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Beyond Garki
monitoring malaria

Monitoring malaria epidemiology and control in Ethiopia and Uganda

Baseline survey
October-November 2012

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Cover photo: A household interview at Guba, Halaba Special district, South Nations, Nationalities and Peoples Region, Ethiopia

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Acronyms and abbreviations

ACT	artemisinin-based combination therapy
ETC	exit trap collection
HBR	human biting rate
HLC	human landing collection
iCCM	integrated community case management
IPTp	intermittent preventive treatment in pregnant women
IRS	indoor residual spraying
ITN	insecticide treated net
LLIN	long lasting insecticidal net
LTC	light trap collection
MIS	Malaria Indicator Survey
NPV	negative predictive value
PHI	proportionate hole index
PPA	Programme Partnership Arrangement
PPV	positive predictive value
PSC	pyrethrum spray catch
RDT	rapid diagnostic test
RMS	room search
U5MR	under-five mortality rate
WHO	World Health Organization

Executive summary

Background

The scale-up of malaria interventions seems to have contributed substantially to decline of the disease in many countries, although other factors may also have been involved. Changes in demographic, socio-economic, political, technological and environmental factors may also had an impact on malaria, in addition to changes in vector and parasite populations, resulting in changing patterns of transmission. However, the trend in malaria is not uniform across countries. Understanding of the heterogeneity of transmission, disease outcomes, vector habits and other factors, as well as monitoring impacts of interventions across a range of settings, will help in adapting control strategies accordingly and preventing resurgence.

Beyond Garki is a project led by Malaria Consortium to monitor changes in the epidemiology of malaria within the context of the implemented interventions, to assess conditions for reducing transmission below its critical level and to make recommendations to adapt prevention and control measures to observed changes. The changes are monitored to provide an evidence base to guide policies and strategies. Currently, four sites in Ethiopia and Uganda are being monitored with funding from UK aid through the Programme Partnership Arrangement (PPA). Here, we report the results of a baseline survey that will be used for comparison with subsequent surveys and longitudinal data, and may support adaptation of control strategies.

Methods

We selected four fixed sites in Ethiopia and Uganda to monitor changes over time: Aduku (Apac district) and Butemba (Kyankwanzi district) in Uganda, and Hembecho (Boloso Sore district) and Guba (Halaba Special district) in Ethiopia. We collected baseline data during the October-November 2012 transmission season, including malariometric and entomological variables, morbidity, treatment-seeking behaviour, demographic and socioeconomic variables and coverage of interventions. The aim was to use the data for comparison with future surveys and monitor epidemiological changes to support adaptation of control strategies. The study was designed as a regular cross-sectional survey in fixed study populations, complemented by longitudinal collection of climatic and morbidity data at health facilities. The components of the study include a) household surveys covering 1,521 households in the four sites; b) malariometric and serological surveys; c) entomological surveys including insecticide resistance studies; d) health facility-based morbidity studies; e) climatic studies; and f) mathematical modelling of transmission. This report covers the household, malariometric and entomological components.

Results

General observations

The findings showed a number of interesting patterns of malaria in relation to coverage and use of interventions, including low prevalence in areas that previously had high endemicity, such as Apac in northern Uganda, wide variations in coverage and use of insecticide treated nets (ITNs) and the association between the use of ITNs and infection risk. In Ethiopia, *Plasmodium vivax* was the dominant parasite species during the survey period. In both countries, significant variations were observed in malaria prevalence among small, adjacent villages showing localised transmission.

Malaria prevalence in the baseline survey in 2012 varied from 1.4% in Guba (Ethiopia) to 9.9% in Butemba (Uganda). The most common species was *P. vivax* in Ethiopia (52-67%) and *P. falciparum* in Uganda (95-100%). Rapid diagnostic tests (RDTs) and microscopy were frequently used in most sites for malaria diagnosis. Half and three-quarters of children with fever for whom treatment was sought received a malaria diagnostic test in Uganda and Ethiopia, respectively. RDTs that were in use accurately determined negative febrile patients.

However, the positive predictive values of the RDTs were not as high as their negative predictive values.

Serological studies

Serological analysis of blood samples from Uganda showed the age-specific sero-conversion rate in Butemba (Kyankwanzi district) was much higher than that in Aduku (Apac district), indicating higher transmission intensity. Historically, Apac had intense malaria transmission, but transmission has declined substantially in recent years. This could be due to multiple factors, but one major difference with Kyankwanzi district is that Apac district has been under indoor residual spraying (IRS) for a number of years, initially with pyrethroids and more recently with the carbamate insecticide bendiocarb.

Vector distribution and biting habits

Entomological studies confirmed that densities of *Anopheles gambiae* s.l. and *A. funestus* s.l. varied among the sites. The most common vector species in Aduku was *A. funestus* s.l., followed by *A. arabiensis*. In Butemba, the most abundant vector species was *A. gambiae* s.s., with *A. arabiensis* constituting a much smaller proportion. *A. gambiae* s.l. (presumably *A. arabiensis*) was collected in very few numbers in Ethiopia.

In Uganda, the majority of human-vector contact occurred indoors, ranging from 83% for *A. funestus* s.l. to 93% for *A. gambiae* s.l., which is encouraging for likely effectiveness of ITNs or IRS. However, most human-vector contact with *A. funestus* s.l. occurred before 22:00, assumed to be the bedtime for most villagers. The majority of human-vector contact with *A. gambiae* s.s. and *A. arabiensis* occurred indoors after midnight, confirming the potential efficacy of ITNs against these two species. The early biting tendency of *A. funestus* s.l. requires further investigation. A previously less important *Anopheles* species complex (*A. coustani* s.l.) was found biting humans in considerable numbers in Apac, Uganda. Like *A. funestus* s.l., *A. coustani* s.l. was observed to feed early in the evening. The vectorial status of the members of *A. coustani* s.l. is unknown in Uganda, although a study in Kenya showed they contribute to malaria transmission. Since *A. coustani* s.l. is a complex of species, a further study using molecular analysis is needed to determine the species involved and their infectivity to elucidate their vectorial status in Uganda.

Vector resistance to insecticides

World Health Organization insecticide susceptibility tests and molecular analyses showed *A. arabiensis* was more susceptible to pyrethroids (both deltamethrin and permethrin) than *A. gambiae* s.s. in Uganda. *A. arabiensis* and *A. gambiae* s.s. were susceptible to the carbamate insecticide bendiocarb and the organophosphate insecticide pirimiphos-methyl.

In Butemba, *A. arabiensis* was susceptible at the knockdown resistance (*kdr*) gene locus but *A. gambiae* s.s. exhibited high *kdr*-L1014S frequencies (>91%). In Aduku, high (81%) *kdr*-L1014S frequencies were observed in *A. gambiae* s.s. collected by various trapping techniques, while those from World Health Organization tests showed a moderate level (30%). In contrast, low frequencies were observed in *A. arabiensis* adults collected through various trapping techniques, while adults reared from larvae showed a moderate frequency (25%).

Insecticide treated net ownership and use rates

ITN ownership did not vary by socioeconomic status; 56-98% and 68-78% of households owned at least one ITN in Ethiopia and Uganda, respectively. In Uganda, 7% of nets were purchased by households but the nets were untreated. The percentage of purchased nets did not vary significantly among socioeconomic levels.

The population with access to long lasting insecticidal nets (LLINs) ranged between 25% in Guba and 68% in Hembecho, both in Ethiopia. Among people with access to nets, use rates were high in three of the sites (except Guba) ranging from 69% to 76%; in Guba the use rate among those with access to ITNs was 33%. Although there were clear differences in LLIN use rates among the sites, there was no inequity between the sexes except in Aduku, where significantly more females used LLINs.

The most frequent reasons for not using nets were 'net too old or torn', 'net too dirty' and 'net not hung'. Malaria prevalence was generally

higher in individuals who did not use ITNs in all sites, although a statistically significant difference was observed only in Aduku.

Indoor residual spraying

IRS coverage ranged from 84% to 96% in the three sprayed sites. Out of the three sites where IRS was implemented, prevalence of infection was significantly lower in individuals living in sprayed houses compared with those in unsprayed houses only in Guba ($p = 0.042$). Among individuals who slept in sprayed houses, prevalence of infection was significantly lower in those who used LLINs in Aduku ($p = 0.026$), indicating that LLINs are more protective in this site than they are in the other sites.

Intermittent preventive treatment in pregnant women

The level of use of intermittent preventive treatment of pregnant women (IPTp) was low in Uganda: only 7-8% of pregnant women took the recommended minimum of three doses. However, 28% and 44% of women who had given birth during the previous year before the survey took two doses of IPTp in Aduku and Butemba, respectively. IPTp is not implemented in Ethiopia.

All-cause under-five mortality trends

Data from interviews with women aged 15-49 years showed that the all-cause under-five mortality rate has been declining in both countries over the past two decades.

Knowledge of malaria

The majority of household heads in Aduku (93%) knew malaria is caused by mosquito bites. In Butemba, half of respondents had a belief that malaria is contracted by drinking dirty water. In Guba, Ethiopia, only 39% of respondents correctly identified mosquito bites as the cause of malaria.

Undernutrition

There was a considerable degree of undernutrition in children in all sites; the problem was most severe in Ethiopia, where two-thirds of the children were classified as stunted (inadequate height for age). In one of the Uganda sites (Butemba), more than a quarter of the children were stunted; stunting was not determined in the Aduku site but the percentage of underweight children was similar to that in Butemba.

Conclusion and recommendations

The findings of the baseline survey indicate that malaria epidemiology seems to be changing compared with earlier published data, and it is essential to have more data to understand how much the changes are attributable to control interventions and other factors. Continued regular monitoring will help to better interpret changes, identify determinants, modify strategies and improve targeting of interventions to address increasing heterogeneity of transmission.

Malaria transmission during October-November 2012 in all locations was low or moderate, including in previously holoendemic areas, but there was wide variation between and within study sites. Intensified vector control and effective treatment seem to have played key roles in bringing endemicity down over recent years, as reflected in the results of serological analyses. Coverage of ITNs is still not to the required level, as the highest proportion of the population with access was just over two-thirds, while in the rest of the study populations access was below 50%. However, except in one site with very low malaria transmission in Ethiopia, ITN use rates among household members were quite high. A substantial level of purchase of nets was observed in Uganda, and this should be encouraged, but these nets were untreated. In Ethiopia, there were no purchased nets.

Although one of the main vectors of malaria (*A. arabiensis*) seems to be mainly susceptible to pyrethroids, the more efficient vector *A. gambiae* s.s. has developed a high degree of both phenotypic and genotypic resistance against pyrethroids, at least in the Butemba site. Resistance management strategies need to be worked out to prolong the efficacy of LLINs.

This study showed individuals using ITNs are at a significantly lower risk of contracting malaria in at least one site, which is encouraging in spite of increasing pyrethroid resistance.

Most human-vector contact still occurs indoors, especially with both *A. gambiae* s.s. and *A. arabiensis*. However, there is a tendency of early biting of *A. funestus* s.l. in one of the sites in Uganda. As no *A. funestus* s.l. from this site was found to be infected with *P. falciparum*, there is a need to carry out molecular identification of this complex. More data are needed to determine the resting habits of vector species in both countries. Furthermore, more studies are required to understand the role of a previously less important anopheline mosquito, *A. coustani* complex, as it was found to bite humans in considerable numbers in Uganda.

This study provides important baseline information for future surveys. However, more investigations will reveal the impact of the various interventions on malaria transmission through in-depth analysis and modelling.

Recommendations

Low malaria prevalence was observed in some sites that previously had high endemicity but there was substantial variation between sites.

- Malaria control efforts should be sustained to reduce transmission further, maintain the gains and prevent resurgence.
- The findings will serve as a baseline against which results of future surveys or other studies could be compared. Although results from a small number of study sites may not be nationally representative, the approach provides more comprehensive information on a range of potential determinants of malaria rates than more geographically extensive surveys and surveillance, and they will provide a basis for and may prompt further investigations of some of the observations. It is necessary to continue monitoring the epidemiological changes.
- Malaria control strategies should be adapted to the changing patterns and heterogeneity of transmission which may require a thorough epidemiological stratification and selective targeting of interventions.

Additional recommendations related to other key findings of the baseline survey are below.

- A tendency of early biting in *A. funestus* s.l. in Apac requires further investigation.
- The impact of pyrethroid resistance in *A. gambiae* s.s. in Uganda on effectiveness of LLINs should be studied further. Pre-emptive rotation should be considered by IRS programmes.
- Non-pyrethroid IRS may be considered when feasible and where other measures have inadequate impact, or in areas where there are major obstacles to achieving high ownership and use of LLINs.
- Robust continuous distribution systems are needed to replace LLINs and maintain high coverage.
- Health services should investigate whether low LLIN coverage in Guba is indicating a similar problem elsewhere and focus efforts on creating a culture of net use.
- Ministries of health should create conditions to make LLINs available in the commercial market at affordable prices and educate communities on the benefits of LLINs over untreated nets.
- Appropriate communication programmes are needed to address existing malaria knowledge gaps in communities.
- Special attention should be given to improving the IPTp supply system within the health services in Uganda and to community education to create demand.
- Improving maternal and child nutrition should receive due focus by health services.

Introduction

In the past decade, a general downward trend in the burden of malaria has been reported worldwide. Malaria mortality rates decreased by 47% globally and by 54% in the World Health Organization (WHO) African Region between 2000 and 2013[1]. During the same period, prevalence of infection in children aged 2-10 years decreased from 26% to 14%[1]. Factors believed to have had significant impact include the scale-up of key vector control interventions, availability of rapid diagnostic tests (RDTs) and effective treatment with artemisinin-based combination therapy (ACT)[2,3]. Simultaneously, changes in demographic, socioeconomic, political, technological and environmental factors have also impacted on malaria, as have changes in vector and parasite populations, resulting in changing patterns of transmission. The inevitable interactions between these factors will necessitate periodic and timely changes in strategies. However, the trend has not been uniform. In some areas with high baseline transmission and/or where high coverage levels have not been achieved, the malaria burden has not declined[4-7].

The changing epidemiology of malaria requires the adaptation of interventions to address shifts in geographical, behavioural and demographic risk characteristics, especially as transmission declines and becomes more clustered[8]. A deeper understanding of possible determinants of change is critically important. Local knowledge of the burden and features of the disease will be important to adapt interventions and maintain cost-effectiveness and equity. Features that need to be monitored include changes in vector habits and insecticide resistance; parasite infection patterns and drug resistance; climatic, socioeconomic and demographic changes; gaps or issues in implemented interventions; and effectiveness and relevance of some control strategies.

Furthermore, a good surveillance system is essential to identify the most at-risk populations and geographical areas and to assess trends and impact of interventions[9]. In addition to surveillance and monitoring of empirical data, appropriate mathematical models are useful to understand malaria transmission dynamics. Examining various scenarios, including the extent to which a set of interventions can reduce malaria to low levels, could help to use resources optimally.

Detailed epidemiological studies have been carried out with the aim of understanding the likelihood of interruption of malaria transmission in Africa. One of the best examples of such studies was epidemiological research undertaken in the Garki Project during 1969-1976 in a lowland rural Sudan savannah in northern Nigeria[10]. The goal was to test the effects of indoor residual spraying (IRS) and mass drug administration and to develop and test a mathematical model of transmission. Although potent interventions were applied, interruption of transmission was not achieved. Part of this failure was attributed to non-uniform exposure to sprayed surfaces due to at least partial genetically determined outdoor resting populations of *Anopheles gambiae* s.l. However, the model constructed by the project proved useful for planning malaria control.

More recently, a malaria model was developed, which has been proposed for use in elimination scenario planning[11,12]. Models could be used to extrapolate realistic predictions in larger geographical areas for selective control planning and evaluation of effectiveness of interventions in bringing down transmission to a low level. Data from the present project could be used to validate such models and to stratify areas for optimum impact within available resources. The project that is the subject of this report was named Beyond Garki to recognise the contribution of the Garki Project to our understanding of malaria epidemiology in Africa.

Beyond Garki is a project led by Malaria Consortium to monitor changes in the epidemiology of malaria within the context of the implementation of various interventions, to assess conditions for reducing transmission below its critical level and to make recommendations to adapt prevention and control measures to observed changes. The project is implemented in collaboration with the Ethiopian Public Health Institute, Ministry of Health of Ethiopia and Ministry of Health of Uganda, and regional/district health offices in the study sites. The changes are monitored to provide an evidence base to guide policies and strategies. Currently, four sites are being monitored with funding from UK aid through the Partnership Programme Arrangement (PPA).

The focus of the study is on understanding epidemiological changes over time in relation to interventions implemented, as well as environmental and socioeconomic factors. Currently, health services in most countries do not routinely monitor the types of data the project collects. There are irregular nation-wide studies/surveys such as Demographic and Health Surveys (DHS) and Malaria Indicator Surveys (MIS) in most countries. However, these serve to generate a snapshot of only a subset of the variables and do not necessarily provide detailed epidemiological information on factors driving transmission and impacts of interventions.

Here, we describe the project and the study design and present data on several variables, including malaria epidemiology; vector behaviour and insecticide resistance; demographic and socioeconomic factors; treatment-seeking behaviour; and coverage of interventions in the study sites from a baseline survey carried out in October and November of 2012. Three more rounds of surveys were carried out up to November 2014. A subsequent report will present the detailed results of these surveys and other data in comparison with the baseline survey.

Methods

Study sites

A 'study site' in the context of the project is defined as a 'health centre and the catchment population in selected villages around it'. Two study sites were selected per country in Ethiopia and Uganda, representing different epidemiological settings in rural environments (Table 1).

Ethiopia

Most areas below 2,000 m above sea level in Ethiopia are considered malarious. An estimated 60% of the population lives in areas at risk of malaria transmission[13]. Both *Plasmodium falciparum* and *Plasmodium vivax* are common. The 2011 MIS showed that, nationally, malaria prevalence was 1.3% in areas below 2,000 m; 77% of the positive slides were *P. falciparum* infections[14]. There is marked seasonality in transmission and geographic variation in intensity. Many areas are epidemic-prone. *Anopheles arabiensis* is the main vector species[15]. *A. pharoensis*, *A. funestus* and *A. nili* are considered secondary vectors. Resistance of *A. arabiensis* against DDT and pyrethroids is widespread in the country[16].

Ethiopia's organised malaria control began in 1959 when the Malaria Eradication Service was established a year after a major epidemic claimed an estimated 150,000 lives[17]. A blanket DDT spraying campaign was used until the early 1970s, when the eradication strategy was abandoned and replaced with a control programme[18]. The programme, based on selective spraying and treatment of cases, continued until the mid-1990s, after which the specialised service was gradually integrated into the general health services.

There has been a substantial increase in coverage of key interventions in the country. More than 64 million long lasting insecticidal nets (LLINs) were distributed through mass campaigns between 2005 and 2014[13]. IRS is also implemented in many areas. Through the expansion of basic health services, mainly health posts, diagnostic and treatment services have increased over the years.

Uganda

Malaria is highly endemic in approximately 95% of the country where 90% of the population lives. The MIS in November and December 2009 reported that 42%

Table 1: Beyond Garki study sites in Uganda and Ethiopia

Country	Region	District	Study site	Coordinates of health centre: latitude, longitude	Average altitude of study site
Uganda	Northern region	Apac	Aduku	1°59′33.51″N, 32°43′8.26″E	1,051 m
	Central region	Kyankwanzi	Butemba	1°8′33.86″N, 31°36′8.79″E	1,107 m
Ethiopia	SNNP* region	Boloso Sore	Hembecho	7°8′59.08″N, 37°39′42.05″E	1,702 m
	SNNP region	Halaba Special	Guba	7°17′6.88″N, 38°13′1.09″E	1,878 m

**SNNP = Southern Nations, Nationalities and Peoples*

of children under the age of five tested positive for malaria with microscopic diagnosis[19]. *P. falciparum* is responsible for 99% of malaria cases. The disease accounts for 25% to 40% of outpatient visits and nearly half of inpatient paediatric deaths[20]. The main malaria vectors are *A. gambiae* s.s., *A. arabiensis* and *A. funestus* s.s.[19,20].

Although IRS was implemented in limited sites as part of the WHO pilot programme between 1959 and 1963, the operation was not scaled up[21]. Treatment of cases remained the only malaria control measure for many years. The Malaria Control Unit was established in 1995 and grew into the National Malaria Control Programme.

The main preventative interventions in Uganda are LLINs, IRS in selected districts and intermitted preventive treatment in pregnancy (IPTp). Uganda has scaled up effective case management and, in some regions, village health teams have been trained to test and treat common childhood illnesses, including malaria, through integrated community case management (iCCM). In 2009, 47% of households owned at least one insecticide treated net (ITN) compared with 16% in 2006[19,22]; this increased to 60% in 2011[23]. These combined efforts are believed to have resulted in reduced transmission in many areas[24].

Up to 10 districts in northern Uganda have been under IRS in the past 6-7 years with support from the US Government's Presidential Malaria Initiative[20]. Starting from 2014, more northern and eastern districts were added, while the operation ended in others (including Apac) due to a decline in transmission. A significant reduction in malaria prevalence was achieved in children living in sprayed areas[25]. Meanwhile, more than 21 million LLINs were distributed in a recent nation-wide mass campaign.

Study components

The study included cross-sectional surveys conducted repeatedly in the selected sites, and longitudinal collection of meteorological and morbidity data at health facilities. The main components were household surveys, malariometric and serological surveys, entomological surveys, health facility-based morbidity studies and climatic studies.

Household surveys

The household surveys included interviews with household heads and women aged 15-49 years using handheld devices: smartphones with Pendragon Forms 5.1 (Pendragon Software Corporation, Chicago, IL US) or tablets with Open Data Kit (https://opendatakit.org) (Figure 1). Data were collected on variables indicating socioeconomic status, prevention methods, knowledge about malaria and ITN ownership and use. Women of child-bearing age (15-49 years) were interviewed using a subset of questions during the household questionnaire. Questions included a birth history of the number of children born who were alive and dead and protection against malaria during previous and current pregnancies.

In addition, a number of questions were asked about children (aged six months to 10 years) who had a fever at the time of the questionnaire or in the previous two weeks to determine site-specific treatment-seeking behaviour and treatment availability in relation to childhood febrile illness, and to look at the prevalence of diagnosis-based treatment and commonly prescribed drugs for malaria. A two-week time restriction was applied because the accuracy of recollection is much lower over a longer time period. The subset of questions was completed separately for each child if multiple children had had a recent febrile illness.

Malariometric surveys

Each member of the sampled households (except infants under six months) was given a subject card and asked to visit a malariometric testing site within

Figure 1: Household survey

Figure 2: Malariometric survey

the village to obtain anthropometric measurements and collect blood samples (Figure 2). Bodyweight, temperature, height and upper arm circumference were measured for children under five. Thick and thin blood films for microscopy, dry blood spots for serology and blood samples for haemoglobin measurement using the HemoCue machine (Hb 301, Ängelholm, Sweden) were obtained for all subjects. RDTs (CareStart™ *Pf*-HRP2/pan-pLDH by AccessBio USA in Ethiopia and SD Bioline in Uganda) were used to test subjects with body temperature 37.5°C and above or a history of fever in the previous 48 hours. Treatment was provided for mild and moderate anaemia and uncomplicated malaria cases at the field sites; severe cases were referred to the site's health centre.

Slides were stained with Giemsa and examined by two independent microscopists for presence/absence of asexual parasites and gametocytes and species identification. In the case of discrepant results, a third microscopist examined the slides for a final verification.

Serological studies

Serological analysis of dry blood spots from the Uganda sites was carried out to determine antibody responses to assess malaria transmission intensity over an extended period of time. The antibody response of individuals against merezoite surface protein 1_{19} ($MSP\text{-}1_{19}$) was determined using an enzyme-linked immunesorbent assay. Serum obtained from the dried blood spots on filter papers was analysed at the Medical Research Council laboratory in Uganda for total IgG antibodies using *P. falciparum* antigen $MSP\text{-}1_{19}$ (CTK Biotech, US, Cat. No. A3003) following previously described methods[26,27].

Entomological surveys

Anopheles mosquitoes were sampled to determine species composition, densities, behaviour and insecticide resistance using light trap collection (LTC), exit trap collection (ETC), room search (RMS), pyrethrum spray catch (PSC) and human landing collection (HLC) in 12 selected houses in each site (Figure 3). *Anopheles* mosquitoes were identified using morphological features, classified into different abdominal stages as unfed, freshly fed, half-gravid and gravid, and individually packed in microtubes for molecular analysis, which were carried out at Rothamsted Research in the UK[28]. Genomic DNA was extracted using the Livak method. *A. gambiae* s.l. samples were analysed to determine whether they

Figure 3: Entomological survey
Five trapping methods were used: a) LTC to collect host-seeking mosquitoes indoors; b) ETC to collect mosquitoes leaving the house after (un)successful attempt to feed; c) indoor RMS to collect mosquitoes resting indoors; d) PSC to collect mosquitoes resting indoors using an insecticide and white sheets spread over the floor surface; e) HLC to collect host-seeking mosquitoes throughout the night indoors (18:00-06:00) and outdoors (18:00-00:00).

were *A. gambiae* s.s. or *A. arabiensis*[29,30]. In addition, the majority of samples were analysed for the knock down resistance (*kdr*) L1014S mutation[31][i]. Sporozoite rates were determined for a subset of anophelines identified in the field as *A. gambiae* s.l. or *A. funestus* s.l.[32]. Positive samples identified by qPCR were further verified by a nested PCR[33].

A. gambiae s.l. mosquitoes in the Ugandan sites were tested for insecticide resistance against commonly used insecticides using WHO susceptibility test procedures[34]. In Ethiopia, not enough anopheline mosquitoes could be collected to perform the tests. Adult *A. gambiae* s.l. females aged one to three days – collected as larvae from local breeding sites – were used in all tests. The mosquitoes were exposed to insecticide-impregnated papers for one hour. Mortality rates were calculated after a 24-hour observation period. The percentage mortality was used to determine insecticide resistance following updated WHO guidelines[35]. Mortality above 98% is indicative of a susceptible population, mortality between 90 and 98% of suspected resistance; mortality below 90% is classified as a resistant population.

The knockdown effect of insecticides was assessed using median knockdown time (KD50) following exposure (i.e. the time in minutes for 50% of the mosquitoes to be knocked down). Pyrethroid insecticides are known for rapid knockdown, and a delay in knockdown could indicate reduced susceptibility of the insect against the insecticide. The interpretation of the knockdown times observed in the present survey is not straightforward because of lack of a susceptible population with a known KD50 to serve as a positive control.

Other study components

Automatic weather stations (BWS200 automatic weather station, Campbell Scientific, Stellenbosch, South Africa) were installed in all four sites to gather hourly meteorological data, including precipitation, temperature and relative humidity. The data will be used in conjunction with results of the entomological and malariometric studies in modelling malaria transmission under different scenarios. Outpatient morbidity data on every suspected or confirmed malaria patient seen at the health facility in each site have been compiled for further analysis.

Sampling strategy and sample size

All households in the villages around the health centre were enumerated, geo-referenced and included in the sampling frame, with the required sample selected randomly. The units of the study had different levels depending on the type of analysis required and the indicator being measured. Sites, households and individuals were all considered study units.

For the household survey, including the malariometric and serological surveys, the sampling units were households. For the purpose of sample size determination, the malariometric survey was considered the primary component of the study, and measurement of prevalence of malaria infection (all species) in children below 10 years of age was used as the main variable of interest. The following assumptions were used: a) children below 10 years of age among total population = 35%; b) average household size = 4.7 (Ethiopia), 4.9 (Uganda); c) baseline prevalence in children under 10 years of age = 5% (Ethiopia), 50% (Uganda); d) statistical significance level = 95%; e) statistical power = 80%; f) reduction to be detected = 50%; g) design effect = 1.2; and h) non-response rate = 10%.

Based on these assumptions, the calculated sample sizes were 571 and 234 households per site in Ethiopia and Uganda, respectively. For entomological studies, 12 households were selected randomly from the sampling frame in each site.

Data entry and analysis

Household data were exported from Pendragon Forms to Microsoft Excel (Microsoft Corporation) and then to Stata (StataCorp LP, College Station, Texas, USA) and cleaned using Stata after removing names of individuals. All other data were collected using paper forms and were entered in EpiData v3.1 (The EpiData Association, Odense, Denmark) using double entry. The data were analysed using Stata versions 12 and 13 and Microsoft Excel. Data analysis for the present report focused on malaria infection prevalence by site, age group, sex and socioeconomic status; fever and malaria; anaemia prevalence; anthropometric

[i] Additional assays are underway for the *A. funestus* s.l. complex, *kdr* L1014F and acetylcholinesterase (Ace-1) target site mutation 119S.

measurements; malaria prevention in pregnancy; treatment-seeking behaviour and access; under-five mortality rates (U5MRs); vector species composition and density; vector habits; insecticide resistance; ITN ownership and use; LLIN durability; impact of IRS; and knowledge of malaria and its prevention in sampled households.

Household survey data

Chi-squared tests were used where appropriate to assess significant differences between groups of interest, taking into account clustering at household level. Most of the analyses were site-specific and combining data from different sites was avoided as much as possible. Principal components analysis was used to calculate a wealth index for each household, computed separately for each country. This was used to study the association of socioeconomic status with various epidemiological variables.

In order to study the gaps between ownership and use, ITN use rates were calculated taking into account access within each household. Access of household members to LLINs was calculated for each study site assuming one net for two people. LLIN use rates among those who have access were then estimated for each site.

Malaria infection rates

Infection prevalence data were analysed in relation to potential household or individual risk factors such as coverage and use of prevention methods, housing conditions, demographic factors and socioeconomic status.

Undernutrition

Undernutrition was studied using the anthropometric data for children under five. A Stata *ado* file ZSCORE06 developed by Jef Leroy (Boston College Department of Economics) was used to calculate anthropometric z-scores using the 2006 WHO Child Growth Standards[36]. The proportions of stunted, wasted and underweight children were determined by study site. Stunted children were those whose height for age was below minus two standard deviations from the median of the WHO Child Growth Standards[37]. Wasted children were those whose weight for height was below minus two standard deviations from the median of the WHO Child Growth Standards. Children whose weight for age was below minus two standard deviations from the median of the WHO Child Growth Standards were classified as underweight.

Anaemia

Anaemia was classified as mild, moderate or severe based on the concentrations of hemoglobin (Hb) as follows: a) mild anaemia: for non-pregnant women, Hb 10.0-11.9 g/dl; for pregnant women and children under 5, Hb 10.0-10.9 g/dl; for men: Hb 10.0-12.9 g/dl; b) moderate anaemia: Hb 7.0-9.9 g/dl; c) severe anaemia: Hb <7.0 g/dl[38].

Serological studies

Optical density values were analysed in Microsoft Excel using a macro file provided by C Drakeley, London School of Hygiene & Tropical Medicine. Normalised optical density values were used for data analysis using a Stata procedure provided by C Drakeley. A cut-off value of 0.177 was used to determine seropositive samples. Age seroprevalence curves were generated using methods described by Corran et al.[39]. Data for children below two years was excluded to avoid potential bias caused by maternal antibodies[40].

Under-five mortality rates

Data from women's interviews were used for the analysis of under-U5MR. First women were classified into five-year age groups: 15-19, 20-24, … , 45-49. Women below 15 or above 49 were not included. Then the mean parity – that is, the average number of live births per age group – and the parity ratio were calculated. The proportion of children dead among live births in each age group was then calculated and the U5MR was estimated. A variant of the Brass indirect method[41] was used to calculate the U5MR using the summary birth history dataset provided by women of child-bearing age, which included age of mother, total number of live births and total number of deaths[42]. The method assumes the age of the mother is a proxy for the age of her children and hence how long they are exposed to the risk of dying. The probability of dying by an exact childhood age is calculated from the proportion of children who

have died among women of a certain age group. From there the U5MR is obtained using model life tables. Mortality rates were not calculated for the most recent period to the survey date (namely, data from women aged 15-19) because of the selection effect where women from lower socioeconomic classes tend to start childbearing early and their children face above average mortality risks; and because random errors are larger for estimates based on the reports of young women, since they have fewer children ever born.

Entomological data

Various entomological parameters were estimated, including species compositions and indoor resting and biting habits. Human biting rates (i.e. the number of bites per person per night) were calculated taking into account the number of collectors working simultaneously, the number of collection nights and the assumed night-time behaviour of the local populations. It was assumed that an average villager in each of the sites spends one hour on average outdoors between 18:00 and 22:00, and all villagers are indoors after 22:00 hours.

Ethical considerations

Ethical clearance was obtained from the appropriate review boards (Uganda: UNCST 1348; Ethiopia: 3-10/819/05). In addition, written consent was obtained from respondents for interviews, from all subjects who participated in malariometric surveys and from household heads for entomology sentinel houses.

Results

Study population

A total of 1,521 households with complete records were obtained in the first survey from the four sites in Ethiopia (average 540 per site) and Uganda (average 221 per site). Household response rates were 94.6% and 94.4% in Ethiopia and Uganda, respectively.

In total, 8,079 people were registered. Nearly all the registered household members were usual residents, ranging from 97.2% in Aduku (Apac district, Uganda) to 100% in Guba (Halaba Special district, Ethiopia). Females constituted 51.6-53.2% of the populations in the study sites. Blood samples for microscopy and serology and body temperature measurements were obtained for 66% of registered study subjects. Blood samples for haemoglobin measurements were obtained from 62% of registered subjects. The highest percentage of blood samples for microscopy and serology were obtained from Hembecho (Boloso Sore district, Ethiopia) (68%) and the least from Butemba (Kyankwanzi district, Uganda) (60%). In total, 1,421 women of child-bearing age (15-49 years old) were interviewed.

Population pyramids for the study sites showed the typical age structure observed in developing countries (Figure 4). In the two Ethiopian sites, there was a clear under-representation of the 20-30 year old age group (both sexes), probably indicating migrations to other areas in pursuit of work. A similar pattern was observed in the male population of Butemba. The proportion of children under five ranged from 11.2% in Hembecho to 19.5% in Butemba.

The average household size in both Hembecho and Guba sites in Ethiopia was 5.2. In Uganda, the average household size was 5.0 in Aduku and 5.3 in Butemba.

Ownership of selected household assets

There was a substantial variation between the two countries in terms of ownership of some household assets. As an example, ownership of mobile phones and radios was much higher in Ugandan sites than in Ethiopian sites. Households owning a mobile phone were 75.1% and 23.9% of the total sampled in Uganda and Ethiopia, respectively. Households owning a radio were 74.4% and 27.2% in Uganda and Ethiopia, respectively.

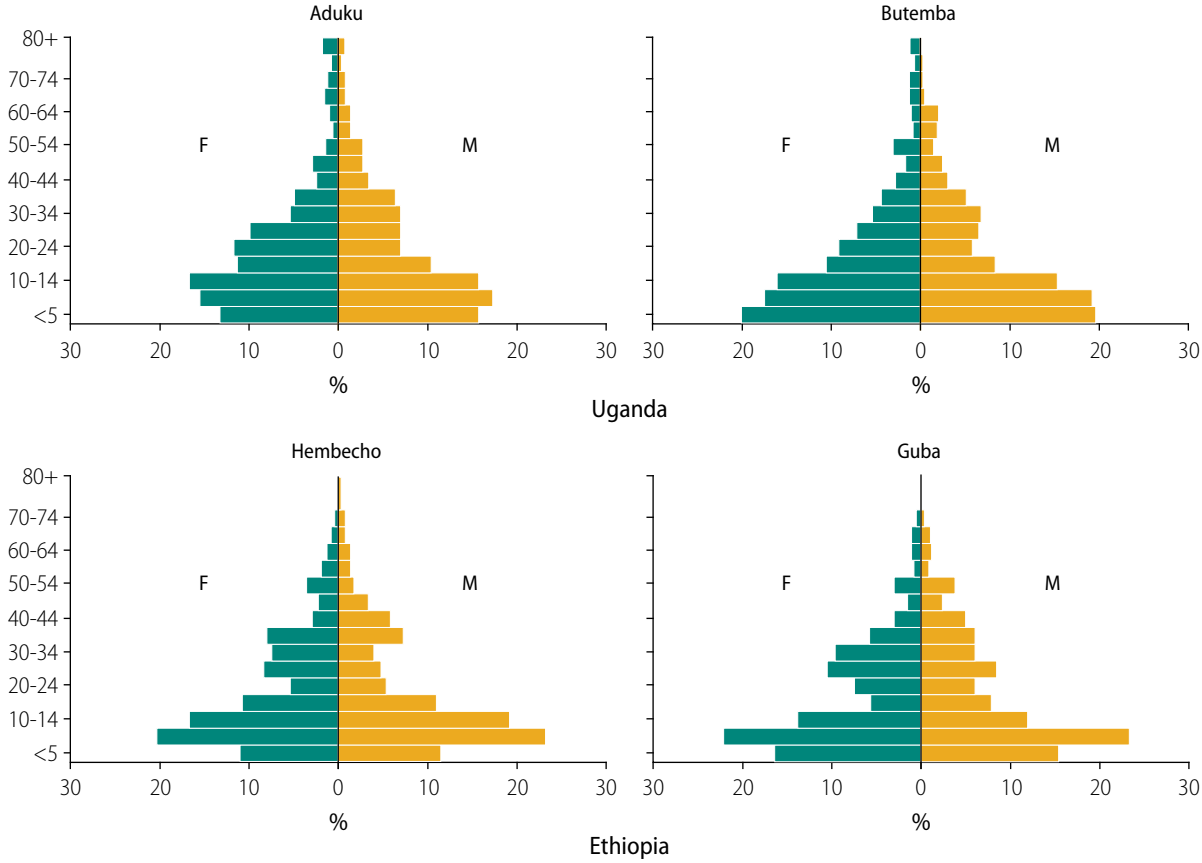

Figure 4: Population pyramid of sampled households in the four study sites
F = Female; M = Male

Malaria prevalence

Prevalence of malaria infection varied between the four sites, ranging from 1.4% in Guba to 9.9% in Butemba (Figure 5). Malaria prevalence rates [95% confidence limits] for Aduku, Butemba, Hembecho and Guba were 3.4% [2.1%, 5.5%], 9.9% [7.4%, 13.1%], 2.5% [1.9%, 3.2%] and 1.4% [0.9%, 2.0%], respectively. The dominant *Plasmodium* species in Ethiopia was *P. vivax*, which accounted for 52-68% of all infections. In Uganda, *P. falciparum* accounted for 95-100%. A breakdown of the malaria prevalence data from both countries indicated that there was significant variation among the various villages within the catchment area in all sites (indicating localised transmission) except in Aduku where the catchment area is relatively smaller and transmission is homogenous (data not shown).

Malaria prevalence varied among age groups in most sites (Figure 6). Risk of infection tended to be higher in older subjects in Aduku than it was in Butemba and Hembecho. The age group pattern in both sites of Ethiopia was typical of low endemicity, whereas the pattern in Butemba (Uganda) reflected moderate endemicity. Aduku exhibited a pattern somewhat between low and moderate endemicity. The seemingly high prevalence in the under-one age group in the Hembecho site was due to a small sample size (one infection out of seven infants).

Subjects with fever had higher infection prevalence than those without fever in Butemba ($p = 0.016$) and Guba ($p < 0.001$). History of fever, however, had no association with malaria infection risk in all sites, except in Guba, where subjects with history of fever were more likely to be positive ($X^2 = 30.86$, $p < 0.001$).

Individuals with fever or history of fever were tested with RDT for the purpose of providing antimalarial treatment. The positive and negative predictive values of RDTs for these cases were calculated for each site using the slide positivity rates determined by microscopy. The negative predictive value (NPV) of

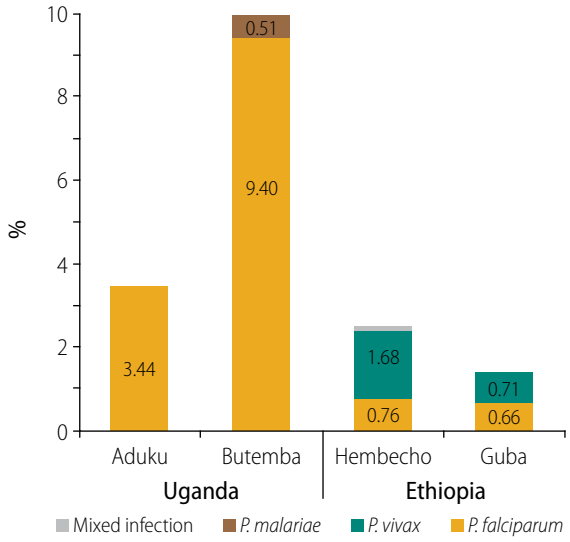

Figure 5: Prevalence of malaria infection and composition of *Plasmodium* species in study sites, October-November 2012

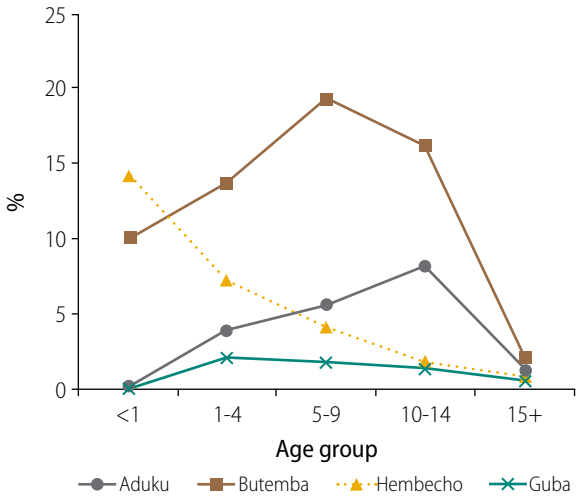

Figure 6: Malaria prevalence by age group (all species)

RDT for *P. falciparum* was high (≥99%) in all three sites with sufficient sample sizes for this analysis (Aduku, Butemba and Hembecho). The positive predictive value (PPV) of RDT was low in all three sites (≤44%). RDT results for *P. vivax* infection exhibited a similar pattern of high NPV (≥98%) and low PPV (≤40%) for the two sites in Ethiopia.

There was no statistically significant difference in infection rates among subjects in households headed by persons with different educational levels in all sites. Similarly, no statistically significant difference was observed in infection rates among subjects in households with different socioeconomic status levels in all sites.

Age-specific seroprevalence

A total of 1,123 samples were analysed for the antigen MSP-1_{19} from Uganda, 602 for Aduku and 521 for Butemba. Overall seropositivity (i.e. the proportion of individuals with a positive antibody response to MSP-1_{19}) was higher in Butemba (45.7%) than in Aduku (22.9%), indicating higher overall exposure to malaria. The seroconversion rate (λ) was higher in Butemba compared with Aduku, indicative of higher transmission intensity (Figure 7). The proportion of seropositive individuals increased with age in both study sites, while parasite prevalence decreased in older age groups, indicating the role of acquired immunity and long-lasting antibody response (Figure 8).

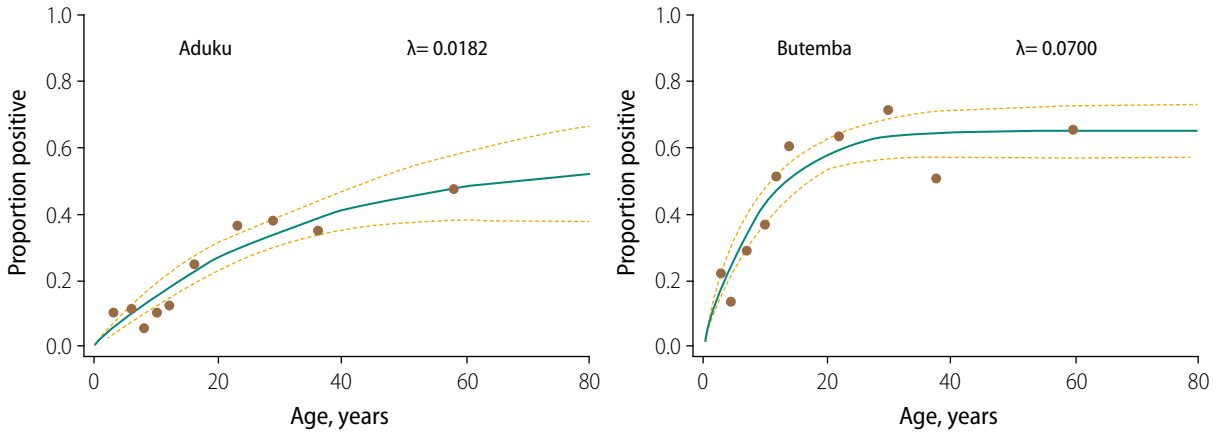

Figure 7: Age-specific seroprevalence for *P. falciparum* anti-MSP-1_{19} antibodies
Dots, continuous lines and broken lines represent data, fitted estimates and 95% confidence intervals, respectively. λ is the seroconversion rate. The 0-2 years age group was omitted because of distortions caused by presence of maternal antibody in high endemicity settings[40]

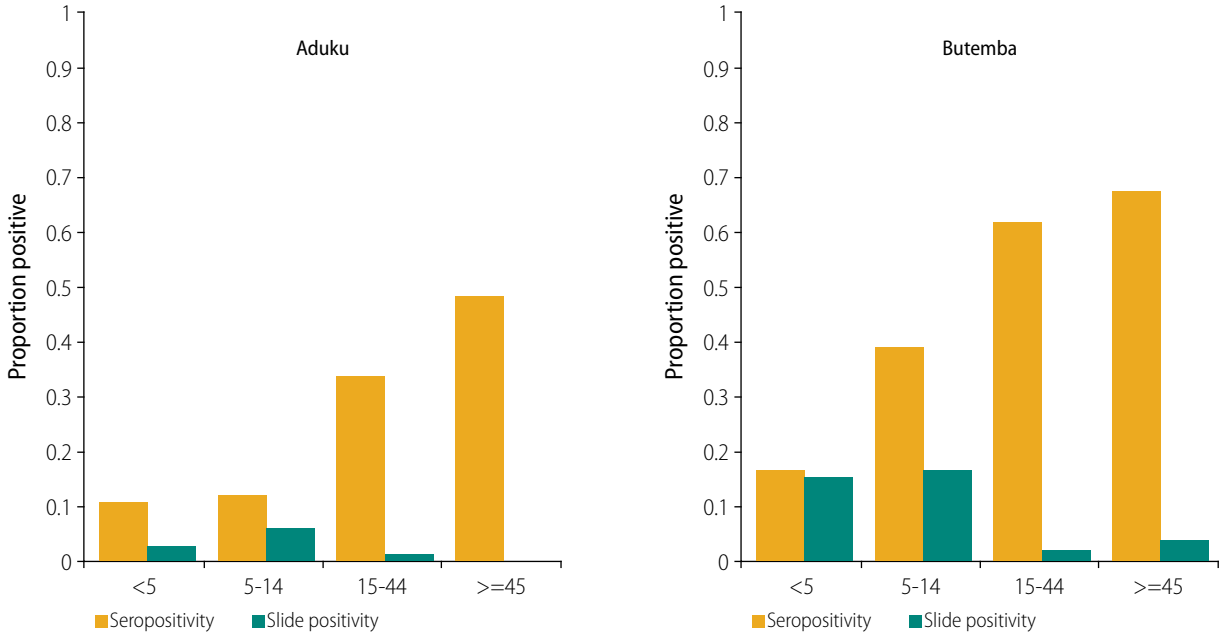

Figure 8: Proportion of seropositive and slide positive *P. falciparum* individuals by age group in Aduku and Butemba in Uganda

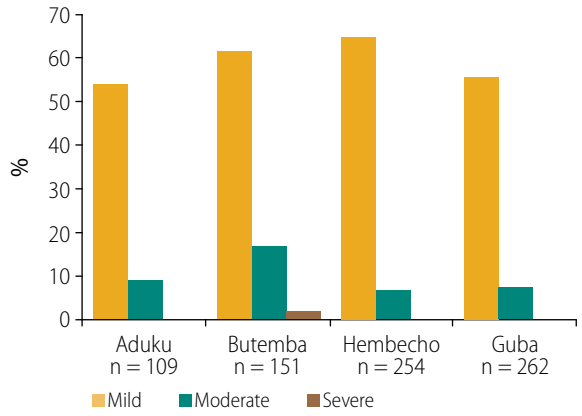

Figure 9: Prevalence of anaemia

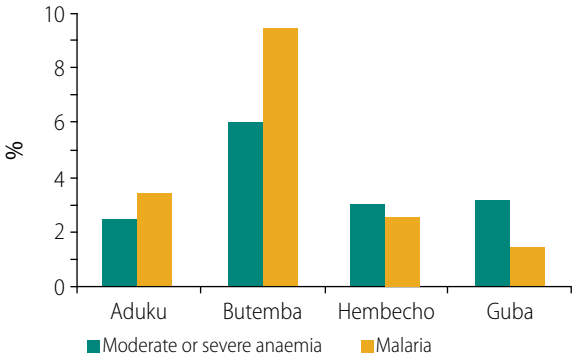

Figure 10: Prevalence of moderate or severe anaemia and malaria

Anaemia prevalence and malaria infection

Prevalence of anaemia by site

There was a uniformly high prevalence of mild anaemia in all sites, ranging from 53% in Aduku to 65% in Hembecho (Figure 9). However, substantial variation was observed in prevalence of moderate and severe anaemia, ranging from 2.4% in Aduku to 6.0% in Butemba.

It appears that the variation in moderate or severe anaemia correlates well with variations in malaria prevalence (Figure 10).

Mosquito density and species composition

In Uganda, a total of 1,670 and 315 anopheline females were collected using the various trapping techniques in Aduku and Butemba, respectively; in Ethiopia, only 22 *A. gambiae* s.l. females were collected from both sites, which were probably *A. arabiensis* (five in Hembecho and 17 in Guba).

In Aduku, the most common vector species was *A. funestus* s.l. (53%); *A. arabiensis* constituted 17% of the collection. In Butemba, *A. gambiae s.s* was the dominant species (83%) followed by *A. arabiensis*

(6%); no *A. funestus* s.l. was collected (Table 2). In Aduku, 29% of all collected anophelines belonged to the *A. coustani* complex. Molecular identification of *A. funestus* s.l. is yet to be completed.

We also assessed the number of anophelines collected through different trapping methods in the Ugandan sites (Figure 11). The majority in both sites were collected by LTC. Almost no mosquitoes were collected by RMS and PSC in Aduku, which could indicate that mosquitoes were not resting inside houses or were being killed after resting on walls, likely because of IRS. In Butemba, 25% of the mosquitoes were collected by ETC and 9% by PSC.

When catches were standardised by the number of collection hours (i.e. by dividing the number caught by the number of hours the trapping method was used) in both sites, relatively fewer anophelines were collected by indoor HLC than by LTC (data not shown).

Variations in species composition were also observed when different collection methods were used (Table 3). In Aduku, the majority of *A. gambiae* s.s. and *A. arabiensis* were collected by LTC, and a substantial proportion were collected by ETC. A large majority (87%) of *A. funestus* s.l. were collected by LTC. Other anophelines (mainly *A. coustani* complex) were primarily collected by using LTC, although some were also collected by using HLC (8%), indicating that they are attracted to human hosts for a blood meal. Proportionally fewer *A. funestus* s.l. were collected by ETC compared with *A. gambiae* s.s or *A. arabiensis*.

In Butemba, most of the *A. arabiensis* were collected by LTC. Most *A. gambiae* s.s. were collected by LTC, but a considerable number were also collected by ETC and PSC. Most of the other anophelines in Butemba were captured by ETC.

Table 2: Relative proportion of anopheline species collected in Uganda sites, October 2012

Anopheles species	% of all collections	
	Aduku	Butemba
A. gambiae s.s.	1	83
A. arabiensis	17	6
A. funestus s.l.	53	0
A. coustani s.l.	29	0
Other *Anopheles* species	0	10
Unidentified *Anopheles* species	0	1

Table 3: Relative proportion of different anopheline species caught by the various trapping methods

	Percentage of each species caught by collection method							
	Aduku				Butemba			
Method	*A. gambiae* s.s.	*A. arabiensis*	*A. funestus* s.l.	Others*	*A. gambiae* s.s.	*A. arabiensis*	*A. funestus* s.l.	Others
LTC	54.3	60.3	87.2	89.9	68.6	84.3	-	6.3
PSC	0.0	0.7	0.2	0.0	10.7	5.2	-	0.0
HLC (In)	0.0	4.7	5.4	6.0	2.3	5.2	-	0.0
HLC (Out)	6.2	1.8	4.0	1.7			-	-
RMS	0.0	0.0	-	-	0.4	5.3	-	0.0
ETC	39.5	32.5	3.1	2.5	18.0	0.0	-	93.8
Total (n)	16	279	891	484	261	19	0	32

*Primarily *A. coustani* s.l.

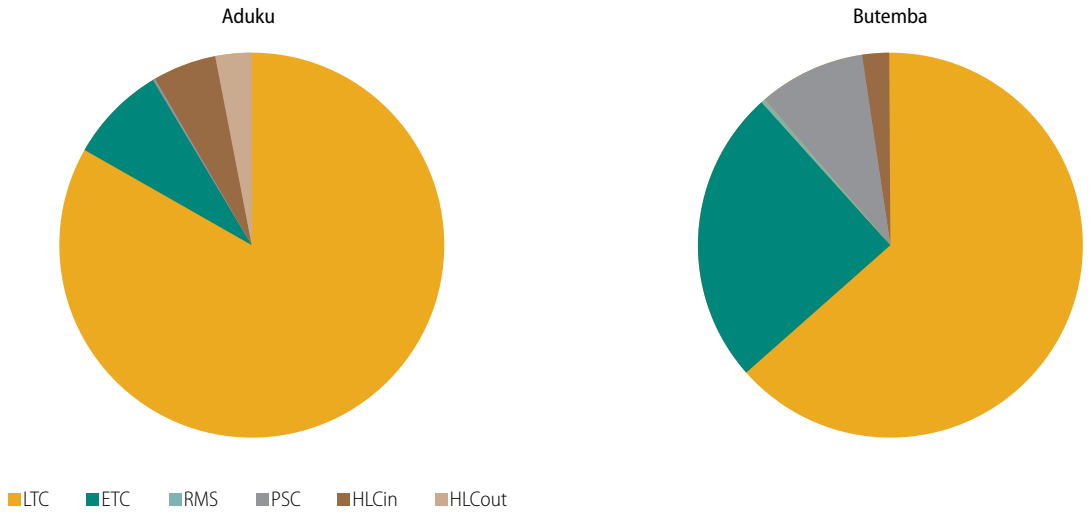

Figure 11: Relative proportion of anopheline species collected in the two study sites in Uganda in October 2012 using the different collection methods
LTC = light trap catch; ETC = exit window trap catch; RMS = room search catch; PSC = pyrethrum spray catch; HLCin = human landing catch indoors; HLCout = human landing catch outdoors

Table 4: Relative densities of anopheline mosquitoes by gonotrophic stage, caught exiting houses (number in exit trap collections) at two sites in Uganda, October 2012

Anopheles species	Site		Unfed	Blood fed	Half-gravid	Gravid	Not recorded	Total
A. gambiae s.l.	Aduku	Exiting	81	2	2	12	0	97
		Resting indoors	1	1	0	0	0	2
		% found resting indoors of total	**1.2**	-	-	-	-	**2.0**
	Butemba	Exiting	40	3	3	2	0	48
		Resting indoors	5	13	9	3	0	30
		% found resting indoors of total	**11.1**	**81.3**	-	-	-	**38.5**
A. funestus s.l.	Aduku	Exiting	24	1	1	2	0	28
		Resting indoors	1	1	0	0	0	2
		% found resting indoors of total	**4.0**	-	-	-	-	**6.7**
Other *Anopheles* species	Aduku	Exiting	5	1	0	0	6	12
		Resting indoors	0	0	0	0	0	0
		% found resting indoors of total	-	-	-	-	-	**0.0**
	Butemba	Exiting	22	6	1	1	0	30
		Resting indoors	0	0	0	0	0	0
		% found resting indoors of total	**0.0**	-	-	-	-	**0.0**

Vector resting habits

To study the degree of endophily (i.e. the proportion of mosquitoes resting inside houses after feeding or attempting to feed), we calculated the percentage caught using ETC out of all mosquitoes collected by using ETC, PSC and RMS (Table 4). *A. gambiae* s.l. in Butemba exhibited a much higher level of endophily than in Aduku (38.5% versus 2.0%). The large majority of the mosquitoes in Butemba (97.4%), were identified as *A. gambiae* s.s., with the remainder being *A. arabiensis*. In Aduku, the sample consisted mainly of *A. arabiensis* (93.5%).

Conversely, only a small proportion of unfed *A. gambiae* s.l. (11%) in Butemba was collected resting indoors of the total unfed as the majority were exiting. More data will be needed to correctly interpret these

observations in terms of exophilic tendency before and after feeding. Although the sample size is not large, it should be noted that most of the blood-fed *A. gambiae* s.l. in Butemba (13/16) were found resting indoors.

Vector biting habits

No mosquitoes were collected with human landing catches in Ethiopia, and only results for Uganda are presented (Table 5).

In Butemba, the only vector species caught by HLC was *A. gambiae* s.l. However, only seven mosquitoes were caught indoors, which was not a sufficient number to deduce any feeding patterns. In Aduku, the *A. gambiae* s.l. identified were 17 *A. arabiensis* and 1 *A. gambiae* s.s. These were observed to feed primarily after midnight indoors (Figure 12). The number collected was too few to determine any behavioural differences between the sibling species.

A. funestus s.l. was observed to feed much earlier in the evening both indoors and outdoors. This observation, however, requires confirmation and, as the molecular analysis of *A. funestus* s.l. has not been completed to confirm if the species under question is an important vector, the result should be interpreted with caution. Additionally, *A. coustani* s.l. showed a similar feeding pattern to *A. funestus* s.l.

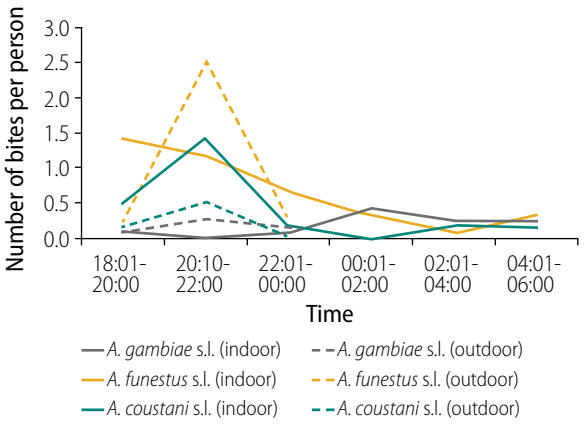

Figure 12: Nocturnal biting cycle of *A. gambiae* s.l., *A. funestus* s.l. and primarily *A. coustani* s.l. in Aduku, Apac district, indoors (18:01-06:00) and outdoors (18:01-00:00), October/November 2012

The human biting rate (HBR) for all anopheline species combined in Aduku was 7.3 anopheline bites per person night (Table 6). *A. funestus* s.l. was the largest contributor to the HBR followed by *A. coustani* s.l. and *A. gambiae* s.l. In Butemba, the HBR was 0.5 bites per person night. If given equal opportunity indoors and outdoors during 18:00-00:00, *A. gambiae* s.l. showed a clear preference for biting outdoors (75%) in Aduku. In contrast, *A. coustani* s.l. clearly preferred biting indoors (24%), whereas *A. funestus* s.l. readily fed both indoors and outdoors.

Table 5: Number of anopheline females collected by HLC indoors and outdoors in Uganda

Site	Species	Indoors	Outdoors
		18:01-06:00	18:01-00:00
Aduku	*A. gambiae* s.l*	0	1
	A. gambiae s.s.	0	1
	A. arabiensis	12	5
	A. funestus s.l.	48	36
	A. coustani s.l.	29	8
Butemba	*A. gambiae* s.l		
	A. gambiae s.s.	6	0
	A. arabiensis	1	0
	A. funestus s.l.	0	0
	A. coustani s.l.	0	0

Note: In each site, collections were carried out for 12 nights (in 12 houses). In each house and at each indoor and outdoor collection site, one human bait was used at a time.
** For one sample no result was obtained in molecular analyses*

Table 6: HBR for each species in study sites in Uganda

Site	Details	*A. gambiae* s.l.	*A. funestus* s.l.	*A. coustani* s.l.
Aduku	Sample size (indoors + outdoors)	19	84	37
	Total HBR per person per night*	1.15	4.04	2.10
	% human-vector contact occurring indoors	92.7	83.0	92.1
	% feeding on humans before 2200 hours (in + outdoors)	12.7	64.9	76.2
	% feeding outdoors if given equal opportunity (exophagy)**	75.0	48.0	24.2
Butemba	Sample size (indoors and outdoors)	7	0	0
	Total HBR per person per night	0.52	0	0

Note: HBR is expressed as the average number of bites per person per night, assuming an average villager spends one hour outdoors during 18:00-22:00.
** It was assumed an average villager spends one hour outdoors between 18:00 and 22:00 and the remainder of the night indoors*
*** Only collections between 18:00 and 00:00 were used for both indoors and outdoors for calculations of the extent of exophagic habit of the vector independent of night time habits of humans*

Nevertheless, the majority of human-vector contact for all three species occurred indoors, ranging from 83% for *A. funestus* s.l. to 93% for *A. gambiae* s.l. Assuming that by 22:00 all residents would be under an ITN, the proportion of contact before this time could be used as a proxy for the potential risk of malaria exposure. The largest proportion of human-vector contact for *A. funestus* s.l. (65%) and *A. coustani* s.l. (76%) took place before 22:00. For *A. gambiae* s.l., only 13% of the human-vector contact occurred before 22:00. As mentioned above, the results for *A. funestus* s.l. should be interpreted fully only when species data are available.

Infection rates in mosquitoes

A subset of anophelines were assessed for sporozoite infection (Table 7). Although not all samples have been analysed, none of 264 *A. funestus* s.l. samples tested was positive for sporozoites in Aduku, while 15.3% and 9.1% of all *A. arabiensis* and *A. gambiae* s.s. had *P. falciparum* infection, respectively. In Butemba, none of the few *A. arabiensis* was found infected but 6.3% of the *A. gambiae* s.s. tested were positive.

Insecticide resistance

Phenotypic resistance

Phenotypic resistance against a number of insecticides was assessed in the Ugandan sites. Many of the *A. gambiae* s.l. samples from the tests were subjected to molecular testing.

In Aduku, sample sizes were not sufficient due to the low number of available mosquitoes and hence results are inconclusive (Table 8). The majority of the mosquitoes tested were *A. arabiensis* (86%) while the remainder were *A. gambiae* s.s., with species distribution largely similar between tests. The tests indicate *A. arabiensis* was susceptible to the pyrethroids (deltamethrin and permethrin),

Table 7: Percentage of *A. gambiae* s.l. (data for *A. arabiensis and A. gambiae* s.s. presented separately where available) and *A. funestus* s.l. infected with *P. falciparum* by site

Site	% *P. falciparum* infected mosquitoes (number of samples tested)*		
	A. gambiae s.l.		*A. funestus* s.l.
Aduku	13.2% (151)		
	A. arabiensis	*A. gambiae* s.s.	0% (264)
	15.3% (124)	9.1% (11)	
Butemba	5.7% (279)		
	A. arabiensis	*A. gambiae* s.s.	--
	0% (14)	6.3% (253)	

**The number of mosquitoes tested is indicated in brackets*

bendiocarb and pirimiphos-methyl. Although sample sizes for *A. gambiae* s.s. were inadequate, resistance was not detected against bendiocarb and pirimiphos-methyl. Mosquitoes used for DDT tests were not available for molecular analysis but all of the few *A. gambiae* s.l. mosquitoes tested were susceptible.

In Butemba, two tests were conducted with sufficient replication against bendiocarb and pirimiphos-methyl, and no resistance was detected. Between 92% and 99% of test mosquitoes were *A. gambiae* s.s., with the remainder identified as *A. arabiensis*. However, resistance against pyrethroids and DDT was detected in Butemba in a subsequent survey round in April 2013 (data not shown).

Kdr L1014S genotype frequencies

In Butemba, *kdr* L1014S (kdr-east) frequencies were 94.7% and 91.5% in *A. gambiae* s.s. from collections and susceptibility tests, respectively, and no susceptible individuals were observed. The few *A. arabiensis* analysed (n = 14) were all susceptible at the *kdr* L1014S locus.

In Aduku, *A. gambiae* s.s. collected by various trapping techniques showed a high frequency of *kdr* L1014S (80.8%), whereas those from the resistance tests showed a moderate level (30.0%), yet sample sizes were between 13 and 15 individuals only. *Kdr* L1014S genotype frequencies for *A. arabiensis* varied between 3.0% and 24.7% (Table 9).

Table 8: Insecticide susceptibility test results using *A. gambiae* s.l. in Uganda, October 2012

Aduku					
Class	Insecticide	% *A. arabiensis* out of *A. gambiae* s.l.	No. of *A. gambiae* s.l. tested	Median KD50 (min)	Average mortality 24h (%)
Pyrethroid	Deltamethrin	89	20	28	100
	Permethrin	76	20	32	100
Carbamate	Bendiocarb	89	20	18	100
Organophosphate	Pirimiphos-methyl	88	20	57	100
Organochloride	DDT	No molecular data	20	29	100
Butemba					
Class	Insecticide	% *A. gambiae* s.s. out of *A.gambiae* s.l.	No. of *A. gambiae* s.l. tested	Median KD50 (min)	Average mortality 24 h (%)
Carbamate	Bendiocarb	99	59	13	100
Organophosphate	Pirimiphos-methyl	92	60	>60	100

Table 9: *Kdr* L1014S genotype frequencies (%) for *A. gambiae* s.s. and *A. arabiensis* and number of mosquitoes tested and identified as RR, RS and SS, in Butemba and Aduku

Site	Type of collection method	*A. gambiae* s.s.				*A. arabiensis*			
		Frequency (%)	RR	RS	SS	Frequency (%)	RR	RS	SS
Butemba	Collections	**94.7**	101	12	0	**0.0**	0	0	7
	Resistance tests	**91.5**	93	19	0	**0.0**	0	0	7
Aduku	Collections	**80.8**	10	1	2	**3.0**	0	15	237
	Resistance tests	**30.0**	4	1	10	**24.7**	21	5	69

Note: Results are presented for adults collected through the various collections and adults from resistance tests reared from field-collected larvae.
S = susceptible allele; R = resistant allele

Ownership and use of ITNs

ITN ownership

ITN coverage varied between countries and sites. Hembecho had the highest coverage, whereas Guba had the lowest (Table 10). The mean number of sleeping places per household ranged from 1.9 in Guba to 2.5 in Aduku. Guba in Ethiopia had the least number of LLINs per household and also the least number of LLINs used during the previous night out of the ones that were available[ii]. In contrast, Hembecho had the highest proportion of sleeping places covered with LLINs. Net ownership did not vary with socioeconomic status.

LLIN brands found and durability

PermaNet® 2.0 (Vestergaard-Frandsen) and Olyset® net (Sumitomo Chemicals) were the most abundant net types in all four sites (Figure 13). Netprotect® (Intelligent Insect Control) was also common in Aduku, but not at the other sites.

Durability of the LLINs found in the households was estimated from the average age of nets of different brands together with the proportionate hole index (pHI) of the nets – that is, the sum total of holes in the net, weighted by the size of those holes[43]. The average age of nets was determined through the household questionnaire in which respondents were asked when the nets were obtained. The average age of the nets was higher in Ugandan sites than in Ethiopian sites (Figure 14). The Butemba site was covered by a universal distribution campaign with Olyset® nets in early 2010.

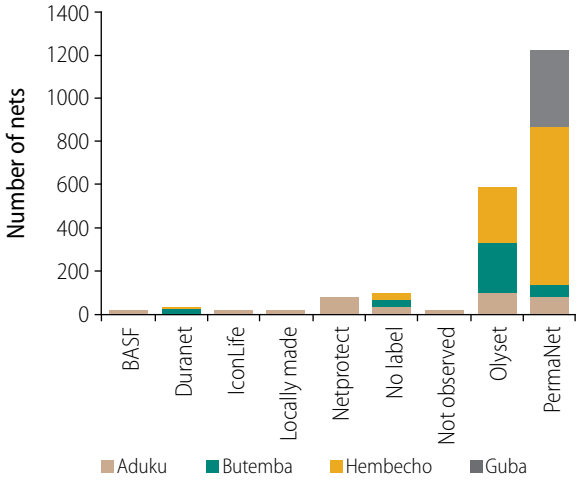

Figure 13: Types of nets found in households

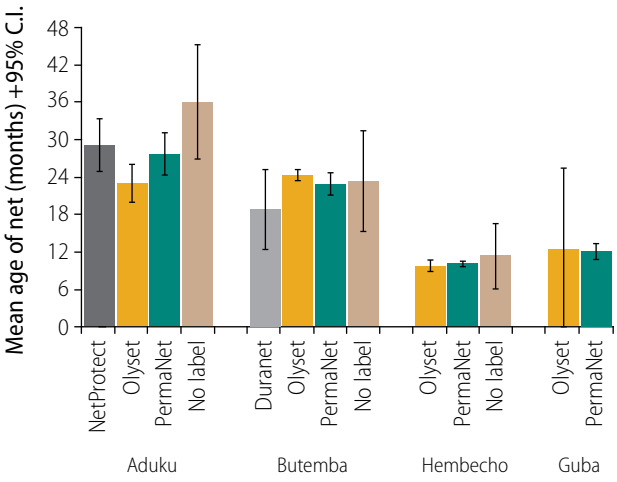

Figure 14: Estimated average age of commonly used LLINs and other (unlabelled) nets by study site

Table 10: ITN ownership and use rates

Indicator	Uganda		Ethiopia	
	Aduku	Butemba	Hembecho	Guba
% households with at least 1 ITN for 2 people	24.3	30.3	24.1	3.6
% households with at least 1 ITN	77.5	67.8	98.2	55.8
% households with at least 2 ITNs	19.5	42.2	73.3	9.8
Number of ITNs per household	1.1	1.5	1.9	0.7
Number of residents per household	5.0	5.3	5.2	5.2
Number of sleeping places	2.5	2.1	2.1	1.9
Number of persons per sleeping place	2.0	2.5	2.5	2.7
Number of ITNs used the previous night	0.9	1.2	1.5	0.2

[ii] Virtually all ITNs found in households in all sites were LLINs, so the results will be presented using the latter acronym.

Table 11: Number of holes of different sizes on nets of various brands and assessment of their fabric integrity in relation to estimated age of the nets

Site	LLIN brand and estimated age (months)	No. nets	Mean holes per net	% holes by size category				% nets by quality*			Mean hole index	% nets with at least 1 hole
				S	M	L	XL	Good	Accep-table	Torn		
Aduku	Netprotect®											
	<12	3	0	0	0	0	0	100	0	0	0	0
	12-23	6	6.2	78	16	5	0	67	33	0	93	50
	24-35	40	2.9	53	28	9	9	76	16	8	229	38
	36+	17	5.8	47	27	7	19	53	18	29	765	88
	Olyset®											
	<12	5	0	0	0	0	0	100	0	0	0	0
	12-23	28	2.8	70	21	5	4	75	21	4	105	46
	24-35	49	3.3	68	19	7	6	80	10	10	178	47
	36+	15	4.8	48	26	22	4	69	15	15	598	46
	PermaNet®											
	<12	5	1.4	100	0	0	0	100	0	0	1	40
	12-23	15	0.6	56	33	0	11	87	13	0	43	13
	24-35	20	3.7	36	11	25	29	80	0	20	793	30
	36+	31	1.2	51	30	19	0	87	10	3	53	19
Butemba	Olyset®											
	<12	10	0.3	0	100	0	0	90	10	0	7	10
	12-23	11	2.6	48	34	17	0	82	9	9	111	36
	24-35	179	2.7	54	29	11	6	83	8	8	172	41
	36+	28	2.4	60	32	8	0	89	4	7	62	43
	PermaNet®											
	<24	9	0.6	100	0	0	0	100	0	0	0.6	22
	24+	48	2.6	41	26	22	11	75	6	19	283	33
Hembecho	Olyset®											
	<12	126	0.5	51	40	9	0	93	7	0	14.5	13
	12-23	119	0.3	42	32	18	8	95	4	1	28.6	8
	24+	5	3.6	15	62	15	8	33	33	33	245	40
	PermaNet®											
	<12	321	0.4	53	43	3	1	97	3	0	7.5	15
	12-23	402	0.1	51	41	5	2	99	1	0	3.4	3
	24+	11	5.4	32	53	15	0	60	20	20	227	45
Guba	PermaNet®											
	<12	128	0.02	67	33	0	0	100	0	0	2.1	2
	12-23	142	1.7	34	38	22	6	81	6	13	352	29
	24-35	41	2.3	32	36	24	8	73	10	17	477	37
	36+	32	5.8	27	38	26	8	43	13	43	683	69

**Based on WHO Guidance Note for Estimating the Longevity of Long-Lasting Insecticidal Nets in Malaria Control, September 2013: a) LLIN in 'good' condition (pHI 0-64): no reduction of efficacy compared with an undamaged net; b) LLIN in 'acceptable' condition (pHI 65-642): effectiveness somewhat reduced but still provides significantly more protection than no net at all; c) LLIN 'torn' (pHI 643+): protective efficacy for the user is in serious doubt and the net should be replaced urgently.*

The fabric integrity of any mosquito net will decline over time, but the rate of this decline can be considerably different between nets, and is influenced by a range of factors, including the brand of net, the type and weight of the material, frequency of use and the net care practices of the household. Based on the pHI, each net was categorised as 'good', 'acceptable' or 'torn' based on WHO-recommended classifications per LLIN brand and 'age group' (Table 11)[43].

It was found that LLINs of similar age belonged to different net categories based on their pHI. Although sample sizes were not always large, data from Aduku showed that 53%, 69% and 87% of Netprotect®, Olyset® and PermaNet® older than three years were classified as 'good', respectively. At Butemba, 89% of Olyset® nets older than three years and 75% of PermaNet® older than two years were still 'good' to be used; in Ethiopia (Guba), 43% of PermaNet® nets were still 'good' after three years of use. The results are not conclusive to assess differences in durability between different brands. Further results should be obtained in subsequent survey rounds.

Purchasing of nets

Purchasing of nets was evident in Uganda but not in Ethiopia. In Uganda, 42 mosquito nets were purchased by the households themselves out of 606 owned (7%). None of these nets was identified as a LLIN (15 were not LLINs and 27 were of unknown type). Thirty-one were bought from open market, 10 from shops and one from a pharmacy. The proportion of nets purchased was not higher in the upper (richer) socioeconomic groups – that is, wealth does not correlate significantly with greater net purchasing (Table 12).

ITN use rates

ITN use as estimated from the percentage of individuals who slept under a net the previous night ranged from 14.2% in Guba to 68.2% in Hembecho (Figure 15).

Access of household members to LLINs was calculated for each study site assuming one net for two people. The LLIN use rates among those who have access were then estimated for each site. The data show there was variation between and within countries in terms of access to nets. Hembecho had the highest access rate and Guba had the lowest (Figure 16).

Among people with access to LLINs, use rates were high in three of the four sites (ranging from 69% to 76%), with the exception of Guba in Ethiopia, where only a third of those with access used a LLIN the previous night. There was no inequity between the sexes in LLIN use for any site, except for adults in Aduku, where significantly more females used an LLIN (OR = 1.6; 95% CI=1.1-2.2).

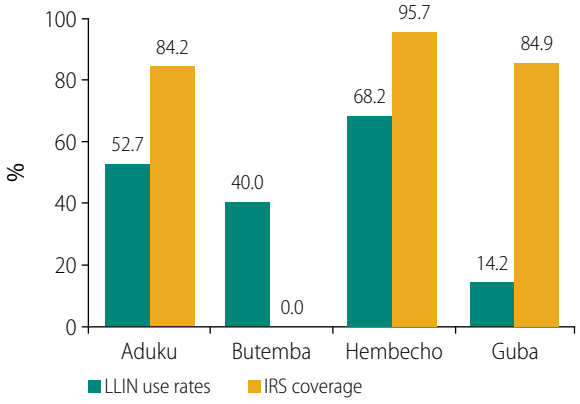

Figure 15: LLIN use rates (% who slept under an LLIN the previous night) and IRS coverage rates (% of households sprayed in the previous 12 months) by site

Table 12: Net purchase as percentage of total nets owned, by socioeconomic status

Wealth quintile	Aduku			Butemba			Hembecho		Guba	
	Bought	Total owned	%	Bought	Total owned	%	Bought	Total owned	Bought	Total owned
Lowest	2	17	12	3	53	6	0	152	0	72
Second	1	31	3	5	75	7	0	126	0	82
Third	8	77	10	6	74	8	0	163	0	120
Fourth	7	78	9	0	60	0	0	280	0	40
Highest	7	76	9	3	65	5	0	291	0	34
Total	25	279	9	17	327	5	0	1,012	0	348

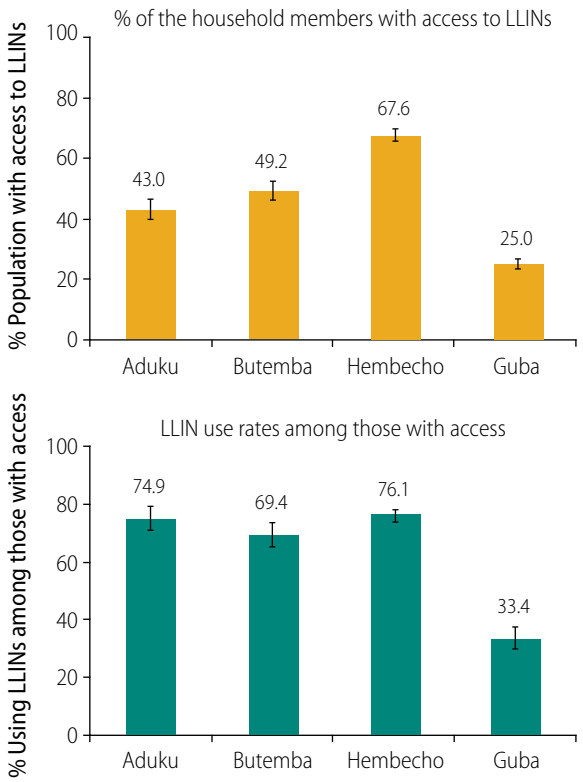

Figure 16: Access to and use of LLINs among household members in the four study sites in Ethiopia and Uganda. Error bars indicated 95% confidence intervals.

Reasons for not using nets

The reasons given for not using nets that were owned varied considerably between countries, and also between sites within countries (Figure 17). The most common explanations across both countries were that the net was too old or torn, or that the net was not hung up over the bed. Other reasons in Uganda included 'net causes allergic reaction', 'net too dirty', 'don't like smell' and 'net not treated'. In Ethiopia other reasons given included 'don't like smell', 'usual user did not sleep here', 'net not available last night' (e.g. being washed), 'don't know how to hang net', 'no malaria now', 'no mosquitoes now' and 'no chemical on the net'.

Difference in LLIN use rates among various age groups and sexes

There were clear differences between the sites in ITN use rates (Figure 18) but no inequity between the sexes in LLIN use for any site, except for adults in Aduku, where significantly more females used an LLIN (OR=1.6; 95% CI = 1.1-2.2). Numbers of infants (<one year old) were too low to show a significant difference

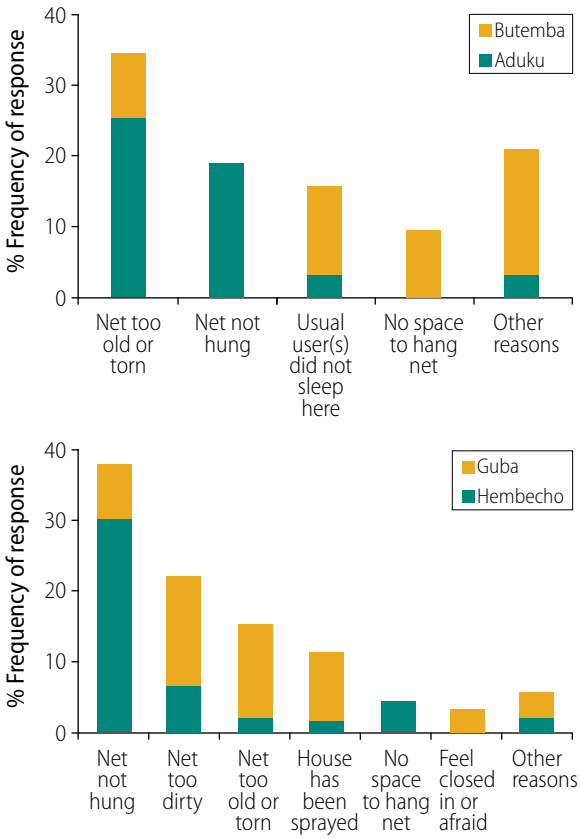

Figure 17: Explanations given for not using nets owned in Uganda sites (top chart) and Ethiopia sites (bottom chart). Values expressed as a percentage of the total responses in that country.

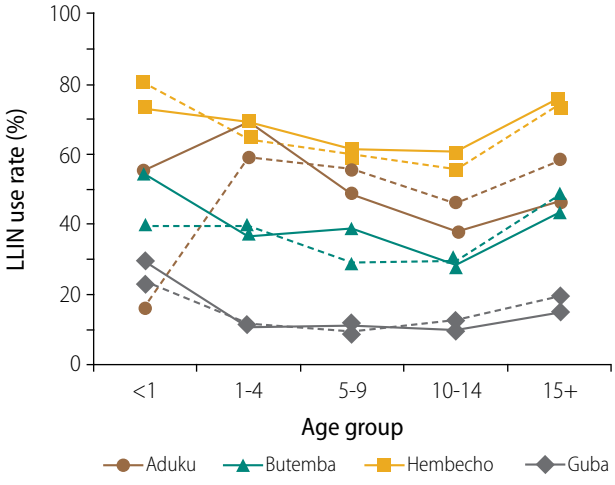

Figure 18: LLIN use rate (% of individuals who used an LLIN the previous night out of all individuals in the age group) by sex and age group
Solid line = males; dashed lines = females

between the sexes at any site. At all sites, LLIN usage was highest in the <one-year-old age group and among adults (15+ years old), with lower usage rates in all other child age groups.

Indoor residual spraying coverage

No IRS was carried out in Butemba. The remaining three study areas contained households that had been sprayed in the past 12 months, with or without concurrent ITN use. Coverage of IRS was consistently high across the villages within each study site. On average, 84% of households in Aduku had been sprayed within the past 12 months, 96% in Hembecho and 85% in Guba (Figure 15).

Prevalence of infection by use of preventive measures

ITN use and infection risk

Individuals who had used LLINs the night before the survey had significantly lower malaria infection prevalence in Aduku (2.3% versus 5.7%; $p = 0.020$) (Figure 19). No statistically significant difference was observed in the other sites.

Open eaves and infection risk

There was no statistically significant difference in malaria infection prevalence between houses with open/partially open or closed eaves in all sites. However, in Guba (the site with the lowest ITN ownership and use rates), houses with open eaves had slightly higher risk of infection with *P. falciparum*, although this was not statistically significant ($p = 0.087$).

Living in an unsprayed house and infection risk

Out of the three sites where IRS was implemented, prevalence of infection was significantly lower in individuals living in sprayed houses compared with those in unsprayed houses only in Guba ($p = 0.042$). Among individuals who slept in sprayed houses, the prevalence of infection was significantly lower in those who used LLINs in Aduku ($p = 0.026$), indicating that LLINs are more protective in this site than they are in the other sites. No such association was observed in the other two sprayed sites.

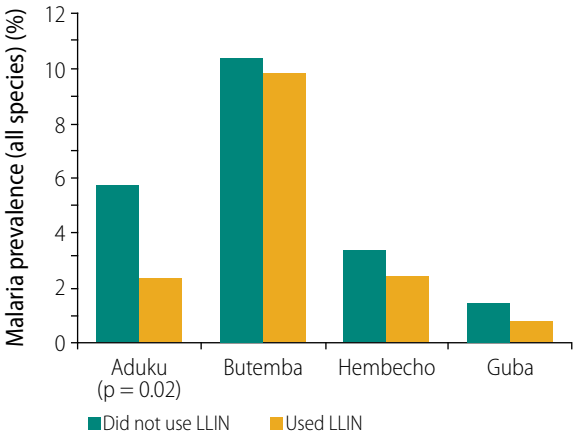

Figure 19: Prevalence of malaria infection (all species) (%) in individuals who used and did not use LLINs the previous night

Spray status of houses and ITN use rates

The relationship between IRS coverage and net use is inconsistent between sites. In Aduku there was no significant difference between net use in IRS households (47% [349/741]) versus unsprayed households (53% [57/107]). In Guba, net use was significantly lower in sprayed houses (12% versus 21%, $X^2 = 14.5$, $p < 0.001$). In Hembecho the trend was reversed – net use was higher in households that received IRS (65%) compared with households that had not been sprayed (50%, $X^2 = 6.4$, $p = 0.010$).

Prevention of malaria in pregnancy

Overall, IPTp use was more common in Butemba compared with Aduku ($X^2 = 15.1$, $p = 0.004$), and 36.9% of the women who had given birth within two years preceding the survey took two doses of IPTp, compared with 20.5% in Aduku (Table 13). The proportion of women who took three or more doses was around 7-8% in both sites (IPTp is not implemented in Ethiopia).

Knowledge of malaria

A large majority of respondents in Uganda identified mosquito bites as the cause of malaria (93% in Aduku and 77% in Butemba), whereas in Ethiopia the percentages were relatively lower (65% in Hembecho and 39% in Guba) (Table 14). In Butemba, although around three-quarters of the population correctly

identified mosquito bites as the cause of malaria, the belief that malaria is contracted by drinking dirty water was widespread (50% of respondents).

In Uganda, 89% and 79% of respondents had heard information about malaria in Aduku and Butemba, respectively, whereas messages about malaria were less frequently heard in Ethiopia ($X^2 = 158.6$, $p < 0.001$) (61% in Hembecho and 36% in Guba) (Table 15). The main sources of information in the Uganda sites were radio and health workers, whereas in Ethiopia community leaders and health workers were mentioned most often.

Febrile illness in children and treatment-seeking behaviour

A higher proportion of children under 10 years of age had fever or history of fever in the two Ugandan sites compared with in the Ethiopian sites (Table 16). In Uganda, treatment was sought for more than 90% of all children with fever, whereas in Ethiopia this ranged between 62 and 80%. In Aduku, first treatment was most commonly sought from privately owned clinics, pharmacies or drug shops, while in Butemba similar proportions were treated in the public and private

Table 13: Utilisation of intermittent preventive treatment of malaria by women who gave birth the two years before the survey in Uganda (IPTp is not part of the national malaria control strategy in Ethiopia)

		IPTp (%)				
Site	n	None	1 dose	2 doses	≥ 3 doses	Don't know
Aduku	39	53.8	10.3	20.5	7.7	7.7
Butemba	84	20.2	25.0	36.9	7.1	10.7

Table 14: Percentage of respondents mentioning causes of malaria

Response mentioned	Uganda		Ethiopia	
	Aduku (n=233)	Butemba (n=211)	Hembecho (n=547)	Guba (n=532)
Through mosquito bites	93	77	65	39
By drinking dirty water	0	50	22	26
Contact with a malaria patient	0	1	18	3
From cold weather	10	5	23	10
From eating corn or maize	0	1	8	2
Through bad smell	0	0	4	17
Don't know	2	10	17	26
Other	0	11	4	1

Table 15: Percentage of respondents who indicated hearing messages and information about malaria

Country	Site	% respondents who heard information about malaria	Main sources of information for respondents who heard messages (% times source was mentioned)						
			n	Radio	Village health worker	Health worker	Community leader	Family or friends	Leaflet or poster
Uganda	Aduku	89	204	86	9	67	4	9	14
	Butemba	79	166	82	10	27	7	8	1
Ethiopia	Hembecho	61	331	4	69	62	40	12	0
	Guba	36	187	33	47	21	17	7	0

Note: The main sources of the messages are shown by percentage of times mentioned (other minor sources mentioned include church or mosque, LLIN distributor, newspaper, television, drama performance and town announcer).

Table 16: Prevalence and treatment of fevers in children (10 years or younger) by study site

Site	Aduku	Butemba	Hembecho	Guba
Number of children with fever	35	59	42	76
% children with recent fever	10.3	15.8	4.4	7.3
% febrile children for whom treatment was sought	94.3	91.5	61.9	80.3
% febrile children for whom treatment was sought at public facilities	33.3	48.0	72.2	91.8
% febrile children given any antimalarial (% ACT use)	77.1 (55.6)	72.9 (79.1)	35.7 (86.7)	73.7 (41.1)
% febrile children treated with antimalarial within 24 hours of onset of fever	44.4	53.5	6.7	1.8
% of febrile children for whom treatment was sought who were tested	51.6	60.4	76.9	91.8
% of the tests that were done in public health facilities	62.5	75.0	100.0	96.4

sectors. In Ethiopia, first treatment was primarily sought in the public sector.

Antimalarials were given to the majority of children with fevers in all sites with the exception of Hembecho, where only 36% of children received an antimalarial. ACT use varied across the sites (in Ethiopia chloroquine was used as well as ACT as it was first-line treatment for *P. vivax* malaria). For approximately half of the children who were given an antimalarial in Uganda, treatment was started within 24 hours following onset of fever. In Ethiopia, only 2-7% of children who received an antimalarial started the treatment within 24 hours of the onset of a fever. Approximately 52-60% and 77-92% of children with fever who sought treatment received a malaria diagnostic test (either RDT or microscopy) in Uganda and Ethiopia, respectively.

In Butemba, the majority of tests were done in the public sector and these were RDTs performed by village health teams who had been trained to test and treat uncomplicated malaria through the iCCM programme implemented in this district. In Aduku, almost all tests performed were RDTs. At the Ethiopia sites, malaria diagnosis was carried out predominantly in the public sector at health centres using microscopy, with a limited number of tests conducted at health posts using RDTs. However, at Hembecho, about half of the tests at health centres still used RDTs.

Undernutrition

Undernutrition was an important problem in both countries. However, a far greater proportion of children under five in the Ethiopian sites (29-32%) were underweight compared with in the Ugandan sites (6-7%). The Ethiopia sites had the highest percentage of children affected by undernutrition. Approximately two-thirds of children in the Ethiopian sites were stunted (Figure 20). Wasting, which is an indicator of acute undernutrition, was most prevalent in the Guba site (16.8%) and affected a much lower proportion of children in the Hembecho site (3.4%). In the Butemba site of Uganda, more than a quarter of children under five were stunted (no data were collected on stunting and wasting in Aduku). In Butemba, 8.1% of children were affected.

Under-five mortality rates

Summary birth histories of 1,503 women were available: 1,041 women completed birth histories in Ethiopia and 462 in Uganda. Of these, 88% were retained in the analysis. Reasons for exclusion included women were less than 15 years old or more than 49 years old (10%); the number of infants born was not specified (1%); and there were more deaths than live births reported (<1%). A greater percentage of women were excluded from the Uganda dataset than from the one from Ethiopia (17% versus 9%).

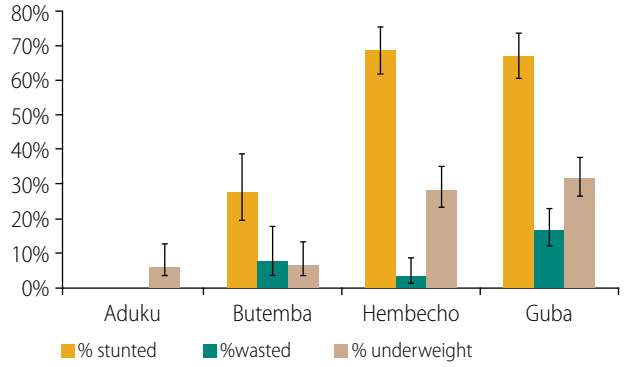

Figure 20: Percentage of stunted, wasted and underweight children under five years of age in study sites in Uganda (Aduku and Butemba) and Ethiopia (Hembecho and Guba). Error bars indicate 95% confidence intervals. Height measurements were not taken at Aduku resulting in absence of stunting and wasting data.

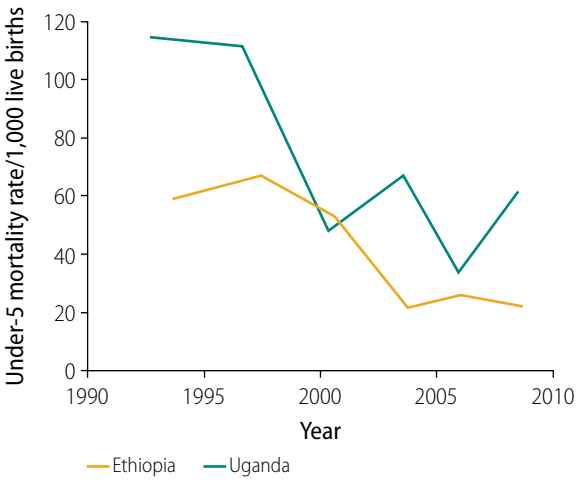

Figure 21: Under-five mortality rate estimated from birth history data in Ethiopia and Uganda

There was a difference in age and education of the women analysed across the two countries. Uganda had a significantly higher proportion of women aged less than 25 years compared with Ethiopia (41% versus 21%) and a significantly lower proportion of women with no education (22% versus 83%).

In total, 3,317 live births were reported in Ethiopia and 1,101 in Uganda, and the average number of live births was approximately 3.5 and 2.9 in Ethiopia and Uganda, respectively (Table 17). Eighty-three percent of all women analysed had ever given birth, and the percentage of women who had ever given birth increased with age. The number of births also increased with age. Among live births, 136 deaths were reported in Ethiopia and 68 in Uganda, giving the percentage dead among live births 4.1% in Ethiopia and 6.2% in Uganda.

The Ethiopia data show a steady increase in mean parity from an average of 0.2 births to approximately five births for women aged 45-49 years. Similarly, for the percentage of dead children among live births, the percentage increases from 0% to approximately 8% in the older age group. In Uganda, parity increased with age; however, the percentage of deaths increased with age of mothers.

The trend observed for Ethiopia is a reduction of almost 60% in the U5MR from approximately 60 deaths per 1,000 live births for the years prior to 2000 to approximately 23 deaths per live births post 2000 (Figure 21). In Uganda, there is also a large reduction observed in U5MR pre- and post-2000. Pre-2000, the U5MR was approximately 113 per 1,000 live births, whereas post-2000 the rates fluctuated between 34 and 67.

Table 17: Data used for calculation of under-five mortality rates from women's interviews in Ethiopia and Uganda

Country	Age group of women	Number of women	Mean parity of women	Number of live births	Number of deaths	% of children dead
Ethiopia						
	15-19	66	0.2	11	0	0
	20-24	130	1.7	191	3	1.6
	25-29	222	3.1	684	16	2.3
	30-34	216	4.1	885	18	2.0
	35-39	184	4.9	898	49	5.5
	40-44	72	5.7	413	32	7.7
	45-49	49	4.8	235	18	7.7
	Total	939	3.5	3,317	136	4.1
Uganda						
	15-19	62	0.5	31	0	0
	20-24	99	1.4	139	7	5.0
	25-29	81	2.8	228	7	3.1
	30-34	57	4.1	236	15	6.4
	35-39	46	5.2	238	11	4.6
	40-44	23	5.7	131	16	12.2
	45-49	17	5.8	98	12	12.2
	Total	385	2.9	1,101	68	6.2

Discussion

We carried out a comprehensive survey in four sites in Ethiopia and Uganda to gather baseline data for future surveys aimed at monitoring changes in malaria epidemiology and effectiveness of interventions over time. Malaria transmission during October-November 2012 in all locations was low or moderate, including in previously holoendemic areas.

Malaria prevalence in the Ugandan sites was much lower than historically recorded, particularly in Apac[44]. It is likely that the combined use of both IRS and LLINs led to the observed decline. However, due to the limited number of study sites, the findings may not represent national trends. As baseline transmission levels vary across sites, it is inappropriate to compare the four study sites; however, the highest malaria prevalence was observed in the site that was not under IRS in Uganda. The point prevalence data may not represent the actual average situation in a site. There is considerable seasonality of transmission in both countries as well as inter-annual variations. Especially in Ethiopia, the variations are likely to be governed by meteorological conditions[45,46]. It is not always possible to accurately determine the peak of the transmission seasons correctly. In Ethiopia, prevalence rates were generally comparable with rates reported in the 2011 MIS[14]. In Uganda, however, the rates were much lower than rates reported in the 2009 MIS[19].

The age group pattern in Ethiopia is typical of low endemicity where all age groups are similarly affected. The pattern in Butemba (Uganda) indicates moderate endemicity, whereas the other two sites are somewhat in-between the Guba and Butemba patterns. These results are worth noting, especially in terms of the lower than expected endemicity in Aduku (Uganda).

It has been previously reported that Apac had a high entomological inoculation rate[44]. Probably as the result of intensified vector control using both IRS and LLINs, transmission in Apac seems to have declined substantially. The observed higher prevalence in older age groups is also supportive of a recent change in transmission to a lower intensity, presumably as a result of reduced immunity over recent years.

Serology data can indicate systematic changes in transmission intensity by looking at the age-seroprevelance distribution[40]. Serological data confirm a higher level of transmission in Butemba compared with Aduku in Uganda. The age group pattern of infection prevalence also indicates a relatively high transmission situation in Butemba. The model does not fit some of the younger age groups well in Aduku, which could reflect transmission changes due to the ongoing IRS programme, which started in 2007 in this site. Similarly, in Butemba, the youngest age group shows a lower than expected seroprevalence rate, which could owe to the LLIN mass distribution campaign in 2010. However, small sample sizes in younger age groups could also account for these findings, and additional serology data from subsequent rounds and additional analyses of samples from Ethiopia, as well as use of other antigens, can provide more robust datasets to analyse the interventions in terms of transmission changes.

Entomological results provided important information on the situation of vectors. Very low densities of *A. gambiae* s.l. mosquitoes were observed in Ethiopia at both sites, so no resting or feeding habits could be deduced. *A. gambiae* s.s. dominated in Butemba, whereas in Aduku *A. funestus* s.l. and *A. arabiensis*

were the main vectors. Most human-vector contact with *A. gambiae* s.l. occurred indoors in Aduku, Uganda, which shows use of interventions against indoor-biting vectors should be considered effective. However, the early feeding observed in *A. funestus* s.l. requires confirmation with data from subsequent surveys. A study during 2001-2002 in Apac showed that a large majority of malaria transmission was sustained by *A. funestus* s.l. and occurred after 23:00[44]. Whether or not the earlier feeding observed here indicates a shift in feeding behaviour remains to be investigated. Even though early and outdoor feeding could jeopardise vector control efforts, malaria prevalence in Aduku was low at the time of the survey (3.4%). Furthermore, none out of 264 *A. funestus* s.l. tested positive for *P. falciparum* infection.

A substantial number of *A. coustani* s.l. was collected by HLC, suggesting attraction to humans. Its status as a malaria vector is unknown in Uganda. A study in Zambia found a high degree of anthropophily in *A. coustani* s.l.[47] and it was reported to be contributing to transmission in Kenya[48]. Molecular analysis will be needed to determine the particular species involved and infectivity rates.

The use of insecticides for IRS (and LLINs with strong repellent/deterrent effects) has the potential to drive malaria vectors outdoors. *A. funestus* s.l. is typically regarded as a highly endophilic mosquito, and thus controllable by IRS. The low number of blood-fed *A. funestus* s.l. in Aduku and the very few *A. funestus* s.l. collected in Butemba suggest control measures seem to be effective against these mosquitoes. The percentage of *A. funestus* s.l. found resting indoors was very low in Aduku; this is likely to be a reflection of control measures rather than evidence of a behavioural shift as a result of IRS, given that the percentage of other species found indoors was also very low. Other anophelines seem to be exhibiting deliberate exophily – that is, they are avoiding using houses as resting sites. *A. coustani* s.l. and *A. pharoensis*, two species that represent most of these other anophelines caught, have previously been classified as exophilic[49].

Insecticide resistance is increasing in many areas of Uganda[50,51]. We observed high *kdr* L1014S frequencies in *A. gambiae* s.s. and low frequencies in *A. arabiensis*, a finding in line with another study from eastern Uganda[52]. Further studies will be needed to confirm the magnitude and impact of insecticide resistance in both countries. No tests were done in Ethiopia due to small sample size but widespread resistance to DDT and pyrethroids has been reported[16,53]. Resistance against pyrethroids and DDT seems to be increasing at a rapid rate in both countries, probably due to the increase in the distribution and use of LLINs in recent years.

Regarding diagnosis and treatment, there are variations observed in both countries, especially around utilisation of services. In Uganda, seeking treatment in the private sector is common. These findings are in line with the MIS 2009, which observed that, for 82% of fevers of children under five, treatment was sought, and 56% of children with a fever were taken to the private sector[19,54]. In Ethiopia, a great majority of febrile children were taken to public health facilities for treatment.

As part of the malariometric survey, febrile subjects were tested with RDTs, which provided an opportunity to compare with subsequent microscopy results. We found the RDTs used had high negative predictive value, but their positive predictive value was uniformly low in all sites. This shows negative results with the RDTs for febrile patients can be a good indicator of absence of infection but a positive result may not always be a reliable indicator of presence of infection. These results may be explained by the nature of the RDTs that test for the presence of parasite antigens that circulate in the blood for several weeks post-infection[55,56].

Prevention of malaria in pregnancy was one of the interventions implemented in Uganda. The survey results indicated that IPTp uptake was low in Uganda and needs to be strengthened if the current recommendation of at least three doses is reached[57]. The rather low coverage or utilisation levels are in line with findings from the Demographic and Health Survey in 2011, which found that 25% of women reported having taken at least two doses of IPTp[23]. While data for Aduku were similar to the national average in 2011, in Butemba a higher proportion of women reported using IPTp, probably because of activities of various projects in this region working to improve its uptake.

Knowledge of malaria varied among the sites. Households in the Ugandan sites had overall better knowledge about malaria compared with in the Ethiopian sites. However, in Butemba, other causes of malaria were frequently cited, unlike in Aduku, where most respondents correctly identified mosquitoes as being the only cause of malaria. This could be due to behavioural change communication campaigns that accompanied the IRS programme. In Guba, knowledge about the cause of malaria needs to be improved through appropriate community education.

Universal coverage is defined differently in the two countries: two ITNs per household in Ethiopia and one ITN for two people in Uganda. In one of the Ethiopian sites (Hembecho), the goal was nearly achieved, with 1.9 ITNs per household; ownership in the second site (Guba) was the lowest of all sites (0.7 ITNs per household). The cause of the low ownership rate in Guba requires more investigation. In Uganda, although the percentages of sleeping place covered with ITNs were 44% and 71% in Aduku and Butemba, respectively, the percentages of households with one ITN for two people were 24% and 30%, respectively. However, uniformly high IRS coverage was observed in the sprayed sites.

In one of the four sites (Aduku), individuals who had slept under an ITN the night before the survey had lower risk of infection than those who had not. This could be due to low levels of pyrethroid resistance in *A. arabiensis* in that site. Lack of association in ITN use and malaria in the other sites does not necessarily translate to lack of effectiveness. It may just mean that the mass effect of the ITNs was confounding the result, that people who did not use nets might have been 'protected' by all other nets in the villages due to mortality effects on mosquito vectors.

It is not clear whether ITN and IRS have an additive or synergistic action when used in combination. The current WHO recommendation is that, where LLIN coverage is high and they remain effective, IRS may have limited utility in reducing malaria morbidity and mortality, unless the combined use is for resistance management[58]. In Guba, where both ITN ownership and use rates were low, malaria infection risk was higher in individuals living in unsprayed houses than in those in sprayed ones, indicating the potential benefit of the combined use of both interventions in such situations.

Among the important observations in the present survey was the high level of undernutrition in the study sites. Undernutrition during the critical first 1,000 days of a child's life could have devastating consequences by increasing morbidity and mortality and development of the child. Recently, stunting (or low height for age) has been chosen as a key indicator to measure global and national progress towards reduction of undernutrition[37]. We found undernutrition was a particularly severe problem in the Ethiopia sites, where two-thirds of children were classified as stunted. Critical nutrition interventions should be strengthened to address both maternal and child undernutrition. These include, among others, promoting optimal breastfeeding practices, micronutrient supplementation, reducing incidence of low birth weight and prevention of disease.

Conclusion and recommendations

The findings of the baseline survey indicate malaria epidemiology seems to be changing compared with earlier published data, and continued regular monitoring will help modify strategies and improve targeting of interventions to address increasing heterogeneity of transmission. More investigation will be needed to reveal the impact of the various interventions on malaria transmission through further in-depth analysis and modelling, which will make use of data gathered in subsequent survey rounds. The use of a mathematical model will also be useful to predict risk patterns and heterogeneity in larger areas in order to help in epidemiological stratification and evidence-based targeting of appropriate interventions.

The study provides important baseline variables against which future changes in malaria epidemiology can be compared, including changes in vector behaviour, malaria endemicity, immunological profiles, risk patterns by demographic and socioeconomic profiles and climate. It also provides information that can be used to correlate control inputs to results.

Low to moderate malaria transmission intensity was observed in all sites, including in previously holoendemic area. Prevalence of infection varied between and within study sites. In Ethiopia, it seems *P. vivax* is becoming a predominant malaria species as transmission has declined over recent years.

Although subsequent surveys and more analysis will be likely to show the impact of various interventions, intensified vector control and effective treatment seem to have played key roles in bringing endemicity down over recent years, as reflected in the results of serological analyses. High coverage of nets was observed in three of four sites but it is still not at an ideal level, as the highest proportion of the population with access was just over two-thirds, while in the rest of the study populations access was below 50%.

However, except in one site with low malaria transmission in Ethiopia (Guba), ITN use rates among household members were quite high. The study also showed there is willingness to buy nets, at least in the Uganda sites. In Ethiopia there were no purchased nets; more data will be needed to understand whether this is because of insufficient demand or an under-developed supply system or both.

The WHO insecticide susceptibility tests and subsequent molecular analysis of resistance markers showed *A. arabiensis* is somehow susceptible to pyrethroids, whereas *A. gambiae* s.s. has developed a high degree of both phenotypic and genotypic resistance against pyrethroids.

This study showed that individuals using ITNs are at a significantly lower risk of contracting malaria in at least one site, which is encouraging in spite of increasing pyrethroid resistance.

Most human-vector contact still occurs indoors, especially with both *A. gambiae* s.s. and *A. arabiensis*. However, there is a tendency of early biting of *A. funestus* s.l. in one of the sites in Uganda. As no *A. funestus* s.l. from this site was found to be infected with *P. falciparum*, molecular identification is required. More data are needed to determine the resting habits of vector species in both countries. Furthermore, more studies are required to understand the role of a previously less important anopheline mosquito, *A. coustani* complex, as it was found to bite humans in considerable numbers, especially in Uganda.

The rate of malaria diagnosis using microscopy and RDTs has been strengthened in all sites. RDTs have been found to have a high level of negative predictive value for fever cases, indicating that service providers should pay attention to other causes of fever when RDT negative results are reported for patients, especially as malaria endemicity levels decline.

More investigations will reveal the impact of the various interventions on malaria transmission through in-depth analysis and mathematical modelling using some of the parameters of the entomological, malariometric and climatic studies.

Recommendations

Low malaria prevalence was observed in some sites that previously had high endemicity but there was substantial variation between sites.

- Malaria control efforts should be sustained to reduce transmission further, maintain the gains and prevent resurgence.
- The findings will serve as a baseline against which results of future surveys or other studies could be compared. Although results from a small number of study sites may not be nationally representative, the approach provides more comprehensive information on a range of potential determinants of malaria rates than more geographically extensive surveys and surveillance, and they will provide a basis for and may prompt further investigations of some of the observations. It is necessary to continue monitoring the epidemiological changes.
- Malaria control strategies should be adapted to the changing patterns and heterogeneity of transmission which may require a thorough epidemiological stratification and selective targeting of interventions.

Additional recommendations related to other key findings of the baseline survey are below.

- In Uganda, most human biting by *A. gambiae* s.s. and *A. arabiensis* occurred indoors late at night when people are likely to be under nets. However, a tendency of early biting in *A. funestus* s.l. in Apac requires further investigation. Low vector densities in Ethiopia did not allow any assessment of biting habits.
- A high level of vector resistance against pyrethroids was observed, especially in *A. gambiae* s.s. in Uganda. The impact of this resistance on effectiveness of LLINs should be studied further. IRS programmes should closely monitor resistance against non-pyrethroids and implement pre-emptive rotation.
- There was a high coverage of IRS using carbamates in areas under spraying in both countries and it seems to have a good impact. Non-pyrethroid IRS may be considered when feasible and where other measures have inadequate impact, or in areas where there are major obstacles to achieving high ownership and use of LLINs.
- Coverage of ITNs was below country targets in most of the study sites. Although the required levels may have been restored in recent mass campaigns, these findings highlight the need for robust continuous distribution systems to replace nets and maintain high coverage.
- ITN use among those with access was good in all sites, except in one study site in Ethiopia (Guba). Health services should investigate whether there is a similar phenomenon in other areas and the possible causes, and should focus efforts on creating a culture of net use through appropriate behavioural change communication.
- No nets had been bought by households from commercial outlets in Ethiopia while in Uganda a substantial level of purchase of untreated nets was observed. Ministries of health should create conditions to make LLINs available on the commercial market at affordable prices and educate communities on the benefits of LLINs over untreated nets.
- Knowledge of household respondents about malaria and its cause varied among sites and was low in some cases. Appropriate communication programmes are needed to address existing gaps.
- Although the use of at least one dose of IPTp by pregnant women was relatively high in Uganda, the level of use of the recommended repeated doses was below target and should be strengthened. Special attention should be given to improving the supply system within the health services. Community education is also required to create demand.
- There was a considerable level of undernutrition in the study sites. Improving maternal and child nutrition should receive due focus by health services.

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