International Journal of Biology Sciences

ISSN Print: 2664-9926
ISSN Online: 2664-9934
IJBS 2024; 6(1): 176-182
www.biologyjournal.net
Received: 18-11-2023
Accepted: 25-12-2023

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First detection of *Anopheles stephensi* (Deptera: Culicidae) in Gash Barka region: Eritrea

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DOI: https://dx.doi.org/10.33545/26649926.2024.v6.i1c.203

Abstract

Background: *Anopheles* (An) *gambiae* and (An) *funestus* are the dominant vectors in Africa. These vectors inhibit at the rural area and as a result malaria was being restricted to the rural areas. Recently, (*An*) *stephensi* as a competent vector of both *Plasmodium* (*p*). *vivax* and *P. falciparum* appear to be better adapted in urban and peri-urban areas of East African countries. So far, *An. Stephensi* found to inhibit in Djibouti, Ethiopia, Sudan, and Somalia and further distributed in Nigeria and Kenya, since 2012. If the invasion of *anopheles stephensi* continue across the African continent, additional 126 million people from urban areas will be at risk of malaria infection. Gash Barka (GB) Region has shared a border with Ethiopia and Sudan (*An. Stephensi* prevalent areas). So the current surveillance aimed to ascertain the presence of *An. Stephensi* in GB region using morphological and molecular identification.

Method: Cross sectional entomological survey was conducted during the period Sep-Dec 2022 in three towns of Gash Barka Region (*Vis*. Tesseney, Agurdet and Gulj) to assess the presence of *An. stephensi*.

Result: This is the first report in Eritrea to identify the invasive malaria vector *An. stephensi*. Cement basin was the principal breeding habitat in the study site. It was accountable for more than 90% of the immature *Anopheles* species. Other habitats include plastic/metal basin barrels, clay pots and overhead tanks were found with immature mosquitos.

Conclusion: *An. Stephensi* co-exists with native vector species in the study sites. Further research is needed to determine its relative role in malaria transmission in the Region. Heath authorities need to revise the existing vector control strategies to target *An. Stephensi* alongside the native malaria vector species.

Keywords: WHO, *P. falciparum*, *An. stephensi*

Introduction

According to World Health Organization (WHO) annual report 2021, an impact limitation was occurred in 2015-2020. The progress of malaria control and elimination was remained the same. The report revealed African region as a whole missed the milestone of the Global Technical Strategy for malaria (GTS) 2020, incidence and mortality reduction by 38% and 40%, respectively. In Botswana, Comoros, Eritrea and Madagascar, the malaria mortality rate were increased by 40% or more (WHO, 2021) [32]. In 2021 alone there were 247 million cases and 619,000 deaths globally. The sub Saharan Africa (SSA) were responsible for the 96% of the cases and 96% of deaths (WHO, 2022) [34]. *Anopheles arabiensis*, a member of the *Anopheles gambiae* species complex, is the principal malaria vector in East African countries like Djibouti, Sudan, Somalia, and Ethiopia, as well as in some countries of the Arabian Peninsula, such as Yemen and Saudi Arabia (WHO, 2022) [34]. Twenty years ago, a study conducted in Eritrea investigated the distribution of Anopheles mosquitoes throughout the country. The study identified thirteen species, and *Anopheles arabiensis* emerged as the most dominant vector, accounting for more than 80% of the total (Shililu *et al.* 2007) [20]. A similar study conducted in the Anseba region of Eritrea in 2020 also revealed the same result; *Anopheles gambiae* was found to be the dominant vector in that particular location (Yenus *et al*., 2021) [36]. It is believed that *An. arabiensis* is a rural malaria vector breeds in a variety of breeding habitats like streams, animal hoof prints, river edges, in both temporary and permanent ground pools (Yenus *et al*., 2021, Shililu *et al*., 2007, Sinka *et al*., 2010, Himeidan and Rayah 2008) [36, 20, 24, 16].

In Africa, malaria has been restricted to the rural areas due to the vectors breeding behavior (Robert *et al*., 2003) [18]. But this time the invasive *An. Stephensi* has been established in Africa region since 2012. It was first detected in Djibouti in 2012 (Faulde *et al*., 2014) [12]. Since then, the vector was identified from urban and rural sitting in Ethiopia, Sudan and Somalia in between 2016 and 2019 (Ahmed *et al*., 2021, WHO 2022 [34], Carter *et al*., 2018) [3, 34, 10] and further detected from Nigeria (2020), Yemen (2021) and Kenya (2022) (WHO, 2023) [31]. Based on the experience observed from Djibouti, *P. falciparum* incidence might be increased by 50% in Ethiopia due to the invasive *An. Stephensi* (Tadesse *et al*., 2021, Hamlet *et al*., 2022) [27, 2]. If the invasion of *Anopheles stephensi* continue across the African continent, additional 126 million people from urban areas will be at risk of malaria infection (Sinka *et al*., 2020) [25].

Recently, (*Ae*) *stephensi* as a competent vector of both *Plasmodium* (p). *vivax* and *P. falciparum* appear to be better adapted in urban and peri-urban areas of East African countries (Al-eryani *et al*., 2023) [6]. It primarily breeds in domestic and peri-domestic household (HH) water containers including water tanks, tyres, basins either plastic or cement, construction sites, barrels and other human-made habitats (Singh *et al*., 2021, Thomas *et al*., 2016) [22, 28]. *An. Stephensi* is the primary vector of malaria in Asia (Sinka *et al*. 2011) [23]. Several strategies have been employed in countries endemic with *An. Stephensi*, predominantly targeting the aquatic stages due to the confirmed resistance of this vector to the four major insecticide classes: organochlorines, pyrethroids, organophosphates, and carbamates (Enayati, *et al.* 2020) [11]. In India, Larva control through chemicals and biological means (larvivorous fish and bacteria) are commonly used. Temephos and *Bacillus thuringiensis var israelensis* (Bti) are the widely used larvicides (Subbarao, *et al*., 2019) [26].

According to Al-Eryani *et al.* 2023 [6], the emergence and spread of *An. Stephensi* threatens the progress in malaria control in the Horn of Africa and SSA.

Gash Barka (GB) Region is one of the six Regions in Eritrea. 95% population of the region are living in malarias area. This region is responsible for more than 80% of the total cases produced in Eritrea. According to the annual report from the Communicable Diseases Control (CDC): Eritrea, there was a 57% increase in malaria cases observed between 2015 and 2019, paradoxically contradicting the goals of the 2020-Enitiative: a 38% and 40% reduction in incidence and mortality, respectively. GB records high transmission with annual parasite index of 57.7 in contrast to the other Regions 3.6 in Maekel, 3.8 in Anseba, 9.7 in Debub, 13.8 in southern red sea (SRS) and 7.4 in Northern red sea regions (NRS).

GB Region has shared a border with Ethiopia and Sudan where *An. Stephensi* was incriminated in 2016 and 2019, respectively. The current surveillance effort aims to determine the presence of *An. Stephensi* in the GB Region, using both morphological and molecular identification.

Methodology

Study design

Cross sectional entomological survey during the period Sep-Dec 2022.

Study area: Gash-Barka is one of the most malarias zones in the country. It has an altitude range of 142 to 2,702 meters above sea level; it is divided into sixteen administrative sub-zones; and it has mainly a July-September rain season with a small rain season in April/May. The zone is one of the malaria zones in the country. It has a total population of 910, 082, of which 95% (864, 578) live in malaria-endemic areas. Based on recent 2022 malaria data, *Plasmodium falciparum* is the dominant parasite species in the zone, accounting for 88% of infections. *Plasmodium vivax* follows with 12%, and the remaining percentage comprises mixed infections involving either Pf, Pv, or other species. The study was conducted in three towns of the Region: Teseney, Omhajer and Agordet.

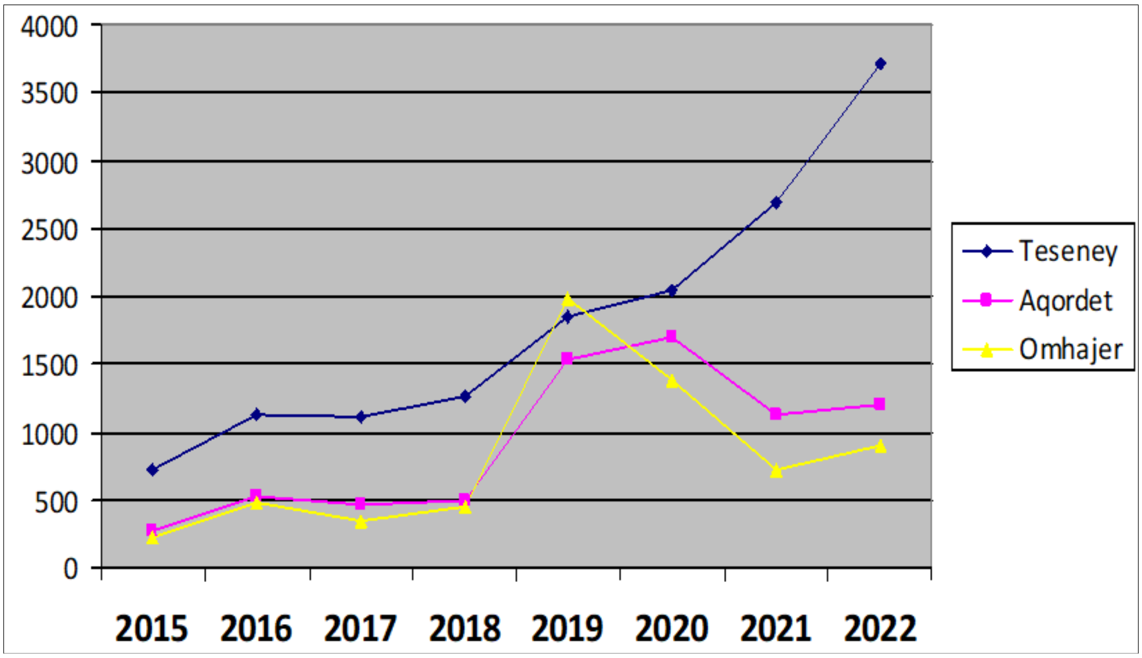

Fig 1: Trend of confirmed malaria cases in Tesseney, aqordat and Omhajer from 2015-2022

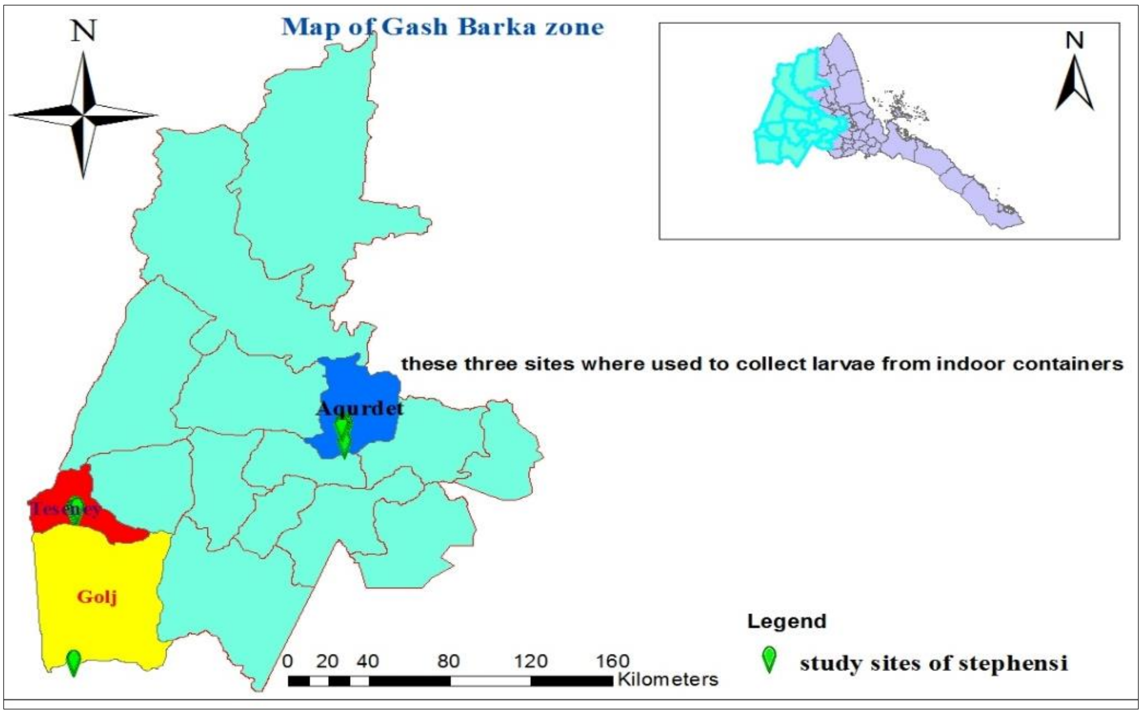

Fig 2: Map of Eritrea, Gash Barka Region and study towns (Aqurdet, Teseney and Golj)

Tesseney

Tesseney, located in the western part of the Gash Barka Region (GBR), is known as a land port due to its role as a center of trade between Eritrea and its neighboring countries, Sudan and Ethiopia. Tesseney has a total population of 30, 000, and its economy relies heavily on farming and animal husbandry. The Gash River runs alongside the town, increasing its vulnerability to malaria transmission. Tesseney is located at 14°19'23" N, 36°54'43" E, at an elevation of 600 meters above sea level (masl). A dramatic increment in malaria cases was observed between 2015-2022. The number of malaria cases in Tesseney increased more than fivefold, jumping from 727 in 2015 to 3,702 in 2022.

Agordat

Agordat is the third largest town in GBR, with a population of 22,048. This town has maximum population movement for trade and other economic matters. The economy is heavily driven by agriculture and animal raising, even though malaria cases are increasing. The Gash Barka River, flowing through the town, provides permanent breeding grounds for mosquitoes, contributing to the persistent malaria epidemic despite extensive efforts against both adult and immature mosquitoes. An outstanding achievement was observed in malaria reduction in Eritrea between 2000-2015, but paradoxically a significant increment has been observed since 2015 for unknown reasons. In this town, malaria cases were increased by 624% between 2015-2020 (From 272 to 1699). Akordat located at 15,551174" N and 37.886464" E at an elevation of 615 masl.

Omhajer

Omhajer is indeed the closest town to both Ethiopia and Sudan with a population of near 10, 000. Omhajer's location at the confluence of borders with Sudan and Ethiopia, combined with unrestricted movement across them, makes it susceptible to cross-border epidemics. Additionally, its proximity to the Tekeze River further increases this vulnerability. A dramatic change was observed in malaria cases between 2015-2019. It increased by nearly eight fold from 255 to 1985, despite a slight fall in malaria cases was shown in 2022. Omhajer located at 1584569 N, 37 + 247269 E at an elevation of 603.

Study Population

Larva of *Anopheles* mosquito.

Sampling Technique and sampling size determination

To identify *Anopheles* mosquito breeding in households (HH), we inspected wet water-holding containers for *Anopheles* larvae and pupae. Collected specimens were then reared to adulthood for morphological and molecular identification.

Any water-holding containers were inspected for the presence of immature mosquitoes (Larva or pupa). To maximize the detection of *Anopheles* mosquito breeding, we inspected all readily accessible water-holding containers within local households (HH). These included basins (Cement, plastic, metal), discarded tins and tires, clay pots, barrels, hand washing basins, leaky faucets, gutters, and other potential breeding sites. A sufficient number of *Anopheles* mosquito immature stages were collected and reared at the malaria entomology laboratory under controlled, optimal environmental conditions.

Data Collection

During the present survey, only immature mosquitoes were used. Larvae and pupa from indoor and outdoor containers were collected either by pipetting or dipping methods. Dipping was done in accordance with the larva searching strategies recommended by WHO (WHO, 1975) [30]. Pipetting was used in small containers not suitable for dipping. The water was first emptied into an enamel tray and local materials and all the larvae and pupae were collected in a vials. The collected larva and pupa were brought to the mosquito rearing facility at Tesseney town, where they were placed in trays and raised to adult. Each enamel tray containing larvae or pepae was labeled by habitat type. The larvae were allowed to develop in the water that was drawn from the field, to maintain the same aquatic environment. The pupae were sorted and transferred with pipettes from the enamel trays to beakers with modest volumes of water, then kept inside cages. A dissecting microscope was used to identify emerging adults to the species using identification key. All identified specimens were preserved individually in Eppendorf tubes for further analysis and were sent to the national malaria control program for further molecular identification.

Data Analysis

CSPro7.2 and statistical package for social science (SPSS) software version 21 were used as data entry and analysis respectively. Descriptive statistics were calculated for both variables, and a chi-square test was performed to assess the significance of the association between them. A p-value of less than 0.05 was considered to indicate a statistically significant association. Estimates were given in percentages, odds ratio and confidence intervals.

Ethical Clearance

Ethical clearance was first obtained from the ethical review committee at the Eritrean Ministry of Health. Upon entering the selected study area, permission was obtained from the sub-zonal administration, municipalities, and local administrations. Additionally, verbal consent was obtained from all household heads.

Result

Malaria morbidity

During the last eight years 28,315 malaria cases were reported from both study areas. *Plasmodium falciparum* (pf) accounts for more than 80% of the cases and the rest were *Plasmodium vivax* (pv) and mixed infection with both pf and pv. ver the past eight years, malaria cases in GB increased nearly fivefold, from 1,224 in 2015 to 5,806 in 2022. (Fig 1).

Habitat types: Their positivity and productivity for *Anopheles* and other mosquito type during a 4-month study period with 8 collection rounds, a total of 828 containers were inspected from three urban sites in Gash Barka zone (Teseney, Aqordet, and Omhajer). Of these, 186 (22%) were positive for at least one of the three mosquito types. And more than 94% of the total positive containers were found harboring *Anopheles* species (Table 1). The positivity of containers and the number of larva per container were almost the same at all the study sites. Table 3 shows that cement basins and barrels (plastic or metal) were the most common breeding sites for mosquitoes, accounting for over 62% of positive traditional water containers inspected during the study. Although widely used at all study sites, clay pots accounted for only 3% of the total positive mosquito breeding containers.

Table 2: During the study, 18, 607 larvae and 1, 225 pupae were collected from various container types, including cement basins, plastic basins, 200-liter barrels (plastic and metal), flower vases, clay pots, and overhead tanks. Of these, 8,212 were *Anopheles* mosquitoes, 7,046 *Culex* mosquitoes, and 4,349 *Aedes* mosquitoes. Cement basins stood out as the most productive breeding site, contributing a staggering 87% of collected *Anopheles* mosquitoes, followed by 45% of *Culex* and 50% of *Aedes* species. 200-liter plastic or metal barrels emerged as the second-most productive breeding site, contributing 12% of *Anopheles*, 31% of *Culex*, and a notable 42% of *Aedes* mosquitoes collected during the study. Table 4 reveals that all three mosquito species, *Anopheles*, *Aedes*, and *Culex*, were found to co-breed in the same habitats.

Prevalence of *Anopheles stephensi*

Figure 3 reveals that 383, 242, and 185 female *Anopheles* mosquitoes were reared for identification from Omhajer, Tesseney, and Agordat, respectively. Each adult mosquito emerged from the pupa was identified to species level using morphological characters under a dissecting microscope two to four days of post-emergence. During morphological identification: 368 (96.1%) from Omhajer, 174 (71.9%) from Tesseney and 105 (56.8%) from Aqordet were found to be *An. Stephensi*. All the distinct features of *An. Stephensi* was observed.

The present result shows the invasive malaria vector known as *An. Stephensi* is well-established at all the study sites.

Table 1: Habitat types and their positivity to *Anopheles* larvae in selected sites of the Gash Barka region

Container type	No. of inspected n (%)	Positive for any mosquito larva	Positive for *Anopheles* larva n (%)
Cement basin	178	116 (65%)	109 (94%)
Plastic barrel	312	31 (10%)	16 (52%)
Metal Barrel	143	24 (17%)	10 (42%)
Flower vase	84	2 (2%)	2 (100%)
Clay pot	63	5 (8%)	1 (20%)
Metal tank	45	7 (16%)	5 (55%)
Shower vase	3	1 (33%)	0 (0%)
Total	828	186 (22%)	143 (77%)

Table 2: Larvae of *Anopheles* and culicines collected from various habitat type in

Container type	No. of positive containers	No. of containers positive for both mosquito type	No. of *Anopheles* collected	No. of *Culex* collected	No. of *Aedes* collected	No. of pupa
Cement basin	116	38 (31%)	7136	3166	2165	681
Plastic barrel	31	12 (26%)	444	1408	815	380
Metal Barrel	24	5 (21%)	546	800	999	151
Flower vase	2	0	5	200	50	1
Clay pot	5	0	7	195	30	0
Metal tank	7	1(14%)	74	500	240	4
Shower vase	1	0	0	578	50	8
Total	186	56	8212	7046	4349	1225

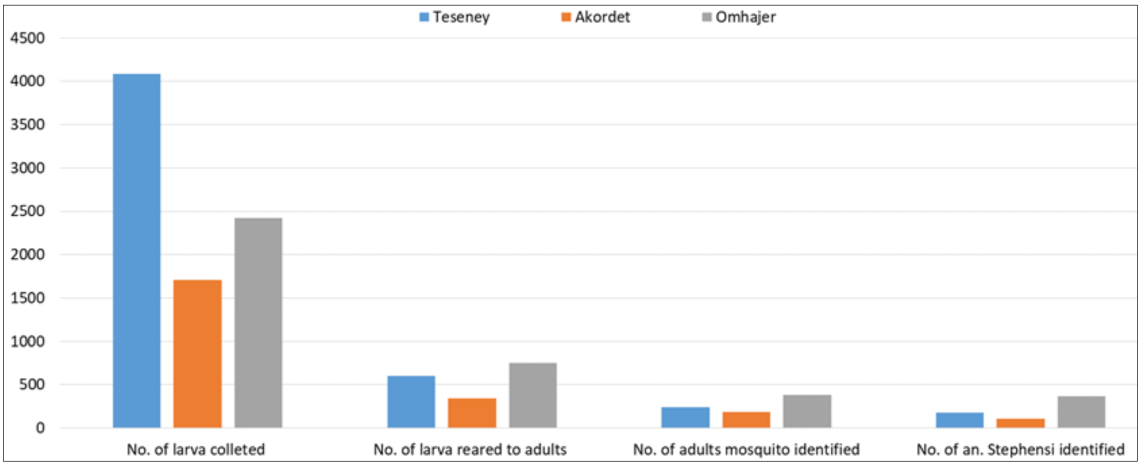

Fig 3: Comparison of *Anopheles* mosquito larvae collected, reared to adult and identified as *An. Stephensi*

Discussion

The result of the present study presented the morphological identification of *An. stephensi* in the urban sites of western part of Eritrea. This Region shared a border with Ethiopia and Sudan. *An. stephensi* was previously identified in almost all the neighboring countries, in Djibouti in 2012 (Faulde *et al*., 2014) [12], Ethiopia in 2016 (Carter *et al*., 2018) [1], and later in western Ethiopia in 2022 (Hawaria *et al*., 2023) [15], in Central Sudan, 2019 (Ahmed *et al*., 2021) [3], in Somalia (Somaliland) 2019 (Ali *et al*., 2022) [27], Kenya (Ochomo *et al*., 2023) [17] and in the in the Middle East like in Yemen (Allan *et al*., 2023) [8] and Saudi Arabia (Al Ahmad *et al*., 2011) [5].

The surveillance targets only immature mosquito. No CDC light trap and other adult collection methods were deployed. It was only conducted to ascertain the presence of *An. Stephensi*. Based on the recommendation from WHO, to incorporate Integrating *An. stephensi* surveillance into the existing malaria vector monitoring system in Eritrea yielded significant results. Surveys conducted in four out of six regions confirmed the presence of this mosquito with a very high prevalence in all urban sites surveyed within Gash Barka region. Molecular identification performed in Sudan by the national malaria control program (NMCP) confirmed the presence of *Anopheles stephensi* in the samples. Unfortunately, detailed information necessary for phylogenetic analysis and comparison with neighboring countries was not provided. Finding phylogenetic similarities with neighboring countries might not be essential in this case, as it's possible that multiple introductions of different clades occurred simultaneously in various parts of Africa. (Ochomo *et al*., 2023) [17]. The recent surge in malaria cases observed in our study areas over the past five years may be linked to the emergence of this invasive vector. This mosquito species has been demonstrated to be a highly efficient transmitter of both *Plasmodium falciparum* (Pf) and *Plasmodium vivax* (Pv), the parasites responsible for the most severe forms of malaria. A study from Ethiopia suggested that an increment of 50% in malaria incidence could have happened in areas where *An. Stephensi* is prevalent (Hamlet *et al*., 2022) [2]. A 2020 study by Sinka *et al.* predicted that an additional 126 million people in African urban areas could be exposed to malaria due to the spread of this invasive vector. The 2013 outbreak in Djibouti serves as a stark reminder of how quickly malaria cases can surge. With fewer than 3,000 cases reported in 2013, the number skyrocketed to over 49,000 just five years later (Seyfarth *et al*., 2019) [19], highlighting the importance of vigilant mosquito control measures.

An. stephensi shares the same breeding habitat with *Aedes* (*Ae.*) *aegypti*. These mosquitoes prefer HH water holding containers. Cement basins emerged as the dominant breeding site across all study areas, with over 87% of *Anopheles* immatures collected from them. A study in India identified overhead tanks as the dominant breeding site for *Anopheles stephensi*, with over 78% of mosquito larvae collected from this source (Thomas *et al*., 2016) [22]. All three mosquito species (*Anopheles*, *Culex*, and *Aedes*) were found co-inhabiting the same breeding sites across the study areas, with a relatively even distribution observed. In contrast, a study by Thomas found very limited co-inhabitation of *Anopheles* with other mosquito types in overhead tankers (Less than 5%). This contrasts with our findings of widespread co-inhabitation across different breeding sites.

Unplanned urbanization is creating mosquito-friendly infrastructure, leading to a surge in threats like arboviruses and, more recently, malaria transmitted by *An. Stephensi*, posing significant risks to urban residents. These factors, coupled with climate change creating favorable breeding conditions, pose a significant threat for the spread and establishment of *An. stephensi* across Africa. (Ochomo *et al*., 2023) [17]. Furthermore it was believed that this vector is resistant to the four classes of insecticides. In Ethiopia, resistance testing revealed that *Anopheles stephensi* mosquitoes are highly resistant to pyrethroids, carbamates, and organophosphates, which are the main classes of insecticides used for mosquito control (Balkew *et al*., 2020) [19].

Our understanding of *Anopheles stephensi*'s resting and biting behavior remains limited, despite its growing importance as a malaria vector in Africa. This knowledge gap, as highlighted by Mnzava, hinders effective control strategies (Mnzava *et al*., 2022) [36]. The finding that *An. Stephensi* bites outdoors challenges the effectiveness of traditional control methods like insecticide-treated nets (ITNs) and indoor residual spraying (IRS), which primarily target indoor-resting mosquitoes (Ochomo *et al*., 2023) [17].

A community-based intervention targeting the *Aedes aegypti* mosquito, the primary vector of dengue fever, was implemented in Anseba Region, Eritrea. The effectiveness of the intervention, which involved door-to-door education and community mobilization for mosquito breeding site elimination, was assessed through a reduction in both mosquito populations and reported dengue cases. The community-based approach achieved an impressive 10-fold reduction in larval indices, suggesting its potential as a valuable tool for controlling dengue fever transmission in similar settings (Yenus *et al*., 2021) [13]. This intervention would be a choice of intervention against *An. stephensi*, as this vector has the same breeding behavior with *Ae. aegypti*. In addition to this, a social and behavioral change guidance for *An. Stephensi* in Africa was developed and disseminated, which targets to promote the utilization of core interventions (ITS, IRS and prompt care-seeking for fever) and larval source management (Household larviciding, community larviciding, regulatory finding and removing standing water and covering water storage container.

Conclusion and Recommendation

Anopheles stephensi co-occurred with various native vector species, including [*Culex* and *Aedes*], at all three study sites. Further research is needed to determine its relative role in malaria transmission in the region. Given the co-existence of *Anopheles stephensi* with native malaria vectors, health authorities urgently need to revise existing control strategies. This comprehensive approach must simultaneously target both dominant and newly identified vectors to effectively manage their behavior and prevent disease transmission.

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