Blood Meal Sources and Feeding Behavior of Anophelines Mosquitoes in Bure District, Northwestern Ethiopia

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Abstract

Background: Mosquito blood meal sources determine their own feeding rates, adult survival, fecundity, hatching rates, and developmental times. The only female *Anopheles* mosquito takes blood meals from humans, birds, mammals, or other vertebrate animals for egg development. Studies of host preference patterns in blood-feeding anopheline mosquitoes are crucial to incriminating malaria vectors. However, the human blood index, foraging ration, and host preference index of anophelines mosquitoes were not known so far in Bure district Ethiopia.

Methods: The origin of blood meals from all freshly fed and a few half-gravid exophagic and endophagic females collected using the center for disease control and prevention light trap catches were identified as human and bovine using Enzyme Linked Immune Sorbent Assay. Human blood index, forage ratio, and host feeding index were calculated.

Results: A total of 617 specimens belonging to *An. arabiensis* (n = 209), *An. funestus* (n= 217), *An. coustani* (n= 123), *An. squamosus* (n= 54) and *An. cinereus* (n= 14) were only analyzed for blood ELISA. 577 of the overall specimens were positives for blood antigens of the host bloods. All anopheline mosquitoes assayed for blood meal sources had extremity mixed blood meals sources than single blood meals. The FR for a human was slightly > 1.0 than bovine for all *Anopheles* species. HFI for each pair of vertebrate hosts revealed that humans was a bit preferred blood meal source to bovine for all species (except *An. squamosus*), but did not exhibit a marked host selection.

Conclusions: All assayed anopheline mosquitoes for blood meal ELISA had a mixed feed which tends to diminish the density of gametocytes in the mosquito stomach, thereby reducing the chance of fertilization of the female gamete and reduce the chances of malaria vector becoming infected. Moreover, *An. coustani* was the only species that had only human blood meal alone mean that this species has a potential to transmit the disease. Therefore, combination zooprophylaxis should be reinforced as a means of vector control because the study sites are mixed dwelling.

1. Introduction

Malaria is transmitted by the blood feeding of infectious female *Anopheles* mosquitoes [1, 2] with a complex parasite life cycle, which depends on both humans and mosquitoes [3, 4]. In Ethiopia, malaria is the leading health problem [5] and the nature of malaria transmission is seasonal and unstable [6], vary with elevations, temperature, and rainfall [7, 8].

In Ethiopia, over 42 species of *Anopheles* were identified [9, 10], but *Anopheles arabiensis* is the principal malaria vector while *An. pharoensis*, *An. funestus* and *An. nili* are secondary vectors [11, 12].

Understanding of the biology and behavior of *Anopheles* mosquitoes can help to understand how malaria is transmitted and can aid in designing appropriate control strategies [13]. Each species of *Anopheles* has own blood feeding, host preference, biting, flight range, and host selection behavior [14, 15].

The blood feeding behavior of malaria vectors is an important parameter in malaria epidemiology [3]. This behavior can influence vectorial potential [1] and depending on the vertebrate host groups with which the mosquito makes contact and influence the spatial distribution of a disease [16]. The most successful malaria vectors feed commonly on humans, and secondarily on cattle and other domestic animals, depending on host availability [3]. Host choices and subsequent feeding success depend on host availability [14, 17] including host accessibility, density, host defense mechanisms, host size, and proximity to mosquito habitats [18, 19], environmental factors, flight behavior and feeding periodicity of the mosquitoes [20]. Intervention through long-lasting insecticide treated nets (LLINs) and insecticide residual spraying (IRS) determine the successful feeding and oviposition nature of malaria mosquitoes [21].

Preference of anophelines to feed on humans can be estimated using human blood index (HBI). HBI represents the proportion of blood meals derived from humans in mosquito vector [22]. The study of host-feeding pattern is an essential part of understanding of the epidemiology of disease transmitted by the arthropods [23, 24]. Host preference studies have also been used to monitor the effectiveness of vector control programs by observing a reduction in blood-feeding behavior, and have served as evidence of control failure [25,26,27].

Totally, anopheline exhibits a wide range of host preferences, such as humans, cattle, sheep, horses, pigs, dogs, cats, birds, reptiles and other mammals [28, 29, 30]. Particularly, animal feeding vectors are known by suppressing the human blood meal source and reducing the level of infection in the local vector population [31,32]. However, HBI results do not always reflect host preference [33,34]. Therefore, several authors proposed different indices to separate preferential versus opportunistic feeding patterns of mosquitoes [23,35]. The forage ratio (FR), which is measures of host selection patterns, i.e., quantifies vector selection of a particular vertebrate host rather than other available hosts [33]. It is helping to show the attribute of only one host preference [23] and it does not require a full host census [36]. The other parameter is the feeding index (FI), which helps to compare the observed proportion of blood feeds to one host with another host divided by the expected comparative proportion of feeds on the two hosts [23, 16].

Generally, the knowledge of the HBI, blood-feeding preferences and pattern of a mosquito species provides insight into its vector potential [16,37] and the epidemiology of diseases transmission [23, 24, 28, 38] and allows designing and implementing efficient strategies for vector control [22, 28,29]. In our study, the HBI, FR and host preferences index (HFI) of anophelines mosquitoes were not known so far. Therefore, this study was conducted to determine the abdominal status, HBI, FR and HFI of Anophelines mosquitoes in Bure district, Northwest Ethiopia.

2. Materials And Methods

2.1 Sstudy Area

Longitudinal study was conducted in Bure district, northwestern of Ethiopia, from July 2015 to June 2016. Geographically, Bure district is situated at an altitude ranging from 700 (Blue Nile gorge) to 2,350 m.a.s.l. (Fig. 1). Socio-economically, the majority (85%) of the populations are farmers who grow maize, teff (*Eragrostis teff)*, pepper, potatoes, wheat, millets, followed by beans & peas, sunflower, niger, spices, vegetation's, and others; and the rests are merchants (6.8%) and others (non-governmental organizations, civil servants) (8.2%). Animals such as cattle, sheep, hens, mules, and donkeys are reared by the farmers. Additionally, both modern and traditional bee-keepers were present. The majority of the population in the district live in houses made of mud and corrugated iron roofs.

The majority of Bure district has a subtropical zone (Woina-Dega) climate with annual mean minimum and maximum temperature of 9.9 °C and 29.2 °C, respectively, and 2,000 mm mean annual rainfall range being 1,350–2,500 mm. The major rainy season of the district is from July to September, and a small amount is obtained from May to June and from October to December. The rest of the month (January - April) are dry seasons [39].

The study was conducted in three rural villages: Bukta, Workmidr and Shnebekuma, from July 2015 - June 2016. The description of the detail of the three villages is found elsewhere [40]. Totally, these villages are malarious, bed nets were distributed for the three villages once per 3-years before malaria infestation begins, on the first week of September. Moreover, anti-malaria chemical spraying (IRS) (Deltamethrin, K-Othrine Flow) was administered to the three villages according to the national spraying operation guidelines [11].

2.2 Survey of Vertebrate Hosts

Humans and domestic animals (hens and mammals) census report were obtained by interviewing the head of households during house to house visits in the district. The numbers of humans and domestic vertebrates in neighboring houses were not counted. Potential blood hosts for *Anopheles* from PSCs were not included due to the presence of very low number of engorged *Anopheles.*

2.3 Determination of Abdominal Status of *Anopheles* Mosquitoes

The abdominal conditions of *Anopheles* were determined as the result of blood digestion and ovarian development using standard keys as unfed, freshly fed, half-gravid, and gravid [41]. At the end, freshly fed and half gravid of the Anophelines was taken into Jima University for blood meal ELISA (Enzyme Linked Immune Sorbent Assay) test.

2.4 Identification of the Blood Meal Sources of *Anopheles* Mosquitoes

The origin of blood meals from all freshly fed and a few half-gravid indoor resting, exophagic and endophagic females collected using the center for disease control and prevention (CDC) light trap catches (LTCs) were identified as human and bovine using ELISA [42]. For all collected mosquito species, only freshly blood fed and half-gravid of *An. arabiensis*, *An. funestus*, *An. coustani*, *An. squamosus* and *An. cinereus* were assayed using peroxidase-conjugates for human and phosphatase - conjugated goat anti-bovine IgG for bovine.

That is, only the abdomen of fresh blood fed and half-gravid *Anopheles* species were cut with sterile forceps and placed in a labeled eppendrof tube, separately. After adding 100 µl PBS, the abdomen was crashed using electrical pestle. Finally, the pestle was rinsed with 100 µl PBS to have a total of 200 µl final volumes. Then 100 µl of homogenate, 100 µl positive (animal serums, at 1/ 100 in PBS) and 100 µl negative (from a laboratory colony of *An. arabiensis* adults, not fed with blood) as a control were added in 96-ELISA plates according to the prepared ELISA-sheet. Plates were covered (to avoid contamination and evaporation) and incubated (helps the marker to become attached to well surface) at room temperature for 2 hours. After incubation, the wells contents were discarded and banged five times on the tissue paper (to avoid the remained content) and washed (ELISA-washer) three times with 200 µl PBS-Tween-20 (to remove unattached marker). About 50 µl human peroxidase conjugate (1° attaches to sample) (Lot No: 023M4782; Batch No: 023M4782; Product No: A0170) was added; plates were covered and incubated for one hour at room temperature. Plates were washed by ELISA-washer three times with 200 µl PBS-Tween-2, and 100 µl of ABTS [2, 2'-azino-bis (3- ethylbenzothiazoline-6-sulphonic acid] was added in each plate/ well and incubated for thirty minutes for human blood detection. ABTS peroxidase substrate was prepared from peroxidase of substrate solution-A (Lot No: 080775; Product Code: 50-64-00) and from peroxidase substrate - solution-B (Lot No: 080831; Product Code: 50-65-00). For Bovine blood sources, 50 µl bovine phosphatase conjugate (Lot No: 062M4761V/ Sigma-aldrich.com) was added, then covered and incubated for one hour at room temperature. The wells were washed three times with 200 µl PBS-Tween-20 by ELISA-washer and 100 µl of pNPP (Cat No: 0421-01; Lot No: H4014-VG96) substrate was added in each plate and incubated for one hour. Finally, positive samples, included positive control were changed into blue green colors for human blood (peroxidase) and dark yellow reactions (phosphatase) for bovine blood (visually seen). Immediately, using ELISA reader, the value of each plate was determined at 405 nm wavelength. Samples were considered positive if absorbance values exceeded two times the mean of three negative controls, unfed mosquitoes.

2.5 Data Analysis

Data were entered and cleaned using 2007-Microsoft Excel and analyzed using SPSS software package version-20.0 (SPSS, Chicago, IL, USA). HBI and bovine blood index (BBI) were calculated as the proportion of the mosquitoes fed on human and bovine blood meals out of the total blood meals determined/ tested [22]. Mixed (human + bovine) blood meals were added to the number of a human and bovine blood meals when calculating the overall HBI and BBI. The presences of significant difference between HBI and BBI, and indoor and outdoor HBI/ BBI were checked by Independent- T-test. ($p < 0.05$). Variation among blood meal sources (host types) for *Anopheles* mosquitoes was analyzed separated by one-way ANOVA. Tukey HSD was run for mean separation variation (in ANOVA) was separated by Tukey test (HSD) ($p < 0.05$). Before applying mean comparison, normality of blood meal sources (host types), HBI and BBI data were checked and data were log transformed [log 10(x + 1)]. Every statistical test significance was considered at $p < 0.05$ during the analysis. Foraging ratios (FRs) were determined to obtain the proportion of blood meals from occurring in human and cattle only. FRs were calculated as the percent of female *Anopheles* mosquitoes (five species as described in result area) containing blood of a particular host divided by the percent of the total available host population represented by the particular host [35] as follows:

$$FR: (NAE/NTE)/(NAP/NTP)$$

Where, FR: foraging ratio of Anopheles species,

NAE: number of engorged female mosquitoes containing blood from host-1

NTE: total number of engorged females,

NAP: number of hosts of type one in the collection area, and

NTP: total number of hosts of all types in the collection area.

Foraging ratio of 1 indicated neither a selective bias nor avoidance of a particular host animal (opportunistic = equally feeding); FRs significantly > 1 indicated a selective bias, and values < 1 indicated avoidance of a host in favor of other available hosts [35, 23]. However, in our study, the percentage of the FRs was calculated for only human and cattle, and comparison were made between the two hosts. The host preference indices (HFI), which is defined as the observed proportion of feed on one host with respect to another divided by the expected comparative proportion of feeds on these two hosts [23, 16], the formula as follows:

$$HFI = (Nx/Ny)/(Ax/Ay)$$

Where 'Nx' and 'Ny' are the mean numbers of blood meals taken from hosts 'x' and 'y' per study site, respectively, and 'Ax' and 'Ay' are the mean numbers of hosts 'x' and 'y' per study site, respectively. An index of 1 indicates equal feeding on the two hosts. An HFI > 1 indicate that host 'x' was preferentially fed upon, whereas a value < 1 indicated that host 'y' is preferentially fed upon [16]. HFIs were calculated for each pair of hosts (Humans: Cattle) [23].

3. Results

3.1 Abdominal Status of Female *Anopheles* Mosquitoes

The overall abdominal status of each adult female *Anopheles* mosquito is presented in Table 1 and Fig. 2. Of 4,703 *Anopheles* mosquitoes collected, the higher proportion of mosquitoes were unfed (69.7%), followed by fed (24.5%), gravid (3.9%), and half-gravid (1.9%).

Overall, 56.0% (n = 646/ 1153) fed Anopheline mosquitoes were from indoor and 44.0% (n = 506/1153) were from outdoor collection. The proportion of half-gravid (HG) mosquitoes collected outdoor (n = 51, 56.7%) was greater than HG mosquitoes collected indoor (n = 39, 43.3%). Collection method comparison indicated that over 99.6% of unfed (UF), fed (F), HG, and gravid (G) were collected by LTCs while the remaining catches were by Pyrethrum Spray Catches (PSCs). However, artificial pit- shelters (APSs) was not fully productive at all (Table 1). Because of the unsuccessful catches by PSCs and APS, the degrees of exophily and endophily behavior were not determined.

For blood ELISA, 617 specimens (LTs and PSCs) belonging to *An. arabiensis* (n = 209), *An. funestus* (n = 217), *An. coustani* (n = 123), *An. squamosus* (n = 54) and *An. cinereus* (n = 14) were only analyzed.

3.2 Compositions and Abundances of Potential Vertebrate Hosts for *Anopheles* Mosquitoes

A total of 3,803 hosts was recorded from 324 surveyed houses in three villages. Of these, hosts from Bukta accounted for 39.2%, Shnebekuma 33.0% and Workmidr for 27.8%. Hosts included: bovines (40.0%), humans (37.7%), sheep (16.0%), donkeys (0.8%), mules (0.7%), chickens (4.0%) and dogs (0.7%). From all, a higher proportion of human and cattle hosts were recorded in the study area ($p > 0.05$) (Table 2).

3.3 Blood Meal Indices of *Anopheles* Mosquitoes

Table 3 shows the blood meal origins and HBI of *Anopheles* mosquitoes by site and collection method. Overall, 617 *Anopheles* mosquitoes (fed and HG) belonged to five species (*An. arabiensis*, *An. funestus*, *An. coustani*, *An. squamosus*, and *An. cinereus*) were tested by ELISA. Of these, 94.5% (n = 583) were positive for host blood antigen and the rest 5.5% (n = 34) were unidentified. From 583 positive samples (LTs and PSCs), the largest proportion (93.5%, n = 548) was from LTCs and the least proportion (1.0%, n = 6) was from PSCs. Out 577 positive samples (LTs), the majority (54.7%) of them was from indoor collection.

Of 208 tested *An. arabiensis*, only 94.7% were positive. Of these positive blood meals, 91.8% had mixed blood meal (LTs-in and out) and 3.0% had only bovine blood meal origin. No single *An. arabiensis* specimen had blood from human only. However, the indoor and outdoor HBI (t = 1.587; df = 22; p = 0.127), BBI (t = 1.406; df = 22; p = 0.173), and overall HBI and BBI of *An. arabiensis* had not shown any statistically significant difference between them (t = -0.05; df = 22; p = 0.961).

The result of this study revealed that 213 tested *An. funestus* specimens, only 95.8% of them were positive for blood meal-ELISA. Out of the total positive specimens, 91.5% had mixed blood meal (LTs-in and out), and the remaining (4.2%) was only bovine. From mixed blood meals, most (54.9%) of them were from indoor collection and 40.8% were from outdoor collection. However, a single bovine blood meal was found from mosquito specimen collected indoor. The overall (single plus mixed) HBI of *An. funestus* was 91.5%, which was slightly less than the overall BBI (95.8%). However, the indoor and outdoor HBI (t = 1.322; df = 22; p = 0.2), BBI (t = 1.355; df = 22; p = 0.189), and overall HBI and BBI of *An. funestus* had not shown any statistically significant difference between them (t = -0.168; df = 22; p = 868).

A total of 122 specimens of *An. coustani* were tested for blood-ELISA. The majority of them (92.6%) were positive for blood feeding, and the rest was bovine (4.1%) and human blood meals only (1.6%). Of these, the overall mixed blood meal (LTs-in and out) was 86.9%. For all positive samples, 49.2% were from indoor collection, and 43.4% were from outdoor collection. *An. coustani* was the only species that had only human blood meal alone. However, the indoor and outdoor HBI (t = 0.546; df = 22; p = 0.591) and BBI (t = 0.662; df = 22; p = 0.515), overall HBI and BBI *An. arabiensis* had not shown any statistically significant difference between them (t = -0.043; df = 22; p = 0.966). Similar to this species, both *An. squamosus* and *An. cinereus* had not shown any statistically significant difference between indoor and outdoor HBI and BBI, the overall HBI and BBI (p > 0.05).

3.4 Foraging Ratio and Host Feeding/ Preference Index of *Anopheles* Mosquitoes

Foraging ratio values and feeding preference index of *Anopheles* mosquitoes are presented in Table 4. Humans and cattle were the most common vertebrate hosts in the study area (Table 2). The FR for a human was slightly > 1.0 for all *Anopheles* species. Similarly, the FR for cattle was slightly > 1.0 for all *Anopheles* species. Calculation of the HFI for each pair of vertebrate hosts revealed that humans were a bit preferred blood meal source to bovine for all species (except *An. squamosus*), but did not exhibit a marked host selection (Table 4).

4. Discussion

In this study, over 95% of Anopheline mosquitoes were collected by LTCs. This is identical with other findings that, more mosquito vectors were trapped while host seeking than resting [43,44,45,46,47]. Of the 4,703 collected mosquitoes, the largest proportions (69.7%) were unfed. Consistent with our study, Fornadel *et al.* [48] in Zambia, Bashar *et al.* [49] in Bangladesh and Getachew *et al.* [47] in Ethiopia collected most of the unfed *Anopheles* mosquitoes using LTCs. These unfed mosquitoes are stimulated and attracted by light generated by incandescent bulbs from light-traps [49,50]. As a result, mosquitoes were caught while searching for their blood meals before they took blood. However, contradicts to this study, Animut *et al.* [51] collected most of the fresh fed Anophelines species than unfed using light traps in Ethiopia. The catches of higher numbers of fresh fed mosquitoes using light traps could be due to recapturing of mosquitoes after repeatedly feeding behavior [52].

The present study revealed that no single *An. arabiensis* specimen had a human blood meal origin. It is inconsistent with reports of Massebo *et al.* [53] (8.0%), Animut *et al.* [51] (33.7%), Yewhalaw *et al.* [54] (6%), Getachew *et al.* [47] (50.7%) and Ngom *et al.* [55] (40.1%) in Ethiopia and Senegal. On the other hand, the presence of single bovine blood origins of *An. arabiensis* (2.9%) was very minimum as compared to Massebo *et al.* [55], Animut *et al.* [51], Yewhalaw *et al.* [54] and Getachew *er al.* [47] reports; which found 7.1%, 38.2%, 23.5%, and 20.9% of bovine blood meals in Ethiopia, respectively (using LTs). This probability due to the presence of large number of human populations as like as cattle for blood meal sources, which divert more *An. arabiensis* to feed both human and cattle.

In the present study, the majority of *An. arabiensis* had a mixed blood meal which was higher when compared to other records [47,51,54, 55] who reported 65%, 13.2%, 1.6%, and 4.4% in various parts of south and South west of Ethiopia, respectively. The highest proportion of mixed feeding implies that the sites are mixed dwellings (humans and cattle) [28,56]. The practice of having human, cattle, hens, donkeys, mule, etc in the same house (one house) was confirmed during this survey, which contributed for having higher mixed feed by providing alternative hosts (Personal obsr.). Moreover, it is also probabibily associated with the incidence of very high disturbances [33] or climatic factors [49,56]. Generally, in this study the proportion of mixed blood meals were higher than single feeding, implying that *An. arabiensis* has plasticity feeding behavior in the area. Other findings also strengthen this reality too [57,58,59,60].

The overall HBI and BBI, and indoor and outdoor HBI and BBI of *An. arabiensis* had not shown any statistically significant difference between them indicating its opportunistic feeding behaviour in the area. Similar feeding preferences are reported from southern Ethiopia where people and livestock either share the same houses or where cattle are kept separate but close to houses during the night [51,61].

The result of this study demonstrated that most of *An. funestus* had a mixed blood meal (humans and cattle) but no single human blood meal was detected as similar to *An. arabiensis*. The absence of single human blood meal source is in agreement with Massebo *et al.* [55], on the other hand it is contradicting to other studies [62,63], who reported extremely high single HBI for *An. funestus* in Kenya (90.8%, 99.5%) and Cameroon (98%), respectively. Therefore, our result indicated that *An. funestus* changes its blood meal sources from only human [58,64] to both cattle and human. Various reports indicated that *An. funestus* now found to feed blood of both humans, goats, calves, chickens, cows, dogs and goats and equines [57,58,59,60], is depend on availability of host types.

The overall (single plus mixed) HBI and BBI, and indoor and outdoor HBI and BBI of *An. funestus* had not shown any statistically significant difference between them, which indicating its opportunistic feeding behaviour in the area. However, the overall (single plus mixed) HBI (91.5%) and BBI (95.8%) of *An. funestus* were greater than as compared with other findings made in Kenya (HBI = 25.2%; BBI = 57.7%) [59] and in Ethiopia (HBI = 86.0%; BBI = 14.3%) [65]. These equal proportions of blood meal sources in our study were due to the mixed dwelling activities carried out by the three villages (in Bure district).

The blood ELISA result of *An. coustani* indicated that, the majority (92.6%) of them had mixed blood meals, and the rest had only bovine (4.1%) and human blood meals (1.6%). Though proportionally different, Muriu *et al.* [59] reported 71.4% and 5.4% of *An. coustani* that fed human and bovine blood alone in Kenya, respectively. In southwest Ethiopia, Getachew *et al.* [47] also reported that *An. coustani* with 3.3% (2/59) human and 92.8% (64/69) bovine blood meals alone. In our study *An. coustani* with human blood meals was found in CDC-LTs, which is corroborated with Getachew *et al.* [47]. On the contrary, Yewhalaw *et al.* [54] didn't detect any *An. coustani* with human blood in southwest Ethiopia. Though this species still is not well confirmed as a malaria vector in Ethiopia only, many findings in Ethiopia [54,66,67], Cameron [68], and Kenya [69,70] reported *An. coustani* with malaria parasites (*Plasmodium spp.*). Therefore, all these results suggested that *An. coustani* would be the responsible malaria transmission in Bure district.

Moreover, the FRs for human were > 1.0 for all of the anophelines species, except *An. squamosus*. Based on the HFIs for each pair of vertebrate hosts, humans were relatively the preferred blood source for all tested species, except for *An. squamosus*. The limitation of this study is that blood meal sources of *Anopheles*

mosquitoes (blood ELISA) from sheep, donkeys, mules, hens, and dogs were not determined though they were recorded in the study area. Being this, HFI could not be calculated for the aforementioned vertebrate hosts.

Conclusions

The animal census survey indicated that human, bovine, sheep, donkeys, mules, hens, and dogs were the common vertebrate hosts in the study area; however, the proportion of human and bovine were significantly high. Therefore, *Anopheles* mosquitoes have many alternative blood meal sources. Houses were traditional (made of mud) and served as cooking, sleeping and tethering livestock, result in increased indoor temperature. Hence, this microclimate attracts more mosquitoes and provides more access to blood meal sources. Being this, a relatively high proportion of indoor feeding (indophagic) mosquitoes were recorded.

All the Anophelines mosquitoes assayed for Blood ELISA indicated the presence of high proportion of mixed blood meals (humans and cattle) which are very important than single human meals because mixed feed tends to diminish the density of gametocytes in the mosquito stomach, thereby reducing the chance of fertilization of the female gamete and reduce the chances of malaria vector becoming infected [33,49,59,55,71]. Moreover, among assayed Anophelines mosquitoes, only *An. coustani* had single human blood; implying that his species may be linked with malaria transmission. Therefore, proper investigation is required to be more certain on its role as a malaria vector. Further confirmation is needed to whether the existed intervention activity is fully effective or not to *An. coustani*. Totally, combination zooprophylaxis should be reinforced as a means of vector control working because the study sites are mixed dwelling.

Abbreviations

MoH; Ethiopian Ministry of Health; PMI's: President's of Malaria Imitative; LLINs: Long Lasting Insecticide Treated Nets; IRS: Insecticide Residual Spraying; HBI: Human Blood Index: FR: Forage Ratio; FI: Feeding Index; HFI: Host Preferences Index; WHO: World Health Organization; ELISA: Enzyme Linked Immune-sorbent Assay; CDC: Center for Disease Control and prevention; LTCs: Light Trap Catches; BBI: Bovine Blood Index; HG: Half Gravid; PSCs: Pyrethrum Spray Catches; APSs: Artificial Pit- Shelters; UF: Unfed; F: Fed, G: Gravid.

Declarations

Ethical Clearance

A collection of mosquitoes was carried out after obtaining ethical approval from the ethical review committee of Addis Ababa University (Reference No.: CNSDO/382/07/15), Amhara Health Regional Bureau (Permission Reference No.: H/M/TS/1/350/07) and the Head of the Bure District Health Office (Permission Reference No.: BH/3/519L/2). Moreover, informed consent was obtained from the selected households.

Consent for publication

No applicable.

Availability of data and materials

All data (generated and analyzed) presented in this study are available from the hand of corresponding author with a reasonable request.

Competing interests

Addis Ababa, Jima and Mizan-Tepi Universities financed this research work, but have any role in research design and data collection, conducting research, and data analysis. Therefore, all authors declare no computing interest.

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Authors' contributions

TA: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper. DY: Conceived and designed the experiments; contributed reagents, analyzed and interpreted the data; wrote the paper; EG: Contributed reagents and materials, analysis data; Wrote the paper.

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Tables

Table 1

Abdominal status of female *Anopheles* mosquitoes by place and method of collection in Bure district, Ethiopia

Blood digestion stage	**Place of collection**			**% Abdominal status by method of collection**			
	Indoor (LTs & PSCs), n(%)	Outdoor (LTs), n(%)	Total (%)	LTs, n(%)	PSCs, n (%)	APTs, n (%)	Total (%)
UF	1023(31.2)	2253(68.8)	3276 (69.7)	3267 (99.7)	9(0.3)	0	3276 (69.7)
F	646(56.0)	507(44.0)	1153 (24.5)	1145 (99.3)	8(0.7)	0	1153 (24.5)
HG	39(43.8)	50(56.2)	89(1.9)	89(100.0)	0	0	89 (1.9)
G	89(48.1)	96(51.9)	185(3.9)	183(98.9)	2 (0.1)	0	185(3.9)
Total	1797(38.2)	2906(61.8)	4703 (100)	4684 (99.6)	19 (0.4)	0	4703 (100)

Note: Unfed (UF), Fed (F), Half-gravid (HG), and Gravid (G).

Table 2

Mean difference between hosts

Identified Hosts	(M ± SE)
Human	4.419 ± 0.07[a]
Bovine (cattle)	4.681 ± 0.100[a]
Sheep	1.881 ± 0.093[b]
Donkeys	0.166 ± 0.045[b]
Mules	0.180 ± 0.046[b]
Hens	1.417 ± 0.252[b]
Dogs	0.2658 ± 0.05973[b]

Table 3

Blood-meal sources of *Anopheles* mosquito species by site and method of collection in Bure district, Ethiopia

Species	Total tested, negative and positive for Blood ELISA				Numbers of Tested (by LTs and PSCs) Positive for Blood Antigen (%)			Single Blood Meals (by LTs)				Mixed Blood Meals (both human and bovine blood meals)			Overall (single + Mixed) numbers & % from LTs only	
	Total Tested Specimens from		Total LTs		LTs		PSCs	Human, Only, n (%)		Bovine only, n (%)		LTs, n (%)		PSC, n (%)	HBI, n (%)	BBI, n (%)
			Tested Un know (%)	Tested positive (%)	In n (%)	out n (%)	n (%)	In	Out	In	Out	In	Out			
	LTs	PSCs														
An. arabiensis	208	1	11 (5.3)	197 (94.7)	124 (60)	73 (35.1)	0 (0.0)	0	0	6 (2.9)	0	118 (56.7)	73 (35.1)	0 (0.0)	191 (91.8)	197 (94.7
An. funestus	213	4	9 (4.2)	204 (95.8)	117 (54.9)	87 (40.8)	4 (100)	0	0	9 (4.2)	0	108 (50.7)	87 (40.8)	4 (100)	195 (91.5)	204 (95.8
An. coustani	122	1	9 (7.4)	113 (92.6)	60 (49.2)	53 (43.4)	1 (100)	2 (1.6)	0	1 (0.8)	4 (3.3)	57 (46.7)	49 (40.2)	1 (100)	108 (88.5)	111 (91)
An. squamosus	54	0	3 (5.6)	51 (94.4)	24 (44.4)	27 (50)	0 (0.0)	0	0	0	12 (22.2)	24 (44.4)	15 (27.8)	0 (0.0)	39 (72.2)	51 (94.4
An. cinereus	12	2	2 (16.7)	10 (83.3)	6(50)	4 (33.3)	1 (50)	0	0	0	0	6 (50)	4 (33.3)	1 (50)	10 (83.3)	10 (83.3
All Total	609 (100)	8 (100)	34 (5.6)	577 (94.7)	333 (54.7)	244 (40.1)	6 (75)	2 (0.3)	0	16 (2.6)	16 (2.6)	314 (51.6)	228 (37.4)	6 (75)	544 (89.3)	574 (94.3

Table 4

Foraging Ratio (FR) and Host Preference/ Feeding Index (HFI) of *Anopheles* mosquitoes in Bure, from July 2015 – June 2016

Hosts	Vertebrate Host, n (%)	Mosquito species	HBI	BBI	Total FR		HPI Human: Bovine
					HBI	BBI	
Human	1435 (48.6)	*An. arabiensis*	91.8	-	1.88	-	1.03
Cattle	1520 (51.4)		-	94.7	-	1.84	
Human	1435 (48.6)	*An. funestus*	91.5	-	1.88	-	1.01
Cattle	1520 (51.4)		-	95.8	-	1.86	
Human	1435 (48.6)	*An. coustani*	88.5	-	1.82	-	1.03
Cattle	1520 (51.4)		-	91	-	1.77	
Human	1435 (48.6)	*An. squamosus*	72.2		1.49	-	0.81
Cattle	1520 (51.4)		-	94.4	-	1.84	
Human	1435 (48.6)	*An. cinereus*	83.3	-	1.71	-	1.06
Cattle	1520 (51.4)			83.3	-	1.62	

Figures

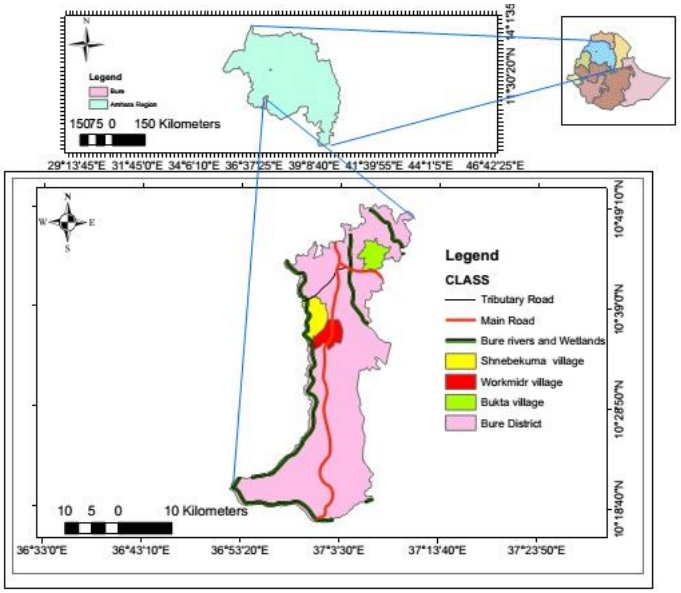

Figure 1

Map of the study area. a) Ethiopia, b) Amhara region, and c) Bure district. 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.

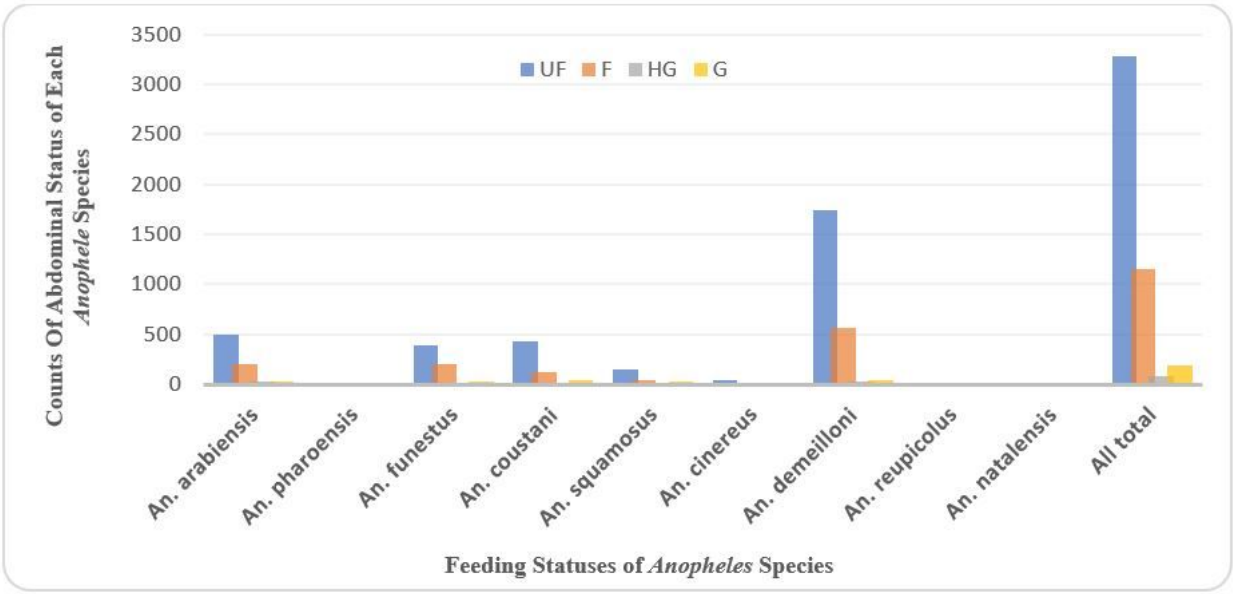

Figure 2

Abdominal statuses of Anopheles mosquito species in Bure district, Ethiopia

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