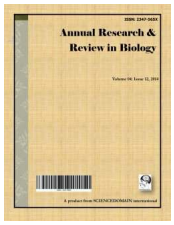

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Species Composition and Infection Rate of Mosquito Vectors Following the Indoor Residual Spraying Exercise in three Communities in Awka North L.G.A of Anambra State, Nigeria

Aribodor Dennis Nnanna[1], Okezie Godson Nwabueze[1], Ugwuanyi Ifeoma Kosisochukwu[1*], Aribodor Ogechukwu Benedicta[2] and Ike Edith Amauche[3]

[1]Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
[2]Department of Zoology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
[3]Ministry of Health, Awka, Anambra State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors ADN, OGN and UIK did the study design and wrote the protocol. Authors ADN, OGN, UIK, AOB and IEA managed the experimental process. Authors ADN, OGN, UIK and AOB did the statistical analysis and literature searches while analyses of the data obtained from the study was done by authors ADN, OGN and UIK. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Mosquitoes constitute important vectors of diseases of public health importance. The study was carried out to determine the species composition of man-biting mosquitoes and their infection rate following indoor residual spraying (IRS) in order to currently assess the epidemiology of mosquito-borne diseases in the study communities.
Study Design: A six-month study was carried out in Urum, Mgbakwu and Isuaniocha communities

**Corresponding author: E-mail: ik.ezugbo-nwobi@unizik.edu.ng;*

in Awka North L.G.A where no information exists on the infection rates of mosquitoes following the indoor residual spraying exercise. Sample collection was carried out between June and December 2015 using Knockdown and Human Landing Catches (HLC) techniques.

Methodology: Adult indoor and outdoor man-biting mosquitoes were collected in the three study communities. Ten houses were randomly selected in each community using Simple Random Sampling method. Adult female mosquitoes were morphologically identified and dissected following standard keys and procedures.

Results: A total of 684 indoor adult biting mosquitoes comprising of 3 species were collected. *Culex quinquefasciatus* had the highest distribution of 414 (60.5%) followed by *Anopheles gambiae* 269 (39.3%) and *An. moucheti* 1 (0.2%). One hundred and forty-three (143) outdoor adult biting mosquitoes comprising 4 species were collected. *Aedes aegypti* had the highest distribution of 104 (72.7%) followed by *Ae. albopictus* 33 (23.1%), *Ae. africanus* 4 (2.8%) and *Ae*. *simpsoni* 2 (1.4%). There was a significant difference among the species of the indoor and outdoor biting mosquitoes collected in the communities (P<0.05). Female mosquitoes dissected for infection showed zero infection rates.

Conclusion: Our data demonstrates that the IRS exercise may have been effective in reducing mosquito infection rates in the study area. This work may provide an entomological baseline data required for evaluation and implementation of future vector control interventions in the study area.

Keywords: Mosquito; species composition; infection rates; IRS; Nigeria.

1. INTRODUCTION

Mosquitoes constitute important vectors of diseases of public health importance. Mosquito surveillance is a prerequisite for an effective and efficient mosquito control program. The dynamics of changing mosquito populations provides significant information for evaluating risk potential for the transmission of mosquito-borne diseases [1]. Over 3000 genera of mosquitoes have been recorded worldwide, but the most important man-biting mosquitoes belong to the genera; *Anopheles*, *Culex*, *Aedes*, *Mansonia*, *Haemagogus*, *Sabethes* and *Psorophora* [2]. *Anopheles* species are important vectors of human malaria parasites. They also transmit filariasis, caused by *Wuchereria bancrofti* and *Brugia malayi* as well as certain arboviruses including *Tataguine* and *Guaroa*. *Aedes* mosquitoes are important vectors of yellow fever, dengue and encephalitis viruses. Certain *Culex* species also transmit Bancroftian filariasis and a variety of arboviruses including West Nile Virus and St. Louis Encephalitis Virus. *Mansonia* species transmit *Brugia malayi* and sometimes *W*. *bancrofti*. *Haemogagus* and *Sabethes* species are vectors of yellow fever. *Psorophora* species are important mainly as nuisance mosquitoes, but also transmit arboviruses including occasionally yellow fever [3].

All mosquitoes are similar in that the females take blood meal for the development of their eggs, hence the degree of anthropophily will determine whether they will be good transmitters of diseases or not. Several factors including abundance, biting behaviour, host preference and longetivity have been reported to influence the vectorial role of mosquitoes in disease transmission [4]. Diseases transmitted by mosquitoes constitute serious health, economic and social problems especially in the developing world. Malaria for instance, remains the most important public health parasitic infection and it threatens almost one third of the world population in 103 tropical countries resulting in 207 million of clinical cases and 627,000 deaths [5]. Nigeria is known for high prevalence of malaria and the disease remains one of the leading causes of childhood and maternal morbidity and mortality, low productivity and reduced school attendance in Nigeria [6]. Yellow fever is endemic in tropical and sub-tropical areas of South America and Africa. World Health Organization, estimates that there are 200,000 cases of yellow fever and 30,000 deaths per year, with 90% of the infection occurring in African continent [7]. Dengue fever, which is endemic in more than 100 countries in Africa, America, South East Asia and Western pacific region, has an estimate of 50 million cases worldwide per year [5].

In recent times, Indoor Residual Spraying (IRS) has been adopted and scaled up to protect the entire household and community members who possibly do not have access to treated bed nets in Africa [8]. The Federal Government policy on malaria control in Nigeria focuses on Long-lasting Insecticide treated bed nets (LLINs), IRS,

Intermittent Preventive Treatment (IPT) and environmental management [9]. In line with these strategies, the Nigeria Malaria Elimination Programme (NMEP) in Nigeria has scaled up IRS to achieve 85% coverage in 20% of eligible structures in Nigeria in 2014. To achieve this target, IRS activities was progressively expanded in the seven World Bank Supported Malaria Booster States of Bauchi, Gombe, Kano, Jigawa, Rivers, Anambra, Akwa-Ibom states Nigeria, from 2009-2014 to supplement LLIN and environmental management.

There is paucity of information on the species composition and infectivity of mosquitoes in Awka-north L.G.A. especially after the IRS exercise in Anambra state. Therefore, this study was carried out to determine the species composition of man-biting mosquitoes and their infection rate following indoor residual spraying (IRS) exercise in the study communities.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Urum, Mgbakwu and Isuaniocha communities in Awka North L.G.A of Anambra state, southeast Nigeria (Fig. 1). The study communities lie between latitude 6°26' N/4°05'N and longitude 7°03' E/7°30'E. The area is within the tropical rain forest zone and has marked wet and dry seasons. The wet season (March-October) is about 8 months while the dry season (October-February) is about 4 months. The relative humidity averages 70%, reaching 80% during rainy season with an annual rainfall of about 2000-3000 mm. The temperature ranges from 26ºC-35ºC in the dry season and 22ºC-30ºC in wet season.

The population census of the study communities with respect to the creation of Anambra state in 1991, as projected by 3% to 2013 was Isuaniocha 7,631, Mgbakwu 11,751 and Urum 7,553 [10]. The communities are inhabited mostly by Igbos with few people from other ethnic groups in Nigeria. Most of the inhabitants are farmers, with some petty traders, civil servants, students and technicians. The three communities have streams which serve as a source of domestic and drinking water supply to them. Most of the streams have swamps that could support mosquito breeding.

2.2 Ethical Consideration and Informed Consent

Prior to the study, ethical consideration and approval were obtained from the research ethics committee of Anambra state Ministry of Health. Introductory letters to the community leaders was also obtained from the Head of Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka. Similarly, verbal informed consent of the head of households were obtained after they were made to know the benefit of their participation in the study. Oral consent was obtained from all participants that were involved in sample collection. Malaria prophylaxis with Artemether and Lumefantrine (the recommended Chemoprophylaxis in Nigeria) was provided for the Human Landing Catches (HLC) volunteers once a month throughout the study period.

2.3 Sampling Methods

Adult mosquitoes were collected in the three study communities- Urum, Isuaniocha and Mgbakwu. Ten houses were randomly selected in each location using Simple Random Sampling method. Sample collection was carried out for six months (June-December, 2015) using the following techniques:

2.3.1 Knockdown technique

This technique involved the laying of Calico cloth on the floor and on the surfaces of immovable household furniture. The doors and windows of each room sampled were closed and the houses were sprayed using the pyrethroid knockdown insecticide aerosol, Raid® which contains D-allethrin 0.25%, Tetramethrin 0.15% and Deltamethrin 0.015%. After 20 minutes the Calico cloth was carefully taken out of the house and knocked-down mosquitoes were collected using entomological forceps and places into damped petri dishes. Three houses with nets and three houses without nets were used for the collection of samples using this technique.

2.3.2 Human Landing Catches (HLC)

Human Landing Catches (HLC) was carried out according to World Health Organization standard procedure [11] which adopts the standard human bait collector (HBC) method. In this procedure, a volunteer will sit outdoor from 6 pm-10 pm and collect the mosquitoes that come to bite him. Three trained volunteers were employed for the HLC collection. Torch lights were used to search

for mosquitoes that alight to feed on the volunteers, and test-tube vials were used to collect landed mosquitoes caught.

2.4 Sample Preservation

All the adult mosquitoes that were collected were kept in a labeled 1.5 ml eppendorf tubes containing silica gel desiccant. Samples were stored in the laboratory at room temperature (35℃-37℃).

2.5 Identification of Samples

The mosquitoes were identified using the gross morphology of the species, external morphology of the palps, antenna, proboscis, patches of pale and black scales on the wings and legs and the terminal abdominal segments as described by Gillet [12].

2.6 Dissection of Mosquitoes

Only blood fed indoor-biting mosquitoes collected during the Pyrethrum-based Knock down Collection (PKC) exercise were dissected for parasite infection.

2.6.1 Dissection of *Anopheles* mosquitoes

This was carried out using the pressing method as described by Service [3].

2.6.2 Dissection of *Culex* mosquitoes

Female *Culex* mosquitoes were dissected to check for filarial parasite. The dissection was done under a simple dissecting microscope consisting of x10 objective. The legs and wings were removed from its body using a dissecting needle. The mosquito was then placed in a drop of normal saline on a clean microscope slide and a clean cut was made to separate the head, thorax and abdomen under the x10 objective. The severed head was transferred to a separate drop of saline in another clean microscope slide. This was held in position with a dissecting needle while another fine needle was used to separate the gutter-shaped labium from the other parts of the proboscis to expose filarial parasite if present. This was then examined under the microscope. Infected filarial worms when present, were expected to emerge from the labium into the saline.

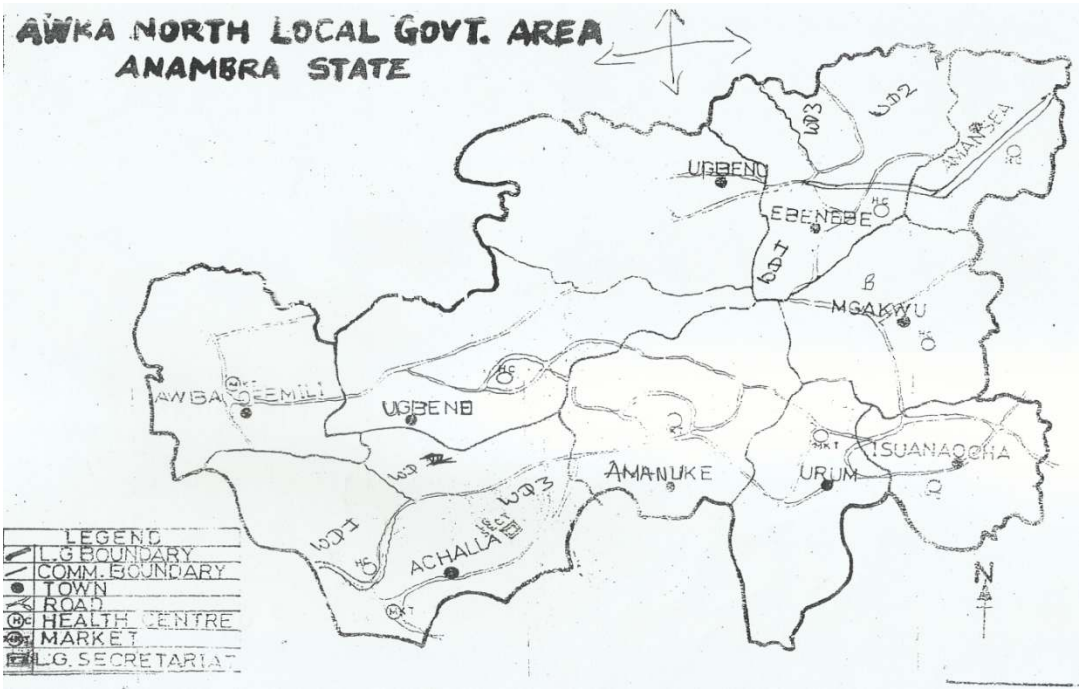

Fig. 1. Map of Awka North Local Government Area, showing Isuaniocha, Mgbakwu and Urum communities

Source: (Awka North Local Government Secretariat, 2014)

2.7 Statistical Analysis

Data obtained from the study were analysed using Statistical Package for the Social Science (SPSS) version 20.0. One Way Analysis of Variance (ANOVA) was used in the data analysis and means were separated using the least significant difference (LSD) values. Statistical significance was set at $P< 0.05$.

3. RESULTS

3.1 Species Distribution of Mosquitoes

A total of 684 indoor man-biting mosquitoes were collected in Isuaniocha, Mgbakwu and Urum communities. Their species distribution is *Anopheles gambiae* 269 (39.3%), *An. moucheti* 1 (0.2%) and *Culex quinquefasciatus* 414 (60.5%). There was a significant difference among the three species of indoor mosquitoes in the communities ($P< 0.05$). The mosquito distribution according to communities was 136 (38.1%) for *An. gambiae*, 1 (0.3%) for *An. moucheti* and 220 (61.6%) for *Cx. quinquefasciatus* in Isuaniocha. Mgbakwu had 81 (39.1%) for *An. gambiae*, 0 (0%) for *An. moucheti* and 126 (60.9%) for *Cx. Quinquefasciatus*. Urum had 52 (43.3%) for *An. gambiae*, 0 (0%) for *An. moucheti* and 68 (56.7%) for *Cx. quinquefasciatus* as shown in Table 1.

A total of 143 outdoor man-biting mosquitoes were collected and they include *Ae. aegypti* 104 (72.7%), *Ae. albopictus* 33 (23.1%), *Ae. africanus* 4 (2.8%) and *Ae*. *simpsoni* 2 (1.4%) as shown in Table 2. There was a significant difference among four species of outdoor mosquitoes in the communities ($P< 0.05$). The distribution of mosquitoes according to communities was 35 (89.7%) for *Ae. aegypti*, 0 (0%) for *Ae. albopictus*, 4 (10.3%) for *Ae. africanus* and 0 (0%) for *Ae. simpsoni* in Isuaniocha. Mgbakwu had 51 (68.9%) for *Ae. aegypti*, 23 (31.1%) for *Ae. albopictus* and 0 (0%) for *Ae. africanus* and *Ae. simpsoni*, respectively. Urum had 18 (60%) for *Ae. aegypti*, 10 (33.3%) for *Ae. albopictus*, 0 (0%) for *Ae*. *africanus* and 2 (6.7%) for *Ae. simpsoni* (Table 2).

The overall species composition of man-biting mosquitoes in Isuaniocha, Mgbakwu and Urum were *An. gambiae* 316 (27.9%), *An. moucheti* 1 (0.1%), *Ae. aegypti* 183 (16.1%), *Ae. albopictus* 78 (6.9%), *Ae. africanus* 4 (0.4%), *Ae. simpsoni* 35 (3.1%) and *Cx. quinquefasciatus* 517 (45.6%) making a total of 1134 as shown in Table 3. There was no significant difference among species of man-biting mosquitoes in the three communities ($P> 0.05$). The distribution by communities was 151 (13.3%) for *An. gambiae*, 1 (0.1%) for *An*. *moucheti*, 70 (6.2%) for *Ae. aegypti*, 0 (0%) for *Ae. albopictus*, 4 (0.4%) for *Ae. africanus*, 0 (0%) for *Ae. simpsoni* and 252 (22.2%) for *Cx. quinquefasciatus* in Isuaniocha. Mgbakwu had 90 (7.9%) for *An*. *gambiae*, 0 (0%) for *An. moucheti*, 80 (7.1%) for *Ae. aegypti*, 39 (3.4%) for *Ae. albopictus*, 0 (0%) for *Ae. africanus*, 20 (1.8%) for *Ae*. *simpsoni* and 151 (13.3%) for *Cx. quinquefasciatus*. Urum had 75 (6.6%) for *An. gambiae*, 0 (0%) for *An. moucheti*, 33 (3%) for *Ae*. *aegypti,* 39 (3.4%) for *Ae. albopictus*, 0 (0%) for *Ae. africanus*, 15 (1.3%) for *Ae. simpsoni* and 114 (10.1%) for *Cx. quinquefasciatus*.

Table 1. Distribution of Indoor man-biting mosquitoes collected in Isuaniocha, Mgbakwu and Urum, in Awka North LGA, Anambra State

Communities	No. of mosquitoes	Species of indoor biting mosquitoes collected		
		An. gambiae	*An. moucheti*	*Cx. quinquefasciatus*
Isuaniocha	357 (52.2%)	136 (38.1%)	1 (0.3%)	220 (61.6%)
Mgbakwu	207 (30.3%)	81 (39.1%)	0	126 (60.9%)
Urum	120 (17.5%)	52 (43.3%)	0	68 (56.7%)
Total	**684**	**269 (39.3%)**	**1 (0.2%)**	**414 (60.5%)**

Table 2. Distribution of outdoor man-biting mosquitoes collected in Isuaniocha, Mgbakwu and Urum, in Awka North LGA, Anambra State

Communities	No. of mosquitoes	Species of indoor biting mosquitoes collected			
		Ae. aegypti	*Ae. albopictus*	*Ae. africanus*	*Ae. simpsoni*
Isuaniocha	39 (27.3%)	35 (89.7%)	0	4 (10.3%)	0
Mgbakwu	74 (51.8%)	51 (68.9%)	23 (31.1%)	0	0
Urum	30 (21%)	18 (60%)	10 (33.3%)	0	2 (6.7%)
Total	**143**	**104 (72.7%)**	**33 (23%)**	**4 (2.8%)**	**2 (1.4%)**

Table 3. Species composition of mosquitoes collected in Isuaniocha, Mgbakwu and Urum, Awka North LGA, Anambra State

Genus	Species	Distribution			Sub-total	Total
		Isuaniocha	Mgabkwu	Urum		
Anopheles	*An. gambiae*	151 (13.3%)	90 (7.9%)	75 (6.6%)	316 (27.9%)	317 (28%)
	An. moucheti	1 (0.1%)	0	0	1 (0.1%)	
Aedes	*Ae. aegypti*	70 (6.2%)	80 (7.1%)	33 (3%)	183 (16.1%)	300 (26.5%)
	Ae. albopictus	0	39 (3.4%)	39 (3.4%)	78 (6.9%)	
	Ae. africanus	4 (0.4%)	0	0	4 (0.4%)	
	Ae. simpsoni	0	20 (1.8%)	15 (1.3%)	35 (3.1%)	
Culex	*Cx. quinquefasciatus*	252 (22.2%)	151 (13.3%)	114 (10.1%)	517 (45.6%)	517 (45.6%)
Total		**478 (42.2%)**	**380 (33.5%)**	**276 (24.3%)**	**1134**	**1134**

Table 4. Infectivity rate of blood-fed female *Anopheles* mosquitoes in Isuaniocha, Mgbakwu and Urum, Awka North LGA, Anambra State

Communities	*An. gambiae*	*An. moucheti*	Number dissected	Number infected
Isuaniocha	87	1	88	0
Mgbakwu	46	0	46	0
Urum	16	0	16	0
Total	**149**	**1**	**150**	**0**

Table 5. Infectivity of female *Culex* mosquitoes in Isuaniocha, Mgbakwu and Urum, Awka North LGA, Anambra State

Communities	*Cx. quinquefaciatus*	Number dissected	Number infected
Isuaniocha	120	120	0
Mgbakwu	62	62	0
Urum	40	40	0
Total	222	222	0

3.2 Species Distribution of Mosquitoes

On the infectivity rate of man-biting female *Anopheles* mosquitoes in Isuaniocha, Mgbakwu and Urum communities, both *Anopheles gambiae* and *Anopheles moucheti* were not infected with malaria parasites as no sporozoites of *Plasmodium* was found as shown in Table 4 above.

Dissection of man-biting female *Cx quinquefasciatus* mosquitoes collected from Isuaniocha, Mgbakwu and Urum communities, showed zero infection rate for filarial parasite as shown in Table 5 above.

4. DISCUSSION

The indoor man-biting mosquitoes collected in Isuaniocha, Mgbakwu and Urum communities were *An. gambiae*, *An. moucheti* and *Cx. quinquefasciatus*. High incidence of *An. gambiae* and *Cx. quinquefasciatus* have been earlier reported by Aribodor et al. [13] in Awka Etiti, Anambra State, Nigeria. The availability of these species in these communities was due to the presence of ground water pools, domestic containers, poorly drained gutters, plant axils and bushes around household where they breed and readily fly into houses to rest and feed on human host. *An. gambiae* is an important vector of malaria as well as filariasis in Africa, especially in rural communities [14], while *Cx. quinquefasciatus* transmits filarial nematode diseases including loiasis and elephantiasis. The abundance of these species was more in Isuaniocha, followed by Mgbakwu and Urum. *An. gambiae* and *Cx. quinquefasciatus* were largely responsible for indoor biting activities in the study area, exhibiting endophilic behaviour. From the results of this study, *Cx. quinquefasciatus* was found to be the most abundant in the study communities. *Culex quinquefasciatus* was found breeding mostly in sites around household, making it easier for them to fly into houses to feed and rest. This disagrees with the findings of Onyido et al. [15] in Awka, Anambra state, where *An. gambiae* was the most abundant species encountered. The high abundance of indoor man-biting mosquitoes observed in the study could pose a potential danger for epidemics should any arboviral infection be introduced in the study area.

The outdoor man-biting mosquitoes were *Aedes aegypti*, *Ae. albopictus*, *Ae*. *africanus* and *Ae. simpsoni*. All these species of *Aedes* are proven vectors of yellow fever and other arboviruses [3,12,16] and all have been incriminated in yellow fever epidemics in Nigeria [17-19]. The presence of these species in all the breeding habitats identified in the study area shows their wide range of breeding habitat preferences. Available ground water pools and gutters in the communities, exposed domestic containers and axils of pineapple, plantain and cocoyam plants around households ensured their continuous existence. In the present study, these species bred and fed on human host outdoor which explains their anthropophilic and exophilic behaviour. The most abundant was *Ae. aegypti*, followed by *Ae. albopictus*, *Ae. africanus* and *Ae. simpsoni*. From the results, both *Ae. aegypti* and *Ae. albopictus* were more in number than other species. This corroborate with the findings of Onyido et al. [15] in Nnamdi Azikiwe University hostels, Awka, Anambra State. The abundance of *Ae. aegypti* in this study was due to the large number of domestic containers found around households where they readily breed and feed on human host. *Ae. albopictus* which was the second most abundant, have been reported earlier in a study in Awka, Anambra state by Mbanugo and Okpalaononuju [20] to be imported into Nigeria through second hand tyres. *Ae. aegypti* was also reported as the principal tropical mosquito vector of arboviruses causing yellow fever, dengue and chikungunya and also transmits other arboviruses [21]. Mgbakwu was the community with the highest number of outdoor man-biting mosquitoe followed by Isuaniocha and Urum. The presence of these *Aedes* mosquito species poses a serious threat of mosquito-borne disease transmission in the future in these areas unless control measures are applied.

The blood fed man-biting mosquito species dissected for infection rate were *An*. *gambiae, An. moucheti* and *Cx. quinquefasciatus*. None of them were found to be infective with either *Plasmodium* sporozoites or filarial parasites. A study by Aribodor et al. in Awka North and South LGAs of Anambra state found mosquito species to be infected with parasites, contrary to the findings of this study [13]. Another work by Manyi et al. in Makurdi, Benue state Nigeria found sporozoite infection in Anopheles population dissected in the study [22]. Also, another study carried out in South-Central Ethiopia incriminated *An. arabiensis* to be the most important vector of *Plasmodium vivax* and *P. falciparum* along highland altitude transects [23]. The fact that the mosquitoes were blood fed without being infective could be that they fed on uninfected human host or that their chances of picking infection from infected host were low.

5. CONCLUSION

The findings of this study indicated the abundance of both indoor and outdoor man-biting mosquitoes. The fact that this study was done during wet season may have contributed to the abundance of these mosquitoes through the creation of suitable breeding habitats. The IRS exercise may have been effective in reducing mosquito infection rates in the study area. This work therefore provides an entomological baseline data required for evaluation and implementation of future vector control interventions in the study communities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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