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Assessment of the Resting Behavior of *Aedes aegypti* During Dengue Fever Outbreak of Dire Dawa, Eastern Ethiopia

Abate Waldetensai*, Chalachew Sisay, Yosef Asrat, Yared Debebe, Solomon Kinde, Alemnesh H/mariam, Melaku Seyum, Yeweynshet Tesera, Mesfin Wosen, Wondatir Nigatu, AlemayehuTadese, Jemal Mohammed, Geremew Tasew, Araya Eukubay

Ethiopian Public Health Institute, Addis Ababa, Ethiopia

***Corresponding author:**
Abate Waldetensai

abyw.res31@gmail.com

Ethiopian Public Health Institute, Addis Ababa, Ethiopia.

Tel: 251911642445

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Abstract

Background: In depth understanding of the ecology and behavior of *Aedes aegypti* has paramount importance for implementation of innovative vector control strategies. The main objective of this study was to assess resting sites of *A. aegypti* as a risk of dengue fever transmission in Eastern Ethiopia.

Methods: A cross sectional study design was used with simple random sampling techniques in six sites from August to October 2020.

Results: A total of 758 mosquitoes belonging *Aedes* 356 (47%), *Anopheles* 221 (29.2%) and *Culex* 181 (23.9%) genera were collected. 149 houses were confirmed for the presence of *A. aegypti* with adult index 62.1% of which Gendegerada (88%), Goro (75%), Dechatu (58%) and Legehare (53%). *A. aegypti* was found to rest highly in bed, bedroom and living room followed by store room and kitchen. Most *A. aegypti* were collected from indoor walls, ceilings, curtains, roof, underside of furniture and wall hangers. The number of outdoor resting *A. aegypti* was low (84, 23.6%) compared to indoor resting surfaces (272, 76.4%). In a total, 113 (34.7%) fed, 54 (16.6%) unfed and 83 (25.5%) gravid *Aedes aegypti* were collected from indoor whereas 32 (9.8%) fed, 25 (7.7%) unfed and 19 (5.8%) were from outdoor

Conclusion: The presence of diverse resting surfaces in indoor and outdoor and the progressing history of disease outbreak of dengue in the study area may inspire an intensification of regular vector surveillance and applying the appropriate control activities in Ethiopia. Further investigations are needed to see if the populations of this species from other localities in Ethiopia have the same resting behavior.

Keywords: *Aedes aegypti*; Yellow fever; Zika viruses; Chikungunya; Dengue fever; Eastern Ethiopia.

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Introduction

Aedes aegypti is one of the most widely distributed mosquito species and causing a global burden of illness such as dengue, yellow fever, chikungunya, and Zika viruses [1]. The arboviruses responsible for these emerging and/or reemerging diseases have the particularity of being transmitted to humans by the mosquito *A. aegypti* in their urban environment [2]. Dengue occurs in developing countries of the tropics with around 2.5 billion people residing in dengue endemic areas [3]. Currently, dengue is most probably endemic to Ethiopia and repeated outbreaks have occurred to an area where *A. aegypti* are established and other local conditions favor transmission [4]. More currently in Ethiopia, dengue cases were reported from Eastern Ethiopia, Dire Dawa, where the ecology and behavior of the vector is not well understood yet [5,6].

The vector's behavioral and ecological adaptations made it to be an efficient vector of these diseases. *A. aegypti* is known to be well-adapted and complete its entire life cycle within urban areas, close to human dwellings and primarily on humans at a very high frequency. This behavioral trait leads human–mosquito contacts and dengue virus attack rates to be very high [7]. Because of the absence of specific treatment and effective vaccines (except yellow fever) for these diseases, vector control remains the only available control method against them [8]. In most dengue-endemic countries, vector surveillance usually consists of monitoring of *Aedes* species present in natural and artificial resting and breeding sites (larval habitats) in and near houses. Entomological measures as thresholds have been proposed to assess and estimate risk for use as early warning systems to predict dengue outbreaks.

Entomological surveillance needs to be in place to ensure the early detection of virus circulation or to detect the introduction of competent vectors with enhanced and sustained entomological surveillance at national and sub-national levels [7]. This ensures the detection of possible species responsible for the infection and establishes monitoring of insecticide resistance in the local *Aedes spp*. Populations. Entomological investigation is crucial to determine changes in the geographical distribution and density of the vector, evaluate control programmes, obtain relative measurements of the vector population over time and facilitate appropriate and timely decisions regarding interventions [6]. It may also serve to identify areas of high-density infestation or periods of population increase [9]. A number of methods are available for detecting or monitoring immature and adult populations. Selection of appropriate sampling methods depends on the investigation objectives, levels of infestation, available funding and skills of personnel. Controlling biting adult *Aedes* mosquito population is not seems to be easy activities. Along with an increase of emerging zoonotic diseases, there have been range expansions of reservoir hosts and *A. aegypti* densities with the pathogens they harbor. Dengue is the current enzootic mosquito borne viral disease being spread to new area especially in Africa due to increased human migration [6].

Though considerable arboviral diseases like DENGV without full documentation are known to be endemic in Ethiopia, infection remains unreported due to lack of laboratory facilities and inaccessibility of some endemic area. Behavioral study of Aedes mosquitoes that linked to DENGV transmission is limited with unknown vector host interaction status, which in turn makes the disease control programs difficult to design [10,11]. The current challenges in adult Aedes control are due to lack of appropriate vector biology including behavioral information as the virus spread becomes a serious threat in the country. An improved understanding of the relation of entomological factors to risk must be a priority [10]. The complex natural history of vector and arboviral transmission contributes to the difficulty in setting goals and executing effective control. The most cost-effective means of preventing mosquito- borne disease is targeting the adult vector, which transmits the pathogen.

Evidence based *Aedes* mosquito specific chemical-based control strategies have yet to receive the attention they merit, despite their obvious promises. As a result, more entomological information on the subject of the resting behavior and the status of *A. aegypti* in its transmission activity is required to design and implement affordable and safe vector control intervention that aid in prevention of the transmission of virus to new area of Dire Dawa, Eastern Ethiopia. The current study therefore aimed to assess the resting behavior of *A. aegypti* and their abdominal conditions during outbreak of dengue fever in Dire Dawa, Eastern Ethiopia.

Materials and Methods

Study area and setting

The study was conducted in Dire Dawa city which is located at a distance of 515 km east of Addis Ababa, the capital city of Ethiopia. It is located at 9°35 N and 41°51E with an altitude of 1193m above sea level with the average maximum annual temperature of 32.8°C and 637mm average annual rainfall. The municipality has a non-contiguous water supply (every 2 days) and irregular garbage collection [6]. The informal settlements with slums or substandard housing because of a number of people living in precarious sites such as mountains and stream sides are commonly known [12]. Due to the high-water shortage in the city, the people used to store rainwater for use. There is also a lack of sufficient food drainage system and good sanitation, hampering the health of the community of Dire Dawa City [6,5].

Study design

A cross-sectional entomological study design with simple random sampling of the household was done during the major outbreak of dengue in Dire Dawa, Eastern Ethiopia. All lower administrations level (Kebeles) with high and low Health facility-based dengue case reports of the city were investigated on the basis of the house to house visiting. Six collection sites with higher dengue case reports were selected by following randomized block design in consultation with experts from the regional health bureau and health care professionals of Dire Dawa from August to October, 2020. 40 houses from each selected collection site (totally 240) were randomly selected starting from the index houses from where the first dengue fever case and to the next to inspect and collect the vector.

The adult *Aedes* mosquito collection was done by well trained collectors and entomologists in peri-domestic and domestic environments with mechanical mouth and battery operated aspirator techniques during resting time of *A. aegypti*. Under this operational collection technique, all potential resting places of *Aedes* mosquitoes: home indoors (bedrooms and dark places such as clothes closets and other hidden sites and outdoors (plant: leaf, stem and hole; tyre and wall) were systematically searched with the aid of flush light. Following a standard collection routine, densities were recorded as the number of adult mosquitoes per house (females, males, or both) or the number of adults collected per specific resting sites at a time of effort.

Ethical considerations

No ethical review is applicable; the study was conducted during an emergency response and there is no human or animal issues related with. But consents were received from the head of household during visiting their home for mosquitoes collection.

Mosquitoes collection/ Aspirator collection

Standard mechanical mouth and battery operated aspirators were used to collect mosquitoes from all resting premises using a torch light. Systematic searching of resting mosquitoes was employed. The number of mosquitoes collected and the type of resting sites or shelters were recorded. For indoor collection of houses, one collector entered the house (including the kitchen and bathroom) and collected mosquitoes resting in all rooms (if there are more than one room) for 10 minutes, one collector was allowed to collect for 10minutes per collection sites for 2hours. The collectors were alternated between collection sites in different rounds. The collectors who did collection inside the house for 10 min were allowed to collect outside the house (walls and other dark places) for another 10 min. As collection proceeded, each aspirator collection cup was labeled with the house code, collection site and the collection sequence numbers.

Mosquito and *Aedes* species identification

After each Capture session by mechanical/oral and battery operated aspirators, mosquito genera and species were identified immediately in the field based on standard key and stored in cryovial tube filled with 96.2% ethanol 5-10 *Aedes* in one. The mosquitoes were identified using the gross morphology of the species from Ethiopia: external morphology of the pulps, antenna, proboscis, patches of pale and black scales on the wings and legs and the terminal abdominal segments, according to Hopkins [13]. Keys based on adult females were provided to distinguish subfamilies, genera and subgenera [13].

Data management and Analysis

All information collected from the study area were recorded in an excel data sheet each exported to SPSS (version 20) software for statistical analysis. The vector abundance (number of mosquitoes collected per site) was estimated with descriptive statistics.

Results

Mosquito species

A total of 758 mosquitoes under three genera: *Aedes* (356; 47%), *Anopheles* (221; 29.2%) and *Culex* (181; 23.9%) were collected. Of the total, relatively high number, 178 (23.5%) and 172 (22.7%) mosquitoes were collected from Goro and Addis Ketema study village that followed by Gendegerada 132 (17.4%) and Melka Jebdu 108 (14.2%). All mosquito numbers collected from each collection of villages were presented in **Table 1**.

Adult entomological Index

A total of 240 houses (40 houses from each village) were inspected for the presence of adult *A. aegypti*. Of this, 149 houses were confirmed for the presence of *A. aegypti*. The overall adult positivity index was 62.1%. The highest adult indexes were observed in the study villages of Gendegerada (88%) and Goro (75%), followed by Dechatu (58%) and then by Legehare (53%) **(Table 2)**.

Resting sites of *Aedes aegypti*

The mosquito species were also identified by sexes to determine the most responsible vector for the transmission of the arboviral diseases in the study area where the dengue outbreaks repeatedly reported. In a total (356) *A. aegypti,* 272 (76.4%) were collected from indoors with 84 (23.6%) outdoors. High (326, 91.6%) female *A. aegypti* were collected compared to that of male (30, 8.4%). Of female *A. aegypti*, 256 (70.8%) were collected from indoor shown in **Table 3**.

Resting premise types of *Aedes aegypti*

In the current study, the resting premises of *A. aegypti* were also identified from each village and showed on **(Figure 1)**. High numbers of *A. aegypti* were collected from bed/bedroom and living room which was followed by store room and kitchen. Plant was also used as alternative resting sites of *A. aegypti* in addition

Table 1 Species of mosquitoes across all collection villages, Dire Dawa, Eastern Ethiopia.

	Study Villages						
Mosquitoes species	Goro (%)	Gendegerada (%)	Dechatu (%)	Legehare (%)	Addis Ketema (%)	MelkaJebdu (%)	Total (%)
A. aegypti	89 (11.7)	66 (8.7)	47 (6.2)	37 (4.9 (	86 (11.3)	31 (4.1)	356 (47)
Anopheles	74 (9.8)	33 (4.4)	36 (4.7)	2 (.3)	41 (5.4)	35 (4.6)	221 (29.2)
Culex	15 (2)	33 (4.4)	11 (1.5)	35 (4.6)	45 (5.9)	42 (5.5)	181 (23.9)
Total	178 (23.5)	132 (17.4)	94 (12.4)	74 (9.8)	172 (22.7)	108 (14.2)	758 (100)

Table 2 Adult entomological index across study villages, Dire Dawa, Eastern Ethiopia.

Village	Number of *A. aegypti*	House (+)	Inspected houses	Adult Index (%)
Goro	89	30	40	75
Gendegerada	66	35	40	88
Dechatu	47	23	40	58
Legehare	37	21	40	53
Addis Ketema	86	18	40	45
MelkaJebdu	31	22	40	55
Total	356	149	240	62.1

Table 3 Abundances of *A. aegypti* by sex across resting sites, Dire Dawa, Eastern Ethiopia.

	Sex		
Resting sites	**Male (%)**	**Female (%)**	**Total (%)**
Indoor	20 (5.6)	252 (70.8)	272 (76.4)
Outdoor	10 (2.8)	74 (20.8)	84 (23.6)
Total	30 (8.4)	326 (91.6)	356 (100)

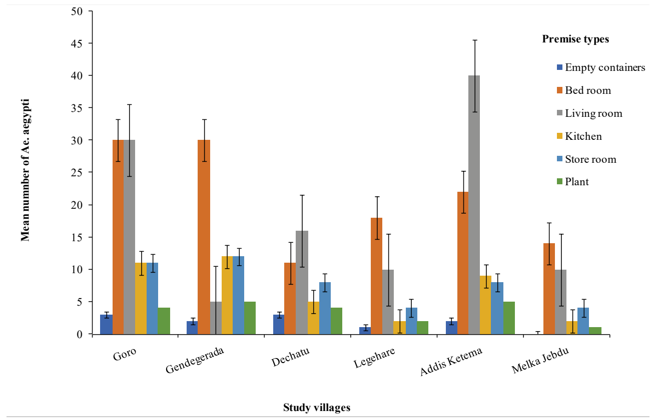

Figure 1 Premise types for resting of *A. aegypti* across study villages of Dire Dawa, Eastern Ethiopia.

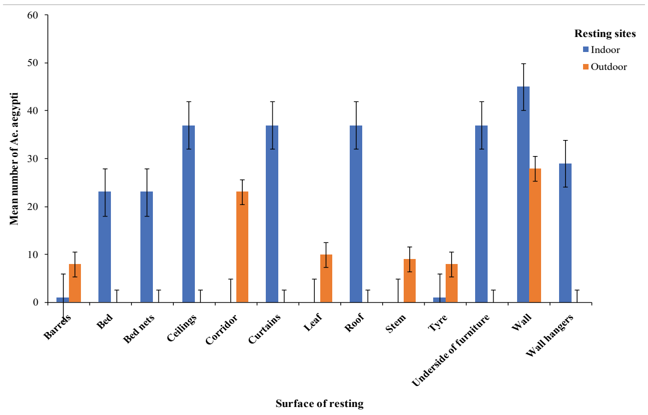

Figure 2 Resting surfaces of *A. aegypti* across resting sites of Dire Dawa, Eastern Ethiopia.

to that of Empty containers. With regard to study villages, high number of *A. aegypti* were collected from living rooms of Addis Ketema and moderate Goro villages. Relatively important *A. aegypti* numbers were collected from bedrooms in Goro and Gendegerada study villages.

The resting surfaces of *A. aegypti* were also identified and presented in **Figure 2** across resting sites. Most *A. aegypti* were collected from indoor walls, ceilings, curtains, roof and underside of furniture as well as wall hangers. The number of *A. aegypti* was low from outdoors when compared to indoor resting surfaces. Though the number of this vector was found to be low, plant leaf and stem, barrel and tyre were also used as resting surfaces in the study sites.

Abdominal condition

Of a total (326) of *A. aegypti* mosquito, 145 (44.5%) fed, 102 (31.3%) gravid and 79 (24.2%) unfed were collected from both indoor and outdoor. From overall fed, 113 (34.7%) were indoor with 32 (9.8%) outdoor. 83 (25.5%) and 19 (5.8%) gravid *A. aegypti* were collected from indoors and outdoors respectively. 54 (16.6%) unfed *A. aegypti* were also collected from indoors with 25 (7.7%) of this vector from outdoor collection. *A. aegypti* abdominal conditions were also showed by each study villages and presented in the **Table 4.**

Discussion

In the current study, the resting preferences, abdominal condition and species of mosquitoes were assessed following the outbreak of dengue fevers. From total 240 houses inspected, for the presence of adult *A. aegypti,* high percent of houses were positive.

Table 4 Adult *A. aegypti* female mosquitos' abdominal status by resting sites across study villages of Dire Dawa, Eastern Ethiopia.

	Abdominal conditions					
	Indoor			Outdoor		
Villages	Fed (%)	Unfed (%)	Gravid (%)	Fed (%)	Unfed (%)	Gravid (%)
Goro	21 (6.4)	10 (3.1)	29 (8.9)	4 (1.2)	4 (1.2)	13 (4)
Gendegerada	16 (4.9)	16 (4.9)	10 (3.1)	5 (1.5)	8 (2.5)	2 (0.6)
Dechatu	9 (2.8)	11 (3.4)	7 (2.1)	7 (2.1)	7 (2.1)	0 (0)
Legehare	18 (5.5)	0 (0)	9 (2.8)	9 (2.8)	0 (0)	1 (0.3)
Addis Ketema	35 (10.7)	17 (5.2)	15 (4.6)	4 (1.2)	6 (1.8)	2 (0.6)
Melka Jebdu	14 (4.3)	0 (0)	13 (4)	3 (0.9)	0 (0)	1 (0.3)
Total	113 (34.7)	54 (16.6)	83 (25.5)	32 (9.8)	25 (7.7)	19 (5.8)

This may be due to aggregation of bites on one or few infected individuals, mosquito biting on multiple viremic individuals to the house, or the dispersal of infected mosquitoes from nearby premises. As the transmission of Arboviral is shaped by the daily mobility patterns of humans living in the houses may experience a high risk of infection [8]. This is because of the availability of oviposition sites for high numbers of infected mosquitoes with preferable house types [14-16].

The present study documents the relative abundance of indoor and outdoor *A. aegypti* captured during the outbreak of dengue. Female *Aedes aegypti* mosquitoes were found to be high in indoor collections than the outdoor collection. This finding is in all the six sites is inconsistent with other study conducted in Kenya [17]. The variation with other previous study might be occurred due to the difference in resting behavior of mosquitoes across different countries. The diurnal biting and most indoor resting behavior of *A. aegypti* in bed, bedroom and living room which was followed by store room and kitchen is a good implication of its capacity to be a responsible vector of dengue vectors [18]. This is mainly because it has adapted to rest in a wide range of resting artificial materials that are mostly located indoor within human dwellings like walls, ceilings, curtains, roof and underside of furniture as well as wall hangers [19]. The indoor *A. aegypti* population significantly elevates human-mosquito contacts and can have a profound effect on the estimate of natural infection and arboviral transmission risk [20]. However, this study result also showed the possibility for this species to rest outdoors in dark and shady areas near its breeding sites [21]. As of the current result, plant was also used as alternative resting sites of *A. aegypti* in addition to that of Empty containers. A population of *A. aegypti* was also resting outdoors in these breeding sites, such as used tires, corridors, plant leaf and stem, wall as well [20].

The use of more indoor and outdoor resting places by *A. aegypti* has important implications for vector control strategies, particularly in an outbreak response context. Thus, it seems essential to target these both indoor and outdoor resting places during insecticides aerial spraying [22]. Besides density at different resting sites being a risk factor for the emergence of dengue, this study showed that *A. aegypti*, feeding pattern were high, important contributing factors that can differentially drive the emergence of dengue. This study also identified the abdominal conditions of collected *A. aegypti* from different resting sites. The majority of *A. aegypti* were fed from indoor collection with moderate gravid and less unfed. In addition to this, the blood feeding status of the vector by itself determines the transmission status of dengue. This data suggest that the vector human contact is high may be due to that female attracted to the residential environment with a larger blood meal supply. This may a great factor for the dengue virus transmission within and/or between communities. The authors concluded that the presence of high number of fed and gravid *A. aegypti* within human population density indicated that there could be high risk of dengue transmission [23].

The number of fed and gravid *A. aegypti* could be also due to high relationships among mosquitoes, human density, and available resting sites in and around house. The predominance of fed mosquitoes might also have been linked with reproductive success of mosquitoes depends on host defensive behavior. This may be sufficient to cause an outbreak of the disease, because a higher human density provides high vector contact rates [24]. The larger proportions of blood fed and gravid female *A. aegypti* is a reflection that most of *A. aegypti* were resting for egg laying. In the current study, the age of the dengue vector to show whether it is parous or nulliparous within gonotrophic cycles and blood sources of the vector were not assessed. The viruses being circulated within the community were also not screened from the vectors that displayed as the limitation of this study.

Conclusion

In conclusion, the firm identification of exact resting places of *A. aegypti* could allow the focal application of insecticides in different mosquito resting sites in an environmental and economically supportable way. *A. aegypti* is well established in the study area, presenting itself as a permanent population with higher numbers inside houses. The presence of diverse resting surfaces in indoor and outdoor and the progressing history of disease outbreak by *Aedes* species in the study area may inspire an intensification of the vector surveillance activities. This study provides the first baseline data on the presence of different resting sites for *Aedes* species, the potential for the emergence of viral diseases in the vulnerable populations and high risk areas based on densities of mosquitoes. The entomological indicators of adult females must be part of an interconnected data set when evaluating and controlling this mosquito. Risk factors such as mosquito density, different indoor and outdoor option resting sites and surfaces, human vector contacts, vector competence and host feeding pattern, when interpreted collectively, may sufficiently inform risk of transmission of Dengue fever virus. Indoor and outdoor resting surfaces should be considered as both breeding sites and resting places of *A. aegypti* in Ethiopia. Further investigations are needed to see if the populations of this species from other localities in Ethiopia and elsewhere have the same resting behavior.

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