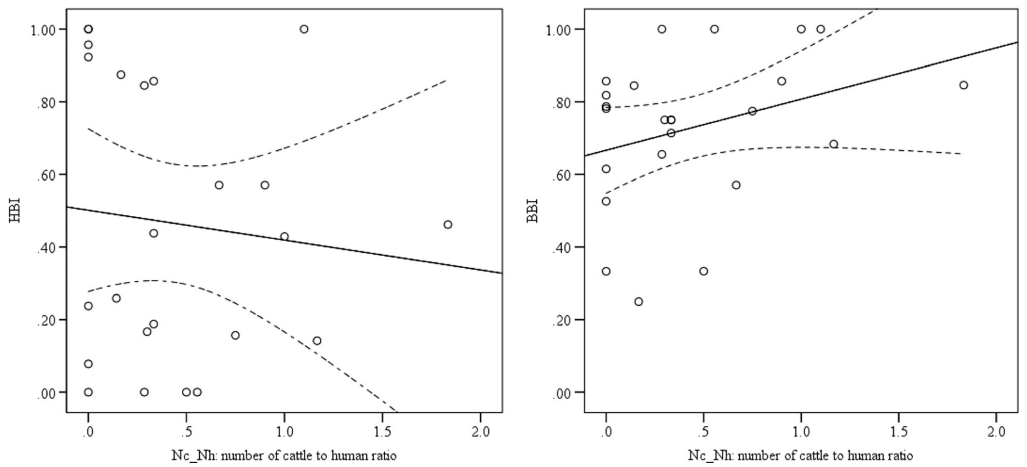

Fig. 5 The relation between human and bovine blood meal indices of *Anopheles marshalli* against the ratio cattle to human in each household. The solid lines represent the linear analysis fit model and dash lines for the 95 % confidence intervals of the mean

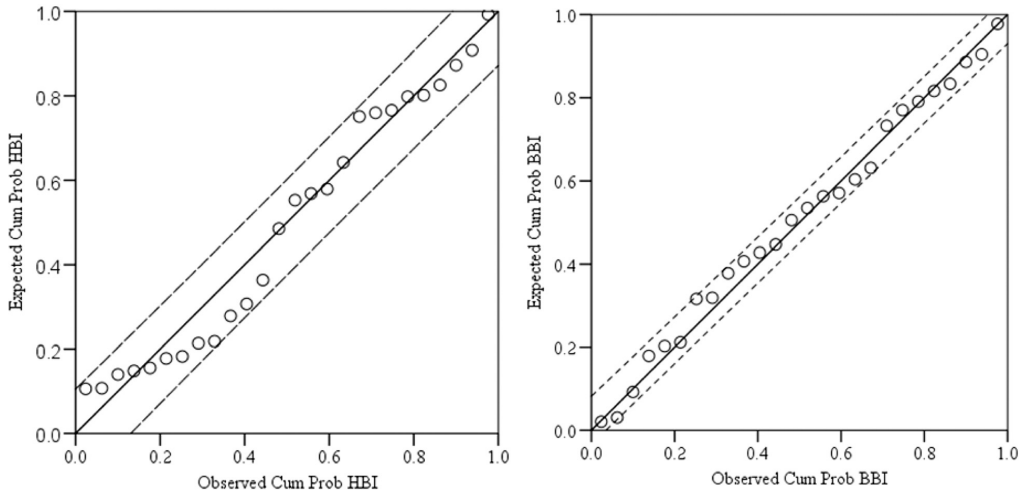

Fig. 6 Comparison between observed and predicted human and bovine blood meal indices of *Anopheles arabiensis* using a linear regression analysis (n = 26, df = 24, $r^2 = 0.97$ for HBI and $r^2 = 0.99$ for BBI)

et al. reported that sharing the house with animals increased the human biting rate of malaria vectors further supporting the pulling potential of animals [15].

Zooprophylaxis strategies can be further strengthened by treating cattle with insecticides (increasing the coverage of insecticides to all blood meal sources) to kill mosquitoes while feeding on animals, thus reducing the vector population and local malaria transmission [10, 13]. Spraying animals with pyrethroid insecticides reduced the incidence of malaria in Pakistan [13]. Habtewold et al. [32] identified two challenges while treating cattle to control *An. arabiensis:* one is the preference of *An. arabiensis* to feed on legs where insecticides washes off easily, and the second is short duration of the action of deltamethrin. Moreover, *An. arabiensis* in the study area is resistant to pyrethroid

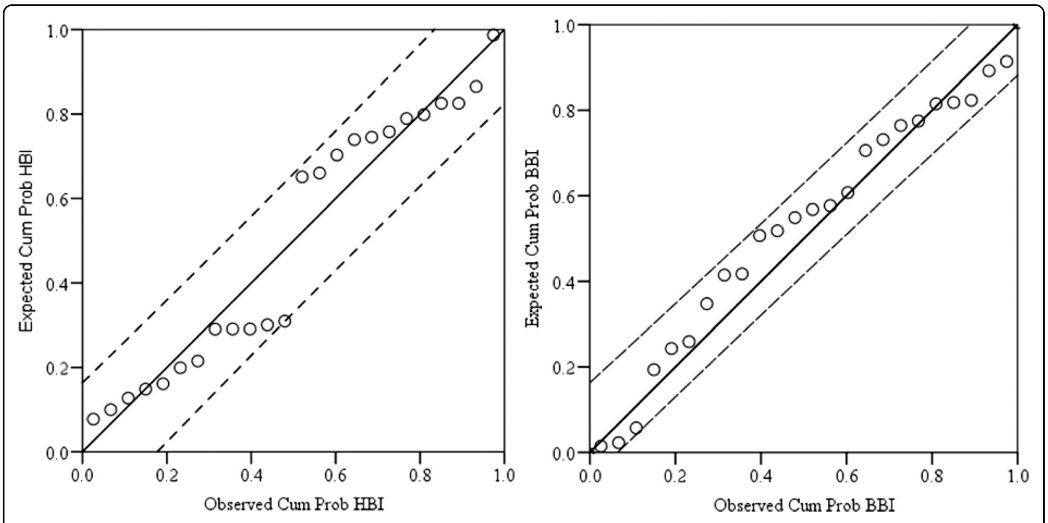

Fig. 7 Comparison between observed and predicted human and bovine blood meal indices of *Anopheles marshalli* using a linear regression analysis (n = 24, df = 22, $r^2 = 0.93$ for HBI and $r^2 = 0.96$ for BBI)

insecticides (the only class of insecticide recommended for spraying animals) including deltamethrin [18]. Alternative longer-lasting chemicals like ivermectin, a systemic insecticide widely used to control endoparasites and blood sucking ectoparasites of animals [33], may be used to control such zoophilic malaria vectors as *Anopheles* mosquitoes are sensitive to low concentrations of ivermectin [34].

The higher proportion of *An. arabiensis*, *An. marshalli* and *An. garnhami* that fed on human blood were from indoor host seeking collections which might be related with the low bed net use rate of the community during the study period [19], and to resistance of *An. arabiensis* to deltamethrin insecticide [18] or early biting behaviours of mosquitoes [35]. But, many mosquitoes had mixed (human/bovine) blood meal origins and had contact with cattle, suggesting that treatment of cattle with appropriate insecticides could be effective for controlling even those malaria vectors biting indoors. Those mosquitoes biting in the early hours of the night might be less affected by the indoor based interventions and more likely bite humans [36]. The role of *An. marshalli* and *An. garnhami* in malaria transmission need to be studied.

The higher bovine blood meal index from indoor resting collections shows the indoor resting preference of *Anopheles* mosquitoes after feeding on cattle outdoors. Thus, the existing indoor interventions such as LLINs and IRS are essential to reduce indoor transmission of malaria and also push mosquitoes out of houses [37]. A few *An. arabiensis*, *An. marshalli* and *An. garnhami* from pit shelters had human blood meals, and it is also important to consider these outdoor resting mosquitoes because they can maintain residual malaria transmission [38].

Conclusion

In this study in southwest Ethiopia, *Anopheles* mosquitoes appeared preferentially zoophilic, feeding on cattle. It is important to consider treatment of cattle with appropriate insecticide to control the zoophilic malaria vectors in southwest Ethiopia. The possibility of using systemic insecticides like ivermectin needs to be considered to control the insecticide resistant malaria vectors in the area.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

FM Project design, conducted field and laboratory work, data analysis and interpretation, wrote the draft of manuscript, MB Project design, laboratory supervision, and manuscript revision, TG Project design and manuscript revision, BL Project design, field supervision and manuscript revision. All authors read and approved the final manuscript.

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Appendices

1. General information collection sheet

1. Household head ----------H.NO---------- Collection method ----------
2. Month ----------Date---------- Sub-village----------
3. Elevation ----------North----------East----------
4. Month ----------NO. Occupant----------Female----------Male ----------
5. No. occupant 1/ Under 5----------2/ Between 5-15----------3/ >15----------
6. Types of house ---------- 1/Holes on the walls ----------2/Holes on the roof ----------
   3/Door fitness ----------4/Window fitness ----------5/No. of cattle ----------

Table I: Morphological identification of anopheline and culicine mosquitoes, and their sex

Mosquito Genera	Number of males	Number of females	Total Number	Remark
Anopheline				
Culicine				

Table II: Morphological identification of female *Anopheles* mosquitoes and determining abdominal stage

Species	Number	Unfed	Freshly fed	Half gravid	Gravid	Remark
An. gambiae						
An. marshalli						
An. funestus						
An. pharoensis						
An. garnhami						
An. tenebrosus						
An. longipalpis						

Table III: Processing *Anopheles* mosquitoes for age grading, blood meal origin determination, sporozoite rate, and speciation of *An. gambiae* complex

Ho. No.	Sub-village	Distance from lake	Collection date	Collection method	Mosquito spp.	Abdominal status	Parity/ ageing	ELISA blood meal origin	ELISA CSPs (+/-)	PCR speciation of *An. gambiae*

I. Insecticide susceptibility test assessment format

Collection site of mosquitoes: ----------

Date of collection: ----------

Mosquito species: ----------

Insecticide tested (%): ----------

Date of impregnation: ----------

Date of expiry: ----------

No. of times the paper was previously used: ----------

Date, month and year of test: ----------

Test condition: Exposure Holding period

Maximum Temp ---------- ----------

Minimum Temp ---------- ----------

Insecticide resistance test results	R1	R2	R3	R4	C1	C2
No. of mosquitoes tested	--------	--------	--------	--------	--------	--------
No. knockdown at (min):						
10	--------	--------	--------	---------	--------	--------
20	--------	--------	--------	---------	--------	--------
30	--------	--------	--------	---------	--------	--------
40	--------	--------	--------	---------	--------	--------
50	--------	--------	--------	---------	--------	--------
60	--------	--------	--------	---------	--------	--------
No dead end of 24 hours	--------	--------	--------	---------	--------	--------
Observed mortality (%)	--------	--------	--------	---------	--------	--------
Corrected mortality (%)	--------	--------	--------	---------	--------	--------

R1-R4 = insecticide impregnated papers, C1-C2 = controls