Host-seeking behavior and hourly biting rhythm of vectors in malaria endemic district of Arba Minch area, Southwest Ethiopia

Melkam Abiye Zeru (melk12ab@gmail.com)
University of Gondar College of Medicine and Health Sciences
Simon Shibru
Arba Minch University
Fekadu Massebo
Arba Minch University

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Abstract

Background

The early and outdoor biting behaviors of malaria vectors are among the key challenges in malaria control. Hence, understanding the host-seeking behavior and the peak biting time of malaria vectors is important in malaria vector control programs. This study assessed the host-seeking behavior and hourly biting activity of malaria mosquitoes in Chano Mille village in Arba Minch district, southwest Ethiopia.

Methods

The first trial was done by keeping cattle together with human that collects the entered mosquitoes in tent, while the other was done by keeping cattle outside a 1 m distance from human collector inside a tent. In both trials, mosquito collation was done inside tents baited by cattle and human using human landing catches (HLC) techniques. Four human volunteers' were recruited and trained to collect mosquitoes in the four tents from 18:00-24:00 for three months. Two tents were selected randomly for human alone and two for cattle baited collections in the first night and then rotated to minimize the variation due to location of tents and collectors skill. The tent trial was done close to the shore of the Lake Abaya to minimize the interference of other animals on mosquito movement. The peak biting hour of malaria vectors was assessed within a Chano village from 18:00-6:00. Mosquito collation was done both indoor and outdoor by HLC. Morphological speciation of Anopheles mosquitoes was done. The sporozoite infectivity status of *Anopheles pharoensis* was examined using enzyme-linked immuno-sorbent assay technique. The data was analyzed using a Generalized Estimating Equations with a negative binomial distribution.

Results

An. pharoensis, An. gambiae complex and *An. tenebrosus* were the three species documented during the trial. Keeping cattle together with human collector inside the tent attracted 42% (P < 0.001) more *An. pharoensis* compared to human alone tent. Also, keeping cattle outside near to a tent with human at 1 m distance, attracted the entering *An. pharoensis* into the tent inside with human by 46% (P = 0.002) than human in a tent with no cattle outside the same tent. The impact was not significant for *An. gambiae* complex and *An. tenebrosus*. A*nopheles pharoensis* and *An. gambiae* complex showed early night biting activity with peak biting from 19.00-20:00 which was significant for both *An. gambiae* complex (P < 0.001) and An. pharoensis (P = 0.015). *Anopheles gambiae* complex was mainly biting humans outdoor in the village.

Conclusions

Finally, keeping cattle within and close to human dwellings could increase malaria vectors bite exposure particularly to the zoophilic malaria vector *An. pharoensis* and, hence deployment of cattle far from human residence could be recommended to reduce the human exposure. The outdoor and early hours

biting behavior of the *An. gambiae* complex could be a threat for success of current indoor based interventions and hence, tools could be designed to reduce this threat.

Introduction

Malaria is a mosquito-borne disease transmitted through the bites of infected female Anopheles mosquitoes (Wiebe et al., 2017). It is prevalent in the tropical and subtropical regions of the world. Since 2000, it is reducing in many malaria endemic countries due to the combined efforts of distribution of long-lasting insecticidal nets (LLINs), effective case management with effective anti-malarial drugs, larval source management (LSM) and indoor residual spraying (IRS) (Bhatt et al., 2015). IRS and LLINs are highly successful where the major vectors are predominantly anthropophilic and endophagic. They are less successful in areas where malaria vectors are exophagic and exophilic (Habtewold et al., 2004). Anopheles arabiensis, the principal malaria vector in Ethiopia, shows flexibility with respect to feeding and resting habits, and seems an opportunistic host feeder (Waite et al., 2017). It exhibits both anthropophagic and zoophagic behaviors (Massebo et al., 2015, 5).The other important malaria vector is An. pharoensis which exhibited mostly exophagic behavior (Kenea et al., 2016).

The early hours and outdoor biting behavior of malaria vectors could be a key challenge for malaria control and elimination, and results residual malaria transmission (Russell et al., 2011). In many malaria-endemic areas residual transmission continues regardless of the wide coverage of LLINs and IRS (WHO, 2016, 8).Residual malaria transmission occurs in the gaps left after use of LLINs and IRS which highlights the need for new strategies in vector control (Chaccour et al., 2015). The early hours biting activities of malaria vectors have been documented in north and south central Ethiopia (Kenea et al., 2016). It is obvious that the species composition and biting behavior of mosquitoes can vary from place to place. Hence, understanding the host-seeking behavior and hourly peak biting activity of malaria vectors are keys for malaria vector control programs.

Livestock are usually kept outdoor close to the human dwelling in the area. These animals may increase human exposure to bites of malaria vectors by attracting mosquitoes towards to human dwellings or reduce the human exposure by diverting mosquitoes away from the human hosts. If the vectors are attracted mainly to animals, it may open an opportunity to target animals to tackle the residual malaria due to the zoophilic vectors. Moreover, if there is a shift in biting hours of the malaria vectors, it may demand the supplementary interventions to protect the people at early hours. Therefore, the aim of this study was to investigate host-seeking behavior and hourly biting activity of malaria vectors in Arba Minch district, southwest Ethiopia.

Materials And Methods

Description of the study area

The study was conducted in Arba Minch district in Southern Nation, Nationalities and Peoples' Regional state (SNNPR) of Ethiopia. The study village is found north of Arba Minch at about 16 km and south of Addis Ababa at about 470 km. The village is located at 6°6.666´ N and 37°35.775´E and at altitude of 1,206masl. It is one of the malarious villages in Arba Minch district. The climate is hot and humid which is suitable for malaria vectors. The inhabitants are mixed farmers as they are ranching cattle and growing crops. They grow cash crops such as mangoes and bananas as the main source of income. LLINs and IRS are the principal malaria vector control tools. An. arabiensis is the principal malaria vector in the area (Massebo et al., 2013). (Fig. 1).

Study design

An experimental study was conducted to investigate the host-seeking behavior of malaria vectors. The first experiment was conducted in the shore of Lake Abaya with a high potential of mosquito breeding from October to December 2016. Four tents were constructed and placed close to the shore of the Lake Abaya (Fig. 2). The tents have mosquito entry points which can be closed and opened by zips. The zip of each tent was closed at day time to prevent damages by wind. During the mosquito collection time (18:00–24:00) the zip was remaining open to allow mosquito entering. Mosquito collection was done until the mid-night due to the high wind wave after mid-night. The shore of the Lake was selected to avoid the interference of other domestic/wild animals by attracting mosquitoes as the experiment was conducted in open area where other wild animals are usually not stay. (Fig. 2.)

The tents were designed to sample mosquitoes by human land catches (HLC) from human alone and cattle baited tents to see the mosquito biting tendency. The distance between tents was 20 m. The tents for human alone and cattle bait were selected randomly on the first night, and then rotated to reduce the bias driven by variation in attractiveness to mosquitoes, collecting abilities, and spatial variation due to location of the tents. Four human volunteers (above 18 years) were recruited from the local people to collect mosquitoes entering the tents. The volunteers who had collection experience, and gave written consent were included. Two persons sat inside tents with calves (one person and calve in each tent). The other two persons sat inside the other two tents (one person in each tent) without calves. There was two collection shifts in one cycle which was completed in eight nights. In the first four consecutive nights, collectors were rotated daily between tents, and after four consecutive night collection, there was two days break for collectors. Then, in the next four consecutive nights, we rotated only calves.

The second trial was done by keeping calves outside the tents at 1 m distance from the tents (Fig. 3). In this case two human collectors sat inside the two tents (one in each tent) separately without calves outside the tent at one meter distance. And inside the other two tents two human collectors sat separately in each tent and two calves (one outside each tent) were kept at one meter distance. In the first night, the tents were randomly selected for calves and humans. In the first four days, the calves were rotated and in the second four days the collectors were rotated to minimize the bias due to location and collection skills.

The third activity was done within the village to assess the biting rhythm and identify the peak biting hours of the malaria vectors. Indoor and outdoor mosquito collection was done in two houses with human occupants using HLCs. In each house, a pair of collectors were interchanged their positions between indoor and outdoor every hour. A pair of collectors at one house in the first night was rotated to the second house in the next collection night and the cycle continues until the end to minimize the bias due to collection skills and attractiveness of the collectors. The distance between the two houses was about 200 m. The collection was done from 18:00–06:00 for seven nights during the study period.

Each collector sat on a chair with the legs exposed from foot to knee and caught mosquitoes as soon as they land on the exposed legs before they start biting by aspirator. Each hour collection was kept separately in labeled paper cups for species identification and further analysis. A strong supervision was made to avoid the bite of mosquito on collectors. (Fig. 3.)

Identification of Anopheles mosquitoes

The collected Anopheles mosquitoes were brought to Medical Entomology Laboratory at Arba Minch University. They were killed via refrigerator and the species were identified through microscope based on morphological characteristics using an identification key(Gillies and Coetzee, 1987). Then, the identified female Anopheles mosquitoes were placed in vials over silica gel for circum-sporozoite proteins (CSPs) test.

Mosquitoes processing for CSPs detection

The CSP detection was performed by Enzyme-Link Immuno Sorbent Assay (ELISA) technique (Beier et al., 1987) at Arba Minch University Medical Entomology laboratory. The head and thorax of female An. pharoensis was used for P. falciparum and P. vivax-210CSP detection. Two separate 96-well micro-titer plates were coated with 50 µl solution of P. falciparum and P. vivax-210 monoclonal antibodies (MAB). The plates were covered and incubated for overnight at room temperature. Then the contents of plates were sucked, emptied and filled with 200 µl blocking buffer (BB) and further incubated for one hour at room temperature. During the incubation time, mosquitoes were grounded individually in 50 µl grinding solution and the final volume brought to 250 µl by rising with 100 µl BB twice. BB was removed from plate after one hour and 50 µl of each homogenized mosquito triturate was added to each of the two test wells. P. falciparum and P. vivax-210 positive samples and a wild-colony of An. pharoensis were used as both positive and negative controls. Plates were incubated for two hours and washed with phosphate-buffered saline (PBS)-Tween 20 twice. Horseradish peroxidase-conjugated monoclonal antibody was then added to each well and incubated for one hour, and the wells were washed three times with PBS-Tween 20. Finally, 80 µl of preoxidase substrate was added per well and incubated for 30 minutes. The wells were observed visually for green color and also their optical density determined at 414 nm in microplate ELISA reader. Sample with green with color and with optical values of greater than two times the average optical density of the negative controls were considered positive.

Outcome variables

The first primary outcome variable was the host-seeking tendency and hourly biting rhythm of malaria vectors to hosts. The secondary outcome variable was the species composition of Anopheles mosquitoes.

Data analysis

The host-seeking tendency of malaria vectors was calculated by A Generalized Estimating Equations (GEE) with a negative binomial distribution. The mean ratio of the number of Anopheles species collected from cattle baited tents and human alone was used to determine the host preference of mosquitoes and the impact of the proximity of the cattle on human exposure. The Anopheles mosquito peak biting time was also determined by GEE with negative binomial distribution. The p-value < 0.05 was used to determine the peak biting hours and the feeding tendency of malaria mosquitoes. The data were entered into excel and analyzed using SPSS version 20.

Ethical Approval

The study was reviewed and approved by the ethical review committee of Arba Minch University (Ref.No.1480/11).The objective of the study was explained to human volunteers. Participation in mosquito collection was based on their willing and only those adequately trained on the collection process was included. Verbal and written consent was obtained from human volunteers. The volunteers for field trial were informed to be alert and catch the coming mosquitoes as soon as landing on their exposed legs. Malaria prophylaxis was given to prevent infection.

Results

Species composition of *Anopheles* mosquitoes from tent experiment

A total of 1593 *Anopheles* mosquitoes belonging to three species were collected during tent experiment. *An. gambiae* complex, *An. pharoensis* and *An. tenebrosus* were the species documented. *An.pharoensis* was the pre-dominant species that accounted for 51.4% (819/1593), followed by *An. gambiae* complex (34.5%; 550/1593) and *An. tenebrosus* (14.1%; 224/1593). About 174 *An. gambiae* complex were collected indoors and outdoors in Chano village using HLCs.

Host-seeking behavior of *Anopheles* mosquitoes in tents with calves

Of the 463 *An. pharoensis* collected during the first trial (calves inside tents), 284 (61.3%) were from cattle baited tents and 179 (38.7%) were from tents with human alone. The mean number of *An. pharoensis* was 3.2 in cattle baited tent/human/night (Wald 95% Confidence Interval (CI): 2.4-4.2) compared to 1.8 in tent with human alone /night (Wald 95% CI: 1.51-2.2).

From 421 *An. gambiae* complex collected, 235 (55.8%) were from tents with human alone and 186 (44.2%) were from cattle baited tents. More *An. gambiae* complex and *An. tenebrosus* were collected from tents with human alone, but it was not statistically significant compared to the cattle baited tents (**Table 1**).

Host-seeking behavior of *Anopheles* mosquitoes when calves are outside the tents

Of the 356 *An. pharoensis* collected, 230 (64.6%) were caught from inside human tents where a calves were outside the tents at 1m distance and 126 (35.4%) were from inside tents where no calve outside the tents. Of 129 *An. gambiae* complex, 68 (52.7%) were collected inside the human tents where a cattle kept outside the tent at 1 m distance and 61 (47.3%) were collected inside the human tents where no cattle kept outside the tents. It was not statistically significant for *An. gambiae* complex (P = 0.8) and *An. tenebrosus* (P = 0.6) (**Table 2**).

Hourly biting activities of *Anopheles* mosquitoes at the shore of the Lake Abaya

Anopheles pharoensis and *An. gambiae* complex showed similar biting activities at early hours of the night with a peak biting activities from 19-20:00 **(Figure 4).** Significantly higher number of *An. pharoensis* (P = 0.015) and *An. gambiae* complex (P = 0.0009) were biting humans at early hours (19.00-20:00) close to the breeding sites.

Indoor and outdoor hourly biting activities of *Anopheles* mosquitoes

The principal malaria vector *An. gambiae* complex showed outdoor biting activities throughout the night. Its outdoor biting activity was peak at the early hours (21:40-22:40) of the night (P< 0.001) **(Figure 5).** Of the 174 *An. gambiae* complex collected inside the village, 87.9% (153/174) were collected outdoor and the remaining 12.1% (21/174) were collected indoor.

Sporozoite rate of *Anopheles pharoensis*

About 792 *An.pharoensis* were tested for CSPs by using ELISA technique, but none of them were positive for *P. falciparum* and *P. vivax* infection.

Discussion

The main objective of this study was to investigate the host-seeking tendency and hourly biting rhythm of *Anopheles* mosquitoes. The presence of cattle with human inside the tents increased the number of *An. pharoensis* by 42% compared to human alone (mean ratio of human alone tent to cattle baited tent was 0.58; P < 0.001. Moreover, Keeping cattle outside the tent at least in 1 m distance from the tents increased the number of *An. pharoensis* inside the tents by 46% compared to human tents with no cattle outside the tents (mean ratio of mosquitoes inside tent with no cattle outside to mosquitoes inside the tent with cattle outside the tents was 0.54; P = 0.002).

The presence of cattle with human inside tent increased human exposure to the bites of malaria vectors, in particular to the zoophilic vector *An. pharoensis*, and hence may get the risk of infectious bites. Moreover, cattle outside the tents at 1m distance also increased the risk of human biting by *An. pharoensis*. This shows that the presence of cattle close to the human dwelling may increase mosquito bites. In another cases keeping cattle in surroundings of houses may give protection against the bites *An.*

arabiensis by diverting host seeking mosquitoes. In contrary, animals may increase the risk of being bitten by attracting mosquitoes to the human if the livestock are close to the houses (Bøgh *et al*., 2002; Habtewold *et al*., 2004). Therefore, for effective uses of zooprophylaxis (the diversion of arthropod vectors to animals), livestock would be kept at a distance from human dwellings at night during peak vector activity(Donnelly *et al*., 2015). Moreover, insecticidal zooprophylaxis would have an impact on malaria vectors density by inducing mortality when feeding on insecticide treated animals (WHO, 2001).

In the present study most *Anopheles* species were biting human mainly at early hours of the night before most people slept under bed nets. Outdoor and early hours biting tendency of malaria vectors has been documented in south-central Ethiopia (Kenea *et al*., 2016). A study in south-west Ethiopia also reported early hour's biting tendency of *An. pharoensis*(Taye *et al*., 2006). We also documented outdoor biting tendency of *An.gambiae* complex throughout the night with a peak biting activity at early hours in the village where IRS and LLINs are the cornerstone interventions implemented. Similar finding has been reported in Jimma zone, southwest Ethiopia(Taye *et al.,* 2016).This outdoor biting behavior of *An. gambiae* complex could bean avoidance behavior to the indoor based key interventions IRS and LLINs. Outdoor and early hours biting behaviors could result persistence of a residual malaria transmission even after high coverage and use of IRS and LLINs. In contrast, a study from Sille in 2006 before 13 years documented late night peak biting activity of *An. arabiensis*(Taye *et al*., 2006*)*. This might be due to the low coverage and usage of indoor based interventions as the intervention intensity was increased since then. Today, vector control interventions like insecticidal zooprophylaxis could be implemented to target outdoor and early hours biting vectors in area where animals kept outdoor.

In this study period, three *Anopheles* species namely, *An. gambiae* complex, *An. pharoensis* and *An. tenebrosus* were documented. *An. pharoensis* was the pre-dominant species in the shore of the Lake; this is due to the presence of permanent water and grasses that grow near to lakeshore during the collection period. This is the ideal places for *An. pharoensis* breeding, and small water bodies created by hoof prints of cattle and hippopotami make the lakeshore a potential mosquitoes breeding site. The species prefer breeding habitats with permanent water body and vegetation. The species is among the widely distributed species in Ethiopia (Taye *et al*., 2016; Kenea *et al*., 2016). No sporozoite infected *An. pharoensis* were recorded. This could be due to the collection of the mosquito close to the breeding sites where the younger mosquitoes might dominant.

This study had several strengths and limitations. HLC method was used for collecting mosquitoes which is the gold standard to estimate the host biting behaviors of malaria vectors. Rotation of human collectors and hosts believed to minimize the bias due to attraction and spatial variation. The tent experiment was conducted at the shore of the Lake Abaya where the density of mosquitoes was high and no interference of other animals. Identification of mosquito was done by using morphological identification key, which might lead misclassification. The human volunteers may miss some mosquitoes during collection and transferring them into paper cups, and this may bias the estimation of the mosquito biting hours.

Conclusion

The presence of cattle with human inside tents increased the number of malaria vectors biting on humans. Keeping cattle close to the human tent increased the number of malaria vectors bites on humans. The principal malaria vector, An. gambiae bite humans mainly outdoors and in the early parts of the night where people are not protected by bed nets. Finally, outdoor and early hours biting behavior of the vectors could threaten the effectiveness of current indoor based interventions and hence, new tools could be designed. Moreover, deployment of cattle far from human residence may be recommended to reduce the human exposure to malaria vectors.

Declarations

Competing interests

The authors have no competing interest

Funding statement

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Ethical statement

The study was reviewed and approved by the ethical review committee of Arba Minch University (Ref.No.1480/11). The verbal and written consent was obtained from each volunteer.

Author's contributions

MAZ participated in the study design, and conducted the field and laboratory work. SS participated in study design and revised the manuscript; FM participated in the study design, laboratory and field supervision, and revised the manuscript.

Availability of data and materials

The data supporting the conclusions in this article is presented in the main paper.

Consent for publication

Not applicable.

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Tables

Table 1: Host-seeking behavior of *Anopheles* mosquitoes in cattle baited and tents with human alone close to the shore of Lake Abaya in Chano Mille, Southwest Ethiopia

Anopheles species	Cattle baited tent		Tent with human alone				
	No	Mean (Wald 95% CI)	No	Mean (Wald 95% CI)	Mean ratio	% variation	P value
An. pharoensis	284	3.16 (2.37-4.2)	179	1.82 (1.51-2.2)	0.58 (1.8/3.16)	42	0.001
An. gambiae complex	186	2.08 (1.52-2.85)	235	2.43 (1.64-3.59)	0.86 (2.1/2.43)	14	0.5
An. tenebrosus	79	0.9 (0.69-1.18)	89	0.82 (0.59-1.14)	0.91 (0.82/0.9)	9	0.34

Table 2: Host-seeking behavior of *Anopheles* mosquitoes inside human tents by keeping cattle outside the tents in 1 m distance and no cattle outside human tents at the shore of Lake Abaya in Chano Mille, Southwest Ethiopia

Anopheles	CBT outside tents		No cattle outside tents				
Species	No	Mean (Wald 95%CI)	No	Mean (Wald 95%CI)	Mean ratio	% variation	P value
An. pharoensis	230	2.4 (1.83-3.1)	126	1.3 (1-1.7)	0.54	46	0.002
An. gambiae complex	68	0.71(0.49-1.02)	61	0.66 (0.38-1.14)	0.93	7	0.8
An. tenebrosus	30	0.3 (0.17-0.53)	26	0.26 (0.16-0.42)	0.87	13	0.6

Figures

Figure 1

The experimental site close to the shoreline of Lake Abaya with a high potential of mosquito breeding habitats, Southwest Ethiopia

Figure 2

Tents for mosquito collection in Chano Mille close to the shore of Lake Abaya, Southwest Ethiopia

Figure 3

Tent experiment where a calve kept at one meter distance from the tent, Southwest Ethiopia

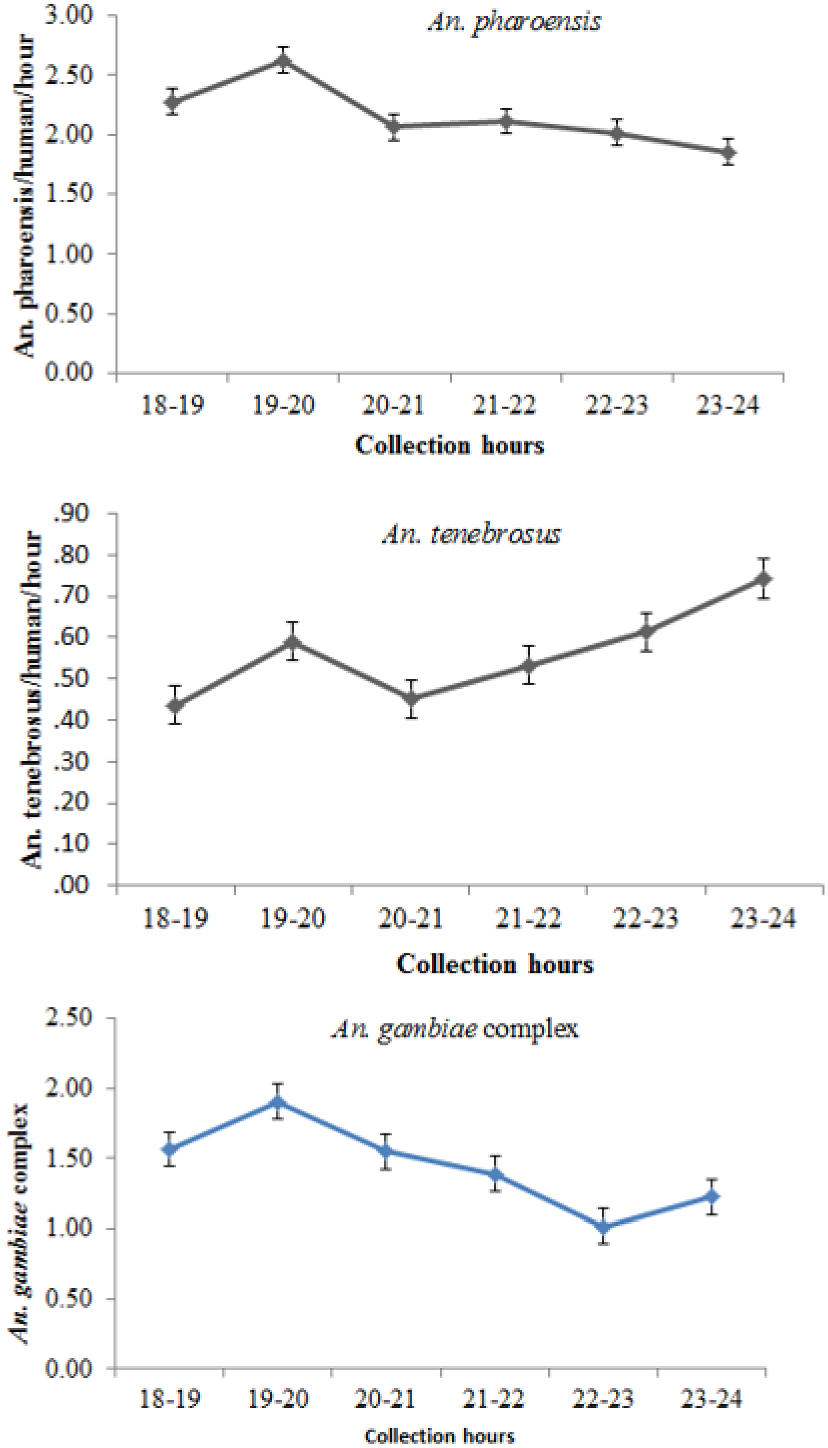

Figure 4

The overall hourly biting activities of the three Anopheles species close to the shore of Lake Abaya in Chano Mille, Southwest Ethiopia

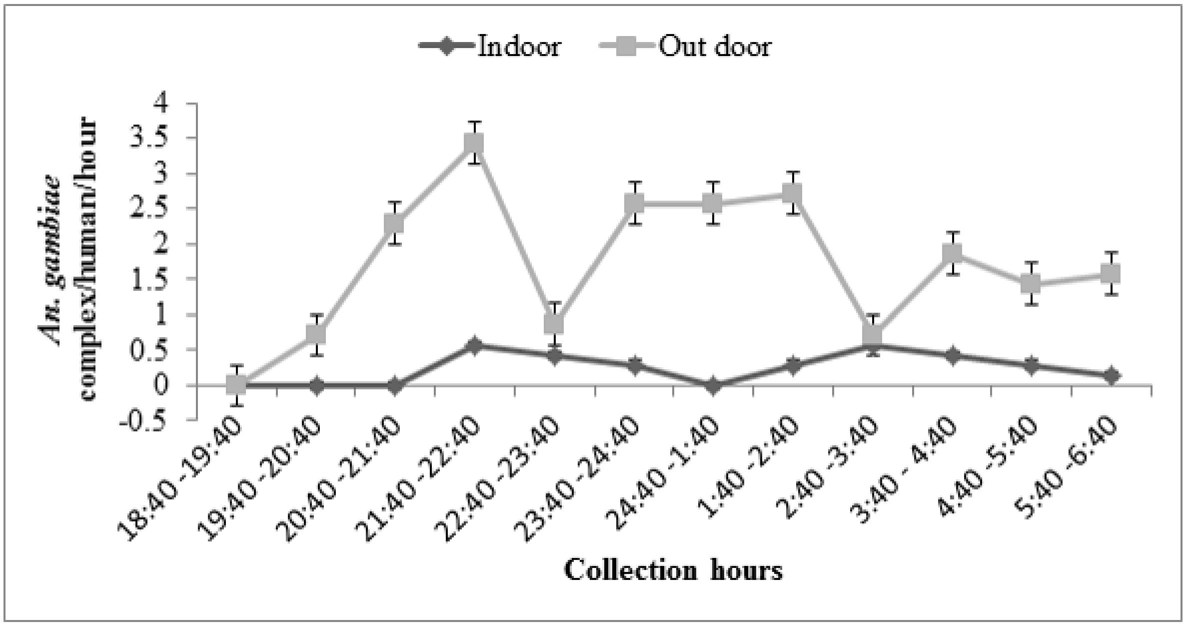

Figure 5

The overnight hourly indoor and outdoor biting activities of Anopheles gambiae complex inside the Chano Mille village, Southwest Ethiopia