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Patterns of human exposure to early-evening and outdoor biting mosquitoes and residual malaria transmission in Ethiopia

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Abstract

Ethiopia has shown a notable progress in reducing malaria burden over the past decade, mainly due to the scaleup of vector control interventions such as long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Based on the progress, the country has set goals to eliminate malaria by 2030. However, residual malaria transmission due to early-evening and outdoor biting vectors could pose a challenge to malaria elimination efforts. This study assessed vector behavior, patterns of human exposure to vector bites and residual malaria transmission in southwestern Ethiopia. *Anopheles* mosquitoes were collected monthly from January to December 2018 using human landing catches (HLCs), human-baited double net traps, CDC light traps and pyrethrum spray catches. Human behavior data were collected using questionnaire to estimate the magnitude of exposure to mosquito bites occurring indoors and outdoors at various times of the night. Enzyme-linked immunosorbent assay (ELISA) was used to determine mosquito blood meal sources and sporozoite infections. A total of 2,038 female *Anopheles* mosquitoes comprising

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Authors' contribution

TD conceived and designed the study protocol, involved in data collection, performed laboratory analysis, analysed the data and wrote the manuscript. ML involved in mapping the study site, AKG, GY and DY involved in designing the study protocol and critically reviewed the manuscript. All authors read and approved the final manuscript.

Competing interests

We authors declare that we have no competing interests.

Ethical approval

Ethical approval for the study was obtained from Institutional Review Board of Jimma University (Ref No. IHRPGD/2075/18). Permission was sought from chief of the study site. Informed consent was obtained from heads of households and data collectors.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Anopheles arabiensis (30.8%), *An. pharoensis* (40.5%), *An. coustani* (28.1%), *An. squamosus* (0.3%) and *An. funestus* group (0.2%) were collected. *Anopheles arabiensis* and *An. pharoensis* were 2.4 and 2.5 times more likely to seek hosts outdoors than indoors, respectively. However, 66% of human exposure to *An. arabiensis* and 39% of exposure to *An. pharoensis* bites occurred indoors for LLIN non-users. For LLIN users, 75% of exposure to *An. arabiensis* bites occurred outdoors while 23% occurred indoors before bed time. Likewise, 84% of exposure to *An. pharoensis* bites occurred outdoors while 15% occurred indoors before people retired to bed. *Anopheles arabiensis* and *An. pharoensis* were 4.1 and 4.8 times more likely to feed on bovine than human, respectively. Based on the HLC, an estimated indoor and outdoor EIR of *An. arabiensis* was 6.2 and 1.4 infective bites/person/year, respectively, whereas *An. pharoensis* had an estimated outdoor EIR of 3.0 infective bites/person/year. In conclusion, *An. arabiensis* and *An. pharoensis* showed exophagic and zoophagic behavior. Human exposure to *An. arabiensis* bites occurred mostly indoors for LLIN non-users, while most of the exposure to both *An. arabiensis* and *An. pharoensis* bites occurred outdoors for LLIN users. Malaria transmission by *An. arabiensis* occurred both indoors and outdoors, whereas *An. pharoensis* contributed exclusively to outdoor transmission. Additional control tools targeting early-evening and outdoor biting malaria vectors are required to complement the current control interventions to control residual transmission and ultimately achieve malaria elimination.

Keywords

Malaria; vector behavior; human behavior; exposure; residual transmission; Ethiopia

1. Introduction

Malaria remains one of the most serious vector-borne diseases, affecting hundreds of millions of people mainly in the sub-Saharan Africa including Ethiopia. Yet unprecedented success has been achieved over the past two decades in reducing the disease burden, averting an estimated 663 million malaria cases in Africa between 2001 and 2015 (Bhatt et al., 2015; WHO, 2015). Vector control is one of the key elements in achieving the remarkable reduction in malaria, with long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) estimated to have averted 68% and 10% of the cases, respectively (Bhattarai et al., 2007; Otten et al., 2009; WHO, 2015).

Similarly, morbidity and mortality due to malaria has remarkably declined in Ethiopia over the past decade as a result of large-scale distribution of LLINs and high coverage of IRS, together with nationwide implementation of artemisinin-based combination therapy (ACT) (FMoH, 2016; Otten et al., 2009; Shargie et al., 2010; Taffese et al., 2018). Based these gains, the country has set goals to eliminate malaria by 2030 and the elimination program is being implemented in 239 selected low malaria transmission districts encompassing six different regions (PMI, 2020). More than 11 million LLINs have been distributed through mass campaigns in 2018 alone to further reduce malaria cases and accelerate the progress towards elimination (PMI, 2019; WHO, 2019).

However, malaria transmission continues to occur and still remains a significant public health problem in Ethiopia despite the progress made in scaling up of the control measures (Abraham et al., 2017; Taffese et al., 2018). This transmission could be attributed to several factors including the spread of insecticide resistance (Messenger et al., 2017; Yewhalaw et al., 2011) and preference of malaria vectors to bite outdoors and in the early evening when people are indoors but unprotected by existing tools (Kenea et al., 2016; Kibret and Wilson, 2016; Yohannes and Boelee, 2012). The current indoor-based malaria vector control interventions such as LLINs offer protection from anthropophilic and endophilic vectors, but have little impact on vector species predominantly feeding on animals and humans outdoors (Durnez and Coosemans, 2013).

In Ethiopia, the primary vector of malaria is *An. arabiensis*. This vector species has a peculiar feature in that it can readily feed on humans to sustain intense malaria transmission (Abraham et al., 2017; Animut et al., 2013; Kibret et al., 2014; Massebo et al., 2013), but often enough on animals to evade the effect of LLINs and IRS, and to maintain residual malaria transmission (Killeen et al., 2017; Massebo et al., 2015). Such dual feeding preference of *An. arabiensis* could pose another challenge to malaria control and elimination efforts as malaria transmission may continue even with a high coverage of the current vector control interventions (Durnez and Coosemans, 2013; Killeen et al., 2017). Moreover, the feeding behavior of *An. arabiensis* could vary in different eco-epidemiological settings depending on several factors including host availability (Fettene et al., 2004; Habtewold et al., 2001) and the genetic structure of the vector itself (Lulu et al., 1998, 1991; Mekuria et al., 1982).

In addition to the vector behavior, human habits and sleeping patterns could also be vital determinants of malaria transmission since exposure to malaria vector bites occurs when unprotected people and vector biting activities overlap in time and space (Edwards et al., 2019; Finda et al., 2019; Monroe et al., 2019). Addressing the challenge of residual malaria transmission on malaria elimination efforts requires better understanding of both the local vector and human behavior. Moreover, quantifying the magnitude of human exposure to infectious mosquito bites which occurs indoors and outdoors is crucial to evaluate the likely success of the current vector control measures (Killeen et al., 2006). However, most vector surveillance activities in Ethiopia focused mainly on vector behavior with less or no attention to human behavior that also contributes to residual malaria transmission. The aim of this study was to assess vector behavior, patterns of human exposure to mosquito bites and residual malaria transmission in southwestern Ethiopia.

2. Materials and Methods

2.1. Study area

The study was carried out in Bulbul *kebele* (7.70285°N; 37.09592°E, altitude 1705 m asl), which is located in Kersa district, Jimma Zone 320 km southwest of the capital, Addis Ababa (Figure 1). The inhabitants mostly rely on subsistence farming, with maize and *teff* being the main cultivated crops in the area. Most houses are mud-walled with roofs made of corrugated iron sheets. Malaria transmission is seasonal in Bulbul area. The transmission peaks from September to October, following the major rains from June to September. Minor

transmission occurs in April and May, following the short rains of February to March. *Plasmodium falciparum* and *Plasmodium vivax* are the two predominant malaria parasite species co-occurring in the area and are transmitted mainly by *An. arabiensis* (Yewhalaw et al., 2009).

2.2. Mosquito sampling

Adult mosquito collections were carried out monthly from January to December 2018. Host-seeking mosquitoes were collected both indoors and outdoors using human landing catches (HLCs), Centers for Disease Control and Prevention (CDC) miniature light traps and human-baited double net traps (HDNT). Indoor resting mosquitoes were collected using pyrethrum spray catches (PSCs).

The HLC was performed in four randomly selected houses per month by adult male volunteers. For each house, two collectors (one indoor and the other outdoor) seated on stools with their legs exposed from foot to knee to capture mosquitoes as soon as they land on the exposed legs before they commence blood-feeding using a flashlight and mouth aspirator (Service, 1977; WHO, 2013). There were two collection shifts: one team worked from 18:00 to 24:00 hr during each collection night, followed by the second team from 24:00 to 06:00 hr. Each hour's collection was kept separately in labeled paper cups. A supervisor was assigned to coordinate the collection activities and watch volunteers not to fall asleep during the collection nights. All collectors were provided with anti-malarial prophylaxis to avoid a risk of contracting malaria during the collection period. Mosquitoes were identified to species the next morning. The CDC light traps were set indoors beside human-occupied bed nets in other four randomly selected houses monthly and paired with outdoor HDNT. Details of the HDNT are described elsewhere (Degefa et al., 2020). Both traps were set from 18:00 to 6:00 hr during each collection night. The PSC was conducted monthly in twenty randomly selected houses from 06:00 to 09:00 hr following standard protocol (WHO, 1995).

All collected mosquitoes were identified morphologically to species or species complexes using a dichotomous key described by Gillies and De Meillon (Gillies and Coetzee, 1987). Female *Anopheles* mosquitoes were further classified as unfed, freshly fed, half-gravid and gravid. Each mosquito was kept individually in a labeled 1.5 ml Eppendorf tube containing silica gel desiccant. Samples were stored at -20°C freezer at Jimma University Tropical and Infectious Diseases Research Center (TIDRC) Laboratory until used for further processing.

2.3. Human behavior survey

Questionnaire survey was conducted in October 2018 in 140 randomly selected households residing in the study area. The residents were asked about the time they went indoors, when they retired to bed, when they woke up in the morning and when they left their houses for outdoor activities. Moreover, data on the ownership and utilization of nets by the households, and the numbers of potential vertebrate hosts available in the study area including human, bovine, goat, dog and chicken were collected using the questionnaire survey.

2.4. Mosquito sample processing

Anopheles gambiae s.l. specimens were identified to sibling species by polymerase chain reaction (PCR), following the protocol developed by Scott *et al* (Scott et al., 1993). Dried head and thorax of the preserved *Anopheles* mosquito specimens were carefully separated from the abdomen and tested for *Plasmodium* circumsporozoite protein (CSP) using sandwich enzyme-linked immunosorbent assay (ELISA) (Beier et al., 1987; Wirtz et al., 1987). The blood meal sources of freshly fed *Anopheles* mosquitoes collected by PSCs and CDC light traps were assayed by a direct ELISA using human, bovine, goat, chicken and dog antibodies (Beier et al., 1988).

2.5. Data analysis

The mean density of host-seeking *Anopheles* mosquitoes was compared between indoor and outdoor location using a generalized linear model based on negative binomial distribution. Season of collection was treated as covariate in the model. Crude biting rate for each anopheline species was determined as the mean number of *Anopheles* mosquitoes collected by HLC per person per night.

Human exposure to malaria vector bites was calculated based on data from both human and vector behavior. Behavior-adjusted human biting rate (aHBR) experienced by unprotected individuals at each time of the night (t) was determined based on the proportion of people reported to have stayed indoors (I) multiplied by indoor biting rate (B_i) plus the proportion of people reported to have stayed outdoors (1-I) multiplied by the outdoor biting rate (B_o) (Killeen et al., 2006). The aHBR per night was then calculated by summing hourly biting rates:

$$aHBR = \sum_{t=1}^{12} [B_{i,t}I_t + B_{o,t}(1 - I_t)] \quad (1)$$

where t = 1 represents the time period from 6:00 to 7:00 pm, t = 2 from 7:00 to 8:00 pm, and continue as such up to t = 12 for the time period from 05:0 to 6:00 am.

The mean biting rate experienced by protected individuals (aHBR_p) per night was calculated by adjusting the indoor biting rates for the sleeping fraction of the population taking into account the personal protection (ρ) provided by LLINs:

$$aHBR_p = \sum_{t=1}^{12} [B_{i,t}(S_t(1 - \rho) + (I_t - S_t)) + B_{o,t}(1 - I_t)] \quad (2)$$

where S_t represents the proportion of people who reported to have retired to bed for sleeping. Personal protective efficacy of 98.3% ($\rho = 0.983$) was assumed for LLINs (DuraNet®) based on findings from experimental hut trials conducted elsewhere (Mahande et al., 2018).

The proportion of human exposure to mosquito bites which occur indoors (π_i) for unprotected individuals was calculated from the mean indoor (B_i) and outdoor (B_o) hourly biting rates as follows (Killeen et al., 2006; Seyoum et al., 2012).

$$\pi_i = \sum_{t=1}^{12} [B_{i,t}I_t] / \sum_{t=1}^{12} [B_{i,t} + B_{o,t}(1 - I_t)] \quad (3)$$

The proportion of human exposure to mosquito bites which occurs during sleeping hours (π_s) for unprotected individuals was determined in a similar way to equation 3, with a numerator calculated as the sum of the products of the mean hourly indoor biting rate ($B_{i,t}$) and the proportion of humans reported to have retired to bed (S) for each hour of the night (t):

$$\pi_s = \sum_{t=1}^{12} [B_{i,t}S_t] / \sum_{t=1}^{12} [B_{i,t}I_t + B_{o,t}(1 - I_t)] \quad (4)$$

The parameter π_i is an indicator of the maximum possible personal protection provided by any indoor interventions, whereas π_s is an indicator of maximum personal protection an intervention such as LLIN could provide during sleeping hours. The proportion of mosquito bites directly prevented using LLIN (P^*) was calculated as the product of π_s and the protective efficacy of LLINs (Killeen et al., 2006; Moiroux et al., 2014; Monroe et al., 2019).

The proportion of residual human exposure to mosquito bites which occur indoors ($\pi_{i,p}$) for LLIN users was calculated by adjusting π_i taking into account the personal protection (ρ) provided by LLIN:

$$\pi_{i,p} = \sum_{t=1}^{12} [B_{i,t}(S_t(1 - \rho) + (I_t - S_t))] / \sum_{t=1}^{12} [B_{i,t}(S_t(1 - \rho) + (I_t - S_t)) + B_{o,t}(1 - I_t)] \quad (5)$$

Human blood index (HBI) was calculated as the proportion of *Anopheles* mosquitoes that fed on human over the total *Anopheles* tested for blood meal origin (Garrett-Jones, 1964). Bloodmeal indices of other non-human vertebrate hosts were also calculated in a similar way. Host abundance was determined from questionnaire survey data as the number of a particular host divided by the total number of all potential hosts (human, cattle, goat, dog and chicken) multiplied by 100. The forage ratio (FR), a measure of host preference by mosquitoes, was determined as the proportion of engorged *Anopheles* mosquitoes which fed on a given host divided by the abundance (proportion) of that particular host in the study area (Hess et al., 1968; Manly et al., 2007). A host was considered to have been preferred if the lower 95% confidence limit for the FR estimate was greater than one and inferred to have been avoided if the upper 95% confidence limit of the FR estimate was less than one. A host for which the 95% confidence interval for its FR included one was considered to have been fed by mosquitoes opportunistically.

The sporozoite rate was estimated as the proportion of mosquitoes positive for *P. falciparum* and/or *P. vivax* CSP over the total number tested. Annual entomological inoculation rate

(EIR) was determined separately for indoor and outdoor mosquito collections as aHBR x sporozoite infection rate x 365 (WHO, 2013). The overall annual EIR was obtained by summing the indoor and outdoor EIRs.

Data were analysed using SPSS version 20.0 (SPSS, Chicago, IL, USA) software package. $p < 0.05$ was considered statistically significant during the analysis.

3. Results

3.1. Mosquito species composition and abundance

A total of 2,038 female anopheline mosquitoes comprising *An. gambiae s.l.* (30.8%), *An. pharoensis* (40.5%), *An. coustani* (28.1%), *An. squamosus* (0.3%) and *An. funestus* group (0.2%) were collected by all methods during the study period (Table 1). The majority (73.1%) of the anopheline mosquitoes were collected outdoors. A total of 278 *An. gambiae s.l.* specimens were analysed for molecular identification of sibling species. Of these, 252 (90.6%) specimens were successfully amplified by PCR and all were *An. arabiensis*.

3.2. Indoor and outdoor *Anopheles* mosquito density

Table 2 shows the results of a negative binomial regression model for the comparison of host-seeking *Anopheles* mosquito density between indoor and outdoor location. Based on the gold standard surveillance method (HLC), *An. arabiensis* was 2.41 (95% CI: 1.46–3.98) times more likely to be captured outdoors than indoors, suggesting this species to display exophagic behavior in the study area. Similarly, the density of *An. arabiensis* was 3.74 (95% CI: 2.07–6.76) times higher outdoors than indoors based on the alternative methods (HDNT vs. CDC light trap). Likewise, the mean densities of *An. pharoensis* and *An. coustani* were significantly higher outdoors than indoors based on both the gold standard and alternative surveillance methods (Table 2).

3.3. Hourly biting activity of *Anopheles* mosquitoes

The crude biting rates of all *Anopheles* species were higher outdoors than indoors throughout the night (Figure 2). The mean indoor and outdoor biting rate of *An. arabiensis* was 2.2 and 5.0 bites/person/night (b/p/night), respectively. The indoor and outdoor biting rate of *An. pharoensis* was 3.5 and 7.6 b/p/night, respectively, whereas the indoor and outdoor biting rate of *An. coustani* was 1.9 and 7.5 b/p/night, respectively. The peak biting activity of *An. arabiensis* was recorded in the evening between 9:00 pm and 10:00 pm and then started to decline when people were indoors. The peak biting activities of *An. pharoensis* and *An. coustani* were observed in the early part of the evening between 7:00 pm and 8:00 pm.

3.4. Human exposure to mosquito bites

For unprotected individuals (LLIN non-users), an estimated 66% and 56% of human exposure to *An. arabiensis* bites occurred indoors and during sleeping hours, respectively (Figure 3). About 39% of exposure to *An. pharoensis* bites and 27% of exposure to *An. coustani* bites occurred indoors for LLIN non-users. Use of LLIN was estimated to prevent 55.2%, 27.8% and 16.8% of exposure from *An. arabiensis*, *An. pharoensis* and *An. coustani*

bites, respectively, which otherwise would occur. For LLIN-users, most (75%) of residual human exposure to *An. arabiensis* bites occurred outdoors while 23% occurred indoors before people retire to bed. Likewise, most (84%) of residual exposure to *An. pharoensis* bites occurred outdoors while 15% occurred indoors before bed time. Similarly, most of the residual exposure to *An. coustani* occurred outdoors (Figure 3).

Results of questionnaire survey showed that 88.5% of the households had at least one LLIN. Over 94% of the study participants reported to have stayed outdoors or between outdoors and indoors until 8:00 pm. About 83% of the respondents reported going to bed by 9:00 pm. The main activities that kept people outdoors include household chore, praying, keeping cattle and social gatherings.

3.5. Blood meal origins and feeding preferences

Table 3 shows the blood meal sources of *An. arabiensis* and other anopheline mosquito species. The HBI and bovine blood index (BBI) and goat blood index (GBI) of *An. arabiensis* were 19.2, 65.4 and 11.5%, respectively. *An. pharoensis* had HBI, BBI and GBI of 16.7%, 66.7% and 5.5%, respectively. Very few fed *An. coustani* were caught and all were positive for bovine. None of the tested anopheline specimens were positive for dog, whereas 1.9% of the tested *An. arabiensis* specimens were positive for chicken. Regardless of higher proportion of humans in the study area compared to other vertebrate hosts, *An. arabiensis* and *An. pharoensis* were 4.1 and 4.8 times more likely to feed on bovine than humans (Table 4).

3.6. Sporozoite rate and Entomological inoculation rate

A total of 2,036 anopheline mosquitoes were tested for *Plasmodium* CSP, of which 6 specimens (3 *An. arabiensis*, 2 *An. pharoensis* and 1 *An. coustani*) were positive (Table 5). The sporozoite rate of *An. arabiensis* from indoor and outdoor HLC was 0.9% and 0.4%, respectively, whereas the sporozoite rate of *An. pharoensis* from indoor and outdoor HLC was 0 and 0.3%, respectively. The sporozoite rates of *An. arabiensis* and *An. pharoensis* from HDNT were 0.6% and 0.4%, respectively. None of the *An. arabiensis* and *An. pharoensis* tested from CDC light trap and PSC were positive. No *Plasmodium* CSP was detected in *An. squamosus* and *An. funestus* group. Based on the HLC, an estimated indoor and outdoor EIR of *An. arabiensis* was 6.2 and 1.4 infective bites/person/year (ib/p/year), respectively, while *An. pharoensis* had an estimated outdoor EIR of 3.0 ib/p/year. HDNT-based EIRs of *An. arabiensis* and *An. pharoensis* were 2.0 and 4.5 ib/p/year, respectively (Table 5).

4. Discussion

This study indicated that *An. pharoensis* was the most abundant anopheline species in the study area followed by *An. arabiensis* and *An. coustani*. Previous studies reported that *An. arabiensis* was the predominant species in different malaria endemic settings of southwestern Ethiopia (Degefa et al., 2015; Taye et al., 2016). The higher abundance of *An. pharoensis* over *An. arabiensis* in this study could be attributed to difference in mosquito breeding habitats. The present study area is located in the Omo-Gibe River Basin

with abundant aquatic vegetations that might have favoured *An. pharoensis*. *Anopheles pharoensis* prefers to breed in vegetated swamps unlike *An. arabiensis* which typically breeds in small, sunlit temporary water pools (Kenea et al., 2011).

Anopheles arabiensis exhibited exophagic behavior, seeking hosts mostly outdoors rather than indoors. Similar findings were also reported from different parts of Ethiopia (Getachew et al., 2019; Kenea et al., 2016; Kibret and Wilson, 2016; Taye et al., 2016). *Anopheles arabiensis* was shown to be preponderantly exophagic even before the scaleup of indoor-based vector control interventions in Ethiopia (Tirados et al., 2006; White, 1974), suggesting that the exophagic behavior of this species might be genetically determined (White, 1974). Moreover, the long-term use of the current vector control interventions (LLINs and IRS) might have further enhanced the proportion of outdoor biting fraction of *An. arabiensis* as observed elsewhere in Africa. For instance in western Kenya, *An. arabiensis* was more likely to bite outdoors (Bayoh et al., 2014; Degefa et al., 2017) when compared with data collected before the scale-up of LLINs (Githeko et al., 1996, 1994). Likewise, *An. pharoensis* showed exophagic behavior in the study area. Similar findings were also reported for this species from different parts of Ethiopia (Kenea et al., 2016; Kibret et al., 2014; Taye et al., 2006, 2016).

In the absence of personal protection by LLINs, human exposure to *An. arabiensis* bites occurred mostly indoors ($\pi_i = 66\%$) despite the outdoor host-seeking preference of this species. This is due to coincidence of humans and the peak biting activities of *An. arabiensis* since most people spend their time indoors when this species is mostly active (Figure 2). A similar phenomenon was documented for other malaria vector species in Africa (Sherrard-Smith et al., 2019). For instance, *An. funestus* and *An. quadriannulatus* did not show preference to bite indoors in Zambia, yet a substantial proportion of human contact with both species was shown to occur indoors in the absence of LLIN use in the country (Seyoum et al., 2012). This highlights the need to consider human behavior to determine the actual magnitude of human exposure to mosquito bites which may occur indoors and/or outdoors.

For LLIN non-users, 56% of human exposure to *An. arabiensis* bites occurred at times when using LLINs is feasible, indicating that the maximum possible personal protection that could be provided by LLIN is 56%. This implies that with only the current indoor-based vector intervention (LLINs), malaria elimination may not be achieved since the remaining exposure to *An. arabiensis* bites could still occur outdoors and/or indoors before people retire to bed. A study conducted in Tanzania also showed that less than half (46%) of all human exposure to *An. arabiensis* bites occurred at times when using insecticide-treated nets (ITNs) was feasible (Govella et al., 2010). Only 28% of human exposure to *An. pharoensis* bites occurred at times when LLINs would be in use if they were available, indicating that the majority of exposure to *An. pharoensis* also occurs outdoors and before sleeping hours.

For LLIN users, most (75%) of residual human exposure to *An. arabiensis* bites occurred outdoors while 23% occurred indoors before people retire to bed. Similarly, most (84%) of the residual exposure to *An. pharoensis* bites occurred outdoors, while 15% occurred indoors before sleeping time. The findings suggest that additional control measures which

can protect against outdoor exposure or which target immature stages of vectors are required to complement the current indoor-based vector control interventions (LLINs and IRS) to interrupt transmission due to exposure to vector bites occurring outdoors and in the early evening hours.

Anopheles arabiensis showed a preference to feed on bovine to humans. The findings of previous studies conducted in different parts Ethiopia showed that the feeding behavior of *An. arabiensis* varied across different geographical locations. The species exhibited zoophagic behavior in some settings (Hadis et al., 1997; Massebo et al., 2015), anthropophilic in other places (Kibret et al., 2017; Tirados et al., 2006; Yohannes et al., 2005) and anthrozoophilic (opportunistic) tendency in some areas (Getachew et al., 2019; Habtewold et al., 2001). Such interpopulation variations in feeding behavior might be due to difference in host availability between different settings (Habtewold et al., 2001; Killeen et al., 2001). Interpopulation genetic variation in *An. arabiensis* might have also contributed to the variation in its feeding behavior between different localities. Subpopulation of *An. arabiensis* with preference to feed on cattle have been shown to correlate with arrangement of 3Ra chromosomal inversion (Lulu et al., 1998; Main et al., 2016). Such phenomenon could increase the proportion of zoophagic fraction of *An. arabiensis* in settings where the 3Ra inversion is documented (Lulu et al., 1998, 1991). Similarly, *An. pharoensis* showed zoophagic behavior, preferring to feed on bovine to other potential hosts available in the study area.

The zoophagic behavior of *An. arabiensis* and *An. pharoensis* can be considered as an opportunity to introduce complementary vector control intervention such as zooprophyllaxis to divert host-seeking mosquitoes from humans (Habtewold et al., 2001; Iwashita et al., 2014). Anthropophilic and endophilic malaria vectors can be controlled by LLINs and IRS, whereas those species predominantly feeding on cattle outdoors could sustain residual malaria transmission despite high coverage of indoor-based vector control interventions. Hence, targeting zoophagic vectors is crucial to achieve malaria elimination. Zooprophyllaxis can reduce malaria transmission by pulling mosquitoes toward dead-end hosts and by reducing vector density if cattle are treated with insecticides (Bulterys et al., 2009; Chaccour et al., 2018).

The estimated indoor and outdoor EIRs for *An. arabiensis* were 6.2 and 1.4 ib/p/year, respectively, indicating the contribution of *An. arabiensis* to both indoor and outdoor malaria transmission. The occurrence of indoor malaria transmission despite high LLIN coverage in the study area might be attributed to the exposure of people to vector bites in the evening before sleeping hours. Resistance of malaria vectors to insecticides (Messenger et al., 2017; Yewhalaw et al., 2011) might have also contributed to the indoor EIR. In addition, *An. pharoensis* had an estimated outdoor EIR of 3.0 ib/p/year, indicating the contribution of this species to outdoor transmission. Although *An. pharoensis* has been considered as a secondary vector in Ethiopia, a recent study revealed similar trends of susceptibility of this species to *Plasmodium* parasite infection as *An. arabiensis* (Abduselam et al., 2016), indicating that *An. pharoensis* could also play a major role in outdoor malaria transmission. Other recent studies have also documented an increasing role of *An. pharoensis* in malaria transmission in the country (Abraham et al., 2017; Kibret et al., 2014).

On the other hand, the EIRs of *An. arabiensis* reported in this study are lower compared to the EIRs of *An. arabiensis* previously reported from different parts of Ethiopia (Abraham et al., 2017; Animut et al., 2013; Degefa et al., 2015; Kibret et al., 2017; Massebo et al., 2013) and elsewhere in Africa (Degefa et al., 2017; Himeidan et al., 2011). This could be attributed to a relatively higher coverage of LLINs in the study area.

The strength of this study is that both vector and human behavior were considered in the calculation of human biting rates and EIRs to better understand where and when exposure to mosquito bites and residual malaria transmission occur. Moreover, this study employed both gold standard method i.e. HLC and alternative methods (CDC light traps and HDNT) for vector surveillance to determine vector density, human biting rates and sporozoite rates. The findings of this study suggest that CDC light trap can be paired with HDNT for routine indoor and outdoor malaria vector surveillance as an alternative tool to HLC. The limitation of the study was that the sporozoite infection rates reported in this study were based on ELISA and the positive specimens were not confirmed by PCR. The proportions of human exposure to mosquito bites were estimated assuming no seasonal changes in sleeping habits of people in the study area, hence night to night differences in sleeping time were not tracked in this study.

5. Conclusion

Populations of *An. arabiensis* and *An. pharoensis* showed exophagic and zoophagic behavior. The majority of human exposure to *An. arabiensis* bites occurred indoors for LLIN non-users, while most of the residual exposure to both *An. arabiensis* and *An. pharoensis* bites occurred outdoors for LLIN users. Malaria transmission by *An. arabiensis* occurred both indoors and outdoors, while *An. pharoensis* contributed exclusively to outdoor transmission. Additional control tools targeting outdoor and early evening biting vectors are required to complement the current control interventions to control residual transmission and ultimately achieve malaria elimination. Further studies are required to comprehend the role of *An. coustani* in malaria transmission in Ethiopia.

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List of abbreviations

aHBR	behavior-adjusted human biting rate
BBI	bovine blood index
CDC	Centers for Disease Control and Prevention

CI	confidence interval
CSP	circumsporozoite protein
EIR	entomological inoculation rate
ELISA	enzyme-linked immunosorbent assay
FR	forage ratio
GBI	goat blood index
HBI	human blood index
HDNT	human-baited double net trap
HLC	human landing catch
ITN	insecticide treated net
LLIN	long-lasting insecticidal net
OR	odds ratio
PCR	polymerase chain reaction
PSC	pyrethrum spray catch

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Highlights

- *Anopheles arabiensis* and *An. pharoensis* showed exophagic and zoophagic behavior
- Most (66%) of human exposure to *An. arabiensis* bites occurred indoors for unprotected individuals (LLIN non-users)
- For LLIN users, most (75%) of the exposure to *An. arabiensis* bites occurred outdoors
- Human exposure to *An. pharoensis* bites occurred mainly outdoors for both LLIN users and non-user
- *Anopheles arabiensis* contributed to both indoor and outdoor malaria transmission while *An. pharoensis* contributed exclusively to outdoor transmission

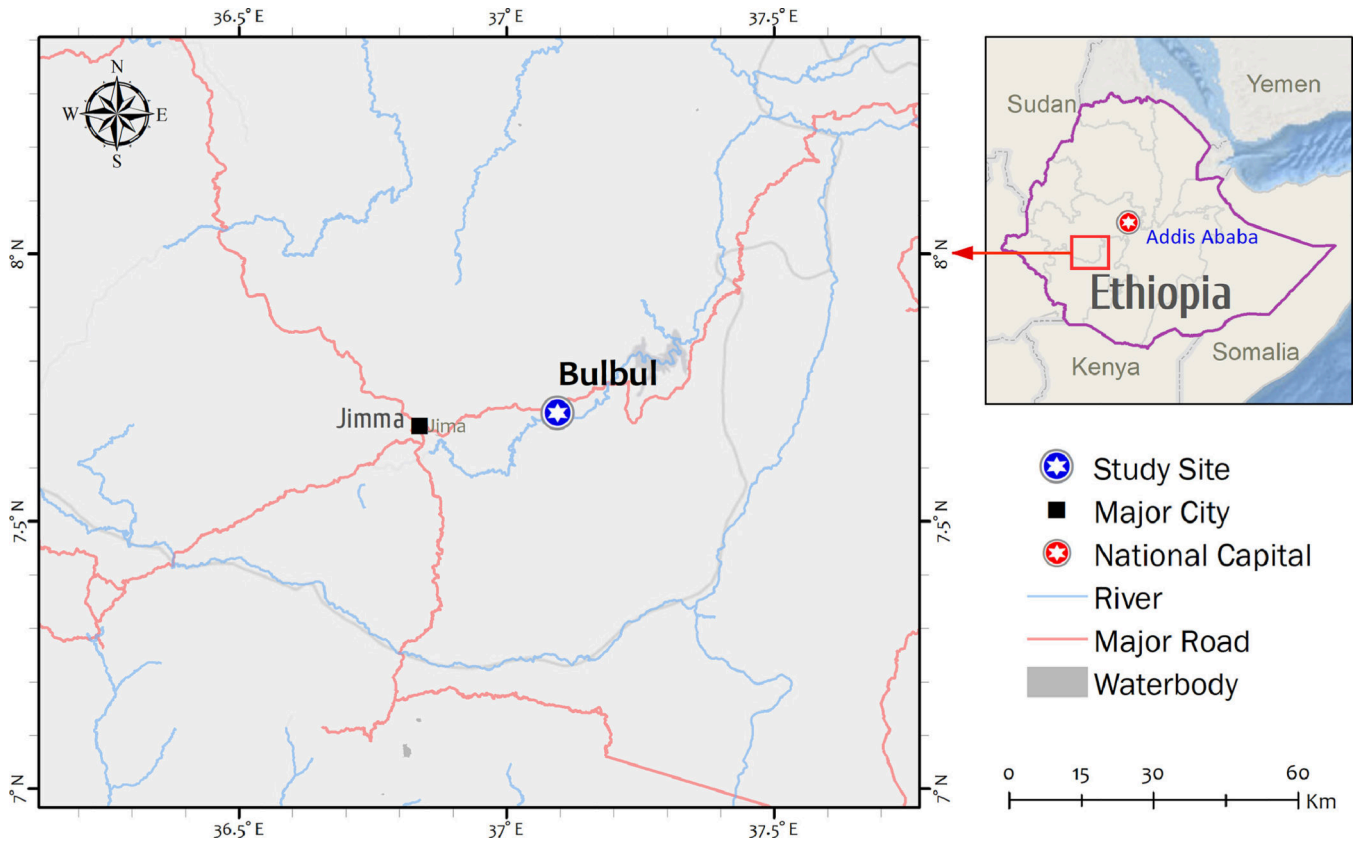


Figure 1:
Map of the study area

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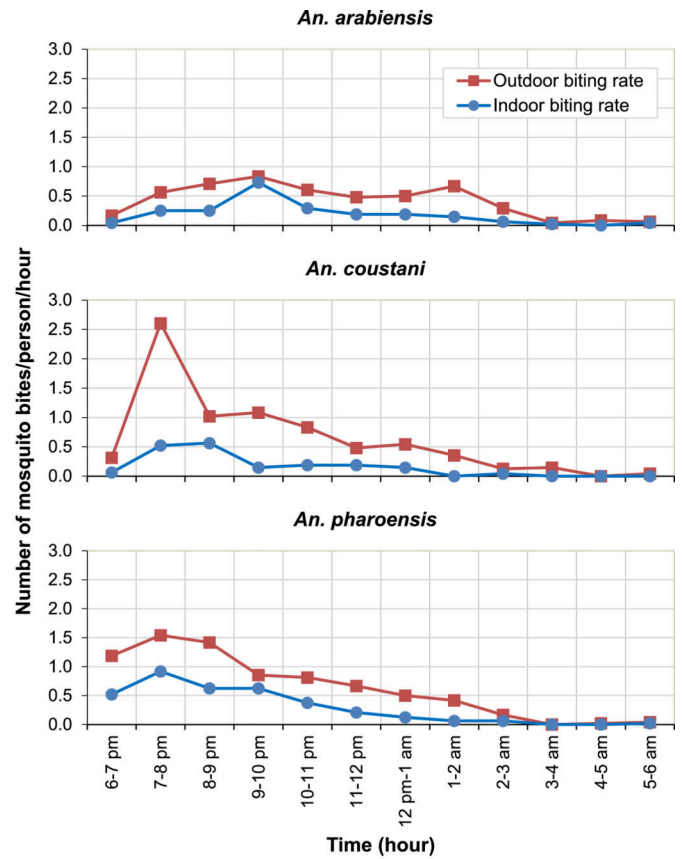
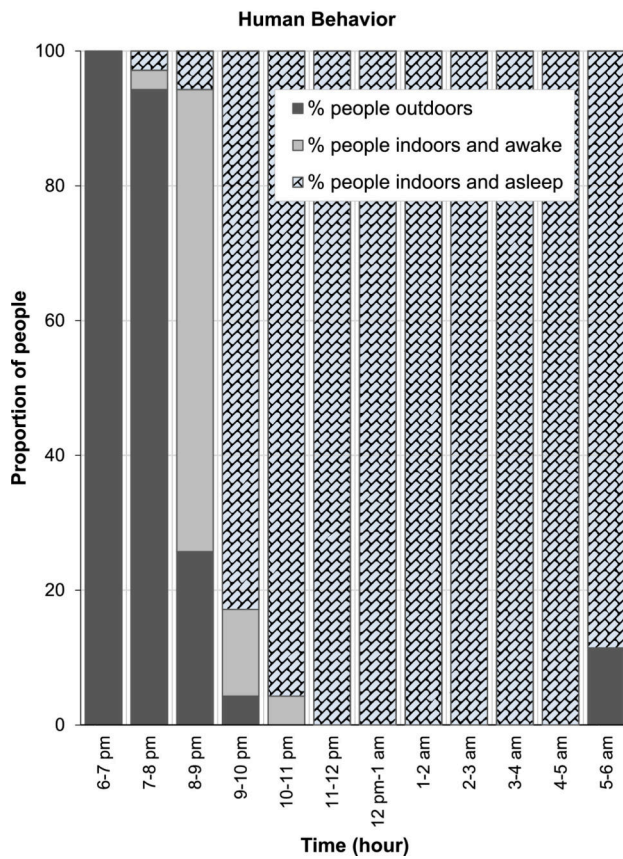


Figure 2: Indoor and outdoor crude biting rates of *Anopheles* mosquitoes with the proportion of people outdoors, indoors and awake, and indoors and asleep throughout the night in Bulbul, southwestern Ethiopia

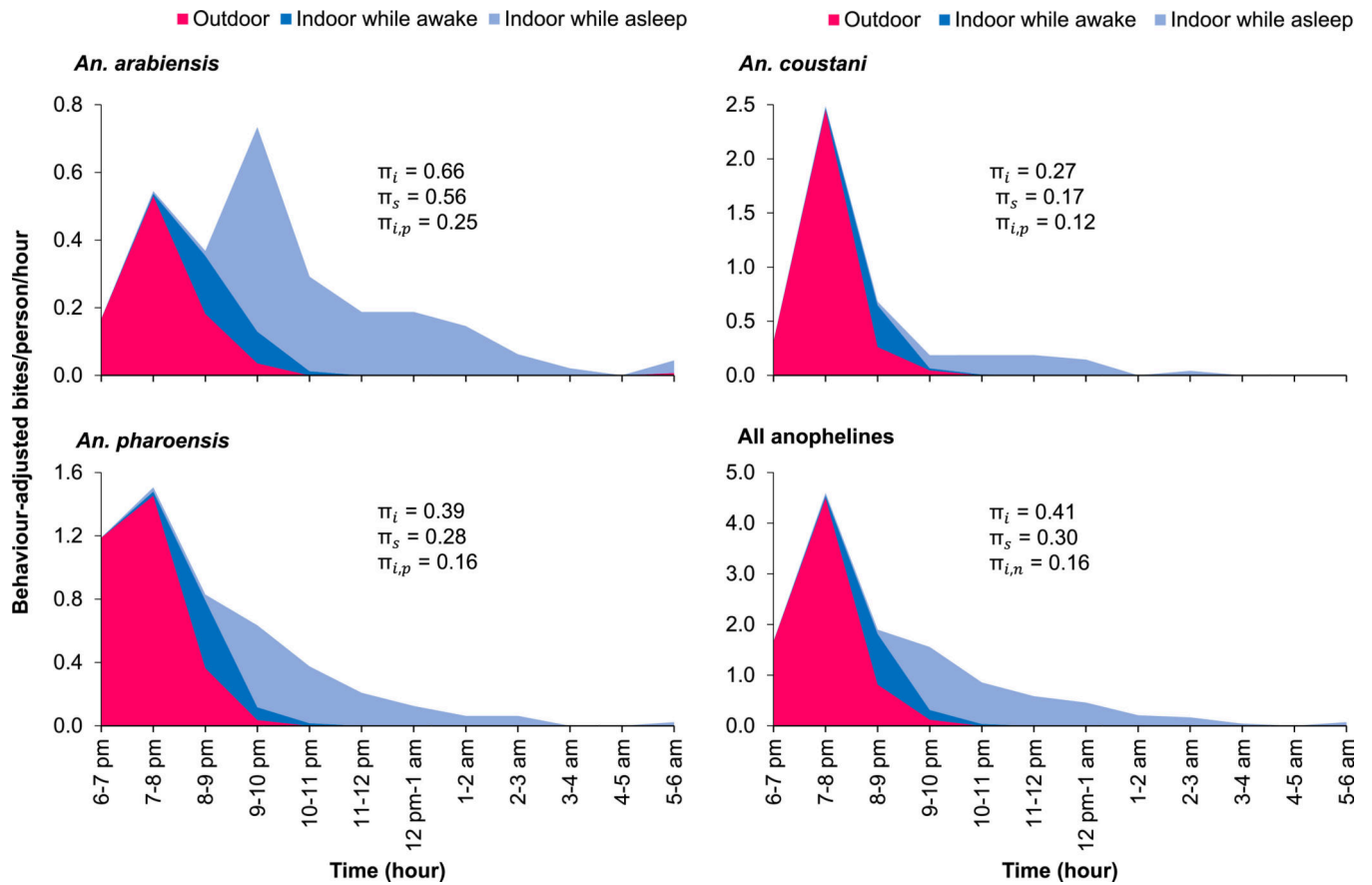


Figure 3: Behavior-adjusted estimates of human exposure to *Anopheles* mosquitoes occurring indoors and outdoors in Bulbul, southwestern Ethiopia

Table 1:

Summary of female *Anopheles* mosquitoes collected from indoor and outdoor in Bulbul, southwestern Ethiopia

Species	Indoor			Outdoor		Total
	HLC	Light trap	PSC	HLC	HDNT	
<i>An. arabiensis</i>	106	72	42	240	168	628
<i>An. pharoensis</i>	170	34	13	366	243	826
<i>An. coustani</i>	89	20	1	362	101	573
<i>An. squamosus</i>	1	0	0	4	2	7
<i>An. funestus</i> group	1	0	0	2	1	4
Total	367	126	56	974	515	2,038

Note: PSC: pyrethrum spray catch, HLC: human landing catch, HDNT: human-baited double net trap

Table 2:

Estimates of a negative binomial regression for the comparison of host-seeking anopheline density between indoor and outdoor location in Bulbul, southwest Ethiopia

Species	Traps	Location	EMM (95% CI)	OR (95% CI)	p value
Standard method					
<i>An. arabiensis</i>	HLC	Outdoor	3.47 (2.48–4.48)	2.41 (1.46–3.98)	0.001*
	HLC	Indoor	1.44 (0.98–2.12)	1.0 ^a	
<i>An. pharoensis</i>	HLC	Outdoor	5.05 (3.65–7.00)	2.48 (1.53–4.00)	<0.0001*
	HLC	Indoor	2.04 (1.42–2.95)	1.0 ^a	
<i>An. coustani</i>	HLC	Outdoor	2.0 (1.12–3.60)	3.71 (2.13–6.45)	<0.0001*
	HLC	Indoor	0.54 (0.28–1.04)	1.0 ^a	
Alternative methods					
<i>An. arabiensis</i>	HDNT	Outdoor	2.34 (1.61–3.40)	3.74 (2.07–6.76)	<0.0001*
	Light trap	Indoor	0.62 (0.39–1.01)	1.0 ^a	
<i>An. pharoensis</i>	HDNT	Outdoor	3.30 (2.32–4.67)	6.61 (3.71–11.77)	<0.0001*
	Light trap	Indoor	0.51 (0.31–0.84)	1.0 ^a	
<i>An. coustani</i>	HDNT	Outdoor	0.96 (0.53–1.74)	8.74 (3.97–19.21)	<0.0001*
	Light trap	Indoor	0.11 (0.47–0.26)	1.0 ^a	

Note: HLC: human landing catch, HDNT: human-baited double net trap, EMM: estimated marginal mean density, OR: odds ratio

^aReference value. EMM was determined using a negative binomial regression model by adjusting for season

Table 3.Blood meal sources of *Anopheles mosquitoes* collected from indoor in Bulbul, southwestern Ethiopia

<i>Blood meal indice</i>	<i>An. arabiensis</i>			<i>An. pharoensis</i>			<i>An. coustani</i>
	Light trap	PSC	Total	Light trap	PSC	Total	Light trap
No. tested	24	28	52	10	8	18	4
Human	4 (16.7)	4 (14.3)	8 (15.4)	1 (10.0)	1 (12.5)	2 (11.1)	0
Bovine	17 (70.8)	17 (60.7)	34 (65.4)	7 (70.0)	5 (62.5)	12 (66.7)	4 (100.0)
Goat	2 (8.3)	2 (7.1)	4 (7.7)	0	0	0	0
Chicken	0	1 (3.6)	1 (1.9)	0	0	0	0
Dog	0	0	0	0	0	0	0
Human+Goat	1 (4.2)	1 (3.6)	2 (3.8)	1 (10.0)	0	1 (5.5)	0
Unknown	0	3 (10.7)	3 (5.8)	1 (10.0)	2 (25.0)	3 (16.7)	0

Note: PSC: pyrethrum spray catch

Table 4:Host preference of *An. arabiensis* and *An. pharoensis* in Bulbul, southwestern Ethiopia

Species	Parameters	Human	Bovine	Goat	Chicken
	Host abundance in the area (%)	39.0	32.2	6.8	22.0
<i>An. arabiensis</i>	Blood index	19.2	65.4	11.5	1.9
	FR (95% CI)	0.49 (0.22–0.77)	2.03 (1.63–2.43) *	1.69 (0.42–3.0)	0.09 (0.0–0.26)
<i>An. pharoensis</i>	Blood index	16.7	66.7	5.5	0
	FR (95% CI)	0.43 (0.0–0.87)	2.07 (1.39–2.75) *	0.81 (–0.74–2.37)	0

Note: FR: forage ratio; CI: confidence interval

* indicates the preferred host

Table 5.

Indoor and outdoor human biting rates, sporozoite rates and annual entomological inoculation rates (EIRs) of *Anopheles* mosquitoes in Bulbul, southwestern Ethiopia

<i>Anopheles</i> species	Parameters	Indoor		Outdoor		Total	
		HLC	Light trap	HLC	HDNT	HLC	ALT
<i>An. arabiensis</i>	No tested	106	70	240	168	346	238
	aHBR	1.8	1.8	0.9	0.9	2.7	2.7
	Pf +ve (%)	1 (0.9)	0	1 (0.4)	1 (0.6)	2 (0.6)	1 (0.4)
	Pf EIR	6.2	0	1.4	2.0	7.6	2.0
	Pv +ve (%)	0	0	0	0	0	0
	Pv EIR	0	0	0	0	0	0
<i>An. pharoensis</i>	No tested	170	34	366	243	536	277
	aHBR	2.0	2.0	3.0	3.0	5.0	5.0
	Pf +ve (%)	0	0	0	0	0	0
	Pf EIR	0	0	0	0	0	0
	Pv +ve (%)	0	0	1 (0.3)	1 (0.4)	1 (0.2)	1 (0.4)
	Pv EIR	0	0	3.0	4.5	3.0	4.5
<i>An. coustani</i>	No tested	89	20	362	101	451	121
	aHBR	1.2	1.2	3.0	3.0	4.2	4.2
	Pf +ve (%)	0	0	0	0	0	0
	Pf EIR	0	0	0	0	0	0
	Pv +ve (%)	0	0	1 (0.3)	0	1 (0.2)	0
	Pv EIR	0	0	3.0	0	3.0	0

Note: HLC: human landing catch, HDNT: human-baited double net trap, ALT: alternative methods, aHBR-behavior-adjusted human biting rate; *Pf*: *P. falciparum*, *Pv*: *P. vivax*; *EIR*: annual entomological inoculation rates