

Malaria in Migrant Agricultural Workers in Dangur District, Benishangul-gumuz, Ethiopia. Entomological Assessment of Malaria Transmission Risk

Sisay Dugassa Lemma (✉ sisaydlw2@gmail.com)

Addis Ababa University <https://orcid.org/0000-0002-6951-3299>

Mathew Murphy

Centers for Disease Control and Prevention Office of Infectious Diseases

Sheleme Chibsa

US Agency for International Development: USAID

Yehualashet Tadesse

The President's Malaria Initiative Private Health Sector Project Abt Associates Inc.

Gedeon Yohannes

ABT Associates Inc

Lena M Lorenz

London School of Hygiene & Tropical Medicine

Hiwot Solomon

Federal Ministry of Health

Delenasaw Yewhalaw

Tropical and Infectious Diseases Center, Jimma University

Seth R. Irish

Centers for Disease Control and Prevention

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Abstract

Background: Ethiopia has made great strides in malaria control. However, this progress has not been uniform and one concern has been reported high rates of malaria transmission in large agricultural development areas in western Ethiopia. Improved vector control is one way this transmission might be addressed, but little is known about malaria vectors in this part of the country.

Methods: To better understand the vector species involved in malaria transmission and their behavior, human landing collections were conducted in Dangur woreda, Benishangul-Gumuz, between July and December 2017. Mosquitoes were identified to species and tested for presence of *Plasmodium* sporozoites.

Results: The predominant species collected was *Anopheles arabiensis* (61.3% of all *Anopheles*), which was also the only species identified with sporozoites (*P. falciparum* and *P. vivax*). *Anopheles arabiensis* was collected as early in the evening as 18:00h-19:00h, and host-seeking continued until 5:00h-6:00h. Nearly equal numbers were collected indoors and outdoors. The calculated entomological inoculation rate for *An. arabiensis* for the study period was 1.41 infectious bites per month. More *An. arabiensis* were collected inside and outside worker's shelters than in fields where workers were working at night.

Conclusions: *An. arabiensis* is likely to be the primary vector of malaria in the agricultural development areas studied. High rates of human biting took place inside and outdoor near workers' residential housing. Improved and targeted vector control in this area might considerably reduce malaria transmission.

Background

The commitment to eradicate malaria from the globe through increased malaria control and treatment has resulted in a remarkable decrease in the number of cases and mortality associated with this disease [1–3]. It is estimated that the scale-up of the major interventions, long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS) and treatment with artemisinin combination therapy reduced malaria cases and mortality by 37% and 60% respectively between 2000 and 2015 [4]. However, malaria remains a serious disease affecting the well-being of people living in the tropical and subtropical countries of the world [5] and progress against malaria has slowed down considerably in the past few years [5].

In Ethiopia, malaria is still an important cause of morbidity and mortality as in other countries in tropical Africa. Despite a prevalence of less than 1% in the country, malaria is important in certain foci that pose a risk for epidemics. The National Malaria Control Program of the Federal Ministry of Health of Ethiopia has recently set an ambitious goal of eliminating malaria from all 565 malarious districts by the year 2030 [6]. In order to achieve this goal, it is important to understand when and where malaria is being transmitted, both at large and small-scale levels.

One of the areas where malaria transmission is of concern is in lowland agricultural development areas. Ethiopia has been practicing an Agricultural Development Led Industrialization (ADLI) since 1991 [7]. As a result, various agricultural development areas have been created in the country. Agricultural development in such areas has resulted in the migration of hundreds of thousands of seasonal workers into areas where vector-borne diseases such as malaria and visceral leishmaniasis are endemic [8].

As these areas offer opportunities for employment, mobile and migrant populations travel to these sites, often during the rainy season. These areas are productive for crops, but also for mosquitoes. Migrant workers may leave mosquito nets in their permanent homes, and stay in temporary, substandard shelters, thus increasing their risk of contracting malaria. Additionally, they may work or remain exposed to mosquitoes in ways and at times that are different from when they are in their hometowns. They may also carry malaria parasites back to their home areas of relatively low malaria risk, complicating the efforts towards malaria elimination in these districts [9].

The biting behavior of mosquitoes is an important risk factor for infection with malaria [10]. Hence, prevention and control measures for the disease should take the site and time of people's exposure to mosquito bites into account. There is very little previous research on malaria vectors in Benishangul Gumuz, western Ethiopia, which is home to a large number of agricultural development areas. In order to establish an effective malaria control program through targeted malaria prevention messages and control interventions, it is essential to understand the mosquito species present in the study area, the venue and times that mosquitoes bite humans and the risk of humans becoming infected with malaria. Our study aimed to provide the entomological context of malaria transmission in agricultural settings in this area in tandem with a second study investigating the human behavior in agricultural development areas (Tadesse *et al.* associated manuscript). Accordingly, our work aimed to determine the host-seeking behavior of *Anopheles* mosquitoes in this area that is one of the agricultural development areas in Ethiopia with substantial migrant human populations from highland areas.

Materials And Methods

Study site

The study was conducted on eight farms in Dangur district (woreda), in Metekel zone, Benishangul-Gumuz region, Ethiopia (Fig. 1). Four of these farms were large-scale farms (larger than 100 hectares [ha]) and four were small scale farms (less than 100 ha). The farms cultivated a range of crops, including sesame, green grams, cow peas, and sorghum. The area has a single rainy season beginning in May that continues until October. The altitudes of the farms range between 751 to 1155 m above sea level. The worker housing on these farms was generally of poor quality, consisting of wooden framed houses with grass or iron sheeting walls.

All data were collected between July and December 2017. The managers of two farms (one small, one large) where mosquito collections were made in July did not wish to continue in the following months, so

these farms were replaced with two other farms.

Mosquito collection

Mosquitoes were collected through human landing collection (HLC), the current gold standard for measurement of human biting activities of mosquitoes and entomological inoculation rates [11–13]. HLCs were chosen to have a collection method that reliably estimated human-vector contact as well as the necessity of a single method that could be used indoors, outdoors, and in night-time agricultural field work sites. HLCs involved the collection of mosquitoes on humans sitting with legs exposed during the collection hours. Each collector was provided with a flashlight, an aspirator to catch biting mosquitoes and one netting-topped polystyrene cup for each 1-hour catch-session. The mosquito holding cups were labeled with the name of the farm, shelter number, time-session and site of collection. These human collectors caught mosquitoes attempting to bite their exposed legs, and kept the mosquitoes sorted by hour of collection so that biting times could be determined. One collector sat indoors and the other one sat outdoors of the same shelter collecting the mosquitoes. The indoor and outdoor collectors exchanged site every hour to reduce biases due to differential attractiveness of the collectors to the mosquitoes. The collectors who conducted human landing collections were locally hired and trained. No personal information was collected about the collectors. The collectors were provided with prophylaxis (mefloquine [Lariam®]) to protect them from getting malaria or treated if they were diagnosed with malaria[14]. The collections were conducted over the course of 12 hours (18.00 h – 06.00 h).

Collections were made at the worker shelter camps in each farm. At each farm, two shelters were chosen, and collections were made inside and outside these shelters during each collection. One collection was made in each site each month, resulting in 16 indoor and 16 outdoor collections made each month. The collections were made between July and December (6 months), resulting in a total of 96 indoor and 96 outdoor collections.

Additionally, outdoor human landing collections were conducted in sites next to workers involved in nighttime work activities in the fields, such as the harvesting of sesame pods. One human landing collection was made in each farm between the months of September and December 2017, resulting in a total of 32 outdoor collections in the fields.

Mosquitoes were killed and identified morphologically using appropriate identification keys [14]. They were then stored in 1.5 ml Eppendorf tubes with silica gel before laboratory analysis.

Laboratory analysis of mosquitoes

Almost all of the mosquitoes morphologically identified as belonging to the *Anopheles gambiae* complex were identified using standard polymerase chain reaction (PCR) to determine the species [15]. In brief, genomic DNA was mixed with the following primers in a 25 µL reaction: AR (5'-AAGTGCCTTCTCCATCCRA-3'; specific for *An. arabiensis*), AG (5' CTGGTTTGGTCGGCACGTTT-3; specific for *An. gambiae* s.s.), QD-b (5'-AGTGTCCAATGTCTGTGAAG-3'; specific for *An. quadriannulatus* species B or *An.amharicus*) and UN (5' GTGTGCCCTTCCTCGATGT-3'; common for all species).

Amplification reactions contained 1 μ L of DNA, 1.5 mM MgCl₂, 10 mM Tris–HCl (pH 8.4), 50 mM KCl, 0.1% Triton X-100, 200 μ M of dNTPs (Amersham, Buckinghamshire, United Kingdom), 25 pmol of primers AR, AG, QD-b and UN and 0.25 U of SilverStar DNA polymerase (Eurogentec, Seraing, Belgium). Amplified PCR products were visualized on 2% agarose gels, stained with ethidium bromide. An *An. arabiensis* strain from the Sekoru colony, maintained at the Vector Biology and Control Research Unit, Tropical and Infectious Diseases Research Center of Jimma University (Jimma, Ethiopia), was used as a positive control.

A subsample of mosquitoes was also analyzed to determine whether sporozoites were present, using established methods [16]. Briefly, heads and thoraces of mosquitoes were separated from the abdomen and were homogenized. ELISA plates were coated with a capture monoclonal antibody. Following aspiration to remove un-adsorbed capture antibody, plates were incubated with blocking buffer to prevent non-specific binding in subsequent steps. The blocking buffer was removed by aspiration and the mosquito homogenate was added to the plates. After 2 hours, samples were aspirated and horseradish peroxidase-linked monoclonal antibody was added. This was then aspirated before the peroxidase substrate solution, ABTS, was added. Absorbance values at 405 nm were obtained 30–60 minutes later using an ELISA plate reader. Positive reactions were those with an absorbance value of greater than two times the average absorbance values of negative control samples.

Behavior-adjusted patterns of human exposure

To understand the risk to humans for infective mosquito bites indoors and outdoors, the biting times of *An. arabiensis* were compared with the outdoor and indoor times of humans as collected by Tadesse *et al.* (associated manuscript). The proportion of the population indoors at each hour was multiplied by the number of mosquitoes collected indoors to estimate the numbers of bites that would have occurred in the absence of any personal protection measure such as insecticide-treated nets. The same procedure was repeated for mosquitoes biting outdoors. The sum of all hours represented the number of *An. arabiensis* bites one person might expect to receive. To estimate the exposure that one person using a LLINs between the hours of 21:00 and 6:00 would receive, the number of mosquito bites expected each hour was multiplied by 0.063, a figure used by Seyoum *et al.* [17] (derived from two studies in Tanzania) to estimate the number of bites that would be received, even when using a net.

Data analysis

Data were entered into Excel (Microsoft office 2007) for cleaning and data summary. Count data were analyzed using generalized linear mixed effects models (glmer – function) with R statistical software version 2.14.2 including the contributing packages MASS, lme4, glht, multcomp (alpha = 0.05) [19]. The biting time was included in the model as a fixed factor and collection round and site were included as random factors in the biting time analysis with Poisson distribution. The over-dispersion between data points that remained after adjustment for all other factors was adjusted by creating a random factor with a different level for each row of the data set.

The parameter estimates of the models were used to predict the mean counts or mean proportions and 95% confidence intervals for the different size farms (CI) by removing the intercept from the models [19]. Multiple comparisons of treatments were also calculated based on the model parameter estimates.

The entomological inoculation rate was calculated as the product of the sporozoite rate and the mean number of *Anopheles arabiensis* collected per person per night.

Results

Mosquito identification and analysis

Over the course of the 6 months of collection, 2,829 mosquitoes were collected. Of these 1,970 (69.6%) were *Anopheles* mosquitoes. Of the *Anopheles* mosquitoes collected, 1,733 (88.0%) were *Anopheles gambiaes.l.* Other *Anopheles* species collected were *Anopheles pharoensis*, *An. coustani*, *An. demeilloni*, *An. squamosus*, *An. pretoriensis*, *An. natalensis* and *An. christyi* (See Table 1).

Table 1

Sporozoite ELISA results for *Anopheles* mosquito species collected from July to December 2017 using HLC in Dangur, Ethiopia

Species	Number tested	Number <i>Plasmodium falciparum</i> positive	Number <i>Plasmodium vivax</i> (Pv210) positive
<i>An. gambiae</i> s.l.	1702	8 (0.47%)	1 (0.06%)
<i>An. coustani</i>	80	0	0
<i>An. pharoensis</i>	42	0	0
<i>An. demeilloni</i>	87	0	0
<i>An. pretoriensis</i>	16	0	0
<i>An. natalensis</i>	2	0	0
Total	1929	8 (0.41%)	1 (0.05%)

Of the mosquitoes morphologically identified as *An. gambiaes.l.*, 120 specimens were randomly selected and tested for species identification. Of the 120 tested, the DNAs of 117 were successfully amplified, and all of these were *An. arabiensis*. Hereafter, *An. gambiaes.l.* mosquitoes are referred to as *An. arabiensis*.

The numbers of *An. arabiensis* collected increased from July, peaking in September, before decreasing to low levels in December. Significantly more *An. arabiensis* were collected in large farms than in small farms (23.77 (95%CI:17.07–60.54) and 2.23 (95%CI:1.63-6.00) respectively; $p < 0.001$) (Figs. 2–3). Significantly fewer *An. arabiensis* were collected in HLCs in fields (mean 2.03 per night, 95%CI: 0-3.06) where night work was taking place than in the outdoor collections made in the same months near workers' shelters (10.09 (95%CI: 4.45–15.74; $p < 0.001$)).

Biting times and locations

An. arabiensis were collected throughout the night in the study area near workers' shelters with the highest numbers collected at 21:00–22:00 and 0:00–2:00. (Fig. 4). The biting times in the fields followed a similar pattern.

The mean numbers of *An. arabiensis* collected indoors (8.72 (95% CI: 4.56–12.88) per person per night) were not significantly different from the number collected outdoors (8.65 (95% CI: 4.70-12.61; $p = 0.61$) over the six months of collection.

Sporozoite rates

In total, 1,929 female mosquitoes were tested for the *Plasmodium* circumsporozoite protein. The only species that tested positive was *An. arabiensis* (1,702 tested). Of these, 8 (0.47%) tested positive for *Plasmodium falciparum* while only 1 (0.06%) was positive for *P. vivax* 210 (Table 1). No mosquitoes tested positive for *P. vivax* 247. The overall *Plasmodium* (any species) sporozoite rate was 0.53%.

The entomological inoculation rate is based on the number of *Anopheles arabiensis* bites one person would receive in one night. In this case, due to the similarity of indoor and outdoor biting rates (see above), the mean of the two rates (not including collections from the fields) was used (8.69). The overall *Plasmodium* sporozoite rate (*P. falciparum* and *P. vivax*) was 0.53%. Therefore, the monthly entomological inoculation rate (EIR) of *An. arabiensis* in the living areas on the farms during the study period was 1.41 infectious bites per person per month (0.0053 sporozoite rate x 8.69 bites per night x 184 days ÷ 6 months).

Behavior-adjusted patterns of human exposure

As reported in the associated paper (Tadesse et al., associated manuscript), the majority of people were outdoors in the early evening. The proportion of people outdoors decreased from 18:00 h to 22:00 h, after which only a small proportion of the population remained outdoors. As a result, despite the near equality of the numbers of *An. arabiensis* that were collected indoors and outdoors, the risk for actual bites from *An. arabiensis* was greatest indoors at night, between 20:00 h and 5:00 h. The risk outdoors was considerably lower but was greatest from 19:00 h-20:00 h (Fig. 4).

When the indoor and outdoor behavior of humans and mosquitoes were combined, the estimated total number of bites one might be expected to receive was 8.81 bites per person per night. When the actual behavior of people working in the farms was calculated, including bednet use by a minority of workers,

the estimated number of bites per person per night was calculated as 7.41. If one used a bednet between the hours of 21:00 h and 6:00 h (the hours during which most people who had bednets used bednets), this number would decrease to 2.49, over three and a half times less than an unprotected individual (Fig. 5).

Discussion

The mosquito literature from Benishangul-Gumuz is extremely limited and we did not find any literature related to the malaria vectors present in Dangur woreda. In this study we identified eight species of *Anopheles*, with *An. arabiensis*, the major malaria vector in Ethiopia, as the most prevalent. Additionally, *An. arabiensis* was the only one found to have *Plasmodium* circumsporozoite protein in the head and thorax, indicating that this is likely to be the most important vector in agricultural areas in Dangur woreda. However, *An. coustani* and *An. pharoensis* were also collected in small numbers and these species have been found to be capable of transmitting *Plasmodium* parasites in laboratory and field studies [20–21].

The biting times of *An. arabiensis* present a challenge for the protection of migrant workers from infectious bites. *An. arabiensis* were found biting indoors and outdoors at nearly all time points, indicating an equal risk for workers staying indoors and outdoors, similar to elsewhere in Ethiopia [22]. Furthermore, biting started as early as the 18:00 h-19:00 h time period and reached a first peak between 19:00 and 22:00. Tadesse *et al.* (associated manuscript) found that at least half of workers went to bed by 21:30, but this would leave them exposed to the first peak for 2.5 hours before sleep. Additionally, LLIN use by migrant workers was found to be quite low, as they often left their nets with their families when they came to the farms for work. One limitation of this study is that the HLCs were completed by 6:00 h, whereas mosquito host-seeking may have continued on beyond this time point, posing further risk for workers in the early morning. Higher mosquito densities in the large farms suggest that more farming activities and hence higher workers' population might enhance the population of the vectors in those farms. Janko *et al.* [23] reported a positive correlation of agriculture coverage and the density of biting *An. gambiae* s.l. For example, they indicated that a 15% increase in agricultural cover was associated with increased probabilities of *An. gambiae* s.l. mosquitoes biting indoors (11.3% (95% UI -15.3 to 25.6) – 19.7% (-12.1 to 35.9)). This range was obtained based on factors such as season (month of surveillance), temperature, and precipitation.

The lower mosquito vector density in the fields compared to outdoors near shelters might be due to higher attraction to places where the host population was higher.

We observed seasonal variations in the numbers of mosquitoes collected that were generally similar to those in other areas in Ethiopia, with an increase in mosquito populations during the main rainy season, and a decrease in population size as the rains end towards the end of the year [24–25]. It is important to notice that the peak of the agricultural activities and hence demand for workers occurs during the time of the year with the largest populations of *An. arabiensis*.

Finally, the detection of sporozoites in *An. arabiensis* allowed us to calculate their. This was found to be 1.41 infectious bites per month in the study area during the 6 months that monitoring was conducted. This figure is similar to EIR found in other parts of Ethiopia. Massebo *et al.* [26] found a yearly EIR of 17.1 in Chano, in the Southern Nations, Nationalities, and People's Region. In both locations annual EIR is well above 1, the threshold below which Beier *et al.* [27] estimated EIRs needed to drop below for substantial reductions of malaria prevalence. However, EIRs in highly malarious areas where vector control has had an impact can often reach much higher levels[28], indicating that improved vector control and treatment for malaria could have an important impact on transmission in these farms. Therefore, it might be very useful for the national malaria control strategy to focus on universal coverage of bednet distribution, including for the mobile and migrant populations and improvement of other vector control measures such as IRS and environmental management in such areas of the country.

Insecticide resistance was not assessed as a part of this study. Further work should be done to evaluate the insecticide susceptibility of *An. arabiensis* in this area. As IRS is not currently an option for protection of many of the workers due to the poor quality of structures or incomplete walls, the susceptibility testing should focus on the evaluation of pyrethroids, chlorfenapyr, and the synergism of pyrethroid susceptibility with piperonyl butoxide.

There were some limitations to our study. Collections were only made over a six-month period, and not over the whole year. While the six months chosen were the primary season for malaria transmission, there may be risks of malaria transmission outside these months. Also, collections in the fields were conducted from September until December, whereas collections in the worker's shelter areas were conducted from July to December. An additional limitation of this study was that the mosquito collections ended at 06:00, and there may be some mosquito biting after this time. The ELISA reactions were considered positive at twice the value of the mean optical density of negative controls and were not re-boiled when positive. Finally, the current recommendations for analysis of human exposure[29] do not take into account mosquito response to human behavior. Outdoor biting mosquitoes that do not find humans outdoor may move indoors to feed on humans, and thus we may have underestimated the risk, for both net users, and, to a greater extent, non-users.

In conclusion, we consider *An. arabiensis* to be the most likely primary vector of malaria in the agricultural development areas surveyed. While the EIRs do not indicate high rates of transmission, the low use of vector control interventions and lack of access for treatment result in a real risk of malaria for workers staying in these locations. Improved malaria prevention and treatment could have a valuable impact on worker health and productivity in these areas. However, it seems likely that the behavior of *An. arabiensis*, particularly outdoor biting and wide range of biting times will also pose challenges to implementing effective vector control.

Conclusion

We consider *An. arabiensis* to be the most likely primary vector of malaria in the agricultural development areas surveyed in this study. While the EIRs do not indicate high rates of transmission, the low use of vector control interventions and lack of access for treatment result in a real risk of malaria for migrant workers staying in these locations. Improved malaria prevention and treatment could have a valuable impact on worker health and productivity in these areas. In addition, the results of this study indicated that proper bednet use between 21.00 h and 6.00 h could reduce an estimated number of bites per person per night by about three-fold. However, it seems likely that the behavior of *An. arabiensis*, particularly outdoor biting and a wide range of biting times will also pose challenges to implementing effective vector control.

Abbreviations

ABTS : 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

ADLI: Agricultural Development Led Industrialization

CI : Confidence interval

DNA : Deoxyribonucleic acid

EIR : Entomologic inoculation rate

ELISA : Enzyme linked Immunosorbent assay

HLC: Human landing catch

IRS: Indoor residual spray

LLIN: Long-lasting insecticidal net

PCR: Polymerase chain reaction

Declarations

Ethics approval and consent to participate

Permissions were sought and obtained from each of the farms where data were collected and local authorities. A letter of support was obtained from the Benishangul-Gumuz Regional Health Bureau. The research was determined to be non-human subjects research by the Centers for Disease Control and Prevention (2017-227), and exempt from IRB review by Abt Associates (#0970).

Consent for publication

Not applicable

Availability of data and material

The data generated and/or analyzed during the current study are available at Abt Associates Inc.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SI, SC, MM conceived the idea of the project. SD, SI, SC, YT, LL, HS, MM designed the study. SD, YT, GY, organized and conducted the field work. DY conducted the blood meal analysis and ELISA work. SD analyzed and interpreted the data. SD, SI, MM drafted the initial manuscript. SD, SI, SC, YT, LL, HS, and MM revised the manuscript. All authors read and approved the final manuscript.

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Disclaimer

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Figures

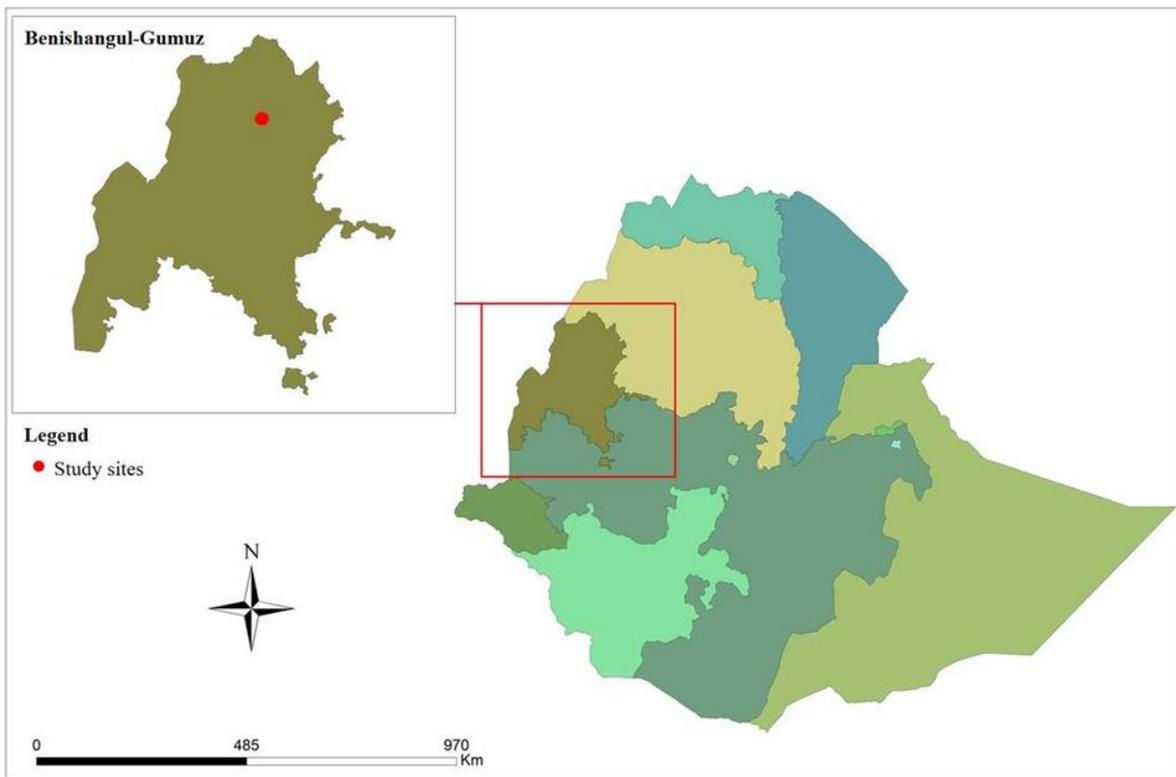


Figure 1

Map of districts in Ethiopia illustrating the location of Benshangul-Gumuz and the location of the sites in this study (red circle). Map produced using ArcMap v. 10.5. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

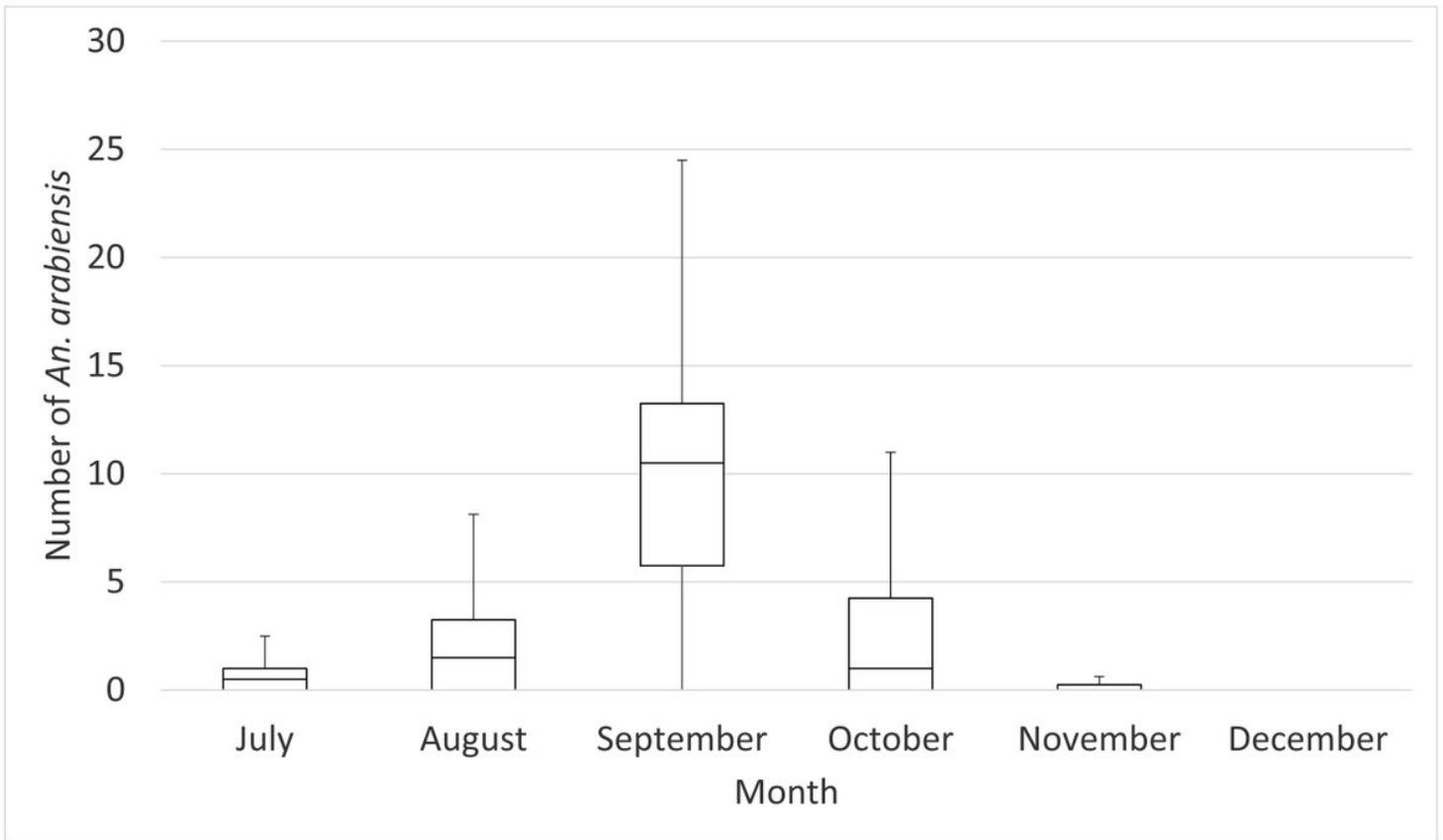


Figure 2

Tukey box-plots showing median and interquartile ranges of the number of *An. arabiensis* collected in indoor and outdoor human landing catches (n=96) at workers' shelters on small farms (<100ha), Dangur woreda, Ethiopia, 2017.

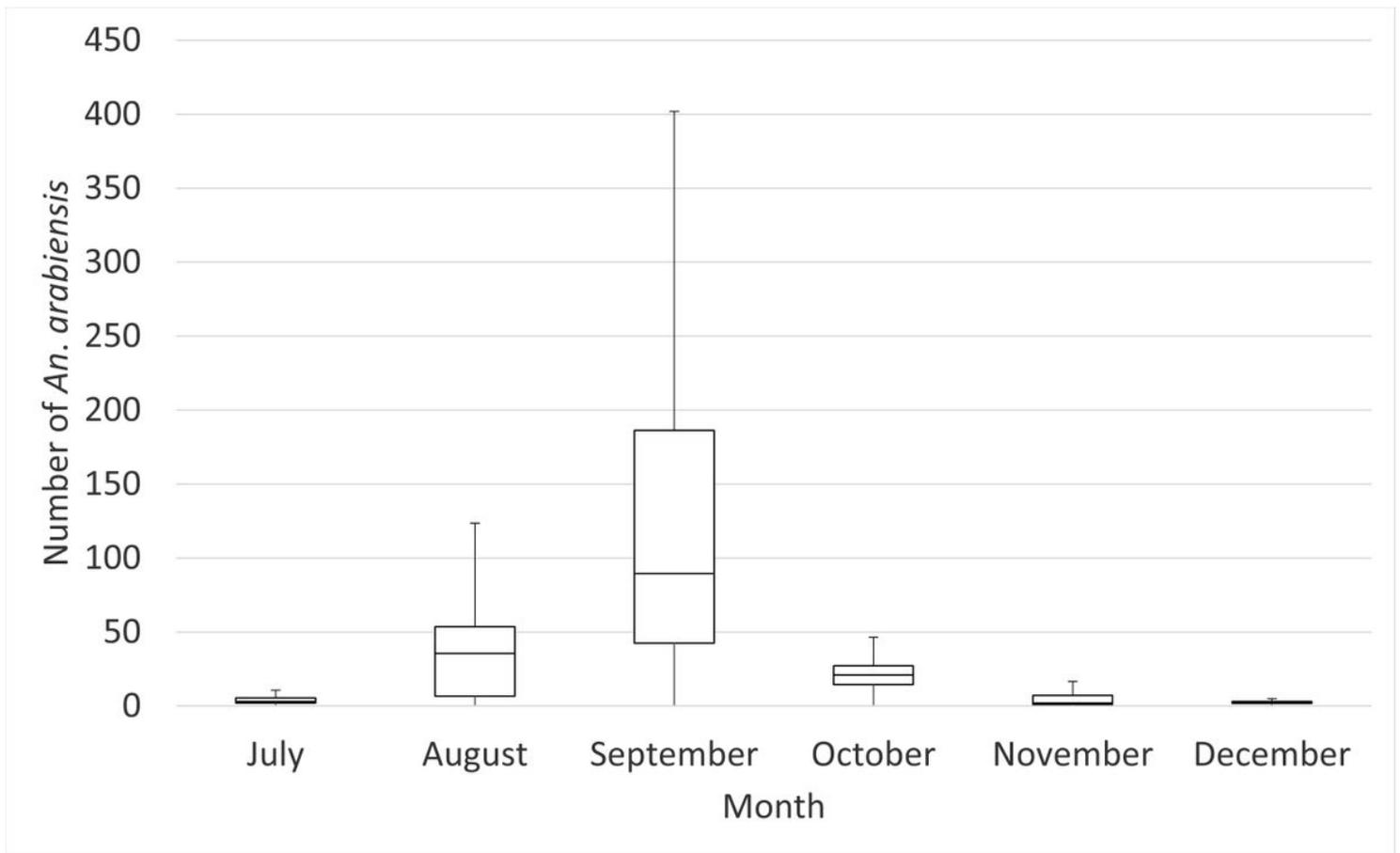


Figure 3

Tukey box-plots showing median and interquartile ranges of the number of *An. arabiensis* collected in indoor and outdoor human landing catches (n=96) at workers' shelters on large farms (>100ha), Dangur woreda, Ethiopia, 2017.

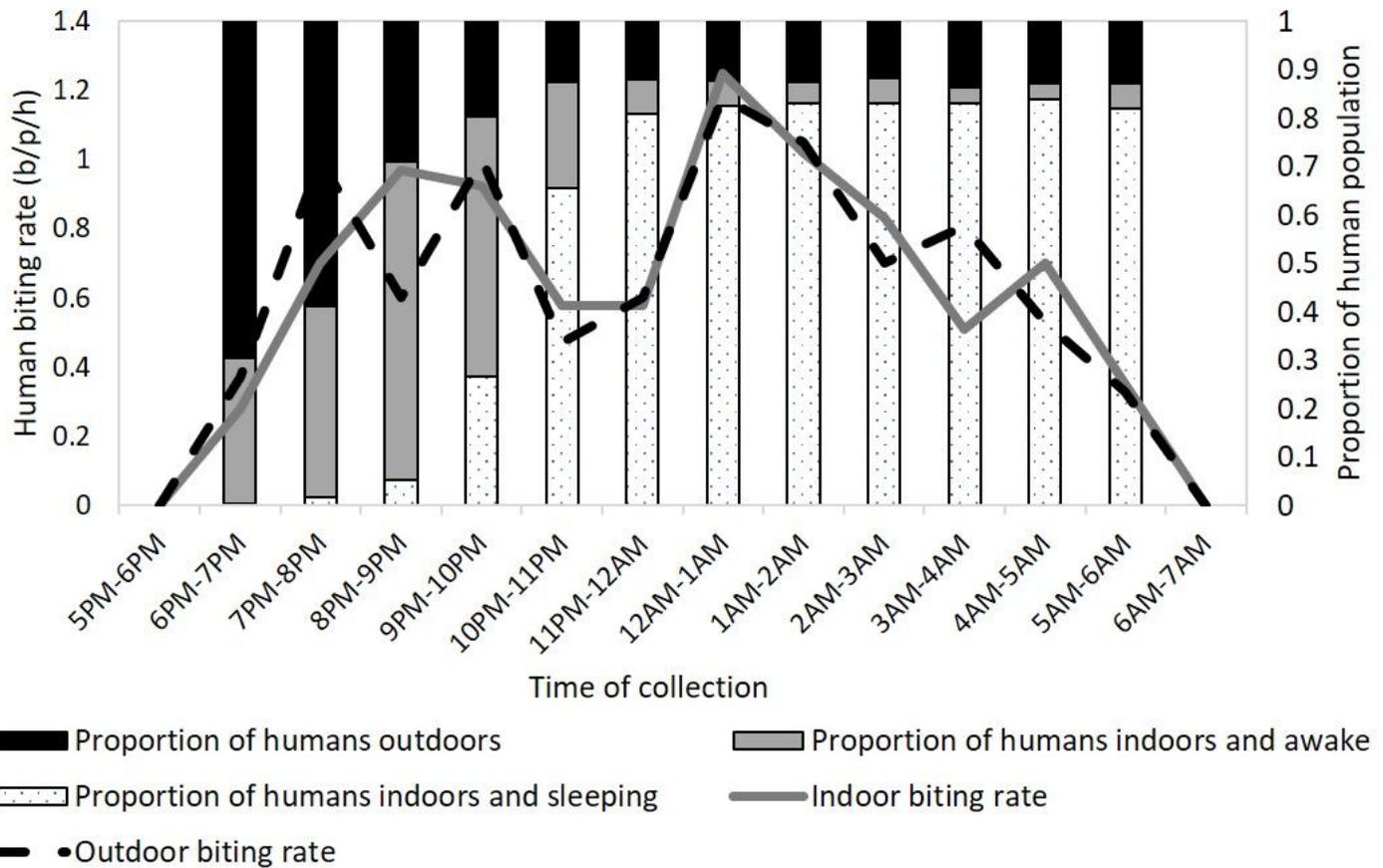


Figure 4

Directly measured hourly biting rate (bites per person per hour) indoors (solid grey line) and outdoors (dashed black line) in worker camps in eight farms in Dangur district (96 indoor collections, 96 outdoor collections) show 48% of directly measured biting occurred indoors. Lines representing human biting rates are overlaid on top of bar charts representing the mean proportion of humans outdoors, indoors and awake, and indoors and asleep each hour of the evening. Approach derived from Monroe et al., 2020 [27].

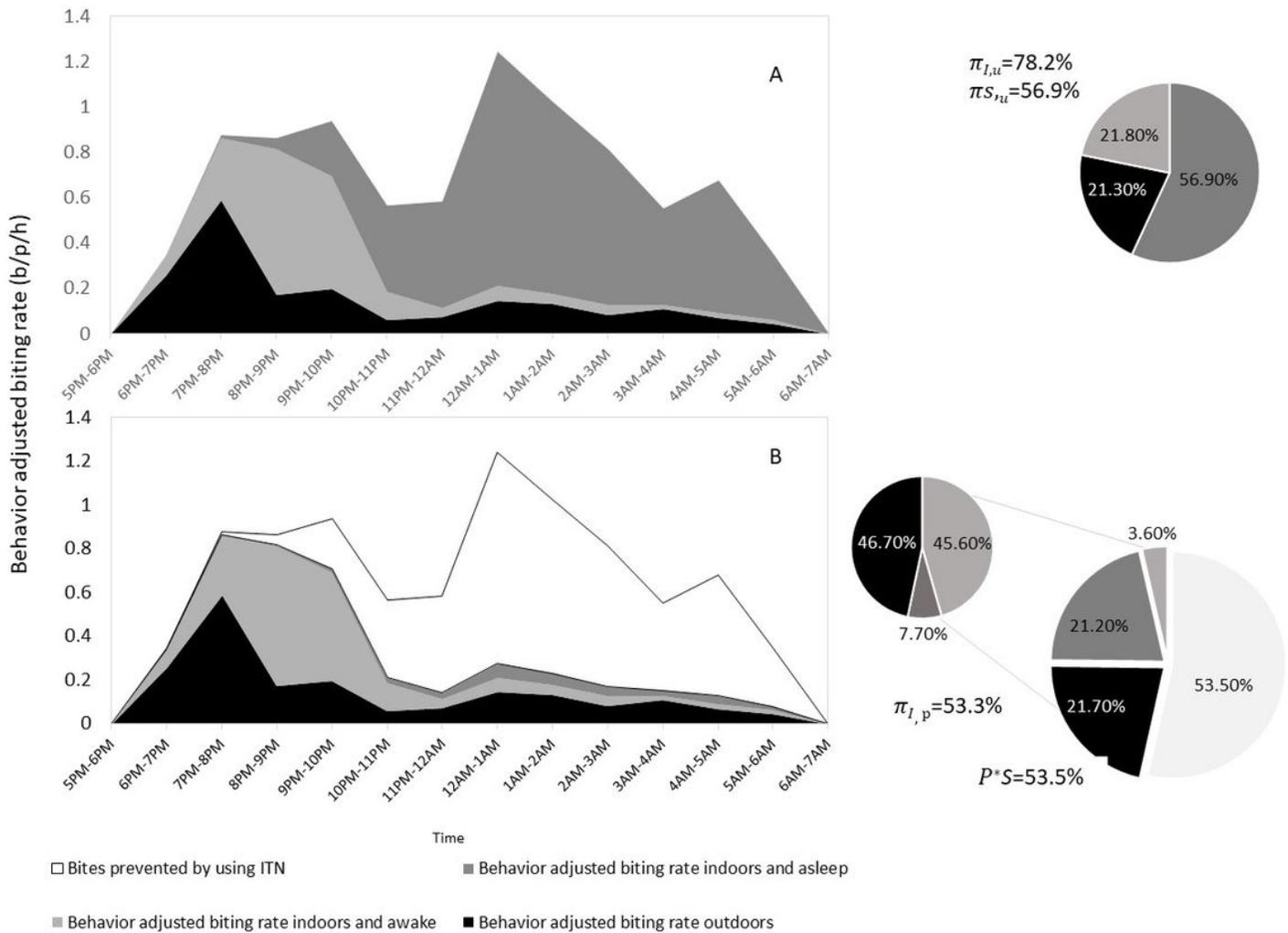


Figure 5

Human and *An. arabiensis* data were combined to derive human adjusted biting rates for ITN unprotected and protected individuals. A) Behavior adjusted biting rates per person per hour for an unprotected individual are presented. The percentage of vector bites occurring indoors for unprotected individuals is 75%, and the percentage of bites occurring while asleep indoors for an unprotected individual is 54%. B) Behavior adjusted estimates of *An. arabiensis* bites per hour per person in the study areas if ITNs were used, using the typical start and end times shown in the study area. The percentage of vector bites that would be prevented by using an ITN is 51%. Approach and calculations derived from Monroe et al., 2020 [27].