Species Diversity and Entomological Inoculation Rate of Anophelines Mosquitoes in an Epidemic Prone Areas of Bure District, Northwestern Ethiopia

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Abstract

Background: Malaria is the leading health problem in Ethiopia. The country has been prevented malaria vectors mostly using long-lasting insecticide-treated nets, the application of indoor residual spraying chemicals, and source reductions. Before interventions, identifying the responsible malaria vector in disease transmission (sporozoite rate) is very vital; hence, the present study was designed to assess species diversity and entomological inoculation rate of *Anopheles* mosquito in Bure district, Northwest Ethiopia.

Methods: Adult mosquitoes were collected from July 2015 to June 2016 using the center for disease control and prevention light traps, pyrethrum spray catches, and artificial pit shelters. Mosquitoes were morphologically identified. Following this, *An. gambiae* s.l was identified molecularly. Head-thorax sporozoite infectivity of the adult female *Anopheles* mosquitoes was assessed using enzyme-linked immunosorbent assays.

Results: Morphologically, nine species of the genus *Anopheles* were identified in the three villages, composed of *Anopheles demeilloni*, *An. arabiensis*, *An. funestus*, *An. coustani*, *An. squamosus*, *An. cinereus*, *An. pharoensis*, *An. rupicolus*, and *An. natalensis*. Of these species, *An. demeilloni* was the most predominant, whereas *An. cinereus*, *An. rupicolus* and *An. natalensis* were the least representative species ($p < 0.0001$). Greater number of adult *Anopheles* mosquitoes were collected in Shnebekuma, non-irrigated villages than non- irrigated village (Workmidr) and irrigated village (Bukta) ($p < 0.0001$). The overall *Plasmodium* infective rate (*P. falciparum* and *P. vivax*) in the district was 0.31%. The overall annual sporozoite rate in non-irrigated villages (Shnebekuma and Workmidr) was 0.35%, whereas zero in irrigated village (Bukta). The overall estimated EIR of *Anopheles* mosquitoes was 5.7 infectious bites /person /year for both *P. falciparum* and *P. vivax* in the district. The annual EIR *Anopheles* species in non-irrigated villages was 5.65 ib/p/y, which was higher than irrigated village (0 ib/p/y).

Conclusions: Both the primary (*An. arabiensis*) and secondary (*An. funestus* and *An. pharoensis*) malaria vectors of Ethiopia were identified in the three villages. Three of *Anopheles* species, *An. arabiensis, An. funestus*, and *An. coustani* were found to be infected only in irrigated villages. Source reduction and proper usage of long-lasting insecticide nets and indoor residual spraying could be implemented in the non- irrigated villages to cut the vector abundance and EIR.

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 Africa, malaria transmission is varied within districts, between seasons [5, 6, 7], between houses and within agricultural zones [6].

Several factors have reported to influencing the vectorial role of mosquitoes in disease transmission including vector density and heterogeneity, resting and blood-feeding behavior, host preference, longevity, susceptibility to *Plasmodium* infection, micro-ecological, socioeconomic factors, poor environmental sanitation, and environmental modification [8, 9, 10, 11]. Therefore, a better understanding of these factors is a precondition in planning effective vector control measures [12].

Moreover, EIR is an important indicator of malaria transmission that helps relate both the human-biting activity of the *Anopheles* vectors and the risk to humans to malaria infection [13, 14]. The dynamics of an EIR are governed by the fluctuation in vector densities and their sporozoite rates [15]; which again influenced by temperature, altitude, rainfall, and urbanization [16].

Therefore, EIR is considered a more direct measure of transmission intensity [16] and its values are used to quantify the impact of vector control measures [17], such as LLINs, IRS, and source reduction [18], malaria endemicity [19] and the risk of an epidemic [20]. Thus, effective vector control measures directly reduce malaria incidence (sporozoite rates) and then EIRs [18, 21, 22].

Generally, understanding the dynamics of malaria transmission in a population is very significant; it provides an insight into the magnitude of the problem and helps to describe when and where the greatest risk occurs. It also helps to facilitate the development and implementation of appropriate control strategies [14, 23, 24].

In Ethiopia, malaria is the leading health problem of the country [25] because three-fourth (75%) of the total area of the country is malarious and more than two-third (approximately 68%) of the total populations live below 2,000 meters above sea level /m.a.s.l/ [26]. In the country, the nature of malaria transmission is seasonal, and unstable [27], vary with elevations, temperature, and rainfall [28, 29].

The major malaria vector in Ethiopia is *Anopheles arabiensis* while *An. pharoensis*, *An. funestus* and *An. nili* are secondary vectors [30, 31]. The Ethiopian federal ministry of health has four key intervention strategies to prevent and treat malaria: 1) spraying houses with insecticides (IRS), 2) mass distribution of LLINs, 3) having lifesaving drugs and 4) treatments for pregnant women [30].

In particular to Amhara region, Ethiopia, malaria is obviously a serious health problem [32, 33, 34]. Bure district is one of the major malaria focus areas in the region known by the high epidemic, which has claimed many lives [29, 35, 36, 37]. Previously conducted epidemiological studies in the region indicated that the prevalence of malaria has shown a sharp reduction in many districts including Bure surrounding [38]. However, information on entomological indices is incomplete in Amhara region [39] and totally absent in the Bure district in particular. Therefore, the present study was designed to assess species diversity, sporozoite and entomological inoculation rates of *Anopheles* mosquito in Bure district, 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. (Figure 1). Socioeconomically, 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 populations in the district live in houses made of mud and corrugated iron roofs.

The majority of Bure districts has 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 months (January - April) are dry seasons [33].

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 are 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 [30].

2.2. Collection, Identification and Processing of Anopheline Mosquitoes

2.2.1. Collection: *Anopheles* mosquitoes were sampled longitudinally from July 2015 - June 2016. Entomological surveys were conducted monthly in each village, for one year using Center for LTCs, PSCs and APSs. In each village, 9 houses for LTCs and 10 houses for PSCs were randomly selected and scattered in near to the breeding sites, in the middle and periphery sides of the village. In parallel, 27-miniature light traps were prepared to collect the outdoor host seeking mosquitoes for the three villages, each had 9- LTCs. Additionally, six APSs were prepared in three villages to collect outdoor resting mosquitoes; each village had two.

Indoor host-seeking *Anopheles* mosquitoes were collected from 6:00 PM (sunset) to 6:00 AM (sunrise) by using miniature LTCs (Model 512; J. W. Hock Co., Atlanta, USA) once per month per house [41, 42]. In the same trends, the outdoor host seeking mosquitoes were collected by LTCs from 06:00 AM to 06:00 PM hrs once per month.

Indoor-resting mosquitoes were collected in the mornings from 6:00 AM to 8:30 AM hrs using PSCs for the study period. Collection was made using white floor sheets, hand lenses, Baygon aerosol (Tetramethrin:0.4% and Permethrin:0.4%; SC. Johnson & Son. Inc, USA), small petri-dishes, paper cups with net covers, forceps, cotton wool, and a torch [43]. Additionally, outdoor-resting mosquitoes were collected in the morning from 6:30 AM - 7:30 AM from APSs (1.5 m depths, 1.0 m width, and 1.2 m length) using a handheld mouth aspirator [44]. The number of human occupants and other potential vertebrate hosts in each surveyed house during the previous night were recorded. Moreover, the house condition of each surveyed house was recorded; including the types of house, type of wall, and number of LLINs used and spray status.

Outdoor-resting mosquitoes were collected from artificially made pit shelters by using a handheld mouth aspirator, paper cup with net covers, cotton wool, torch, and pencil. APSs were constructed under the shade of various dense shrub trees 10 - 15 meters away from the resident villages. APSs had 1.5 m depths, 1.0 m width, and 1.2 m length (1.5 m × 1.0 m × 1.2 m). Approximately 0.5 m from the bottom of each pit-shelter, a 30-cm horizontal deep cavity was prepared in each of the four sides [44]. A collection was made from 6:30 AM - 7:30 AM hrs. Before collection begins, the mouth of each pit shelter was covered with insecticide untreated white net to prevent mosquitoes from escaping and for visibility purpose. Resting mosquitoes was collected for about 10 - 20 minutes in each pit.

Identification: Mosquitoes collected by LTCs, PSCs and APSs were identified morphologically at genus level using taxonomic keys [45,46,47]. *Culex* and male *Anopheles* were recorded and discarded. Moreover, morphological identified and individually preserved *An. gambiae* specimens were identified by species-specific PCR [48] at the Molecular Biology Laboratory of Tropical and Infectious Diseases Research Center, Jima University. DNA was extracted from individual preserved *An. gambiae* complex species based on [49]. Then, DNA amplification was carried out. Following this, gel electrophoresis was carried out [48]. At the end, agarose-gel was placed on UVP (Photo Doc-It- imaging system or UV-Trans- illuminator) to see the nature of the bands (Appendix 1a & b). Those mosquitoes that remained unamplified (without any band on the gel) were tested three times in an independent manner.

2.3 Determination of *Plasmodium* Infection Rate (SIR) and Entomological Inoculation Rate To determine the infection rate, the heads and thoraces of *An. arabiensis*, *An. funestus* group, *An. coustani*, *An. squamosus* and *An. cinereus* females were tested by ELISA for CSP of *P. falciparum*, *P. vivax*-210 and *P. vivax*-247 by the method developed by Wirtz *et al*. [50]. The dried "head-thorax" portion of individual female *Anopheles* was placed in 1.5ml eppindorff tube. 50µl of grinding buffer (GB) was added and grinding was made using electrical-pestle. After completion of grinding, the pestle was rinsed with two 100 µl volumes of GB, to have 250 µl final volumes. Then, 50µl of the capture monoclonal antibodies (MAb) of *P. falciparum* (Cat No: 37-00-24-2; Lot No: 2013pCAP), *P. vivax* (*P.v*-210, Lot No: 060415), and *P. vivax* (*P.v*-247, Lot No: 030276) were added to labeled 96-wells of separate polyvinyl chloride microtiter plates in duplicates (six in number). And then, the wells covered and incubated at room temperature for 30 minutes. After incubation, well contents were removed, banged five times on a paper towel, and then 200 µl BB was added again in each well, covered, and incubated for one hour to block the remaining active binding sites. Well contents were then aspirated and banged five times on a paper towel, and 50 µl of each mosquito triturate (including positive and negative control) was added according to the prepared sporozoite ELISA-sheets and covered and placed to incubate for two hours. For positive controls, *P.f* (fragmented stock solution) for *P.f*-wells, *P.v*-210 (fragmented stock solution) for *P.v*-210-wells and *P.v*-247 (fragmented stock solution) for *P.v*-244-wells, and for negative control unfed laboratory reared species were used. After 2 hrs of incubation, the mosquito triturates were aspirated and the wells were washed twice with 200 µl PBS-Tween

using ELISA-Washer /well washer (Model No: BioTek-WLx 50). Then, 50µl of peroxidase-linked MAbs of *P.f* (Cat No: 37-00-24-3; Lot No: 2013PfHRR), *P.v*-210 (Cat No: 37-00-24-3; Lot No: 060414) and *P.v*-247 (Cat No: 37-00-24-4; Lot No: 030246) were added to respective wells of the microtiter plate, then covered and incubated for one hour. After one hrs incubation, the plates were washed three times with 200 µl PBS-Tween using ELISA-Washer and 100 µl substrate solutions were added in single well and covered and incubated for thirty minutes. Substrate solutions was a mixture of ABTs peroxidase substrate solution-A (Lot No: 080775; Product Code: 50-64-00) and Peroxidase substrate solution-B (Lot No: 080831; Product Code: 50-65-00). Finally, after 30 minutes incubation, absorbance values at 405 nm were recorded from the ELISA plate reader (BioTek-ELx800). Those samples considered positive if the mean absorbance values of the duplicate assays exceeded twice the mean of the three negative controls [50].

2.4 Data Analysis

Data were entered and cleaned using 2007-Microsoft Excel and analyzed using SPSS software package version-20.0 (SPSS, Chicago, IL, USA).

Mean variance between *Anopheles* and *Culex*, between the indoor and outdoor host seeking mosquitoes was tested using independent samples T-test ($p < 0.05$). Variation in mean densities between species and species among villages were analyzed using one-way analysis of variance (ANOVA) ($p < 0.05$). Significant means (ANOVA) were separated using Tukey test (HSD).

Sporozoite rate for each species was estimated as the number of mosquitoes with sporozoite divided by the number of females examined multiplied by 100 [51]. The annual EIRs were estimated by standard method: 1.605 (No of ELISA positive from CDC light trap/No ELISA tested) X (No of *An. arabiensis/ funestus/ coustani* collected from CDC light trap/Total No of CDC light traps) x 365 days. Monthly EIR was estimated by a standard method: 1.605 (No. ELISA positive from CDC light trap/Total No. ELISA tested) x (No*An. arabiensis/ funestus/ coustani* collected from CDC light trap/ Total No number of CDC light traps) x No days per month [52]. Before running Independent-samples T-test and ANOVA, normality of data was first checked and transformed [log10(x+1)]. When significant differences were observed in ANOVA, the Tukey test (HSD-Test) was used to separate the means ($p < 0.05$). All statistical analyses were performed at the 5 % significance level.

3. Results

3.1 Species Diversity and Abundance of Anopheles Mosquitoes

A total of 11,625 female mosquitoes were collected in Bure district using LTs, PSCs and APSs. Of these, 59.5% (n = 6,922) belonged to the genus *Culex* while the rest 40.5% (n = 4,703) were from the genus *Anopheles*. The proportions of the two genera have not shown statistically significant difference (t = 1.165; df = 22; p = 0.257). Morphologically, nine species of the genus *Anopheles* were identified in the three villages, belonged to *Anopheles demeilloni*, *An. gambiae* s.l, *An. funestus* s.l, *An. coustani*, *An. squamosus*, *An. cinereus*, *An. pharoensis*, *An. rupicolus*, and *An. natalensis* (Fig. 2). Of these species, *An. demeilloni* was the most predominant whereas *An. cinereus*, *An. rupicolus* and *An. natalensis* were the least representative species (one-ANOVAs: $F_{8, 99} = 24.593$, $p < 0.0001$).

Moreover, a total of 66 specimens of *An. gambiae* s.l was identified to species by PCR, of which 63 (95.5%) were successfully amplified and identified as *An. arabiensis* (Appendix-1a & b). Only three (4.5%) specimens which were checked for three times were not amplified. Thus, all *An. gambiae* s.l collected in this study were *An. arabiensis*.

The greater number of adult *Anopheles* mosquitoes were collected in Shnebekuma, non-irrigated villages (n = 3,875) than non- irrigated village (Workmidr, n = 472) and irrigated village (Bukta, n = 356) (one ANOVA $F_{2, 33} = 19.202$, $p < 0.0001$). The mean density of each *Anopheles* mosquito by village is presented in Table 1. Significantly higher density of *An. arabiensis*, *An. funestus*, *An. squamosus* and *An. demeilloni* were recorded from Shnebekuma than other villages ($p < 0.05$). In Bukta, only *An. cinereus* was predominant. *An. pharoensis*, *An. rupicolus* and *An. natalensis* were very rare and not distributed uniformly in the three villages; however, the remaining species were abundant in all villages. *An. natalensis* was recorded only in Shnebekuma village, whereas *An. rupicolus* was found only in Bukta and Workmidr villages.

Table 1: Mean density of *Anopheles* species by village, Bure district, Ethiopia (at 3 and 33 degree

of freedom)

Species	Village			F -value	p-value
	Bukta	Workmidr	Shnebekuma		
An. arabiensis	(Mean ± SE)	(Mean ± SE)	(Mean ± SE	16.057	0.0001*
	0.73 ± 0.10^{b}	0.79 ± 0.13^{b}	1.55 ± 0.12^{a}		
An. pharoensis	0.06 ± 0.05	0.06 ± 0.05	0.15 ± 0.071	0.846	0.438
An. funestus	0.51 ± 0.13^{b}	0.45 ± 0.11^{b}	1.48 ± 0.11^{a}	23.935	0.0001*
An. coustani	0.73 ± 0.16	0.49 ± 0.12	1.11 ± 0.20	2.705	0.082
An. squamosus	0.28 ± 0.11^{b}	0.27 ± 0.09^{b}	1.01 ± 0.12^{a}	15.375	0.0001*
An. cinereus	0.47 ± 0.12^{a}	0.03 ± 0.03^{b}	$.25 \pm 0.09^{ab}$	6.356	0.005*
An. demeilloni	0.60 ± 0.10^{b}	0.90 ± 0.20^{b}	2.13 ± 0.10^{a}	33.832	0.0001*
Note: Mean (s) followed by the same letter (s) in the same row are not significantly different from each other at $p < 0.05$, Tukey HSD					

3.2 Sporozoite Rates of Anophelines Mosquitoes

Annual sporozoite rates of *Anopheles* mosquito species (collected by using LTCs) are shown in Table 2. In total, 1,474 (1,466 from light traps and 8 from PSCs) *Anopheles* mosquitoes were tested for *Plasmodium* Circumsporozoite-Protein (CSPs) which consisted of 488 *An. arabiensis*, 350 *An. funestus*, 436 *An. coustani*, 152 *An. squamosus* and 40 *An. cinereus*. Eight specimens from PSCs were excluded for the EIR calculation because they were not infected with sporozoite.

Table 2: Annual sporozoite rates and EIRs of the three *Anopheles* species in three sites in Bure district, Ethiopia (July 2015 - June 2016)

Villages Name	Sporozoite infected mosquitoes	# Collected	# Tested	# positive for –CSPs			Infective Rate				Annual EIR
				CDC-IN	CDC-OUT	Total	*P.f*, n (%)	*P.v*-247, n (%)	*P.v*-210, n (%)	Total (%)	
Bukta (Irrigated Village)	*An. arabiensis*	72	56	0	0	0	-	-	-	0	0
Shnebekuma & Workmidr (Non-Irrigated Village)	*An. arabiensis*	678	432	1	0	0	1(0.23)	-	-	1(0.23)	1.42
	Total	750	488	1	0	1	1(0.20)	-	-	1(0.20)	1.4
Bukta (Irrigated Village)	*An. funestus*	53	20	0	0	0	-	-	-	0	0
Shnebekuma & Workmidr (Non-Irrigated Village)	*An. funestus*	587	330	0	2	2	-	2(0.61)	-	2(0.61)	3.22
	Total	640	350	0	2	2	-	2(0.57)	-	2(0.57)	3.31
Bukta (Irrigated Village)	*An. coustani*	103	67	0	0	0	-	0	0	0	0
Shnebekuma & Workmidr (Non-Irrigated)	*An. coustani*	503	369	0	1	1	-	-	1(0.27)	1(0.27)	1.23
	Total	606	436	0	1	1	-	-	1(0.23)	1(0.23)	1.3
All species in Irrigated Village (Bukta)		228	143	0	0	0	0	-	0	0	0
All species in Non-Irrigated Villages (Shnebekuma & Workmidr)		1,768	1,131	1	3	4	1(0.09)	2(0.18)	1(0.09)	4(0.35)	5.65
All Total		1,996	1,274	1	3	4	1(0.08)	2(0.16)	1(0.08)	4(0.31)	5.7

Annual Sporozoite Rates and EIRs by Village, Bure district, Ethiopia

Village	Species	Total # of collected species	Total # of Tested Specimens	*P.f*, n (%)	*P.v*-247, n (%)	*P.v*-210, n (%)	Total (%)	Annal EIR
Shenbekuma	*An. arabiensis*	588	369	1(0.27)	0	0	0.3	2.9
Shenbekuma	*An. funestus*	544	312	0	1(0.32)	0	0.3	4.3
Shenbekuma	*An. coustani*	389	297	0	1(0.34)	0	0.3	3.4
Workmidr	*An. funestus*	34	18	0	0	1(5.6)	5.6	5.1

Of 1,466 *Anopheles* mosquito specimens examined for CSP, only 4 specimens were positive for CSPs. A single *An. arabiensis* was positive for *P. falciparum* and a single *An. coustani* and two *An. funestus* specimens were positive for *P. vivax*. The overall *Plasmodium* infective rate (*P. falciparum* and *P. vivax*) in the district was 0.31% (4/1,274). Infection rate of *An. arabiensis*, *An. funestus*, and *An. coustani* was 0.2% (1/488), 0.57% (2/350) and 0.23% (1/436), respectively. The two infected *An. funestus* specimens and the single infected *An. coustani* specimen were collected outdoor while the single *An. arabiensis* specimen was collected indoor (Table 3).

All sporozoite-infected *Anopheles* mosquito specimens were collected only from non-irrigated villages. The overall annual sporozoite rate in non-irrigated villages was 0.35%; *An. funestus* accounted for 0.61% (2/330), *An. coustani* accounted for 0.27% (1/369), and *An. arabiensis* accounted for 0.23% (1/432). Of the non-irrigated villages, three infected mosquitoes were found in Shnebekuma (Table 3). Monthly sporozoite rate distribution is shown in Table 3. Infected *Anopheles* mosquitoes were collected in three different months; July, December, and January.

3.3 Entomological Inoculation Rate of Anopheline Mosquitoes

Estimate annual and monthly EIRs of *An. arabiensis*, *An. funestsu* and *An. coustani* are shown in Tables 2 and 3. The overall estimated EIR of *Anopheles* mosquitoes was 5.7 infectious bites /person /year (ib/p/y) for both *P. falciparum* and *P. vivax* in the district. In particular, the overall *P.v* EIR of *An. funestus*, *P.f*

-EIR of *An. arabiensis* and *P.v*-EIR of *An. coustani* were 3.3 ib/p/y, 1.4 ib/p/y and 1.3 ib/p/y, respectively.

Estimates of the EIRs of the three species were also made individually for the three villages (Table 2). The annual EIR of the three *Anopheles* species in non-irrigated villages was 5.65 ib/p/y, which was higher than irrigated village (0 ib/p/y). This variation was true for each species. The overall *P.f*-EIR of *An. arabiensis* was 1.42 ib/p/y in non-irrigated villages and higher than irrigated village (0 ib/p/y). From non-irrigated villages, Shnebekuma had 2.9ib/p/y for *An. arabiensis* while 0ib/p/y. for Workmidr. The overall *P.v*-EIR of *An. funestus* was 3.2 ib/p/y in non–irrigated villages and zero ib/p/y in irrigated village. Of the non-irrigated villages, Workmidr had higher *P.v*-EIR for *An. funestus* (5.1 ib/p/y) than Shnebekuma (4.3 ib/p/y). The *P.v*-EIR of *An. coustani* was 1.23 ib/p/y in non-irrigated villages. Among non-irrigated villages, only Shnebekuma had 3.4ib/p/y for *An. coustani.*

Seasonal variation of an EIR is shown in Table 3. The highest monthly EIR for *An. arabiensis* was 1.6 ib/p/month during the main rainy season; July. Monthly EIR for *An. funestus* was highest (1.7 ib/p/month) in the dry season (January) followed by a December (1.3 ib/p/month). Monthly EIR for *An. coustani*, was 1.1 ib/p/month and detected in December.

Table 3
Monthly distribution of CSP–positive (sporozoite rate) and EIR of the three *Anopheles* species using CDC-LTs in Bure district, from July 2015 - June 2016, [July - Sep = Rainy; Oct- Dec and May -June = Small rainy; and Jan - April = Dry Seasons]

Month of collection	Tested Species	# collected specimens	# Tested Specimens	# positive for *P.f* n (%)	# positive for *P.v*-247, n (%)	# positive for *P.v*-210 n (%)	Sporozoite rate n (%)	EIR
July 2015	*An. arabiensis*	25	14	1(7.14)	0	0	1(7.14%)	1.6
December 2015	*An. funestus*	16	11	0	1 (9.1)	0	1(9.1)	1.3
January 2016	*An. funestus*	39	21	0	1(4.8)	0	1(4.8)	1.7
December 2015	*An. coustani*	44	37	0	0	1(2.7)	1(2.7)	1.1

4. Discussions

The overall aim of this study was to examine the diversity and abundance of *Anopheles* mosquitoes in the three villages. It also helped to determine the sporozoite and entomological inoculation rates of *Anopheles* mosquitoes in the district. A total of nine *Anopheles* species belonging to *An. arabiensis*, *An. funestus*, *An. pharoensis*, *An. coustani*, *An. squamosus*, *An. cinereus*, *An. demeilloni*, *An. rupicolus* and *An. natalensis* were recorded in Bure district. *An. arabiensis*, the main malaria vector in Ethiopia [31, 53, 54], was also among others recorded in such high altitude area (range 2,000–2,157 m.a.s.l), which is in agreement with various findings [55, 56] where *An. arabiensis* had been recorded at an altitude of 2,110 m.a.s.l and 2,170 m.a.s.l, from the outskirts of Addis Ababa and in Tigray region, respectively. Similarly, many studies that conducted in Ethiopia [57, 58] and in Kenyan highland [59, 60, 61, 62] recorded *An. arabiensis* at higher altitude.

In our study area, well-known secondary malaria vectors (*An. funestus* and *An. pharoensis*) of Ethiopia were recorded. *An. funestus* was the second most abundant vector which was recorded in the higher altitude. Previously, *An. funestus*, had been reported from Gojam [63]. However, it is inconsistent with other reports from Ethiopia, where this species had been recorded in areas below 2,000 m.a.s.l [64, 65,66, 67]. The occurrence of this species up to 2,157 m.a.s.l could be attributed to the presence of increased temperature as a result of climate change, land cover and land use changes [62,68,69,70]. *Anopheles pharoensis* was among the least prevalent species in the study area and it had been reported from high altitude areas (between 2,110 m.a.s.l and 2,200 m.a.s.l) of Ethiopia [55,71]. This species is the secondary malaria vector in Ethiopia below 2,000 m.a.s.l [71, 72,73] though not found infected.

An. coustani was another mosquito recorded at 2,157 m.a.s.l in our study. This observation is in line with various reports [55, 60] that made in the highlands of Ethiopia and Kenya. *An. coustani* is a known malaria vector in Kenya [61,67,74,75] and was known to be a suspected vector in Ethiopia [62,76,77]. In the present survey, *An. coustani* was not only abundant (n = 606, 12.9%), but also found infected with *Plasmodium vivax.* All these information indicated that this species is really would be the other potential malaria vectors in Ethiopia in the future.

In the present study, *Anopheles'* mosquitoes were collected in three villages, but highest proportions of adult *Anopheles* mosquitoes were collected in non-irrigated villages (Shnebekuma and Workmidr) than irrigated village (Bukta). This result was not comparable to other findings that made in Ethiopia [78,79] and in Africa [80, 81, 82].

Higher proportions of mosquito in non-irrigated village could be due to the presence of more productive breeding habitats throughout the study period than irrigated villages [83, Personal obsr.]. On the other hands, the lower densities of Anophelines in irrigated villages (and areas near a dam) are probably due to a greater wealth created in the community via irrigation, which helped to construct good houses, resulted in the prohibition of the mosquitoes to enter in the house; thereby less numbers of mosquitoes were collected. Many studies have approved the purpose of well-constructed houses in reducing the abundances mosquito in the house [71,84,85,86]. In the irrigated village (Bukta), the health center is established at the center of the settlement (inhabitants) than non-irrigated villages. Being very near, the villagers may have a better treatment seeking behavior [6] and health experts may give more attention to irrigated surrounding because it is believed to be malaria is usually common in dammed and irrigated area [72,76,79].

In our study, the overall annual CSP rate of all the *Anopheles* species (*P. falciparum* and *P. vivax*) was 0.31%, which is almost comparable to 0.33% reported from Eritrea [87]. However, it was higher than the other studies from Ethiopia (0.12%) [88] and Tanzania (0.02%) [89]. The highest sporozoite rate in our study is due to the presence of a large proportion of the *Anopheles* mosquitoes across the year. These populations had better access to human blood meals [8–11].

The expansion of maize farming (maize pollen) in the district is enhanced mosquito populations that can participate directly in cycles of human biting and malaria transmission [90]. On the contrary, the overall sporozoite rate of all *Anopheles* species in this study is very lower as compared to other reports from different parts of Ethiopia [57,73,91,92,93] and in Africa [82, 94,95]. This very low all over sporozoite rate may be due to the cumulative effects of intensive usage of LLINs and application of IRS, resulted in a decline in human-vector contact, a decrease in the number of mosquitoes and a reduction entomological inoculation rate into people [96–99].

The result of this study showed that the intensity of malaria transmission in the study area is low. In line with the low sporozoite rate, recently made an epidemiological survey from health facilities indicated the reduction of malaria cause in the most Amhara region including Bure-surrounding district [38].

The overall annual *P.f*-sporozoite rate of *An. arabiensis* was 0.2%, which is similar to the 0.2% reported from Eritrea [87] and higher than the 0.06% and 0.01% reported from Ethiopia and Eritrea, respectively [88,100]. Various findings reported relatively higher annual sporozoite rates of *An. arabiensis* in various parts of Ethiopia, 0.3%, 0.28% and 0.3%, respectively [57,92,101]. Similarly, higher annual overall *P. f*-sporozoite rate of *An. arabiensis* has been documented in different parts of Ethiopia [66,76] and elsewhere in Africa [82,94,102, 103, 104].

In this study, sporozoite infected *An. arabiensis* sampled in July as reported by Kibret *et al*. [73]. In this month, usually malaria transmission occurs in the district (in Amhara region and Ethiopia) [26,105] because a small amount of rain is obtained from May to June which is good for both the vectors and the parasite life cycle.

The overall annual sporozoite rate of *An. funestus* was 0.57%, is higher as compared to 0.03%, which was reported from Senegal [106]. However, as it is lower as compared with the other reports in Ethiopia [73] and in Africa [7,82,94,104,107,109].

The current study revealed the presence of *P. vivax*-247 infected *An. funestus* in the district. However, there was no report showing *P. vivax* infected *An. funestus* in Ethiopia until now. *P.v*-247 infected *An. funestus* was collected only from outdoor in December and January. Occurrence of an infection in December is consistent with the major malaria transmission season of Amhara region and Ethiopia [26,105].

In this study, *An. funestus* had a higher sporozoite rate than *An. arabiensis* and *An. coustani*. Previously, Fontaine *et al*.[35] reported that *An. gambiae* s.l (*An. arabiensis*) was the only responsible vector for most of the transmissions of malaria in the highland parts of the Amhara region. However, the presence of infectious *An. funestus* in the current study may suggest that populations of *An. funestus* could be responsible for the transmission of malaria in the highland fringes of Bure district.

Astonishingly, our study found the third infected species, *An. coustani* by *P. vivax*-210. This infectious *An. coustani* specimen was sampled from outdoor in December. Similar to the present finding, Kelel [110] and Yewhalaw *et al*. [76] reported the same *P. vivax*-210 infected *An. coustani* from Gilgel-Gibe hydroelectric dam area, Ethiopia. The overall annual sporozoite rate for *An. coustani* was 0.34%, which was lower than other reports in Ethiopia (0.68%) [77] and in Kenya (1.3%) [74]. Likewise, higher sporozoite rate of *An. coustani* reported from Ethiopia [76] and Kenya [75, 111] than the present study. In Kenya, Taveta district, *An. coustani* was well known vector of malaria [74]. Moreover, the occurrence of infected *An. coustani* in December corresponds with the major malaria transmission season in Ethiopia, in particular Bure district [26]. Generally, until now *An. coustani* was not incriminated as a vector of malaria in Ethiopia, but standing from the current and previous data this species might be a potential malaria vector in Ethiopia. Therefore, it needs a regular entomological surveillance, monitoring and appropriate incrimination of this species to determine its role in malaria transmission in Ethiopia.

The current study revealed the difference in sporozoite rate between villages. All infected mosquitoes were from non-irrigated villages, Shnebekuma and Workmidr. The average annual sporozoite rates of *An. arabiensis* (0.23%), *An. funestus* (0.61%) and *An. coustani* (0.27%) were higher in non-irrigated than irrigated village (nil for all species). Similar to our results, various studies indicated the presence of significantly higher annual sporozoite rate in non-irrigated than irrigated villages in different parts of Africa [5,81,112,113]. Ijumba *et al*. [112], Appawu *et al.* [5], and Sogoba *et al*. [114] also reported highest annual sporozoite rates for *An. arabiensis* and *An. funestus* in non-irrigated than irrigated sites (e.g., rice irrigated area) in Africa. The presence of infected mosquitoes in non-irrigated villages might be associated with the occurrence very large numbers of the adult vectors in the two non-irrigated villages (Shnebekuma, n = 3,875; Workmidr, n = 472) than non- irrigated village (Bukta, n = 356). Non-irrigated villages had more breeding habitats throughout the year (Personal obser.), which helped to increase the vector survival rate by releasing enough moisture to the surrounding areas [114]. Therefore, population dynamics and adult age structure are important determining factors for the ability to transmit malaria [115].

However, contradict to the current result, Kibret *et al*. [116] obtained highest overall annual sporozoite rate of *An. arabiensis* (1.67%) from the irrigated village than non-irrigated villages (0.43%) in Zeway area, central Ethiopia. Similarly, Jaleta *et al*. [117] in Ethiopia and Dolo *et al.* [80], Muturi *et al*. [81] and Mboera *et al*. [82] in Africa found highest annual sporozoite rates for *An. gambiae* s.l and *An. funestus* in irrigated than non-irrigated sites. The absence of sporozoite infected Anophelines mosquitoes in irrigated village is due to the presence improved housing condition and standard of living because of the wealth created by irrigation. Therefore, mosquitoes are unable to enter into the house and further farmers can protect themselves from nuisance mosquitoes using replaceable health-care facilities [6,80,118]. Health education on malaria is the other means of variation [6,119]. Farmers in irrigated village could have access to malaria information because the health center and primary school was established as the hub of the irrigated village (Personal obser.). Moreover, the absence or the presence of little malaria in irrigated community could be the possible reason for the absence infectious mosquitoes in the irrigated village [80,120].

Generally, this study revealed that *P. falciparum* and *P. vivax* were the common *Plasmodium* species in Bure district. This result was in agreement to the epidemiological study conducted in areas surrounding Bure district and in the other Amhara zones that both *P. falciparum* and *P. vivax* were the prevalent parasites [26,38,121, 122].

This study indicated that the combined Entomological inoculation rate for both *Anopheles* species was 5.7 ib/p/y (overall annual EIR). It was extremely lower than those records from similar altitude and altitudes below this study district. Contrary to our study, high infectivity bites per person per year had been reported in Ethiopia [73,117] and elsewhere in Africa [112,118]. In Chad, Kerah-Hinzoumbe *et al*. [123] found 311 ib/p/y; in Ghana, Appawu *et al*. [5] reported 418 ib/p/y and in Malawi Mzilahowa *et al*. [94] reported 183 ib/p/y.

The estimated EIRs of the three *Anopheles* mosquito species varied across villages and seasons in Bure district. The estimated overall annual *P.f*EIR of *An. arabiensis* was 1.4 ib/p/y. It is very lower as compared with those reported by Antonio-Nkondjio *et al*. [111] (16.5 ib/p/y), Mboera *et al.* [82] (728 ib/p/y) and Massebo *et al*. [101] (17.4 ib/p/y) in Cameroon, Tanzania and southwestern Ethiopia, respectively. In this study, the overall annual *P.v* EIR of *An. funestus* was 3.3 ib/p/y. It was lower as compared with other reports [82,111, 107], who reported between 6.5–151.4 ib/p/y, 141ib/p/y and 12ib/p/y in Cameroon, Ghana and Tanzania, respectively. Similarly, *An. coustani* had lower annual *P.v*EIR, which was 1.3 ib/p/y. It is not comparable from Antonio-Nkondjio *et al*. [111] finding, who documented 3.4 ib/p/y in Cameroon.

In our study, infectious mosquitoes were detected only in three months (July, December, and January), though the main malaria vectors were collected in all months. This is highly linked with the given attention (large scale distribution and acceptance of LLINs and the application of IRS) which was offered by the region and the district health offices because all villages in Bure district are malarious. Being this, human-mosquito contact has declined and a reduction sporozoite inoculation rate into people were observed [98,99,124].

Conclusions

A total of nine *Anopheles* species belonging to *An. arabiensis*, *An. funestus*, *An. pharoensis*, *An. coustani*, *An. squamosus*, *An. cinereus*, *An. demeilloni*, *An. rupicolus* and *An. natalensis* were recorded in Bure district. This finding documented important malaria vectors of Ethiopia (*An. arabiensis*, *An. funestus*, *An. pharoensis*). However, higher proportions of adult Anopheles mosquitoes were collected in non-irrigated villages (Shnebekuma and Workmidr) than irrigated village (Bukta). *Anopheles arabiensis*, *An. funestus* and *An. coustani* found to be infected. The CSP-ELISA results indicated that the prevalence of malaria infectious *Anopheles* mosquitoes is very low and this makes Bure district suitable for malaria control and elimination. Annual *P.f*-EIR of *An. arabiensis* (1.42 ib/p/y), *P.v*-EIR of *An. funestus* (3.22 ib/p/y) and *P.v*-EIR of *An. coustani* (1.23 ib/p/y) were higher in non-irrigated villages (Shnebekuma and Workmidr) than irrigated (0 ib/p/y) village (Bukta). Even the distribution pattern of the annual EIRs between villages were highly variable, this indicated full attention was only given to irrigated village. Being this, the annual EIR of each species for irrigated village reached zero. Generally, the obtained infective bites per person per year was low (annual EIRs) which is less than ten (EIR < 10); therefore, the finding implied that the study area is considered to have unstable malaria transmission and had a low malaria transmission intensity [126]; however, not < 1, this implied much work remain to be done.

Abbreviations

EIR: Entomological Inoculation Rate; LLINs: long-lasting insecticides-treated nets; IRS: indoor residual spraying; LTCs: Disease Control and Prevention Light Trap Catches; PSCs: Pyrethrum Spray Catches; APSs: Artificial Pit- Shelters; ELISA: ELISA: Enzyme-Linked Immunosorbent Assay; CSP: circumsporozoite protein; *P.f* : *Plasmodium falciparum*; and *P.v* = *P. vivax*.

Declarations

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

TA and DY conceived and designed the study; TA conducted field work and experiment in the laboratory, analyzed and interpreted the data, wrote the manuscript; and DY and EG supervised in the field and in laboratory. All authors read and approved the final manuscript.

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Availability of data and materials

All data presented in this manuscript (study) are available in the hand of the correspondent authors and will release if necessary, with a reasonable request.

Ethics approval and consent to participate

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 head of the selected households.

Consent for publication

Not applicable.

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Figures

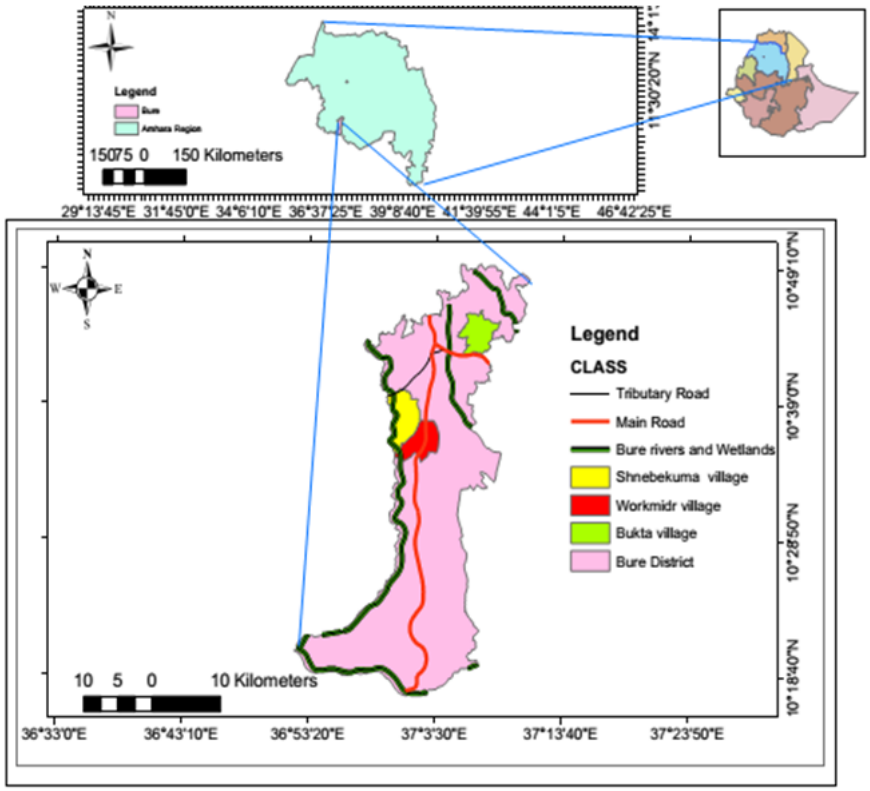

Figure 1

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

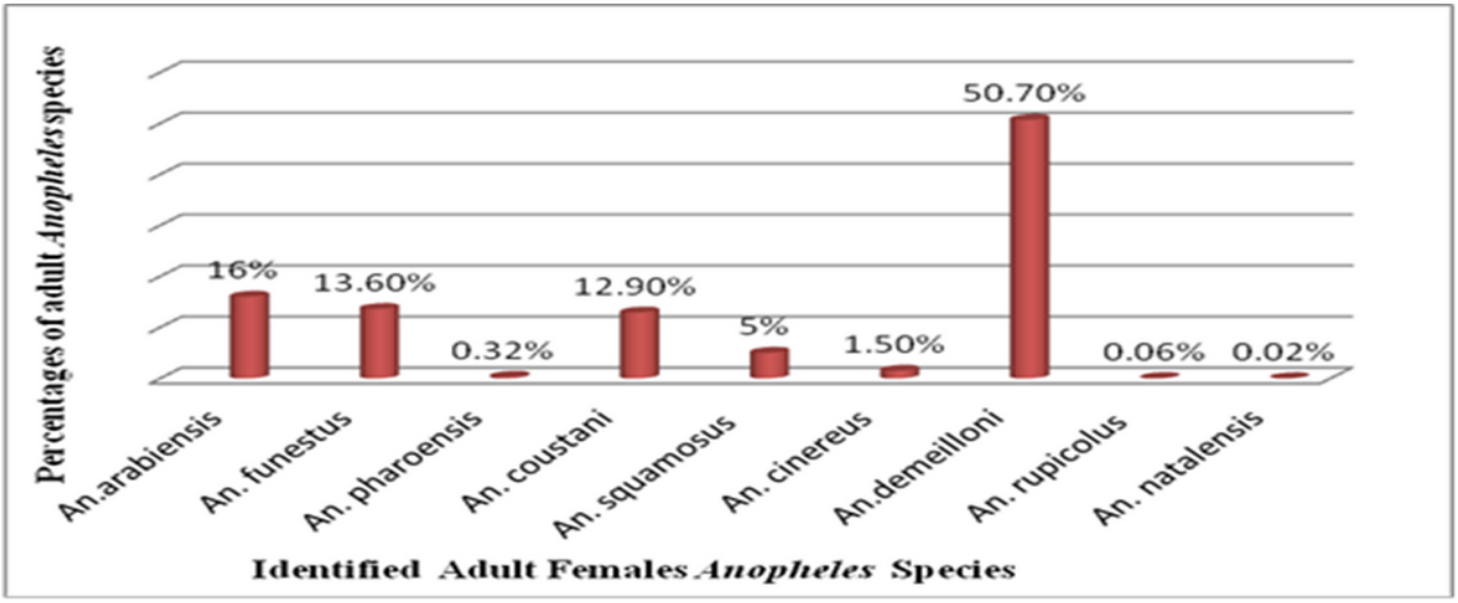

Figure 2

Abundance of adult female Anopheles mosquito species in Bure district, Ethiopia

Supplementary Files

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- Appendix1and2.docx