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College of Health Sciences

School of Biomedicine and Medical Laboratory Sciences

Department of Microbiology, Immunology and Parasitology

Bionomics, Insecticide Susceptibility Status and Community Perception of *Aedes aegypti* in Afar Region, Ethiopia

By

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Declaration

I the undersigned, declare that this dissertation entitled: **Bionomics, Insecticide Susceptibility Status and Community Perception of *Aedes aegypti* in the Afar Region, Ethiopia** submitted to Department of Microbiology, Immunology and Parasitology, Addis Ababa University for the degree of Doctor of Philosophy (PhD) in Tropical and Infectious Diseases is my own original work. I confirm that this dissertation has never been presented in Addis Ababa University or any other University, and all the resources and materials used for the dissertation have been fully acknowledged.

Mohammed Seid Legas (GSR/7760/12) _____ _____

Name of the student Signature Date of submission

Dr. Abebe Animut Signature_____ Date of submission_____

Advisor

Dr. Esayas Aklilu Signature _____Date of submission_____

Advisor

_____

Chair of the department

DEDICATION

To my late father Seid Legas Mitiku, and my late mother Enani Awoke Tesema whom I always remember throughout my life.

Abstract

Background: The recent emergence and re-emergence of *Aedes*-transmitted diseases, such as dengue fever and chikungunya, are public health concerns in Ethiopia, including the Afar Region. Despite the frequent outbreaks of the diseases, control strategies are limited in the country. Due to the absence of licensed vaccines (except yellow fever) against *Aedes*-borne diseases, control of the *Aedes aegypti* that transmits the diseases is a priority. A successful *Ae. aegypti* control strategy, in turn, depends on knowledge of its behavior, insecticide susceptibility status, and vectorial role. Therefore, this research was designed to investigate the bionomics, including the spatial and temporal occurrence and larval/pupal habitat characteristics, adult resting behavior blood meal source, insecticide susceptibility, and viral infection status of *Ae. aegypti*. In addition, the study also assessed the community awareness about *Aedes* mosquitoes and associated risks to chikungunya and dengue fever.

Methodology: Longitudinal study design was employed to collect adult and immature *Aedes* species in Awash Sebat, Awash Arba, and Werer towns of Southern Afar Regional State once per month from May 2022 to April 2023. The larvae/pupae were collected and reared to adults and identified by genus and species morphologically. The physical and chemical properties of their habitats were also characterized. Adult mosquitoes were collected using a Prokopack aspirator between 8:00 to 14:00 hrs and 15:00 to 18:00 hrs both indoors and outdoors of the house. The collected mosquitoes were sorted by sex, date of collection, collection places, and abdominal status and identified using standard morphological keys. Blood meal sources and dengue virus and chikungunya virus infection status of *Ae. aegypti* were determined using enzyme-linked immunosorbent assay and reverse transcriptase quantitative polymerase chain reaction, respectively. Larvae/pupae of *Ae aegypti* were collected and reared to test the insecticide susceptibility status of the species. Non-blood-fed, 3-5-day-old females *Ae. aegypti* were exposed to pyrethroid, carbamate, and organophosphate insecticide impregnated papers in tube tests following WHO standard guidelines. Knockdown rates were noted at 10-minutes interval until one hour. The mortality in *Ae. aegypti* was recorded 24 hrs after 60 minutes of exposure. A total of 384 respondents participated in the questionnaire survey. In addition, three focus group discussions were held to reinforce the questionnaire survey.

Results: A total of 9099 *Aedes* larvae/pupae were collected, of which 4875 (53.6%) were from Awash Sebat, 2687 (29.5%) from Awash Arba, and 16.9% (1537) from Werer. Water-holding tyres harbored the highest number of *Aedes* larvae/pupae followed by water storage

drums. All the *Aedes* larvae/pupae reared to adults were morphologically identified as *Aedes aegypti*. The overall Container Index was 37.9%, House Index 17.1%, Breteau Index 63.9% and Pupal Index 171.7. The occurrences of *Ae. aegypti* larvae/pupae was positively associated with water-holding tyres (AOR 15.89, CI = 3.55-71.09, p <0.001), water storage drums (AOR =19.84, CI = 4.64-84.89, p <0.001) and domestic habitats (AOR = 3.76, CI = 1.27-11.12, p = 0.017). Density of *Ae. aegypti* larvae/pupae was positively correlated with dissolved oxygen ($\beta = 0.523$, p <0.001) and total hardness ($\beta = 0.475$, p = 0.034) of water. A total of 2,745 adult mosquitoes comprising the genera *Aedes,* 1433 (52.2%) *Culex,* 1292 (47.1%) and *Anopheles,* 20 (0.7%) were collected. The proportion of female *Ae. aegypti* in Awash Sebat 611 (36%) was highest as compared to females *Ae. aegypti* in Awash Arba 172 (; 33.8%), and in Werer 59 (11%). A higher proportion of outdoor resting *Ae. aegypti* was caught from tyres rather than other indoor and outdoor resting sites, 314 (37.29%) (X^2 27.374, df = 12; p = 0.007). Seasonal and monthly variation was observed in *Ae. aegypti* collection, where the wet season and the months of August 2022, September 2022, and October 2022 had high *Ae aegypti* density. The overall human blood and bovine blood indices of *Ae. aegypti* were 53/145 (36.6%) and 18/145 (12.4%), respectively. Dengue and chikungunya viruses were not detected from the *Ae. aegypti* examined. The mortality rates of *Ae. aegypti* exposed to 0.1% propoxur were 87% in all the study towns. Similarly, 88% mortality in *Ae. aegypti* was recorded when tested with 0.1% bendiocarb in Awash Sebat and Awash Arba towns. Suspected resistance of *Ae. aegypti* (95% mortality) to 0.05% alpha-cypermethrin was observed in Awash Arba town. However, *Ae. aegypti* collected from all the three towns were observed to be susceptible to 0.05% deltamethrin, 0.75% permethrin, and 0.25% pirimiphos-methyl. The majority of the respondents, 284 (73.9%), heard/knew about chikungunya disease. However, only 24 (8.5%) of the respondents reported that chikungunya is transmitted through bites of infected *Aedes* species. In addition, the majority of the respondents had low knowledge scores (78.1%) about the larval habitats of *Aedes* mosquitoes. Similarly, about 76% of the respondents associated *Aedes* mosquitos' effect with nuisance and biting pain, while 23.7% associated it with diseases risks. Larger proportion of the respondents (73.7%) had low practice scores to avoid *Aedes* bites. Being a health professional (AOR = 7.73, p = 0.020), a student (AOR = 5.21, p = 0.004) and a merchant (AOR = 2.98, p = 0.048) had a sound knowledge compared to housewife regarding larval habitats of *Aedes* species. Respondents aged 29-38 (AOR = 3.74, p = 0.027), university educated (AOR = 4.64, p < 0.001) respondents involved more in the practice to control *Aedes* mosquito bites.

Conclusion: Diverse types of artificial water-holding containers, including tyres, water storage drums, cement tanks, flowerpots, and discarded plastics were positive for *Ae. aegypti* larvae/pupae. Used tyres located at domestic sites were the most preferred *Ae. aegypti* larval habitats and adult resting sites in the study towns. *Ae. aegypti* showed resistance to bendiocarb and propoxur, suspected resistance to alpha-cypermethrin, and susceptible to deltamethrin, permethrin and pirimiphos-methyl. Health professionals, students, and merchants had more knowledge of *Aedes*' larval habitats. Respondents aged 29-38 and university-educated respondents were more involved in controlling *Aedes* mosquito bites. Domestic water-holding containers, which serve as both immature larval habitats and adult resting sites such as used tyres and other potential larval/resting habitats management strategy is recommended in controlling *Ae. aegypti* abundance and hence prevention of *Aedes*-transmitted diseases. In addition, *Aedes* mosquito control strategies should also be designed on the basis of the rising knowledge, risk perception, and prevention practices of the community about *Aedes* mosquitoes and their associated risks towards local chikungunya and dengue fever by targeting people with lower education backgrounds.

Key words; *Aedes aegypti,* Afar Region, Attitude, Chikungunya, Dengue fever, Ethiopia, habitat characteristics, Knowledge, Practice

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Lists of Abbreviations

AAU: Addis Ababa University

AChE: acetylcholinesterase

AD: Adult density

AIPB: Aklilu Lemma Institute of Pathobiology

AHI: Adult house index

ATSB: Attractive toxic sugar bait

asl: above sea level

AUPRC: Africa Union permanent representative committee

BBI: Bovine blood index

BI: Breteau Index

bsl: below sea level

CHIKV: Chikungunya virus

CDC: Center for disease control and prevention

CI: container index

CNaVdp: Voltage- dependent sodium channel

DENV: Dengue virus

EPHI: Ethiopian public health institute

ELISA: Enzyme linked immuno-sorbent assay

FBS: Fetal bovine serum

FGD: Focus group discussion

FDRECC: Federal Democratic Republic of Ethiopia Census Commission

FPH: Female per positive houses

FSH: Female per surveyed houses

GLM: Generalized linear model

GPS: Geographical positioning system

HI: House index

IGR: Insect growth regulators

IIFA: Indirect immunofluorescent assay

IRS: Insecticide residual spray

ISVs: Insect specific virus

Kdr: Knockdown resistance

LLITN: Long lasting insecticidal treated nets

LSM: Larval source management

MoH: Ministry of Health Ethiopia

MoHE: Ministry of higher education

PCR: Polymerase chain reaction

PBS: Phosphate buffer saline

PI: Pupal index

RNA: Ribonucleic acid

ROS: Reactive Oxygen Species

RT-PCR: Real time polymerase chain reaction

SIT: Sterile insect technique

ULV: Ultra low volume

WHO: World Health Organization

List of published articles and prepared manuscript from the findings:

Paper I: Spatio-temporal occurrence and habitat characteristics of *Aedes aegypti* (Diptera: Culicidae) larvae in Southern Afar Region, Ethiopia. 2024; Tropical Medicine and Health, 52(51):1-14 **https://doi.org/10.1186/s41182-024-00612-5**

Mohammed Seid, Esayas Aklilu, Abebe Animut (2024).

Paper II: Resting habitat, blood meal source and viral infection rate of *Aedes aegypti* (Diptera: Culicidae) in Southern Afar Region, Ethiopia. 2025; BMC Infectious Diseases, 25 (346):1-12. **https://doi.org/10.1186/s12879-025-10748-2**

Mohammed Seid, Esayas Aklilu, Yohannes Negash, Andargachew Mulu, Dawit Hailu, Kalkidan Melaku, Abebe Animut (2024).

Paper III: **Susceptibility status of *Aedes aegypti* (Diptera: Culicidae) to public health insecticides in Southern Afar Region, Ethiopia.** 2024; PLoS ONE, 19(8):1-12. **https://doi.org/10.1371/journal.pone.0309335**

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Paper IV: Urban Community knowledge, attitudes, and practices towards *Aedes* mosquitoes and *Aedes*-transmitted chikungunya and dengue fever in the Southern parts of Afar Region, Ethiopia (**Draft manuscript**)

Mohammed Seid, Esayas Aklilu, Abebe Animut (2024).

Definition of terminologies

Attitude: Refers to inclinations to react in certain way to a certain situations; to see and interpret events according to certain predispositions; or to organize opinion into coherent and interrelated structures.

Domestic habitats: are within and around, living houses or buildings, which include the plant and animal in controlled, anthropogenic environment.

Human and Bovine Blood Index (HBI/BBI): Proportion of mosquito blood meals taken from humans or bovines, respectively.

Insecticide: Chemical product (natural or synthetic) that kills insects. Ovicides kill eggs; larvicides kill larvae; pupacides kill pupae; adulticides kill adult mosquitoes. Residual insecticides remain active for an extended period (WHO, 2022a).

Insecticide resistance: Property of mosquitoes to survive exposure to a standard dose of insecticide; may be the result of physiological or behavioral adaptation (WHO, 2022a)

Knockdown: for insecticide bioassays, a knocked-down mosquito is one that cannot stand, cannot fly in a coordinated manner, lies on its back, moving legs and wings but unable to take off or one that can stand and take off briefly but falls down immediately.

Knowledge: is the capacity to acquire, retain and use information; a mixture of comprehension, experience, discernment and skill

Mortality: for insecticide bioassays, mosquitoes mortality is a percentage or proportion of mosquitoes which die after being exposed to an insecticide.

Peri-domestic habitats: are outside, around the perimeter of living houses or buildings. They include the external surfaces of buildings, the ornamental trees and shrubs that characterize the urban and suburban landscape.

Practice: is the application of rules and knowledge that leads to action.

Susceptibility: The degree to which a mosquito population is susceptible to insecticides.

Vector control: Measures of any kind against disease-transmitting mosquitoes, intended to limit their ability to transmit the disease.

CHAPTER ONE: INTRODUCTION

1.1. Background

Aedes-transmitted viral diseases such as yellow fever, dengue fever, chikungunya, and zika have drawn public health attention in several countries of the world (Braack *et al*., 2018). These co-circulating diseases are responsible for several epidemics in recent years. This is mainly due to the rapid increase and geographical expansion of *Aedes* species, particularly *Aedes aegypti* and *Aedes albopictus* (Bhatt *et al*., 2013; Carlson *et al*., 2016; Kraemer *et al*., 2016; Konongoi *et al*., 2018). More than half of the global population is at risk of dengue fever infection (Bhatt *et al.*, 2013). The World Health Organization estimated about 5 million cases and 5000 deaths in relation to dengue fever in 2023 globally (WHO, 2023a). This was an 8-fold increase in the number of cases when compared to the 2019 report. Similarly, about 109,000 cases and 51,000 deaths from yellow fever were reported annually in Africa and South America, of which 90% occurred in Africa during 2018 (Gaythorpe *et al*., 2021). More than 610 million people in 32 African countries live in areas at high risk of contracting yellow fever (Gaythorpe *et al*., 2021).

In Ethiopia, yellow fever caused about 30,000 deaths among over 100,000 infections during the 1960 to 1962 (Serie *et al.*, 1968). Dengue fever outbreaks have been reported in a number of regions including Dire Dawa (Woyessa *et al.*, 2014), Godey (Gutu *et al*., 2021), Humera and Metema (Ferede *et al.*, 2018), Arba Minch (Eshetu et al., 2020), Borena (Geleta, 2019), Amibara and Gewane districts (EPHI, 2021; Mekuriaw *et al*., 2022). In addition, chikungunya outbreaks were reported in Awash Sebat and Awash Arba of the Afar Region (Zerfu *et al*., 2024) and Dire Dawa City and the Somali Region (Geleta *et al*., 2020; Alayu *et al.*, 2021). Such outbreaks could potentially cause unrecognized and/or underreported deaths owing to the highly limited testing facilities in the country.

In addition to causing sickness and death, *Aedes*-transmitted viral diseases cause loss of working time and extra health care expenditure in managing individual patients (WHO, 2012; Weaver *et al*., 2012). This imposes an economic burden, especially in highly affected East African countries, including Kenya, Somalia, Djibouti, Eritrea, and Ethiopia, which are epicenters of outbreaks of viral diseases such as dengue, yellow fever and chikungunya (Usman *et al*., 2016; Langat *et al*., 2020; Gutu *et al*., 2021).

Mosquitoes transmit over 90% of the arthropod-borne viral pathogens *(*Gubler, 2011*;* Li *et al*., 2014), of which *Ae. aegypti* is a primary vector and *Ae. albopictus* a secondary (Almeida *et al*., 2005; Kraemer *et al*., 2015). *Ae. aegypti, Ae. africanus, Ae. albopictus* (Ngoagouni *et al*., 2015) and *Ae. luteocephalus* act as vectors of viral diseases in Africa (Were, 2012). In Ethiopia, although the role of *Ae. aegypti* in transmitting viral infections in humans is not yet determined, some authors reported its high abundance during the outbreaks of yellow fever, dengue fever, and chikungunya (Waldetensai *et al*., 2021a; Waldetensai *et al*., 2023). This suggests the potential of *Ae. aegypti* as a vector of arboviral diseases in the country. Moreover, *Ae. bromeliae, Ae. vittatus, Ae. hirsutus, Ae. simpsoni* complex, *Ae. albopictus,* and *Ae. africanus* may also act as the potential vectors in the country (Woyessa *et al*., 2014; Lilay *et al*., 2017; Ferede *et al*., 2018; Yared *et al*., 2024; Jemberie *et al*., 2025).

Due to its anthropophilic behavior, *Ae. aegypti* inhabits and breeds close to human habitations (WHO, 2009; Powell, 2018). Its larvae and pupae inhabit natural habitats such as tree holes (Dieguez-Fernandez *et al*., 2024) and varieties of man-made water containers, including tyres, mud pots, sinks, polythene sheets, plastic bowls, and buckets that are left outdoors (Wongkoon *et al*., 2007; Brown *et al*., 2011; Hiscox et al., 2013; Getachew *et al*., 2015). It breeds abundantly during the wet season and is strongly associated with rainfall, humidity, and temperature (Gould and Higgs, 2009). Abundance of *Aedes* larvae and associated viral disease transmission risk can be described using indices such as house index (HI), breteau index (BI) and container index (CI) (WHO, 2009; Udayanga *et al*., 2020).

Adult *Ae. aegypti* rests in different habitats. In one instance, it preferred to rest indoors (Dalpadado *et al*., 2022). It was also found resting outdoors, such as in tyres, on vegetation, bricks, and scrap metals in Senegal (Diallo and Diallo, 2020; Diouf *et al*., 2021). Recent studies reported that used tyres are potential outdoor resting and oviposition sites (Diallo and Diallo, 2020; Abdulai *et al*., 2024). Knowledge of the resting behavior of *Aedes* species along with the local environmental factors is the basis for designing an effective control strategy (Roberts and Andre, 1994). However, data on the resting habitats of *Aedes* species is limited in Ethiopia (Waldetensai *et al*., 2023; Yared *et al*., 2024).

A female *Ae. aegypti* feeds on mammalian blood for the development of its eggs (Tenywa *et al*., 2024). The species feeds on human blood during daylight hours (Gubler, 1998; Narayanan *et al*., 2002). In Tanzania, Kenya, Senegal, and Cameroon, the species exhibited a wide range of hosts, such as human, bovine, pig, dog, cat, and rat (Scott *et al*., 1993;

Kamgang *et al*., 2012; Diouf *et al*., 2021; Kamau *et al*., 2023). Knowledge of its blood-feeding preference provides insight into its role as a disease vector (Dye and Hasibeder, 1986) and helps inform efficient strategies for control (Garcia-Rejon *et al*., 2010). The preference of *Ae. aegypti* to feed on humans can be estimated using the human blood index (HBI), which is the proportion of tested mosquitoes that have fed on a human host (Kamau *et al*., 2023). Its preference to feed on other animals is expressed in a similar way. However, the feeding preference of mosquitoes depends on host availability and accessibility. Their blood meal sources can be screened using enzyme-linked immunosorbent assay (ELISA) (Scott *et al*., 1993), precipitin test (Gomes *et al*., 2001), and polymerase chain reaction (PCR) (Siriyasatien *et al*., 2010).

In its attempt to feed on vertebrates, a female *Aedes* mosquito takes viruses, along with blood, from an infected host and transmits the viruses to susceptible hosts, including humans (Vasconcelos *et al*., 1998). Transmission of viral pathogens depends, in part, on the occurrence and abundance of *Aedes* species (Demanou *et al*., 2014), their feeding preference, and replication of the viruses in the mosquito and the vertebrate hosts, including humans (Nisalak *et al*., 2003). Apart from transmitting viruses from infected to susceptible hosts, *Ae. aegypti* maintains the viruses through its generations transovarially (Da-Costa *et al*., 2017; Heath *et al*., 2020). The viral infection status of *Aedes* species can be detected by indirect immunofluorescent assay (IIFA), antigen capture ELISA, and polymerase chain reaction methods (Victor, 2009), where the latter is preferable in terms of sensitivity and specificity (Boonham *et al*., 2014). Viral infection rates of *Aedes* species are traditionally described as the number of a virus species infected species per the total number of the species tested for the virus (Degallier *et al*., 1992; Gu et al., 2003).

Indoor residual insecticide spraying (IRS) and long-lasting insecticide-treated nets (LLITNs) are being used for the control of disease-transmitting mosquitoes (WHO, 2022b). However, these tools are being challenged due to the emergence and spread of insecticide-resistant mosquitoes among others (Moyes *et al*., 2017). Evidence indicates that *Anopheles* and *Aedes* mosquitoes developed resistance to most of the public health insecticides (Mathias *et al*., 2017; Kenea *et al*., 2019; Owusu-Asenso *et al*., 2022). For instance, in malaria-transmitting *Anopheles arabiensis*, insecticide resistance to most of the available insecticides has been reported in Ethiopia, including in the Amibara district of the Afar Region (Messenger *et al*., 2017; Kenea *et al*., 2019). Although, resistance of malaria-transmitting *An. arabiensis* to

insecticides is described adequately, the susceptibility status of *Ae. aegypti* to the insecticides remains poorly investigated in Ethiopia.

Aedes-transmitted viral diseases control strategy depends on surveillance systems including, timely reporting of the cases, inform policymakers, defining funding and research priorities, and increasing awareness among the community (Toan *et al*., 2015). However, these depend on the communities' knowledge, attitudes, and practices in relation to the *Aedes* mosquitoes and their importance as viral disease vectors. Adequate community perception is crucial to reduce viral diseases transmission by reducing available vector habitats and avoiding man-vector contacts, ultimately improving targeted interventions, education programs, and health policy (Gubler and Clark, 1994; Healy *et al*., 2014). Although, recommendation are being forwarded in different countries regarding the importance of understanding community perceptions to *Aedes*-transmitted viral diseases (Whiteman *et al*., 2018), such data remain scarce in Ethiopia.

Several factors contribute to the outbreaks of *Aedes*-transmitted viral diseases, including emerging unplanned urbanizations, improper container disposal, presence of high vector (*Aedes* species) density, and climate change (Ryan *et al*., 2019; Kolimenakis *et al*., 2021). In the absence of a vaccine (with the exception of yellow fever) and a lack of effective drugs against most of the *Aedes*-transmitted viral diseases (Halstead, 2012; Aggarwal and Garg, 2018), vector control remains a priority (WHO, 2014a). In turn, a vector control strategy depends on data on breeding habitat characteristics, adult resting sites, blood meal sources, vectorial role, and insecticide susceptibility status of the local *Aedes* species, as well as perception of the local community. However, data on the aforementioned factors are limited in Ethiopia in general and in Awash Sebat, Awash Arba, and Werer towns in particular, where dengue and chikungunya outbreaks have occurred frequently (EPHI, 2021; Zerfu *et al*., 2024); Amibara district and Awash Sebat health bureaus, unpublished). The control strategies are those used for malaria vector control (Waldetensai *et al*., 2021b). Thus, this study aimed to fill these knowledge gaps by investigating the bionomics (larval/pupal habitat characteristics, adult resting behavior, blood meal sources, and viral infection rates), susceptibility status to public health insecticides, and community perception of *Aedes* species. Therefore, the study aimed to determine physico-chemical characteristics of larval habitats, adult resting behavior, blood meal sources, susceptibility status to public health insecticides,

dengue/chikungunya virus infection rates, and community awareness of *Ae. aegypti* in the Southern Afar Region, Ethiopia.

1.2. Statement of the problem and rationale of the study

In Ethiopia, the history of *Aedes*-transmitted viral diseases dates back to the 1960s (Serie *et al*., 1968). In recent years, there have been repeated outbreaks of viral diseases, including dengue fever and chikungunya, in various parts of the country (Woyessa *et al*., 2014; Ferede et al.2018; Eshetu *et al*., 2020; Gutu et al., 2021; Mekuriaw *et al*., 2022). Dengue fever outbreak has occurred during 2019 in Gewane and Amibara districts of the Afar Region including Awash Sebat and Awash Arba towns. About 588 people were affected by the outbreak. In the case of Awash Sebat and Awash Arba, it was a mixed infection of dengue fever and chikungunya (EPHI, 2021; Mekuriaw *et al*., 2022). Moreover, a sero-prevalence study conducted in Awash Arba and Awash Sebat towns in 2022 revealed that the prevalence of acute chikungunya infection was 47.8% (Zerfu *et al*., 2025). There were also unpublished reports of Awash Sebat, Awash Arba, and Werer town health bureaus during 2021, in which case the people suffered from chikungunya and dengue fever diseases (Amibara district and Awash Sebat town administration health bureaus, 2021, unpublished).

The outbreaks could result from various factors, including increased unplanned urbanization (uncontrolled habitat formation) that enhances breeding of *Aedes* mosquitoes and increased viral transmission. Studies have indicated that there is a variation of *Ae. aegypti* in resting and oviposition habitat preference with local environmental and genetic factors (Dalpadado *et al*., 2022). However, research on this variability of *Ae. aegypti* is limited in Ethiopia, including the Afar Region. Moreover, the intensive use of insecticides targeting malaria vectors could induce insecticide resistance in *Aedes* species where the two cohabit, contribute to increased viral disease outbreaks. There have been reports of *Ae. aegypti* resistance to insecticides commonly used in controlling mosquitoes in Tanzania and Ghana (Mathias *et al*., 2017; Owusu-Asenso *et al*., 2022). Ethiopia has implemented LLINs and IRS since 2005 (WHO, 2017) to control the malaria-transmitting *An. arabiensis*. However the mosquito has developed resistance to the insecticides used in LLINs and IRS (Yewhalaw *et al*., 2011; Balkew *et al*., 2012). Likewise, *Aedes* species, including *Ae. aegypti*, could develop resistance to the insecticides, as the two groups of mosquitoes share similar habitats. However, the susceptibility of *Aedes* species, including *Ae. aegypti*, to the available

insecticides remains uncertain in Ethiopia in general and in the southern part of Afar Region in particular.

Despite increasing seroprevalence reports of *Aedes*-transmitted viral diseases from various corners of Ethiopia, there are no well-established *Aedes*-transmitted disease control strategies in the country. Furthermore, occurrences of *Aedes* mosquitoes that transmit the viruses are poorly investigated in the southern part of the Afar Region. This inspired us to determine the most common *Ae. aegypti* larvae/pupae habitats and adult *Ae. aegypti* resting places, blood meal sources, viral infection rates, and insecticide susceptibility status in the Region. Accordingly, the Spatio-temporal occurrence and habitat characteristic of the *Ae. aegypti* larvae/pupae **(Paper I**); the Resting habitat, blood meal sources and viral infection rate of *Ae. aegypti* (**Paper II**); the Susceptibility status of *Ae. aegypti* to public health insecticides (**Paper III**); and the Awareness of the local community about *Aedes* mosquitoes and their role in dengue and chikunguya diseases transmission (**Paper IV**) were assessed to generate baseline data for policy-makers and researchers in their effort to strengthen control and prevention of *Aedes*-transmitted viral diseases in the Southern part of Afar Region and elsewhere in the country.

1.3. Significance of the study

Data on the bionomics, viral infection rate, and community awareness of *Aedes* species were generated in the Awash Sebat, Awash Arba, and Werer towns of the southern Afar Region, where there have been repeated reports of dengue and chikungunya infections. Thus, the results of the study provided baseline data, which will be useful in developing and implementing effective *Ae. aegypti* control strategies considering its local ecology and behavior (targeting the potential larval/pupal habitats of *Ae. aegypti* to disrupt its life cycle) in the southern part of the Afar Region and in other similar settings in the country. The findings will be insightful in the development and use of insecticide based *Ae. aegypti* control plans in Ethiopia. It will also be used as an input by academics and researchers interested in *Aedes*-transmitted viral diseases management initiatives in the future. Therefore, the overall merit of this PhD dissertation research is to provide baseline data to both the Afar Region and the national health sector, which assist in designing effective vector control strategies, considering the local *Ae. aegypti* breeding habitats, resting behaviors and blood meal sources, susceptibility status to public health insecticides, and the community's knowledge, attitude

and practices towards *Aedes* mosquitoes and associated risks to dengue and chikungunya diseases.

1.4. Research questions

1. What are types, characteristics and prolific months of the larvae/pupae of *Aedes aegypti* in the Awash Sebat, Awash Arba and Werer towns?
2. What are the *Aedes* species inhabiting Awash Sebat, Awash Arba and Werer towns across the months?
3. What are the most common blood meal hosts and preferred resting habitats of *Aedes aegypti*?
4. What are the viral infection rate of *Aedes aegypti*?
5. Is *Aedes aegypti* inhabiting the towns susceptible to public health insecticides?
6. What is the knowledge, attitudes and practices of the inhabitants of the towns in relation to *Aedes* species and *Aedes*-transmitted dengue fever and chikungunya?

1.5. Objectives of the study

1.5.1. General objective

The aim of this study was to investigate larval habitat characteristics, blood meal sources, resting habitats, viral infection rates, insecticide susceptibility status and local community perceptions of *Aedes aegypti* in the Awash Sebat, Awash Arba and Werer towns of the southern Afar Regional State, Ethiopia.

1.5.2. Specific objectives

1. To identify types and characteristics of *Aedes aegypti* larval/pupal habitats in Awash Sebat, Awash Arba and Werer towns*;*
2. To determine resting habitats and blood meal sources of *Aedes aegypti;*
3. To determine viral infection rates of *Aedes aegypti;*
4. To describe susceptibility status of *Aedes aegypti* to public health insecticides; and
5. To assess awareness of residents of the towns towards *Aedes* species and their role as vector of dengue fever and chikungunya.

CHAPTER TWO: LITERATURE REVIEW

2.1. Global situations of *Aedes*-transmitted viral diseases

Several life-threating viral diseases, including yellow fever, dengue fever, chikungunya, and rift valley fever are transmitted by *Aedes* mosquitoes (WHO, 2014a). About 146 countries in the world documented *Aedes*-transmitted viral diseases, among which 111 countries reported dengue fever, 106 chikungunya, 85 Zika, 43 yellow fever, and 39 Rift Valley fever. Most of these countries are situated in tropical and subtropical regions, where environmental conditions favor proliferation of *Aedes* mosquitoes (Leta *et al.*, 2018).

Dengue fever is among the widely distributed *Aedes*-transmitted viral diseases globally, and its incidence has been increasing in recent years (WHO, 2023a). It is primarily transmitted by *Ae. aegypti* and *Ae. albopictus*. Autochthonous dengue transmission has been reported in several countries of Europe, including Croatia, France, and Portugal (Massad *et al*., 2018). This indicates the continued expansion of dengue fever from its historic tropical and subtropical regions geographically (Leta *et al*., 2018). The global spread of dengue might be attributed to the increasing global climate change, urbanization, and increase in the number of long-distance travelers (Giesen et al., 2022). In the Americas, 46 out of 52 countries are suitable for dengue vector reported autochthonous transmission. These trends require control strategies that include effective vector control, established surveillance systems, and awareness creation among the local community (Leta *et al*., 2018). Addressing these strategies requires coordinated efforts from governments, public health organizations, and communities.

In Africa, *Aedes*-transmitted viral diseases overlap in their distributions (Figure 1a) and put about 831 million people at risk (Weetman *et al.*, 2018). In the Sub-Saharan region, dengue fever underscores a significant public health challenge (Eltom *et al*., 2021). The disease occurs in North African countries like Egypt, Mali, and Sudan, indicating its widespread distribution in the continent across different climatic conditions (Humphrey *et al*., 2016). About 10-14% of the Sub-Saharan African population experienced an acute dengue virus infection in the past ten years, and 25% of the population experienced a history of exposure to dengue fever at some point during their lives. This situation reflects poor vector and disease control programs (Eltom *et al.*, 2021).

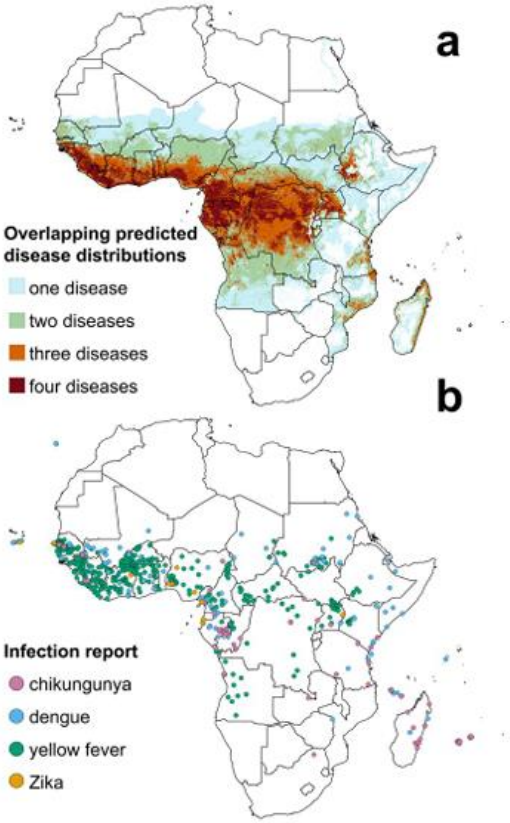

Figure 1: Distribution of dengue fever, chikungunya, yellow fever, and zika in Africa: (a) Areas at risk of one, two, three or all four infections, (b) Locations of reported infections (symptomatic and non-symptomatic) of dengue, chikungunya, Zika and yellow fever (Source: (Weetman et al., 2018).

Yellow fever was first reported in Africa, where it likely adapted to sylvatic vectors and hosts. With time, the slave trade might have facilitated the spread of the virus along with the movement of infected people and infected mosquitoes to new ecological niches in the Americas (Bryant *et al*., 2007). The disease has been reported in 30 African countries, and its incidence rates were high in Senegal and Ghana (Figure 2). Yellow fever cases have been reported from 13 countries of South America and in most of the southern and south-eastern Asian countries where the area is found suitable for the vector (Leta *et al*., 2018). Vaccination campaigns and vector control strategies significantly reduced its incidence in many areas of Africa (Garske *et al*., 2014). However, ongoing vigilance is necessary, especially in endemic regions or where ecological changes might facilitate its resurgence (Cupertino *et al*., 2019).

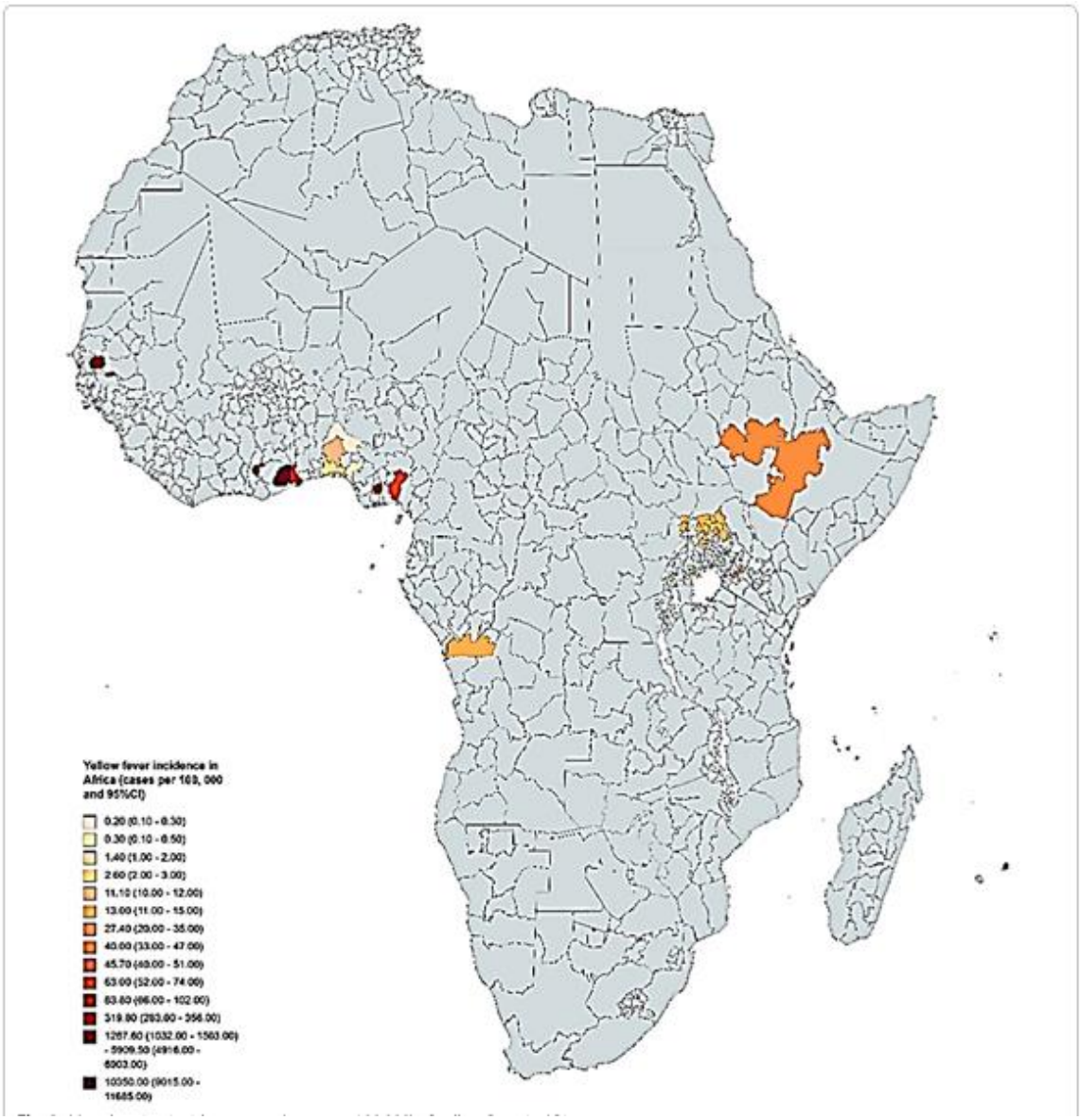

Figure 2: Incidence rates (cases per 100,000) of yellow fever in Africa: Source: (Nwaiwu *et al*., 2021).

Chikungunya virus (CHIKV) was first identified during an outbreak in Tanzania in 1952, and has caused significant outbreaks across various regions, particularly in Eastern and Central Africa (Russo *et al*., 2020). CHIKV infection is asymptomatic in 3-25% of the patients (Weaver and Lecuit, 2015). However, it is a highly contagious disease that can affect up to 70% of the total population in an outbreak affected area (Lourenço *et al*., 2016). It is the most widely distributed disease after dengue fever. All countries of Sub-Saharan Africa are endemic for the vector and the disease (Russo *et al*., 2020). It has already established autochthonous vector-borne transmission in 26 countries/territories. Countries in the Horn of

Africa (Djibouti, Eritrea, Ethiopia, and Somalia) and Southwestern Africa are suitable for the disease (Russo *et al*., 2020; Mude *et al*., 2024; Frezgi *et al*., 2025). Moreover, Southeast Asian countries like Yemen, Saudi Arabia, India, and China have reported cases of the disease (Leta *et al*., 2018).

2.2. *Aedes*-transmitted viral diseases in Ethiopia

Ethiopia has been facing multiple *Aedes*-transmitted viral disease outbreaks in recent years (Figure 3). The outbreaks of dengue fever occurred in different areas of the country. For instance, 11,409 dengue fever suspected cases occurred in Dire Dawa, of which one death was reported in 2013 (Woyessa *et al*., 2014). About 529 suspected dengue cases were diagnosed using the Euroimmune indirect immunofluorescent assay in Arba Minch during 2016, of which 133 cases were confirmed as dengue fever infection (Eshetu *et al*., 2020). A seroprevalence survey in the Metema and Humera districts revealed 114 dengue fever cases out of 600 blood samples of suspected cases in 2017 (Ferede *et al*., 2018). In Gewane district of Afar Region, a total of 1185 dengue fever cases were recorded of which six of them were confirmed positive by RT-qPCR in 2019 (Mekuriaw *et al*., 2022). Prevalence of dengue fever was also studied in Kebridehar, Somali Region, where 101 were suspected and 1 died in 2017 (Gutu *et al*., 2021).

The yellow fever outbreaks have been a serious public health concern in Ethiopia. During the 1960 and 1962 outbreaks in the southwest part of the country, about 100,000 cases and 30,000 deaths resulted (Serie *et al*., 1968). Yellow fever, a viral hemorrhagic disease transmitted by *Ae. aegypti*, is severe and deadly. More recently, a yellow fever outbreak occurred in South Omo area of the country, where it infected close to 22 individuals and killed five of the cases (AUPRC, 2016, unpublished). Moreover, it caused a total of 35 cases and ten deaths in the Wolaita zone (MoH, 2018, unpublished). With its high mortality rate and the potential for rapid spread, the recent outbreaks represent a significant public health threat that requires immediate attention, including vaccination campaigns and vector control measures.

Chikungunya has been reported recently in Awash Sebat and Awash Arba towns during 2022 from a total of 368 febrile malaria suspected cases, of which the prevalence of acute chikungunya infection was 47% (Zerfu *et al*., 2024). CHIK was also reported in Dire Dawa city and the Somali Region of Ethiopia (Alayu *et al*., 2021). Even though, it is less fatal

compared to yellow fever and dengue fever, it can cause debilitating joint pain, rash, and fever affecting public health and quality of life. The multiple outbreaks of yellow fever, dengue, and chikungunya underscore the need for strengthened surveillance, vector control, and public health interventions across the country.

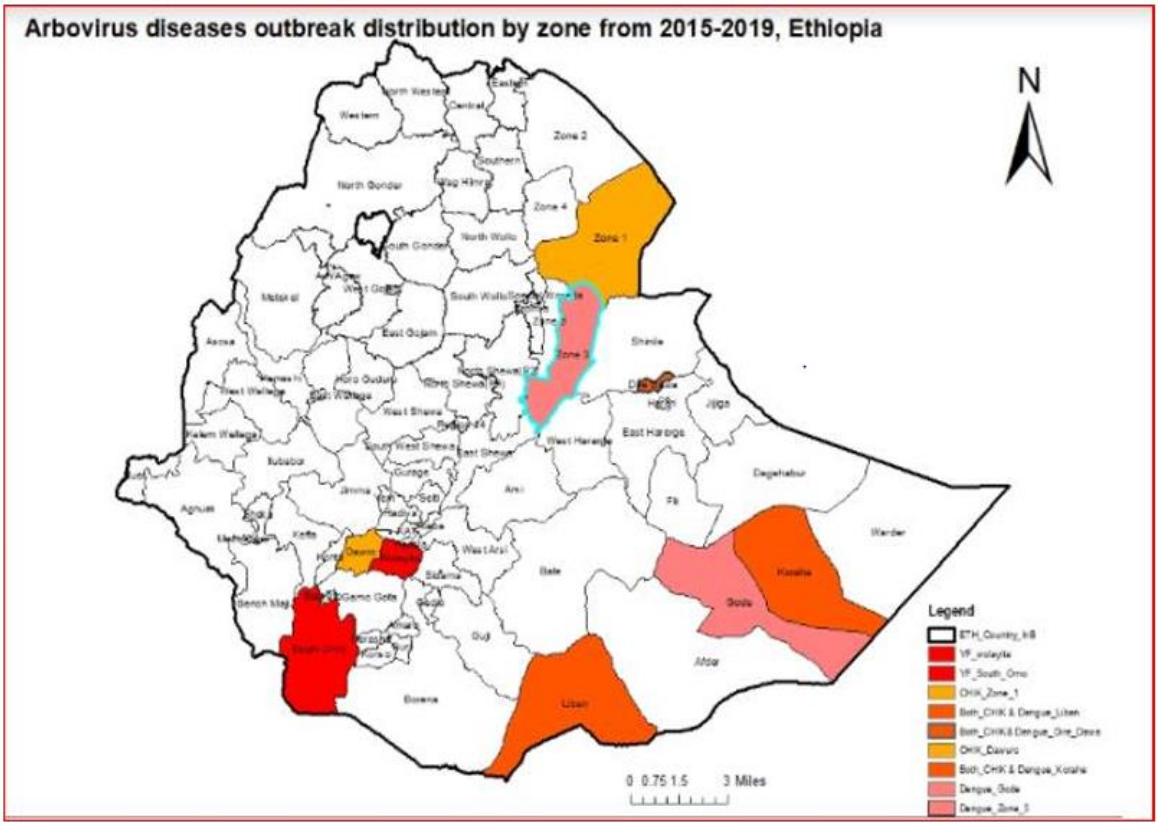

Figure 3: *Aedes*-transmitted viral diseases distribution in Ethiopia (Source: Ethiopian Public Health Institute, 2021).

Despite the increasing reports of *Aedes*-transmitted viral diseases such as yellow fever, dengue, and chikungunya in Ethiopia, their prevalence is likely underreported. This is primarily due to the lack of laboratory facilities and inaccessibility of endemic regions to public health facilities. In addition, vector-borne disease control strategies mainly focus on malaria. No control strategies targeting *Aedes*-transmitted viral diseases are implemented, or such strategies are poorly understood in the country (Waldetensai *et al*., 2021b). This could partly result from the absence of adequate data on the bionomics of *Aedes* mosquitoes and the viruses they transmit. Furthermore, the complex interaction between vector biology and viral disease transmission, along with the clinical similarity of viral diseases and malaria infections, could challenge efforts to set clear goals and implement effective control

programs in Ethiopia. Thus, conducting research into these areas is essential to mitigate the growing threat of *Aedes*-transmitted viral diseases (Leandro *et al*., 2023).

2.3. Bionomics of *Aedes* species

Bionomics of mosquito species refers to a comprehensive study of mosquitoes' interaction with their abiotic and biotic components of the environment (Subbarao *et al*., 2019). Bionomics of *Aedes* species entails understanding the interaction of their eggs, larvae, pupae, and adults with the abiotic and biotic environments as types and characteristics of immature (egg, larvae, and pupae) and adult habitats, the feeding and resting behavior of adults in relation to the local ecology and seasons (Dia *et al*., 2008). The eggs of *Ae. aegypti* resist desiccation and can survive out of water for up to a year (Nimmo, 2015). Thus, understanding the oviposition behavior, including oviposition sites, larval habitats and preference, flight densities, and natural viral infections of *Aedes* species, provides insights to understand the risk of *Aedes*-transmitted viral diseases.

2.3.1. Adult oviposition behavior and habitats of immature stages

Mosquito larval/pupal habitats are the locations where oviposition, larval development, adult emergence, swarming, mating, and resting take place (Mwangangi *et al*., 2009). Oviposition (egg laying) is the process of depositing eggs into water bodies conducive for larval/pupal development and adult emergence. It is one of the most important events in mosquitoes that requires integration of internal and external stimuli (Day, 2016).

Ae. aegypti undergoes a complete metamorphosis in its reproduction (Figure 4). A female *Aedes* mosquito lays 50 to 500 eggs at one time, depending on the species, water or site that will be flooded (Nimmo, 2015). An *Ae. aegypti* lays 100-150 eggs in a single gonotrophic cycle (Nimmo, 2015). The eggs are laid in a variety of water-holding containers such as flower pots, water-storage drums or tanks, and discarded plastic or metal containers, tyres, jars, and buckets (Himatt *et al*., 2015). The eggs hatch to first instar larvae that exhibit wriggling movement to avoid predators and move to oxygen-rich surfaces. Larvae are sensitive to light and often aggregate in shaded areas. A first instar larva will take 5-8.5 days to attain the pupal stage. Larvae are filter-feeders and feed on organic matter, algae, and microorganisms in water. Pupae are non-feeding but highly active and exhibit tumbling motion when disturbed (Anoopkumar *et al*., 2017).

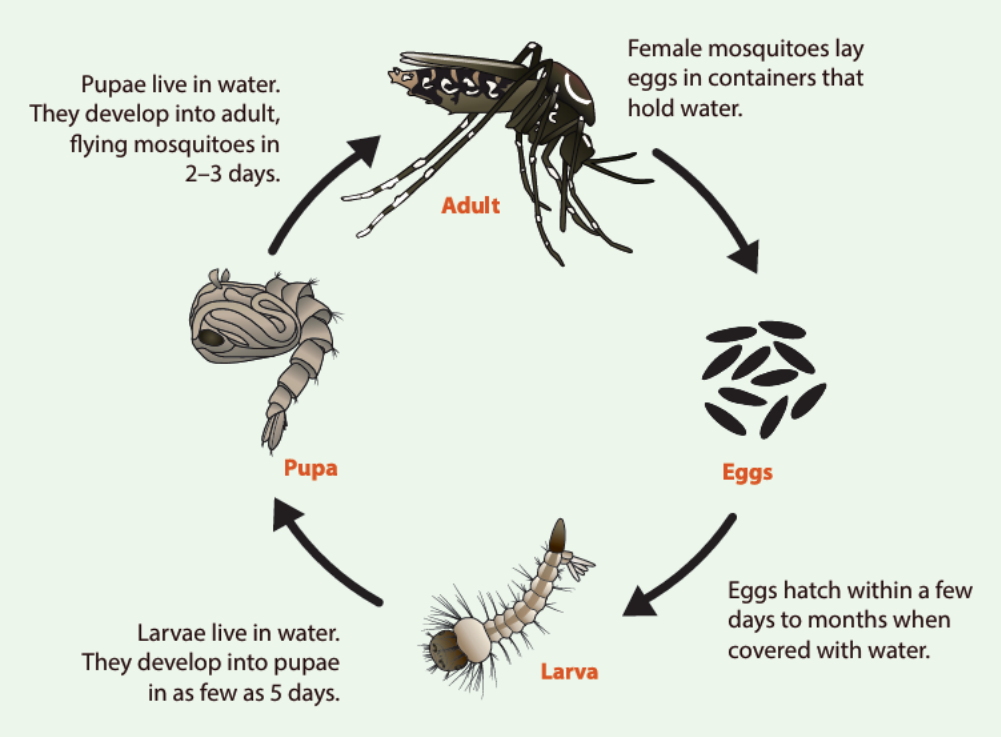

Figure 4: Life-cycle of *Aedes* mosquitoes (Source: CDC, 2022: www.cdc.gov/mosquitoes/about/life-cycles/*Aedes*).

In Ethiopia, *Ae. aegypti* larvae were observed in water-holding discarded tyres, mud pots, discarded sinks, polythene sheet, discarded vehicle parts, plastic bowls, and buckets in Dire Dawa (Getachew *et al*., 2015; Waldetensai *et al*., 2021b), Southern Omo (Waldetensai *et al*., 2023), the Gewane district of the Afar Region (Mekuriaw et al., 2022) and Kebridhar town of the Somali Region (Yared *et al*., 2024).

Oviposition activity mainly occurs during the rainy season due to increased availability of water-filled containers. *Ae. aegypti* has a unique characteristic and adaptation known as skip oviposition. It oviposits its eggs in multiple sites, which decreases larval competition and increases progeny survivorship (Wong *et al*., 2011). In recent years, this behavior has gained significant attention because of its epidemiological importance (Reiter, 2007). However, the principles behind skipping oviposition have become a matter of speculation, as it is mediated by various factors, including chemicals, which originate from the mosquito's larvae, eggs, or both (Guha *et al*., 2014).

Factors including food availability, microorganisms and algae, larval density and competition, presence of predators, and water physicochemical properties influence larval

productivity and oviposition site selection by *Ae. aegypti* (Juliano, 2009; Navarro-Silva *et al*., 2009). In North Carolina, semio-chemicals originated from the larvae and/or eggs of *Ae. aegypti* produced by larval-associated bacteria may help females locate cryptic sites and stimulate egg laying (Ponnusamy *et al.*, 2008). Types of water-holding containers also influence oviposition site preference by *Ae. aegypti*. Usually, *Ae. aegypti* may prefer artificial but, in some occasions, natural containers and receptacles that hold water. In Cameroon, *Ae. aegypti* were observed to breed in ant traps, earthen jars, flower pots, drums, concrete tanks, coconut shells, and discarded tyres (Simard *et al*., 2005). In Tanzania, used tyres were reported to be the most preferred larval habitats of *Ae. aegypti* followed by domestic water storage containers and natural habitats (Mathias *et al*., 2017). The size and color of the larval habitat also influence oviposition site selection. In Thailand, oviposition site selection by *Ae. aegypti* depended on the size and the color of the breeding containers, their use, location, shading condition, water movement, water's pH, temperature, permanence, and the presence of conspecific larvae/pupae (Waewwab *et al*., 2019).

In Brazil, water physico-chemical parameters such as salinity and pH affected *Ae. aegypti* larval survival and growth rate, where extreme values of salinity (greater than 0.1 % of mean salt concentration) and pH (mean pH of < 5 and > 8 values) hindered the presence of larvae in salted water (Medeiros-Sousa *et al*., 2020). In Kinshasa of Congo, habitat dissolved oxygen positively influences the larval density (Mbanzulu *et al*., 2022a). Moreover, in Northern Iran, total hardness of water-holding containers was positively associated with increased larval density of mosquitoes (Nikookar *et al*., 2017).

Temperature is another factor that regulates the productivity of *Ae. aegypti* larvae/pupae in their habitats. In Australia, *Ae. aegypti* exhibited a decrease in larval development time and body size as temperature increased, and there was a reduction in larval survival when the temperatures were extreme, such as 15 ^{o}C or 35 ^{o}C (Tun-Lin *et al*., 2000). In addition, food availability enhances larval development, while competition by intraspecific or interspecific species has significant negative effects on larval development time and adult emergence (Yee *et al*., 2004).

Locations of larval habitats influence productivity of *Ae. aegypti* larvae/pupae. For instance, in Kenya, indoor containers were found more productive for larvae of *Ae. aegypti* (Midega *et al*., 2006). But other studies observed that outdoor water-holding containers were more productive and harbored a higher number of larvae in the indoor (Lutomiah *et al*., 2016;

Islam *et al*., 2019). Black containers located at semi-shady place are preferred oviposition sites of *Ae. aegypti* (Wong *et al*., 2011). Moreover, *Aedes* species may prefer flower pots due to their outdoor location and relative abundance and infrequent cleanings (Philbert and Ijumba, 2013). Therefore, understanding oviposition behavior of *Aedes* mosquitoes may provide new insight into the factors affecting *Aedes* population regulation and aid in predicting their response to control measures (Nylin, 2001).

Containers that harbour water for longer periods tend to have a higher density of larvae/pupae than containers frequently used for drinking and other purposes. Containers with large volumes of water are found to contain a large density of larvae/pupae compared to containers with small amounts of water (Lenhart *et al*., 2006). More breeding activity occurs in containers that are uncovered than in those tightly covered (Maciel-de-Freitas *et al*., 2007).

2.3.2. Adult emergence and dispersal

Immediately after emergence from pupae, *Ae. aegypti* adults rest for the first few hours to allow the exoskeleton and wings to harden (Nelson, 1986). A study in Tamil Nadu, India, revealed that compared to females, male *Ae. aegypti* first undergo a permanent elongation of the terminalia to be able to mate within 2 days of post-emergence (Mayilsamy *et al*., 2024). Moreover, sexual maturity of male *Ae. aegypti* requires about 15-24 hrs whereas female *Ae. aegypti* will copulate and accept sperm after 2 days or older (Gwadz *et al*., 1971; Ramirez-Sánchez *et al*., 2023). Salivary glands in newly emerged females need a period of time to mature, which may explain why blood meals are not accepted during the first 18 to 24 hrs or 20 to 40 hrs (Orr *et al*., 1961).

Ae. aegypti exhibits limited flight ranging, approximately 50 to 200 meters from their breeding site. This restricted movement is influenced by their preference to stay close to human habitats for feeding and breeding. The study conducted in California and Florida indicated that adult *Ae. aegypti* aged 0 to 12 hrs dispersed within 100 m from the release point (Nayar, 1981; Harrington *et al*., 2005). However, in Brazil a female *Ae. aegypti* rarely disperses >200 m from point of emergence or release to locate a blood meal (Maciel-De-Freitas *et al*., 2010). Furthermore, in Maryland, *Ae. aegypti* was shown to be capable of flying 2.5 km in the field, but this could probably reflect its flight potential (Rowley and Graham, 1968). Dispersal of *Ae. aegypti* is affected by the availability and density of houses, hosts, and larval habitats (Brown *et al*., 2017). Thus, knowledge of flight range and dispersal of *Ae. aegypti* is essential to understand *Aedes*-transmitted disease

transmission dynamics among human populations. This is because flight range and dispersal influence mosquito population dynamics, patterns of genetic structure, and pathogen transfer through vector population (Harrington et al., 2005).

2.3.3. Sugar feeding behavior

Sugar provides energy for flight, survival, and mating for *Ae. aegypti* (Foster, 1995). Sugar-poor environments limit the population or survivorship of adult mosquitoes (Gu *et al*., 2011). This could render *Ae. aegypti* evolved to become highly anthropophilic, feeding almost exclusively on humans, with minimal feeding on sugar sources in nature (Harrington *et al*., 2001). Sugar feeding may impact the role of *Ae. aegypti* to transmit pathogens. A laboratory study in Thailand reported that sugar feeding early in life can depress blood meal intake, digestion, and feeding avidity (League *et al*., 2021). However, field studies are needed to determine the extent to which natural sugar feeding (fruits, nectars, honeydew, and others) affects as opposed to artificial sugar.

Sugar feeding positively influences probability of survival, longevity, reproductive physiology, including excitation of the antennal fibrillae, and insemination rates of male *Ae. aegypti* (Chadee and Gilles, 2014). Thus, knowledge of sugar feeding habits in mosquito vectors is an important strategy to formulate attractive sugar baits (ATSB). For instance, the lethality of attractive sugar baits against female *Ae. aegypti* mosquitoes has been demonstrated in laboratory and field settings, but the components that attract mosquitoes are still being studied (Qualls *et al*., 2014).

2.3.4. Mating behavior

Soon after emergence from pupa, many species of mosquitoes initiate mating by forming swarms. Swarm formation is made by males, and females are supposed to enter the aggregate for mating (Mo'awia *et al*., 2014). Swarm formations are usually mediated by chemical stimuli, particularly aggregation pheromones. However, the exact stimuli that trigger swarming in *Ae. aegypti* are not fully understood, although, host odors seem to play a significant role (Triana and Melo, 2024). Variations in swarm size and time of activity further distinguish *Ae. aegypti* from other species. The number of males within a swarm ranges from 5-11 to 20-100 (Cabrera and Jaffe, 2007).

Mating/copulation time of *Aedes* mosquitoes varies with place, species, and season. A study conducted in Trinidad, West Indies, showed that swarming of *Ae. aegypti* peaked in the early-

morning between 6:00 hrs and 8:00 hrs and in pre-sunset from 16:00 hrs to 18:00 hrs both indoor and outdoor sites (Chadee and Gilles, 2014). Understanding the mating behavior of *Ae. aegypti* allows us to widen our view of novel and targeted control strategies aimed at disrupting its reproduction potential and disease transmission. Since males tend to swarm around human hosts, it is crucial to understand the dynamics and role of humans in the mosquito's mating process (Triana and Melo, 2024).

2.3.4. Blood feeding behavior and biting hours

Blood-feeding patterns are important epidemiological drivers of pathogen transmission (Kamau *et al*., 2022). *Ae. aegypti* is generally considered to be highly or exclusively anthropophilic, feeding preferentially on humans, due to the low titer of isoleucine found in human blood (Harrington et al., 2001). However, a study in Thailand using ELISA demonstrated that from a total of 433 fed *Ae. aegypti*, 88% (379/433) were fed on humans, 4.6% (20) human-dogs, 1.4% (6) bovine-humans, 0.5% (2) bovines, 0.2% (1) rats, 0.2% (1) cats and 0.2% (1) fed on chickens (Ponlawat and Harrington, 2005). In addition, a blood meal analysis of *Ae. aegypti* conducted in Kenya using PCR, indicated that from a total of 53 fed *Ae. aegypti*, 47 were amplified out of which humans 51.1% (24/47), dogs 17% (8/47), rats 8.5% (4/47), lizards 6.4% (3/47) cats 4.3% (2/47), goats 4.3% (2/47), bats 2.1% (1/47), tortoises 2.1% (1/47) and mongeese 2.1% (1/47) (Kamau *et al*., 2023). The high host diversity of *Ae. aegypti* is an indicator of its role as a vector of many viral diseases. Thus, it is vital to investigate the host diversity of and feeding preference of *Ae. aegypti* as it has implications in the transmission of zoonotic diseases.

Adult *Aedes* species bite during the early or late hours of the day. Peak biting hours are altered depending on potential host availability (Karunamoorthi, 2013). *Aedes* mosquitoes are normally active within 2 hrs of day-break and at dawn. However, they can bite in the night when there is light. For instance, *Ae. simpsoni* complex species bite mainly in the mid-afternoon, *Ae. africanus* at dusk, and *Ae. vittatus* throughout the day. In Ghana, biting by *Ae. aegypti* peaked within 06:00-08:00 and 18:00-19:00 hrs, whereas *Ae. Vittatus* peaked at 05:00-06:00 hrs. *Ae. aegypti* changed its peak biting activity in the early morning (06:00 and 08:00) and early evening (15:00 and 16:00 hrs) in Greenwich Mean Time (GMT) (Captain-Esoah *et al*., 2020).

2.3.5. Resting behavior

Aedes aegypti is highly adapted to urban areas in and around houses, feeds principally on humans and leads to very high viral disease outbreaks (Liebman *et al*., 2014). Given that *Ae. aegypti* is primarily endophilic (Chadee, 2013) and that both males and females seldom disperse beyond 100 m (Harrington *et al*., 2005), identifying the environmental and behavioral conditions that influence its resting behavior is crucial for devising innovative control strategy.

Previous studies suggest that after an uptake of plenty of blood meal, a female *Ae. aegypti* inhibits its host-seeking behavior and initiates its gonotrophic cycle and shelters in a place and develops eggs mostly indoors (Schoof, 1967; Perich *et al*., 2000; Tainchum *et al*., 2013). Blood-fed, gravid, and unfed females are attracted to non-reflective dark surfaces and remain at rest on dark clothing, bed covers, furniture, doors, walls, ceilings, in darkened areas of the rooms, and on both exposed and unexposed surfaces in close proximity to their larval development site. A study in Trinidad showed that *Ae. aegypti* were found primarily indoors (Chadee, 2013). Even in indoor resting sites, *Ae. aegypti* was found in the living rooms. *Ae. aegypti* predominantly rests indoors, primarily on hanging objects and walls (Perich et al., 2000). They also rest mainly at low heights of house walls (Tainchum *et al*., 2013).

In Africa, *Ae. aegypti* showed elusive resting habits (Trpis and Hausermann, 1975). In Ethiopia, limited studies showed variations in resting behavior of *Ae. aegypti*. For instance, in Dire Dawa, the majority of *Ae. aegypti* collections were made from indoors, especially from house wall surfaces (Waldetensai *et al*., 2021a). On the other hand, the study in the southern parts of Ethiopia showed that *Aedes* species rest outdoor such as under tree leaf (Waldetensai et al., 2023). In south-eastern Senegal, *Ae. aegypti* was preferentially resting in living rooms (Diallo and Diallo, 2020).

Recent observations indicated that applications of indoor residual spraying altered the resting behaviors of *Ae. aegypti* and forced the species to rest in variety of places, including under furniture, in closets, lower walls, and in dark areas which increased intervention costs and provided in-effectiveness against dengue control (Vazquez-Prokopec *et al*., 2010). Identifying resting habitats of *Ae. aegypti* is helpful for the rational use and implementation of vector control tools and appropriate allocation of resources particularly insecticidal space spraying and insecticide treated bed nets during the *Aedes*-transmitted viral diseases outbreak

response. In addition, identifying the environmental and behavioral conditions that influence its resting behavior is crucial for devising innovative control strategies. In Ethiopia, limited studies were conducted on resting and biting behaviors of *Ae. aegypti* and *Ae. simpsoni* complex in the Dire Dawa and Southern Ethiopia (Waldetensai *et al*., 2021a; Waldetensai *et al*., 2023). Adequate data on the resting behavior and blood meal source of *Ae. aegypti* is lacking in the Southern Afar region, Ethiopia.

2.4. Vectorial role of *Ae. aegypti* and associated factors

Vectorial role (vector competence) is defined as the capacity of a mosquito to acquire a pathogen and support its transmission (Souza-Neto *et al*., 2019; Wu *et al*., 2022). It represents all the intrinsic factors (genetic, physical, physiological, and immunological) underlying virus propagation in the mosquito (Kramer and Ebel, 2003). It is a consequence of vector hematophagy, in which the primary purpose is to support egg development and complex and interactions among the mosquito host, the pathogen, and host symbionts (Vasilakis and Tesh, 2015).

Studies from African countries reported that *Ae. aegypti* served as a vector of many viral diseases, including dengue fever, yellow fever, and chikungunya. For instance, in the Central Africa, dengue and chikungunya viruses were detected from *Ae. aegypti*, and it was confirmed that *Ae. aegypti* was the actual vector of the viruses (Paupy *et al*., 2010). Similarly, in Nigeria, out of 193 pools of *Ae. aegyti* 73 pools were positive for yellow fever infection, whereas 150 out of 296 pools were positive for dengue virus in 2015 (Agwu *et al*., 2019). Moreover, in Tanzania, out of 24 pools of *Ae. aegypti*, 8 pools were positive for alfa-viruses and 1 pool for flavivirus (Bisimwa et al., 2016). In Ethiopia, a total of 934 *Aedes* mosquitoes about 77 pools were screened to be dengue and yellow fever negative (Waldetensai *et al*., 2023). Thus, it is imperative to determine the actual vector and the viral diseases being transmitted in the country.

The physiology of *Ae. aegypti* affects its vectorial competence. The midgut of a female *Ae. aegypti* is the primary organ that encounters virus ingested along with blood. The virus then enters the hemolymph and spreads to secondary tissues such as the fat body, trachea and salivary gland. Finally, the virus is released into the salivary tubes and transmitted to susceptible hosts while imbibing blood (Franz *et al*., 2015). In order to multiply in the mosquito, the virus must overcome the tissue barriers associated with the mid-gut and salivary gland and cope with the related antiviral pathways.

A study by Oliveira *et al*. (2017) in Brazil showed antiviral pathways in mosquitoes that limit the spread of viruses. These include reactive oxygen species (ROS) production and the innate immune pathway. However, these reactions appear to be virus specific. The antioxidants in the midgut induced by catalase are triggered after the mosquito has ingested a blood meal (Liu *et al*., 2018). In the mosquito's innate immune pathways, the Toll and JAK-STAT pathways are suggested to regulate DENV infection (McFarlane *et al*., 2014; Jupatanakul *et al*., 2017). The RNA interference (RNAi) pathway also determines the interactions between viruses and their mosquito hosts. The RNAi pathway includes small interfering RNA (siRNA), micro RNA (miRNA) and P-element-induced wimpy test interacting RNA (piRNA) (Ghildiyal and Zamore, 2009). Although miRNAs could potentially be involved in ZIKV mosquito interactions, the direct interaction and specific mechanism are still unclear (Saldana *et al*., 2017).

Endogenous small non-coding RNAs known as miRNAs regulate gene expression through binding to the target mRNA and initiating mRNA degradation by the RNA-induced silencing complex (RISC) (Kumar *et al*., 2018). miRNAs are involved in the replication of viruses in mosquitoes by regulating host factors. The miRNA increased in dengue virus-infected blood fed *Ae. albopictus* and inhibition of the miRNA enhanced viral replication, while overexpression of the miRNA limited viral replication (Yan *et al*., 2014). Another study demonstrated that miRNA could enhance dengue virus serotype 2 infection in an *Ae. aegypti* cell line (Hussain *et al*., 2013). These studies indicate that miRNAs play an important regulatory role in the interactions between viruses and mosquitoes, which may affect the vector competence of mosquitoes. Thus, it is possible that more obvious changes in miRNAs could be observed in specific tissues infected with the virus.

Climatic factors such as temperature, rainfall, and humidity govern *Ae. aegypti* vector competence (Hardy *et al*., 1983). For instance, a warm temperature is important to adult mosquitos' behavior and maturation, especially when the larval development rate is shortened (Focks *et al*., 1993). A higher temperature, especially a daily mean temperature of about more 26 ^{o}C, can reduce infectivity rate of *Ae. aegypti* by altering the its life history traits and vector-virus interactions, reducing fecundity, extending immature development time, and decreasing survival (Carrington *et al*., 2013). In addition, rainfall provides plenty of breeding sites, such as puddles, for mosquitoes, while humidity affects the adult mosquitoes' survival and biting frequency (Banu *et al*., 2015).

The microbiota in the gut of mosquitoes influences key physiological processes related to pathogen transmission (Pike *et al*., 2017). For instance, dengue virus replication was significantly affected by gut bacterial flora in *Ae. aegypti*, the depletion of which by antibiotics rendered mosquitoes to be more susceptible to viruses in Maryland (Xi et al., 2008). Even though, the mechanism remains open for discussion, indirect mechanisms rely mainly on the basal level activation of innate antiviral responses and antimicrobial peptides by the gut microbiota (Ramirez *et al*., 2012). On the other hand, antiviral activity may be directly mediated by antiviral compounds produced by bacteria (Ramirez *et al*., 2014). Besides, there is a massive increase of bacteria in the midgut of mosquitoes after a blood meal and the interference with physiological processes related to the control of midgut homeostasis, such as the production of Reactive Oxygen Species (ROS) and the peritrophic matrix (Rodgers *et al*., 2017). These processes may potentially affect the role of *Ae. aegypti* in transmitting pathogenic viruses and should be further investigated.

Vectorial role of mosquitoes for viral infection and transmission is also influenced by the viriome of the viral particle, which is insect-specific virus (ISVs) and not able to replicate in vertebrate cells. The prevalence of ISVs in natural mosquito populations varies greatly, while cell-fusing agent virus is transmitted vertically and is absent in salivary glands of *Ae. aegypti* (Guégan *et al*., 2018).

2.4.1. Determining dengue and chikungunya viruses infection rates of *Ae. aegypti*

Previously, viral infection rates of *Ae. aegypti* could be determined as the number of a specific virus infected *Ae. aegypti* per the total number of *Ae. aegypti* tested for the virus (Degallier *et al*., 1992; Gu *et al*., 2003). These require identifying the virus using the appropriate diagnostic methods and identifying the mosquitoes' species following standard morphological identification manuals. Currently available methods for detecting dengue and chikungunya viruses from mosquito samples include indirect immunofluorescent assay (IFA), enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) (Hunsperger *et al*., 2014). Each type of test offers unique advantages and disadvantages, and a combination of tests may be employed in order to increase diagnostic confidence (Mardekian and Roberts, 2015). However, polymerase chain reaction is the most widely accepted (Boonham et al., 2014). PCR is a technique of amplification of DNA from a target RNA to produce cDNA through reverse-transcription reaction and hence is also known as reverse transcriptase-polymerase chain reaction (RT-PCR). It has been developed globally for

robust, sensitive detection of dengue and other infectious diseases. This method is simple, sensitive, rapid, and if standardized correctly and can be used for the detection of genomes in human clinical samples, autopsy tissues, or mosquitoes, biopsies. These methods differ in terms of the amplified gene regions of the genome, the way they detect RT-PCR products, and virus typing methods. RT-PCR, being a more sensitive technique, has the advantage of processing a large number of samples at once and can be used both qualitatively and quantitatively (Pawar and Patravale, 2015).

2.5. Global distributions of pathogenic virus transmitting *Aedes* species

Globally, there are over 3500 species of the family Culicidae mosquitoes identified so far (Wilkerson *et al*., 2021). These mosquitoes can be further classified into 112 genera, with four genera repeatedly linked with disease transmission to humans, including *Aedes*, *Anopheles*, *Culex*, and *Ochlerotatus* (Elbers *et al*., 2015). The genus *Aedes* comprises approximately 950 species, in which *Ae. aegypti* and *Ae. albopictus* are the most important viral diseases vectors (Monteiro *et al*., 2019). *Ae. aegypti* has two morphological subspecies (ecotypes); the domestic *Aedes aegypti aegypti* and the sylvan *Aedes aegypti formosus* (Mattingly, 1957). The color of scales on the first abdominal tergite serves as a key to identify *Ae. aegypti* subspecies. The scales in the first abdominal tergite are darker or blacker in *Ae. aegypti formosus*, whereas paler and browner in *Ae. aegypti aegypti* (Futami *et al*., 2020). However, there is substantial overlap, and they can only be identified using molecular methods (Elnour *et al*., 2022).

Aedes aegypti is present in over 188 countries globally (Leta *et al*., 2018), putting more than half of the world's human population at risk of dengue, chikungunya, yellow fever, and Zika diseases. Over the past two decades, increased and frequent outbreaks of these diseases have been reported in different parts of the globe (Leta *et al*., 2018; Sang *et al*., 2022). The expanding epidemiology of *Aedes*-transmitted viral diseases indicates increasing spatial distribution of *Ae. aegypti*. *Aedes aegypti* is highly abundant in Northern Brazil and Southeast Asia including Indian subcontinent and Africa. It also occurs in relatively few areas of Europe and temperate North America (Kraemer *et al*., 2015). *Ae. aegypti* shows a wider geographic distribution than *Ae. albopictus* in Australia, which is confined to the east coast, largely reflecting the known historic distribution of *Ae. aegypti*. *Ae. albopictus* characteristically tolerates lower temperatures and occurs in Southern Europe, Northern China, southern Brazil, northern United States and Japan (Figure 5).

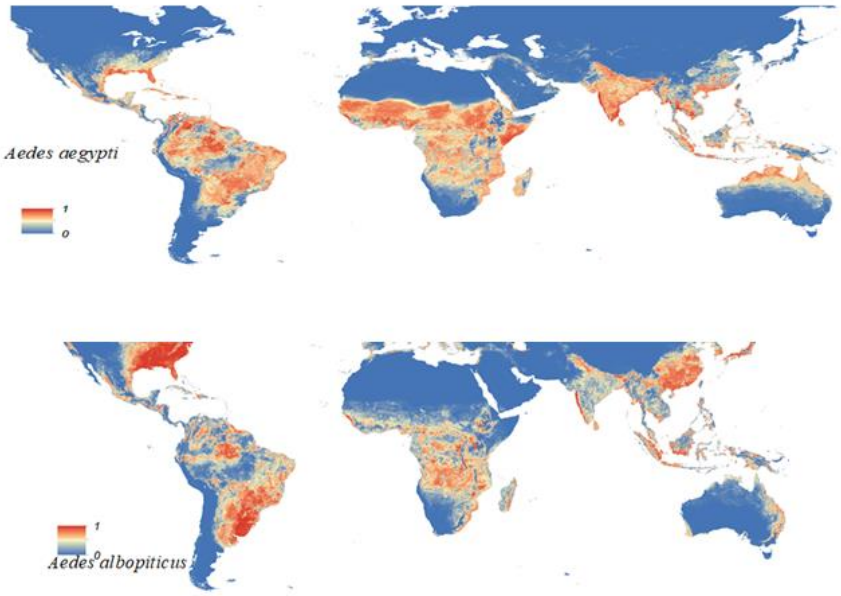

Figure 5: Global distribution of *Ae. aegypti* and *Ae. albopictus,* from complete absence to presence (0 to 1) (Kraemer *et al*., 2015).

Aedes albopictus is also reported in China, United States, West Africa (Nigeria, Cameroon, Gabon), the Central African Republic, Madagascar, and South Africa. *Ae. aegypti* is widely distributed, in Africa (Kraemer *et al*., 2015). About 215 countries of the world are potentially suitable for the existence and development of *Ae. aegypti* and *Ae. albopitcus* (Figure 6).

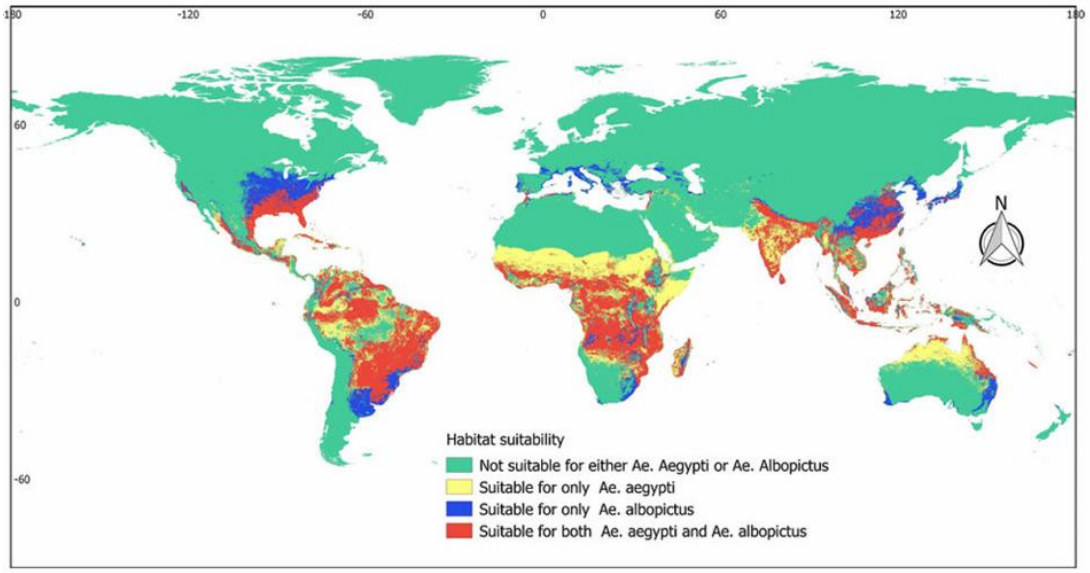

Figure 6: The global habitat suitability for *Ae. aegypti* and *Ae. albopictus* (Leta et al., 2018)**.**

2.6. Control strategies for *Aedes* species

2.6.1. Habitat/environmental management

Currently, different *Ae. aegypti* control options are available for different stages, including eggs, larvae, pupae, and adults. The ultimate goal of each control strategy is to prevent *Aedes*-transmitted viral disease transmissions by reducing mosquitoes densities to avoid vector-human contact (Zhang *et al*., 2024). Environmental management is the manipulation and modification of environmental factors in order to prevent or reduce vector productivity and human-vector contact. The methods include destroying, altering, removing, or recycling non-essential containers that serve as vector habitats (Mahmud *et al*., 2019). *Ae. aegypti* breeds in areas where humans live together, such as in concentration camps, towns, and cities (Mbanzulu *et al*., 2022a; Teillet *et al*., 2024). *Ae. aegypti* eggs and larvae occur in discarded man-made materials that hold water, such as small containers, water tanks, drums, and jerrycans (Karisa, 2018). Container removal is one of the environmental management strategies used to reduce the larval source of *Ae. aegypti* (Gubler and Clark, 1996). This strategy requires active community engagement at large (Morrison *et al*., 2008). In Brazil, removal or management of key containers reduced the density of adult *Ae. aegypti* significantly (Maciel-de-Freitas and Lourenço-de-Oliveira, 2011).

Traps such as ovitraps, Biogent Sentinel traps, and battery-powered mechanical aspirators can reduce the overall vector population and hence should be considered as part of the *Ae. aegypti* control strategy (Kline, 2006). Lethal ovitraps, in Brazil, have shown efficient reduction in *Ae. aegypti* population density (Zeichner and Debboun, 2011).

In Ethiopia, there is no well-established *Aedes* mosquito control strategy, but recently the Ethiopian Public Health Institute (EPHI) prepared guidelines considering environmental (larval source management) and chemical control methods (EPHI, 2021). The larval source management (LSM)) strategy used for the control of malaria vectors can be applied for *Ae. aegypti* that breed in man-made and natural habitats (Tusting *et al*., 2013; Bowman *et al*., 2016). LSM includes permanent alteration to the environment, including landscaping, surface water drainage, filling and land reclamation, coverage of water storage containers with mosquito-proof lids or permanent slabs, and coverage of the water surface with impenetrable barriers to vectors. Furthermore, larval source management is also applicable using habitat manipulation in which a recurrent activity includes water level manipulation, e g., flushing

streams, and drains. On the other hand, changes to human habitation include, actions to reduce human-vector contact, such as installing mosquito screening on windows, door and and other entry points, and using mosquito nets while sleeping during the daytime. Mosquito-proofing of water-storage containers: Water storage containers can be designed to prevent access by mosquitoes for oviposition (EPHI, 2021).

2.6.2. Biological control

Biological control strategies of *Ae. aegypti* include the use of biological predators of mosquitoes (Gan *et al*., 2021). It involves applying living organisms, such as natural predators, parasites, or pathogens that target and control *Ae. aegypti* (Rey e*t al*., 2004). A biological approach is advantageous in that it can control insecticide-resistant mosquitoes. Moreover, it is less costly (Huang *et al*., 2017). However, the approach has its own limitations. Some predators may attack non-target organisms and could disrupt local ecosystems and biodiversity. It requires time for the predator population to grow and control the mosquitoes effectively. Additionally, it also requires infrastructure for breeding and releasing predator organisms, as well as monitoring their effects (Lima *et al*., 2015). Despite these challenges, biological control remains an alternative or a complement to chemical methods in the fight against mosquitoes.

There are some arthropods that feed on *Ae. aegypti* larvae/pupae. The genus *Toxorhynchites* is observed to feed on the larvae of *Ae. aegypti* in the United States (McGregor and Connelly, 2021). In addition, killfish were employed to control *Ae. aegypti* larvae in larger water bodies, such as cisterns or wells in the country (McGregor and Connelly, 2021). The fungi *Beauveria bassiana* and *Metarhizium anisopliae* are used to control *Ae. aegypti* in Brazil (Paula *et al*., 2008). The microsporidian, *Edhazardia aedis*, has been used to control *Ae. aegypti*. This parasite can be transmitted from mother to offspring and significantly affect the egg batch size and emergence rates of *Ae. aegypti* (Becnel *et al*., 1995). The major challenges for using entomopathogenic fungi are delivery to the control *Ae. aegypti* and its longevity in nature (Achee *et al*., 2019).

2.6.3. Insecticide based *Ae. aegypti* control

Insecticides are used in space sprays, indoor residual sprays, and personal protection to kill adult *Ae. aegypti*. The pyrethroid insecticides have been used as adulticides in the form of space spraying, indoor residual spraying (IRS), insecticide-treated surfaces, and attractive

lethal sugar baits (Ridha *et al*., 2023). In Abidjan, Cote d'Ivoire, space spraying of Fludora Co-Max, a combined from flupyradifurone (systemic insecticides) and transfluthrin (pyrethroid), kills adult *Ae. aegypti* effectively during viral disease epidemics. Indoor residual spraying of the insecticide was more effective than outdoor spraying in small-scale studies (Zahouli *et al*., 2023). A study in Peruvian Amazon showed that IRS is promising in reducing the *Aedes* population. For instance, deltamethrin IRS caused significant mortality in *Ae. aegypti* populations (Paredes-Esquivel *et al*., 2016).

A study in Nigeria revealed that kerosene or petroleum oil was effective against *Ae. aegypti* larvae (Ojianwuna *et al*., 2021). The other larvicides include toxins derived from *Bacillus thuringiensis israelensis* (Bti), *Lysinibacillus sphaericus*, and spinosad; insect growth regulators (IGRs) such as methoprene and pyriproxyfen, and surface agents such as monomolecular films and mineral oils (Boyce *et al*., 2013). Bti demonstrated a rapid larvicidal effect, typically against *Ae. aegypti* larvae in their natural habitats in Russia (Khodyrev *et al*., 2024).

In Ethiopia, pyrethroids such as deltamethrin are applied either as residual surface treatments or as space treatments. Space spraying is recommended in emergency situations to suppress an ongoing epidemic or to prevent incipient ones (EPHI, 2021). The intervention study in Dire Dawa in 2021, using propoxur, reduced adult *Ae. aegypti* density significantly (Waldetensai *et al.*, 2021b). In the area, temophos (Abate) had a significant larvicidal effect against *Ae. aegypti* (Waldetensai *et al*., 2021b). Extracts of plants such as *Albizia gummifera* (seeds), *Balanites aegyptica* (fruits), *Hedera helix* (leaves and fruits) and *Warburgia ugandensis* (leaves), and *Millettia ferruginea* showed significant effects against *Ae. aegypti* and *Ae. africanus* larvae (Debella *et al*., 2007). Similarly, a study by Massebo *et al*., 2009, demonstrated that oils extracted from *Ocimum lamiifolium* (leaves) were effective at controlling *Ae. aegypti* larvae in the laboratory (Massebo *et al*., 2009).

Due to their common characteristics, it is recommended to integrate the malaria-transmitting *Anopheles arabiensis* and virus-transmitting *Ae. aegypti* control strategies in Ethiopia, such as IRS (Chadee, 2013), LSM (Tusting *et al*., 2013; Bowman *et al*., 2016), and also biological and chemical larviciding formulations, such as Bti and pyriproxyfen (Mbare *et al*., 2013; Abad-Franch *et al*., 2017).

2.6.4. Genetic control of *Ae. aegypti*

Genetic control of *Ae. aegypti* is either to suppress the populations or to introduce a harm-reducing novel trait to the mosquito (Alphey, 2014). It is considered to be an alternative to chemical insecticides. Sterile male *Ae. aegypti* release technique (SIT) seems feasible to control *Ae. aegypti* (Lees *et al*., 2021). Sterile males are released into the field and mate with fertile females, rendering all their progeny nonviable (Robinson and Hendrichs, 2005). SIT has been implemented against various mosquito species since the 1950s (Dyck *et al*., 2021). In Cuba, sterile males released competed successfully and induced enough sterility to suppress the local *Ae. aegypti* population (Gato *et al*., 2021). Similarly, the technique successfully suppressed the fertility of female *Ae. aegypti* in Sri Lanka (Ranathunge *et al*., 2022) and California (Chen *et al*., 2021).

Incompatible insect technique (IIT) is another genetic method (Crawford *et al*., 2020). In South Miami, Florida, Wolbachia infected males mated with uninfected females, resulting in a significant decline of egg hatching by *Ae. aegypti* females (Mains *et al*., 2019). In Mexico, Wolbachia-based incompatible insect technique is effective and believed to control *Ae. aegypti* and could be implemented as part of integrated, synergistic, and complementary schemes to enhance the efficiency and coverage of control tools (Martín-Park *et al*., 2024). Furthermore, IIT technology was observed to be effective in reducing female *Ae. aegypti* productivity in Australia (Pagendam *et al*., 2020).

Release of insects carrying the dominant lethal gene (RIDL) is also the other genetic approach used in the control of *Ae. aegypti*, in which case the released males are not sterile, but their mating with females results in non-viable or male-biased progeny (Carvalho *et al*., 2015). Other new genetic approaches tested in the laboratory to control *Ae. aegypti* include precision-guided sterile males (PgSIT), which is based on the crossing of two transgenic lines to produce sterile males (Kandul *et al*., 2019), and gene drive, which may allow in the future to drive maleness in target populations (James *et al*., 2018).

2.6.5. Community-based *Ae. aegypti* control

Community-based *Ae. aegypti* control is helpful as it occurs and breeds in and around human habitations (Dzul-Manzanilla *et al*., 2017). For sustainable *Aedes* mosquito control, surveillance should be implemented to confirm the presence of the potential disease

transmitting *Aedes* species (Heintze *et al*., 2007). Active community participation is central for effective control and intervention processes against mosquitoes (Nam *et al*., 2005).

Community-based *Ae. aegypti* control encompasses social mobilization, environmental management, and insecticide-based interventions (Othman *et al*., 2017). Recognizing the complex interplay between mosquitoes, the environment, and human behaviors is critical in ensuring effective prevention and control of *Aedes*-transmitted viral diseases (Ouédraogo *et al*., 2018). It is vital to engage communities from an early stage to ensure culturally appropriate and sustainable interventions are implemented (Zaki *et al*., 2019). Community engagement has resulted in sustainable *Ae. aegypti* control since the 1980s in Thailand. Involving the residents of the community achieved control of the *Ae. aegypti* population through larval source management (Phanthumachinda *et al*., 1985).

Aedes aegypti control at a community level needs awareness creation through education, which is used to ensure people understand the importance of vector control and engage in protective behaviors, such as eliminating immature mosquito habitats (Heyrani *et al*., 2024). This awareness creation enables the community to actively participate (Sousa *et al*., 2022). Approaches in community participation to control *Ae. aegypti* include the top-down and bottom-up approaches. Sometimes, the government provides instructions to the community on how to control vectors; i.e., top-down' participation will be initiated in some situations; communities themselves initiate vector control and update the government. However, bottom-up approaches are rare (Toledo *et al*., 2007).

Aedes aegypti control through community should also be an integrative and collaborative approach, which means ensuring the rational use of available resources through the application of a multi-disease control approach; integration of non-chemical and chemical vector control methods; and integration with other disease control measures (McCall *et al*., 2009). Advocacy is also important during community-based *Ae. aegypti* control, which is social mobilization and legislation; the promotion of the principles in development policies of all relevant agencies, organizations, and civil society; the establishment or strengthening of regulatory and legislative controls for public health; and the empowerment of communities (McCall *et al*., 2009).

The success of community-based *Ae. aegypti* control is influenced by various factors. The government should put clear legislation on residents or property owners that every resident in

the area is responsible to control the breeding of mosquitoes (Allen *et al*., 2023). Moreover, community leaders' attitude towards engaging the community is a key influential factor in the priority as part of *Ae. aegypti* mosquito management. Thus, leaders' attitude and beliefs affect both positively and negatively the success of community participation in *Ae. aegypti* control (Allen *et al.*, 2024). Resources are also needed for appropriate implementation to engage the community. Thus, proper utilization and allocation of budget are mandatory for effective community-based *Ae. aegypti* control. Scarcity of funding as well as limited capacity and competing priorities influence the extent to which the communities are engaged in awareness creation of the local community.

In Ethiopia, there are no national and community-level *Ae. aegypti* control guidelines, although the country has had a long history of malaria and its *Anopheles* vector since 1959 (WHO, 1959). The country has been implementing *Anopheles* mosquito control tools, which can be used to control *Aedes* mosquitoes. Thus, some of the existing *An. arabiensis* control tools such as larval source management (LSM) and insecticide residual spray (IRS) can be adapted for the control of *Aedes* mosquitoes and *Aedes*-transmitted diseases.

2.7. Operational framework for *Aedes* species surveillance

Effective *Aedes* species and *Aedes*-borne disease control strategies consider the following essential factors (Figure 7). In order to ensure focused interventions, the primary task in *Aedes* surveillance is to identify areas suspected of having *Aedes*-borne disease occurrence [(a)] such as reports of human cases or the presence of virus-transmitting *Aedes* specie. Once suspected localities have been identified, adjust outbreak thresholds to account local circumstances[(b)]. The thresholds for declaring an outbreak should be customized to the specific epidemiological and environmental conditions of the area. Factors like mosquito density, past outbreaks, and local climate should be considered to set appropriate response levels.

In addition, classes of the insecticide that do not have known resistance should be utilized[(c)]. For effective control, it is essential to consistently monitor insecticide resistance within local *Aedes* populations. Only insecticides from classes without known resistance should be employed in order to sustain their effectiveness and prevent the proliferation of resistant strains. Then, the timing of re-spraying should be determined by the residual effectiveness of the insecticide[(d)]. The frequency of insecticide reapplication should depend on the longevity

of the insecticide's effect. Some formulations last longer than others, and re-spraying should be done accordingly to maintain effective coverage. Furthermore, indoor space spraying frequency during outbreak[e] is important. In case of an outbreak, indoor spraying should occur at least once a week for a minimum of four weeks. This intensive spraying regimen is critical to interrupting transmission by targeting adult mosquitoes within homes and other structures.

Consistent spraying for prevention[f] is vital. During periods of no outbreaks, spraying should be restricted to two or fewer times per year, depending on based on the assessed need. This preventive strategy aids in managing *Aedes* mosquito populations prior to potential outbreaks. Following this, implementing responsive surveillance systems for quick action is essential[g]. Surveillance systems must be capable of identifying clusters of cases quickly and accurately. A timely response to these clusters can prevent the spread of the virus and help contain outbreaks. Distance of focal activities based on environment and vectors[h] should be determined**.** The geographic range for vector control activities, such as spraying or larval habitat elimination, should depend on the local environment and vector behavior. In urban areas, *Aedes* mosquitoes may breed in close proximity to human habitation, while in rural areas, wider coverage may be necessary.

Responding to suspected or confirmed *Aedes*-transmitted infections[i] should be implemented. Any suspected or confirmed cases of *Aedes*-transmitted viruses should prompt immediate vector control activities to prevent further spread. This includes larval source management, insecticide spraying, and public awareness campaigns. Implementing these measures in a coordinated and timely manner is essential for the effective surveillance and control of arboviral diseases.

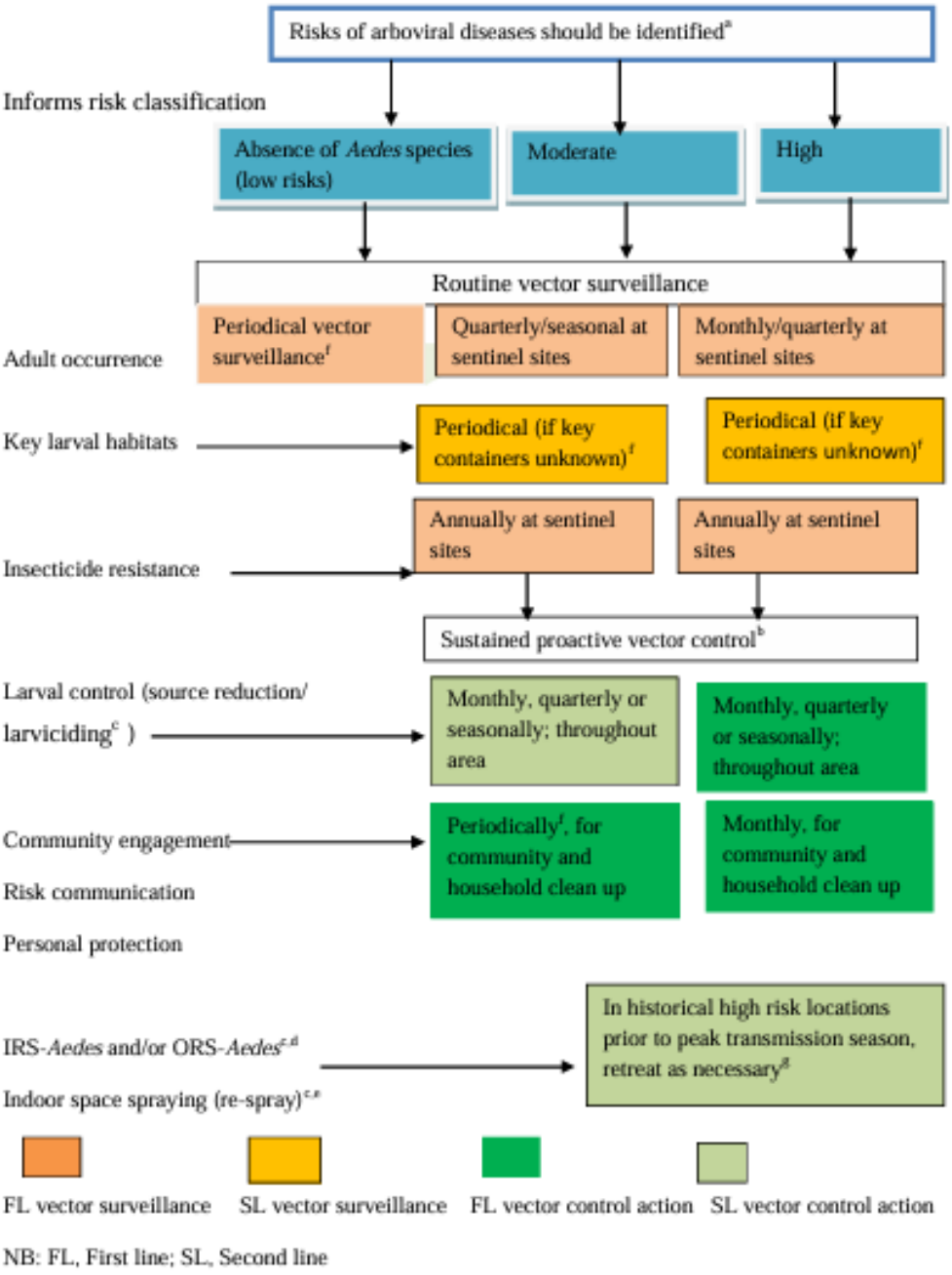

Figure 7: Operational frameworks for vector surveillance and control according to risk of an arbovirus outbreak (WHO, 2023b).

2.8. Current challenges in the control of *Ae. aegypti*

2.8.1. Diverse ecology and behavior

Controlling *Ae. aegypti* has several challenges. One of the challenges is its adaptability to urban environments and ability to breed in a wide range of artificial and natural water-holding containers, which makes it difficult to manage the species (Weetman *et al.*, 2018).

This nature of *Ae. aegypti* breeding in diverse breeding habitats becomes a challenge to eliminate all potential larval sources. In addition, unlike many other mosquito species that bite during the night, *Ae. aegypti* is primarily a daytime biter. This complicates protection efforts because bed nets are less effective, and people are more exposed during the day (Facchinelli *et al*., 2023). Another obstacle in *Aedes*-borne viral disease control is the asymptomatic nature of most of the viruses. This can lead to undetected transmission cycles, making it harder to identify outbreaks early and respond with appropriate vector control measures (Duong *et al*., 2015).

Changing climatic conditions, such as increased temperature and rainfall, are creating environments conducive to *Ae. aegypti* reproduction. Similarly, expanding urbanization with low levels of community awareness about *Aedes* mosquitoes favors suitable habitats for the mosquitoes, particularly in low and middle-income countries, as previously observed (Ligsay *et al*., 2021). Thus, effective vector control depends heavily on public participation, especially for source reduction (eliminating breeding sites). However, maintaining long-term community engagement and compliance with preventive measures can be challenging.

2.8.2. Insecticide resistance

Insecticide resistance is considered the increased ability of insects to withstand or overcome the toxic, killing, or repellent effects of insecticides through natural selection and mutation (WHO, 2012). Insecticide resistance is an area of current research throughout the globe that hinders the effective implementation of vector control tools (Asgarian *et al*., 2023). Resistance to insecticides is a major risk in interventions to control vector-borne diseases (WHO, 2014b). Thus, it is essential to identify the geographical regions where vector resistance to insecticides exists and can make it difficult to control the vector and to improve the induction of innovative tools for vector control.

The global insecticide resistance reports of *Aedes* species to commonly used public health insecticides varied (Asgarian *et al*., 2023). For instance, in Africa, particularly in Central Africa, *Ae. aegypti* and *Ae. albopictus* species were found to be resistant to the pyrethroid deltamethrin (Ngoagouni *et al*., 2016) (Figure 8). The study conducted in Cameroon in 2021 showed that *Ae. aegypti* was resistant to deltamethrin and permethrin insecticides (Djiappi-Tchamen *et al*., 2021). Similarly, the findings observed in Nigeria revealed that *Ae. aegypti* was resistant to deltamethrin (Ayorinde *et al*., 2015). Moreover, in Senegal, *Ae. aegypti*

populations were suspected to be resistant to deltamethrin and resistant to lambda-cyhalothrin. In Tanzania, *Ae. aegypti* showed lower susceptibility to deltamethrin and permethrin and resistance to lambda-cyhalothrin (Mathias *et al*., 2017). However, data on *Ae aegypti* susceptibility status to public health insecticides is lacking in Ethiopia, where seroprevalence of *Aedes*-transmitted viral diseases is increasing in the country, as indicated in the introduction section.

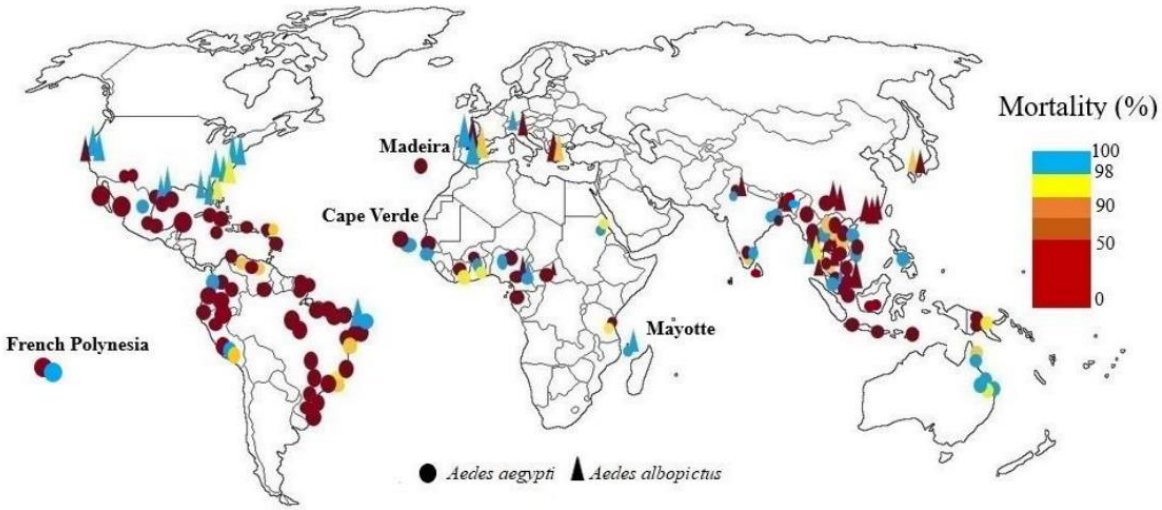

Figure 8: Global distributions of pyrethroids insecticide resistance in *Ae. aegypti* and *Ae. albopictus* in 2022 source: (Asgarian *et al*., 2023).

Basically, four main classes of insecticides (carbamates, organochlorines, organophosphates, and pyrethroids) are currently available, of which pyrethroids are recommended for insecticide-treated nets due to their relatively low toxicity to humans, a rapid knockdown effect on mosquitoes, and low cost (Nauen, 2007). Mutations in the amino acid sequence of the binding site of the insecticide target, as well as metabolic changes, lead to frequent resistance in mosquito populations. This is mainly driven by the high selective pressure stemming from the huge use of pyrethroids in agriculture and the scaling-up of pyrethroid LLINs and IRS for malaria and other mosquito-borne disease control (Liu, 2015).

Prevention of mosquito-borne diseases mainly relies on management of its vectors (Buhler *et al*., 2019). Insecticide-based intervention is the main strategy routinely used to control mosquito populations. Despite the increasing nuisance due to *Aedes* mosquito bites, there have been so far limited control efforts targeting specifically *Aedes* mosquito population in *Aedes*-borne disease endemic countries (Weetman *et al*., 2018). Most vector control efforts are directed against malaria vectors with the massive deployment of insecticide-treated nets,

and it is likely that the scaling up of insecticide-treated nets alongside the intensive use of insecticides in agriculture could be affecting non-malaria vector species and could lead to the development of resistance within *Aedes* mosquito populations (Kamgang *et al*., 2017).

The emergence of insecticide resistance in *Ae. aegypti* mosquitoes in Africa is primarily attributed to factors including frequent utilization of specific insecticide classes (particularly pyrethroids), poor vector surveillance, inadequate control strategies, genetic variations leading to target-site mutations (i.e., knockdown resistance mutation (F1534C), increased metabolic detoxification enzymes within the mosquito, and the widespread use of agricultural insecticides, which can contribute to cross-resistance (Dusfour *et al*., 2019; Konan *et al*., 2021; Sombie *et al*., 2019).

The mechanisms for insecticide resistance are complex and include behavioral and physio logical alterations in mosquitoes (Weetman *et al*., 2018). Behavioral insecticide resistance can be viewed as a temporal, spatial, or topical avoidance of contact with insecticides. Female mosquitoes can adaptively evolve in response to selection pressures imposed by insecticide control tool use as long as sufficient genetic variation in constitutive behavioral resistance traits or inducible behavioral resistance traits accumulates in a given population (Stone and Gross, 2018).

Physiological resistance involves three mechanisms of resistance, including reducing penetration of insecticides through the cuticle, metabolic resistance that is the presence of enzymes that detoxify the insecticide, and target site resistance in which prevention of the insecticide binding or interacting at its site of action as previously observed (Nkya *et al*., 2013). The voltage-gated sodium channel (VGSC) target site of *Ae. aegypti* is the most studied insecticide resistance mechanism. Numerous VGSC knockdown resistance (kdr) mutations have been identified in *Ae. aegypti* worldwide (V1016I, V410L, S989P, I1011V, V1016G, I1011M and F1534C). Among these mutations, three variants (V410L,V1016I and F1534C) have been detected in Africa (Ayres *et al*., 2020). Metabolic resistance occurs through increased insecticide metabolism or sequestration by detoxification enzymes. Several genes of the P450 family, especially carboxy/choline esterases (CCEs) and glutathion-S-transferases (GSTs) have been strongly associated with insecticide resistance in *Aedes* mosquitoes (Sombie et al., 2019). Mutation of these targets is a very effective resistance mechanism, inducing cross-resistance to all insecticides acting on the same target. The target of organophosphates and carbamates is AChE (Sokhna *et al*., 2013).

In Ethiopia, the emergence and spread of vector insecticide resistance to the main classes of insecticides is a serious operational impediment that could compromise the control of mosquito-borne diseases (Messenger *et al.*, 2017). Although many studies have investigated the characterization of malaria vector resistance, there is limited evidence on the *Ae. aegypti* insecticide resistance status in the country. Thus, further studies should be inspired to generate scientific baselines to synthesize applicable chemical control tools in the prevention and control of viral-transmitting mosquitoes.

Different methods are employed to determine the susceptibility and resistance status of *Ae. aegypti* in the laboratory. One of these methods is biological assays. This is usually conducted to confirm the prevalence or level of phenotypic resistance. This can be done by exposing mosquitoes to a fixed dose of insecticides for a given time that is expected to kill all susceptible individuals (WHO, 2006). The resulting dose-response analysis provides outcomes like the concentrations (or exposure times) that kill 50% or 95% of specimens (WHO, 2016b).

Biochemical assays are also another method used to measure the activity or quantify the amount of detoxification enzymes, such as esterases, monooxygenases, and glutathione-S transferases, in wild populations compared with a reference strain (WHO, 2005). These assays require a cold chain to avoid loss of enzyme activities.

Molecular assays are a recent method that provides key data for identifying the causes of resistance in order to implement adequate resistance management strategies (Hemingway *et al.*, 2013). Robust diagnostic tools have been developed for detecting kdr mutations associated with pyrethroid and DDT resistance in *Ae. aegypti* (Moyes *et al.*, 2017). They are now sometimes integrated into resistance monitoring programs. However, molecular assay has challenges due to the lack of validated DNA markers.

2.9. A framework for implementation of insecticide resistance management

A comprehensive framework for insecticide resistance monitoring is crucial for maintaining the effectiveness of vector control programs, especially for mosquitoes like *Ae. aegypti*. Strengthening the capacity of control agencies, building collaborations with research institutions, and using integrated management strategies are key steps in controlling mosquito populations while minimizing resistance development. Long-term surveillance combined with effective data analysis and response strategies can help ensure that insecticides remain

effective and that mosquito-borne disease outbreaks are kept under control (Faucon et al., 2017). The framework encompasses the following stages for insecticide resistance monitoring and management (Figure 9).

Initial Assessment of Resistance Status: Before implementing any vector control strategy, it is essential to assess the baseline insecticide susceptibility of the local mosquito population. This can be done through bioassays, which test the efficacy of different insecticides on mosquito samples collected from the field. WHO tube tests and CDC bottle bioassays are commonly used methods.

Regular Monitoring and Surveillance: Establish a system for continuous or periodic monitoring of mosquito populations to detect changes in insecticide resistance. This should include testing for resistance against all insecticides commonly used in the area for mosquito control (e.g., pyrethroids, organophosphates, carbamates, etc.). Then, collect data from different places and analyse them to understand resistance patterns.

Molecular and Biochemical Tools: In addition to bioassays, it is important to investigate the molecular and biochemical mechanisms underlying insecticide resistance. Techniques like PCR can be used to detect known resistance-associated mutations, such as knockdown resistance (kdr) mutations that reduce mosquito sensitivity to pyrethroids.

Apply insecticide resistance management system: Rotating insecticides helps to prevent or slow down the development of resistance, rotate insecticides from different classes with different modes of action. This reduces the selective pressure on mosquitoes and prolongs the efficacy of available insecticides.

Integrated Vector Management (IVM): Combine chemical control with non-chemical strategies such as biological control, source reduction, and community engagement to reduce the reliance on insecticides alone. This can help mitigate resistance development over time.

Decision Support Systems: Use the collected data to inform decision-making in real-time. Decision support systems that integrate resistance monitoring data can help predict resistance trends and guide the choice of insecticides for vector control programs.

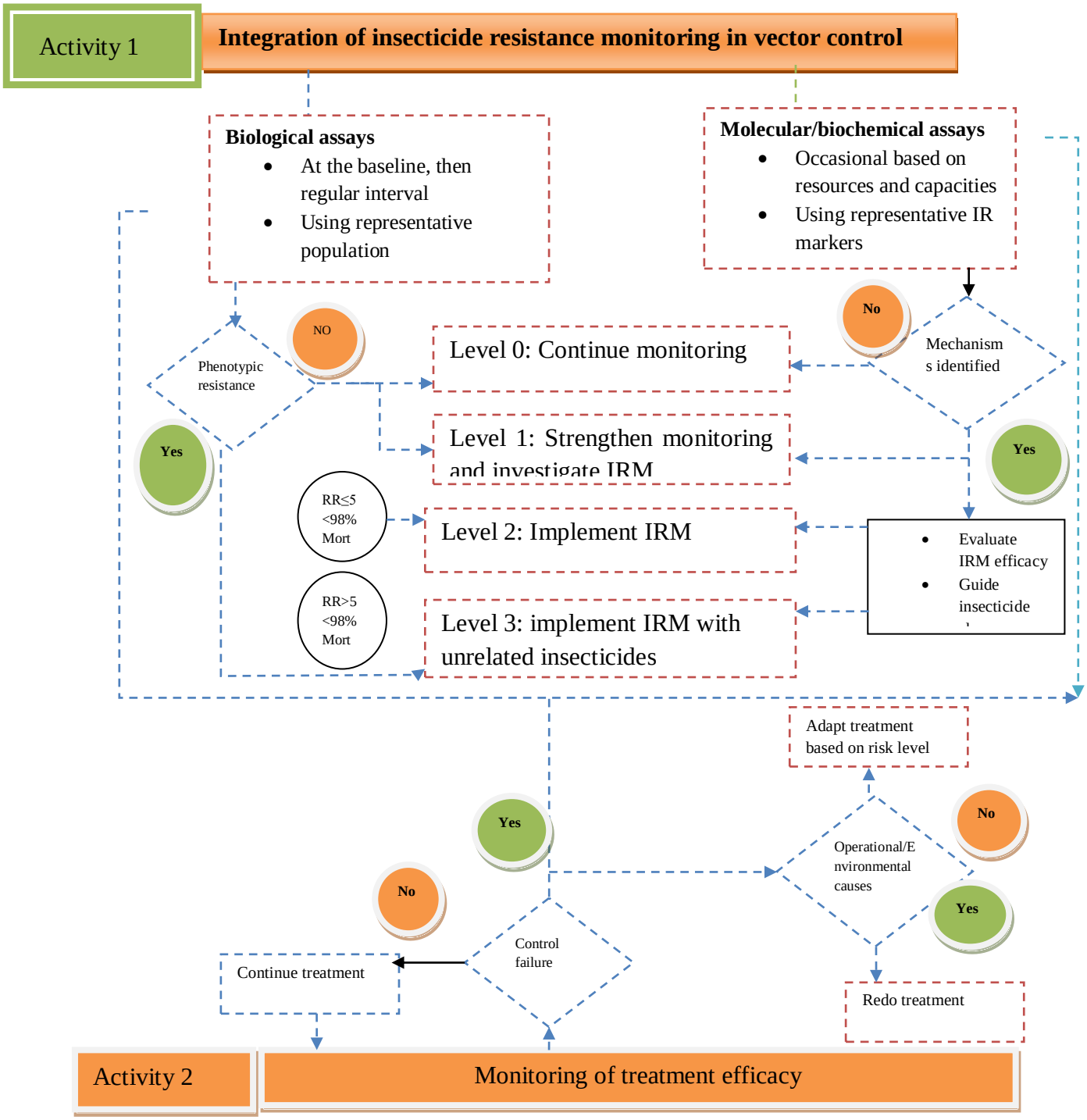

Figure 9: A framework used to implement insecticide resistance mamagement in vector control programs. Source: (Dusfour *et al.*, 2019).

CHAPTER THREE: MATERIALS AND METHODS

3.1. Description of Awash Arba, Werer and Awash Sebat towns

The study on the bionomics, insecticide susceptibility status, and community perception of *Ae. aegypti* was undertaken in Awash Arba, Werer, and Awash Sebat towns, Gabi-Rasu Zone, Afar Regional State, Ethiopia from May 2022 to April 2023. The Gabi-Rasu zone is one of the five zones found in Afar Regional State of Ethiopia. It is bordered by Oromia Region on the south, by the Amhara Region on the southwest, by Hari-Rasu zone on the west, by Awsi-Rasu zone on the north and by the Somali Region on the east. This zone has a total population of 198,751, of whom 108,995 are men and 89,756 women. About 54,328 or 27.33% people are urban inhabitants, while 12.11% are pastoralists (CSA, 2007, unpublished).

Awash Arba town is located at about 226 km in the northeast of Addis Ababa at 9.141111^{o}N, 40.15889^{o}E, 720-1100 meters above sea level (m.a.s.l.), Werer town at 256 km, 9.33453^{o}N, 40.181385^{o}E, 720-1000 m.a.s.l and Awash Sebat at 214 km, 8.98810^{o}N, 40.163936^{o}E, 820-1120 m.a.s.l. (Figure 10). Awash Arba and Werer are administered by the Amibara district and Awash Sebat by the Awash Sebat town administration. Awash Arba and Werer are semi-arid with temperatures ranging from 25 °C to 35 °C and average annual rainfall of 530 mm. Awash Sebat experiences 22.6 ^{o}C to 30.6 ^{o}C annual temperature and 606.6 mm mean annual rainfall (Mekuyie *et al*., 2018).

The climate in the Afar Regional State is broadly divided into wet and dry seasons. The main rainy season (locally referred to as Hagaya) spans from June to September, while the long dry season (locally known as Gilal) comprises the months from October to May (Balehegn *et al*., 2019). The residents of the towns store water in man-made containers such as drums, jerrycans, and cement tanks at their domestic and peri-domestic areas due to scarcity of water. The water in the containers is mainly harvested from rainwater and piped water.

The study sites are found in the middle of the Awash Valley, where infectious diseases such as malaria, chikungunya, and dengue fever are common (Gebru and Ahmed, 2016). Considerable attention has been given by the Regional government to achieve better health in the region, including the three towns (Goshu *et al.,* 2021). The Region has hospitals, health centers, clinics, and health posts in different zones and districts. There is one hospital, one

clinic, five health centers, and a few private clinics in the Amibara district. However, with limited health service delivery in the Region especially in the rural localities.

In Ethiopia, a nation-wide coverage of insecticide such as DDT and Malathion has been implemented mainly in the control of malaria including the Afar Region since 1952 (Fontaine and Najjar, 1958; WHO, 2017). Later on, due to the emergence of insecticide resistance of *Anopheles* mosquitoes to DDT and malathion (Yewhalaw *et al.*, 2011), insecticides such as deltamethrin, permethrin, alpha-cypermethrin, pirimiphos-methyl, bendiocarb, and propoxur have been used for indoor residual spraying or pyrethroids in long lasting insecticide nets for the control of malaria and arboviral diseases in Awash Arba, Awash Sebat and Werer towns (Messenger *et al.* 2017; MoH, 2017). Similarly, since maize, sorghum, and cotton productions are common in the area, agricultural chemicals such as soil fertilizers and agricultural pesticides are being used to increase crop production (Sileshi *et al.*, 2021).

Anthropogenic behavior and communities' ways of life can affect the transmission of vector-borne diseases, such malaria, dengue fever and chikungunya. Anthropogenic factors can affect the spreading of these diseases by directly adjusting the behavior and geographical dissemination of the *Aedes* and *Anopheles* mosquitoes. In addition, movements of people, goods, and services with vehicle play a significant role in the transmission of *Aedes*-borne diseases by facilitating the dispersal of infected *Aedes* mosquitoes and the movement of people who carry the diseases to new locations (Islam and Hu, 2024). In the present study areas, there is a high traffic and people movements particularly in the Awash Sebat and Awash Arba towns following the main road of the neighboring countries including Djibouti, which could contribute the higher transmission of *Aedes*-borne diseases in the towns. In addition, the study towns, people stay outside for handling the livestocks and for other agricultural activities during the daytime which may increase the risk of *Aedes*-bone disease transmission in the towns.

The woods and bush vegetation type was found along the major perennial rivers in Awash Sebat, Awash Arba and Werer towns and their surroundings. These vegetations get evergreen due to continuous water supply from the Awash River and browsed by the livestock during the dry season. Grazing and browsing of livestock in Amibara district is bush land (Hassen 2018). Cattle, Sheep, goat, and Camels are the primary livestock population in income generation, foods supply and financial security for the rural poor population of the region. Sheep and goat have a number of advantages as an integral component of the pastoral and

agro-pastoral production system of the Afar. The Gabi-Rasu Zone has an estimated population of 581,902 cattle, 1,036,987 sheep, 1,665,529 goats, 347,177 camels, 66,580 donkeys, and 39,245 poultry (Endris, 2021).

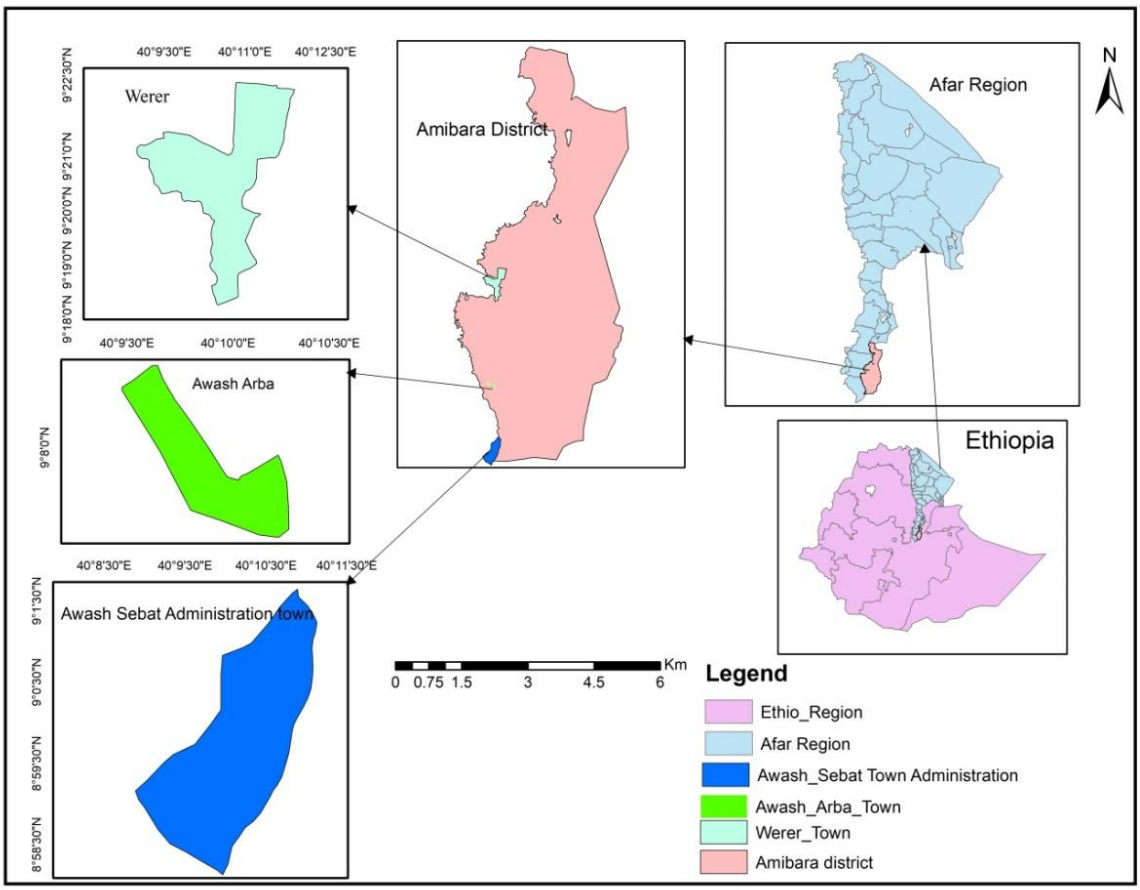

Figure 10: Locational map of Awash Arba, Awash Sebat and Werer Towns, Gabi-Rasu Zone, Afar Regional State, Ethiopia (Source: Ethio_GIS, 2023).

3.2. Study design

The towns were selected purposively in consultation with the respective towns' health bureaus, based on recent dengue fever and chikungunya reports (Figure 11). There were 2238 households in Awash Arba town, 3033 in Werer, and 3149 in Awash Sebat (Amibara District and Awash Sebat Town Health Bureau, unpublished). A longitudinal study design was employed to collect larvae/pupae and adult *Aedes* mosquitoes. A cross-sectional study was undertaken to collect data on the perception of the residents in the towns about *Aedes* mosquitoes and their role as the vector of dengue fever and chikungunya using questionnaires and focus group discussions. *Ae. aegypti* larvae were collected, grown to adults, and tested

for their susceptibility status to public health insecticides. Larval/pupal and adult mosquito collections were carried out from 20 randomly selected houses every month, making 240 house surveys in each town over the course of the year. Intervals between subsequent sampling houses were determined by dividing the total residential households by 240. Accordingly, 9 (2238/240) was the interval for Awash Arba, 12 (3033/240) for Werer, and 13 (3149/240) for Awash Sebat. In each town, the first house was selected randomly from downtown among five houses, and then the subsequent house selected towards the periphery using the corresponding interval for the town.

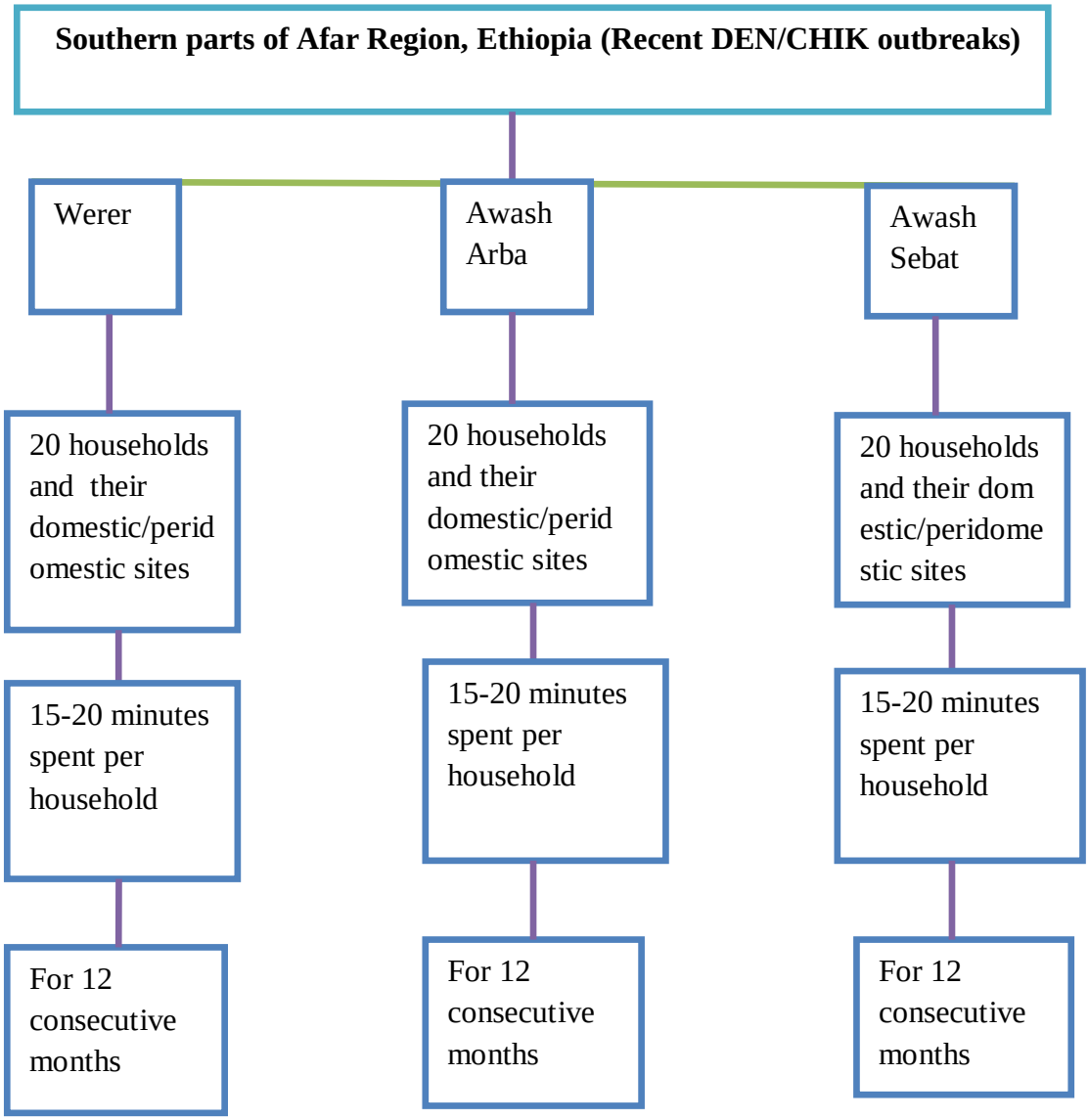

Figure 11: Flow chart depicting the study design and household selection in Awash Sebat, Awash Arba, and Werer towns, Southern Afar Region, Ethiopia, May 2022-April 2023.

A minimum distance of 300 m was kept between the subsequent survey house in each town, assuming the flight range of adult *Aedes* species to minimize overlap (WHO, 1995; Maciel-de-Freitas and Lourenco-de-Oliveira, 2009). The survey sites with respect to each selected house were categorized as: 1) domestic (inside the house rooms and outside the house within a radius of about 50 m) and 2) peri-domestic (outside the house compound between 50 m and 300 m).

3.3. *Aedes* larvae/pupae collection and habitat characterization

The purpose of larva/pupa collection was for the identification and characterization of habitats and to determine *Aedes* mosquitos' species composition. All available water-holding containers, such as used tyres, water storage drums, cement tanks, and flowerpots were inspected for the presence of mosquito larvae and pupae. Larvae/pupae-positive habitats were sampled by dipping, pipetting, and netting, following the World Health Organization guidelines (WHO, 2016a) in a wide range of habitats in indoor and outdoor areas of each selected house in the towns (Figure 12).

A minimum of 10 dips of water samples were collected for larvae/pupae from relatively large water-holding containers using a 350 ml dipper. Pipettes were used to collect the larvae/pupae from small water-holding containers such as discarded plastics. During the surveys, each water-holding container was classified as positive (if it harbored at least one larva/pupa) or negative (if it did not harbor any larva/pupa). Larvae/pupae collected from each container were categorized by genus, counted, transferred to a labeled plastic jar, transported to the temporary field insectary in Awash Sebat town, where they were transferred to white enamel trays, and reared to adults.

During larvae/pupae surveying, habitats were characterized. The physical/environmental characteristics of larval/pupal habitats measured include location (domestic or peri-domestic), presence of vegetation, source of water, usage of water, substrate type, and habitat permanence. Habitat substrate was categorized as mud, sand, gravel with soil, or cement. A habitat was considered permanent if it harbored water throughout the year, semi-permanent if it contained water for approximately 2 to 3 months, or temporary if it stored water for 2 to 3 weeks (Mereta *et al.*, 2013). The distance between the larval/pupal habitat and the nearest residential house and between the habitat and the nearest plant (tree or shrub) was recorded using measuring tape (Kamgang *et al*., 2013). Habitat temperature and pH records were made

at the time of collection using an ordinary thermometer and litmus paper, respectively. Habitat turbidity was determined as clean or turbid by holding a water sample in a clean glass test tube against a white background (Minakawa *et al*., 1999).

Habitat exposure to sunlight was recorded as shaded, partially shaded, or fully exposed visually. About 250 ml of water was collected from each positive habitat in a polyethylene bottle, transported in a cold-box, and analyzed for its chemical characteristics (such as alkalinity, salinity, conductivity, total dissolved solids, dissolved oxygen, and total hardness) at the Chemistry Department, Addis Ababa University. Permission was obtained from the Africa Center of Excellence for Water Management to use materials for the analysis.

The larvae were provided with powdered fish food (®/TM/©2019 Germany) and covered with netting until they emerged as pupae. Then, pupae were transferred to beakers and placed in netting cages (30 × 30 × 30 cm^3) and reared to adults and identified by genus and species morphologically under a dissecting microscope following taxonomic keys (Rueda, 2004; Coetzee, 2020; WHO, 2020).

Figure 12: Selected man-made water-holding containers positive for *Aedes* larvae/pupae in Awash Arba, Awash Sebat and Werer towns, Southern Afar, Ethiopia, May 2022-April 2023: Tyres (A), Cement tank (B), Flower pots (C), and Drums (D).

3.4. Adult mosquito collection, identification and processing

Adult *Ae. aegypti* collection was made in order to determine its resting habitat, blood meal sources, and dengue virus and chikungunya virus infection rates in the towns. The collection of *Ae. aegypti* was carried out using a Prokopak aspirator (John W. Hock Co., Gainesville, FL, USA, Model: 1419) powered by 12-volt rechargeable batteries. Mosquitoes were collected between 08:00-14:00 and 15:00-18:00 hrs along domestic and peri-domestic sites of each selected house (Figure 13). Indoor collection sites included bedrooms, dining rooms, bathrooms, and kitchens. While outdoor collection sites included animal shelters and vegetated areas (Garcia-Rejon *et al*., 2008; Vazquez-Prokopec *et al*., 2009).

Adult mosquito collection, in relation to each selected house, lasted about 15-20 minutes depending on the size of the house and area of its premises. The monthly *Ae. aegypti* density in each area was calculated using the following indices: number of *Ae. aegypti* females per surveyed house (FSH), number of *Ae. aegypti* females per *Aedes* positive house (FPH) (Rodrigues *et al*., 2015), percentage of houses positive for *Ae. aegypti* (AHI), and number of *Ae. aegypti* per surveyed house (AD) (Janaki *et al*., 2022).

The collected mosquitoes were anaesthetized by freezing, sorted by sex, date of collection, location, and abdominal status and identified according to their genera and species using morphological keys (Rueda, 2004; Coetzee, 2020; WHO, 2020). The fresh-fed and semi-gravid female *Ae. aegypti* were preserved individually in 1.5 ml Eppendorf tubes with silica gel for blood-meal source analysis using Enzyme linked immunosorbent assay (ELISA). While the male, unfed, and gravid *Ae. aegypti* were pooled (each pool containing 10 to 30, according to their study site, place of collection, and date of collection) and preserved in 1.5 ml Eppendorf tubes, which were then stored at -80 ^{o}C for dengue and chikungunya virus detection using molecular methods.

Figure 13: outdoor (A) and Indoor (B) collections activities and adult mosquito identifications (C) in the Southern parts of Afar Region, Ethiopia, May 2022-April 2023.

3.5. Blood meal source identification

Blood meal source detection was performed following a previously described method (Beier *et al*., 1988). The abdomen of freshly-fed or half-gravid female *Ae. aegypti* was cut and put individually into a 1.5 ml Eppendorf tube and pounded with 100 µl phosphate-buffered saline (PBS) (Sigma Aldrich, Co., 3050, USA) using a pestle (Figure 14). The pestle was rinsed with 100 µl PBS to make a total volume of 200 µl. Then, 100 µl homogenate was added to a 96-well U-shaped ELISA plate. 100 µl animal sera was added to the plates as a positive control. Moreover, 100 µl homogenate of unfed *Ae. aegypti* (from ALPB laboratory colony) and 100 µl PBS were added to the plate as negative controls. A similar preparation was made on a different plate for the detection of the human blood meal source of the mosquito. The plates were covered and incubated at room temperature for 2 hrs. After incubation, the well contents were discarded and tapped upside-down five times on tissue paper and washed three times with 200 µl PBS-Tween-20 (Tween 20, Sigma Aldrich USA). This was followed by the addition of 50 µl of host-specific conjugate of human peroxidase conjugate (lot no. 040831; catlog no. 474-1002) or bovine phosphatase conjugate (lot no. 120108; catlog. No. 14-12-06) to each well and incubated for 1 hr at room temperature. Plates were washed three times with 200 µl PBS-Tween 20, and 100 µl of ABTS® (Gaithersburg, MD, 20878, USA) was added to each well and incubated for 30 minutes. Finally, positive samples, including positive controls, were detected visually. Immediately, using an ELISA reader, the absorbance value

of each well was determined at a 405 nm wavelength. Samples were considered positive if absorbance values exceeded two times the mean of three negative controls (unfed mosquitoes/PBS-blank solution). Human blood obtained from volunteers and cow blood from abattoirs was used as positive controls.

Figure 14: Laboratory activities during blood meal detection of freshly-fed or half gravid *Ae. aegypti* using ELISA at the laboratory of Vector Biology and Control Research Unit, Aklilu Lemma Institute of Pathobiology, Addis Ababa University.

3.6. Dengue virus and chikungunya virus detection

RNA was extracted from 24 pools of mosquito samples using the TRIzol RNA extraction method as described in (Avramov *et al*., 2024). The final elution volume was 100 µl. The purity and concentration of extracted RNA were assessed by using the Nano drop 2000 (Thermo Fisher Scientific). Extracted RNAs were further pooled into 7 pools in equal proportions based on collection season (dry and wet), sex (males and females), and study towns of *Ae. aegypti*. The pooled RNAs were concentrated by using RNA Clean XP Beads (Beckman Coulter, A63987) and eluted by one-third of the pool volume. In short, 1.8x RNA Clean XP beads were added to the tubes containing the RNAs and incubated at room temperature for 15 minutes. Then, the tubes were put on a magnetic stand, and the supernatant was discarded after the solution cleared. The beads were washed twice with 80% ethanol, and after complete drying of the ethanol, the tubes were removed from the magnetic stand and eluted with DEPEC-treated nuclease-free water with one-third of the starting RNA volume.

Two-step RT-qPCR was used to detect dengue and chikungunya viruses' nucleic acids (Figure 15). First, the pooled and concentrated RNAs were reverse transcribed by using the Supper Script (SSIV) RT kit (Thermo Fisher Scientific, Cat. No. 18090010) following the manufacturer's protocol with the random hexamers option. Then, HotStar Taq DNA polymerase (Qiagene, cat. No. 203203) was used for the detection of dengue or chikungunya viruses from the cDNA using the primers and probes. The sequences of the primers and probes were taken from (Mishra et al., 2019), with slight modification of the fluorophores and quenchers for the probes. As the company we used for the synthesis of the probes did not have the CAL Flour Orange and Black Hole quencher 1 (BHQ1) plus fluorophore and quencher combination, we opted for the available FAM, BHQ1 flourophore quencher combination. Since the experiment was single-plex, this change did not affect the outcome of the result. Probes for dengue fever were DENV3P_probe FAM-CCCAGCGTCA ATATGCTGT-MGB, DENV_3P_QF ACTAGAGGTTAGAGGAGACCCCC, DENV_3P_Q RA GGCGCTCTGTGCCTGGATT, and DENV_3P_QRB TGGCGTTCTGTGCCTGGAAT. For chikungunya, CHIK_NSP2_Probe FAM AAAAGTATCTCCAGGCGG-MGB, CHIK_N SP2_NQF CATCTGCACYCAAGTGTACCA, and CHIK_NSP2_NQR GCGCATTTTGCC TTCGTAATG. The primers and probes were at final concentration of 0.16 µM and 0.08 µM in a total of 25 µl reaction. A previously known clinical sample positive for the viruses and molecular grade water were included as positive and negative control respectively, in the run together with the samples. Thermocycling conditions were 45 cycles of initial denaturation and enzyme activation at 95 ºC for 15 minutes, denaturation at 95 ºC for 10 seconds, annealing and elongation at 60 ºC for 1 minute.

Figure 15: Laboratory activities at Armauer Hansen Research Institute during the RT-PCR detections of dengue and chikungunya viruses from adult *Ae. aegypti:* preparations of reagents for cDNA synthesis.

3.7. Insecticide susceptibility test

Larvae and pupae were collected from positive habitats, transferred into labeled plastic jars (with date of collection and study town), transported to the insectary of the Aklilu Lemma Institute of Pathobiology (ALPB), Addis Ababa University, and reared to adults under standard laboratory conditions. Tetra cichlid floating fish food (®/TM/©2019 Germany) was provided as food to the larvae. The pupae were collected in glass flasks and transferred into (30 x 30 x 30 cm^3) netted cages. Emerged adult *Ae. aegypti* were kept at 27 ± 2 °C temperature and 75 ± 10% relative humidity and provided a 10% sucrose solution until the bioassay tests.

Non-blood-fed, 3-5-day-old female *Ae. aegypti* were tested for their susceptibility status to insecticides following the World Health Organization procedures (WHO, 2016b, WHO, 2022a) (Figure 16). The insecticides belonged to pyrethroids (0.05% deltamethrin, 0.05% alpha-cypermethrin, and 0.75% permethrin), carbamates (0.1% bendiocarb and 0.1% propoxur), and organophosphates (0.25% pirimiphos-methyl) (WHO, 2022a). These were among the insecticide classes commonly used for the control of public health-important mosquitoes in Ethiopia in general and the study areas in particular. Except for alpha-cypermethrin (0.05%) and propoxur (0.1%), we used the WHO's discriminating concentration for the *Anopheles* species insecticide susceptibility test. In each test, 20 female *Ae. aegypti* were exposed to each of the five treatment replicates and two control test tubes, making a total of 140 mosquitoes in a single test. The exposed mosquitoes were observed for 60 minutes at an interval of 10 minutes to record knockdown mosquitoes. After an hour, they were transferred into holding tubes and kept for 24 hrs at 27.0 ± 2.0 °C temperature, 75 ± 10% relative humidity, and were offered cotton wool pads soaked in a 10% sugar solution. *Ae. aegypti* mortality was recorded 24 hrs after 60 minutes of exposure. *Ae. aegypti* susceptibility to alpha-cypermethrin test was repeated in the case of Awash Arba town due to the observation of possible phenotypic resistance to the insecticide. In the case of Werer town, susceptibility to bendiocarb was not tested due to insufficient number of *Ae. aegypti* during the time.

Figure 16: Insecticide susceptiblity tests of *Ae. aegypti* at ALIPB, preparation of female *Ae. aegypti* (A) and exposing *Ae. aegypti* to insecticides (B).

3.8. Assessment of community awareness towards *Aedes* species and dengue/chikunguny -a

3.8.1. Knowledge, attitudes and practices data

Since data on the knowledge, attitudes, and practices of the communities of the towns is scarce, sample size was calculated assuming 50% of the community in each town has knowledge of *Aedes* mosquitoes and *Aedes*-transmitted chikungunya and dengue fever infections, with a 95% confidence level and 5% allowable error. Accordingly, the total sample size was 384. This sample size was proportionally divided among the towns, with 144 for Awash Sebat, 138 for Awash Arba, and 102 for Werer. A household was first selected by random sampling, followed by the systematic random sampling method following the intervals. Proportionally, the interval was calculated for each town using the households described in the previously published work (Seid *et al*., 2024). Thus, the interval for Awash Arba was 5, for Werer 7, and for Awah Sebat 8 to get the subsequent participants.

The questionnaire consisted of (1) socio-demographic information, including participant's age, gender, education level, marital status, family size and the immediate environmental characteristics such as house location, house type, and water storage; (2) knowledge of *Aedes* mosquitoes (larval habitats, resting habitats, biting time, bitten bodies (legs, faces, hand, and other body parts), biting place, and vectorial role), DEN and CHIK, transmission of DEN and CHIK, source of information, and season of outbreak; (3) attitudes of respondents about

Aedes mosquitoes and effects of *Aedes* mosquitoes on their daily life. In addition, questions about their perception of *Aedes*-transmitted CHIK and DEN were asked, and (4) practices: respondents' methods to reduce or avoid mosquito bites and mosquitoes (Figure 17). Scoring of the responses to knowledge, attitudes, and practices questions was performed following a previous study (Mbanzulu *et al*., 2022b). To calculate knowledge (Larval habitats, biting time), attitude and practice scores from the mean, 7, 3, 2, and 7 possible points were given, respectively. A value of 1 was given for a correct response to the knowledge, attitude, and practice questions from the given alternatives, whereas a value of zero was assigned to an incorrect response. Low knowledge, attitude, and practices scores were values less than the mean, and high scores were values equal to or greater than the mean.

Figure 17: Administering knowledge, attitudes and practices questionnaire to the participants in Werer, Awash Arba and Awash Sebat towns, Afar Regional State, Ethiopia.

3.8.2. Focus group discussion (FGD)

In addition to questionnaires survey, three focus group discussions (FGD), one per town, were conducted to facilitate interaction of representatives from diverse backgrounds in relation to *Aedes* mosquitoes and their importance as vectors of dengue and chikungunya. The FGD involved males and females, health professionals, farmers and merchants (Figure 18) and its size varied from 10 to 15 people. It was designed to encourage participation from all members, allowing comprehensive exploration of the community's understanding of *Aedes* mosquitoes and dengue/chikungunya transmission in each town. Open-ended questions facilitated in-depth responses and personal experiences, while closed-ended questions provided quantifiable data for analysis.

Figure 18: Focus group discussion in Werer Town (A) and in Awash Arba town (B).

3.9. Data quality management

Prior to the data collection, a team of supervisors, the principal investigator, and health professionals of the towns conducted a pilot survey. The pilot survey identified the study sites. The principal investigator provided training to the field assistants to ensure familiarity with the study. Data collection forms were prepared for the purpose.

Data collections were made following standard entomological guidelines, recommended reagents and previously used standard operating procedures. Standard morphological keys were used in identifying mosquitoes to their genera and species. Blood source of *Ae. aegypti* was determined using direct ELISA at Aklilu Lemma Institute of Pathobiology laboratory. While viral detection was done using RT-qPCR at Armauer Hansen Research institute, Ethiopia. The logical procedure (validity) and the quality of measurements (reliability) of the data were checked in the field and then entered in a computer database using SPSS versions 20.

3.10. Ethical Issues

This study obtained ethical approval from the Institutional Review Board (IRB) of the Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Ethiopia, with the reference number (ALIPB/IRB, June 13/80/2022). Written permission letters were obtained from the district health bureaus and health centers in the towns. The households were informed about the objective of the study, and their agreements were sought prior to the data collections.

3.11. Data analysis

***Aedes aegypti* Larvae/pupae density:** All data were entered, cleaned and analyzed using SPSS version 20 (SPSS Inc., Chicago, IL, USA). Density of *Ae. aegypti* larvae/pupae per container type was calculated by dividing the total number of larvae or pupae by the number of dips in a particular container type. Larvae/pupae density was log-transformed [log (n+1)] to fit the normal distribution curve and checked for normality by the Shapiro-Wilk test. Independent samples T-test was used to determine the difference in the mean of *Ae. aegypti* larvae/pupae density between two groups of habitat physical characteristics and One-way analysis of variance (ANOVA) for comparisons among more than two groups. When differences were significant in using ANOVA, Tukey's post-hoc test was used for pairwise comparisons of the means (Muturi *et al*., 2007). Pearson correlation was used to assess between *Ae. aegypti* larvae/pupae and habitat chemical characteristics. Bivariate logistic regression was performed to assess associations between habitat positivity for *Ae. aegypti* larvae/pupae and the physical characteristics of their habitats. Variables with p values <0.05 were considered as determinants and analyzed using multiple logistic regression to determine key predictors of physical/environmental variables. In addition, multiple linear regression was used to determine the relations between *Ae. aegypti* larvae/pupae density and habitats' water chemistry. All p values <0.05 were considered statistically significant. Larval/pupal infestation level was computed using indices: House index (HI), Container index (CI), Breteau index (BI) and Pupal index (PI) (Takken and Van-den-Berg, 2019).

$$\text{House index} = \frac{\text{Number of positive house}}{\text{Number of house inspected}} x100$$

$$\text{Container index} = \frac{\text{Number of positive containers}}{\text{Number of containers insepected}} x100$$

$$\text{Breteau index} = \frac{\text{Number of positive containers}}{\text{Number of house inspected}} x100$$

$$\text{Pupal index} = \frac{\text{Number of pupae}}{\text{Number of house inspected}} x100$$

Resting behavior and blood meal sources of *Aedes aegypti*: The Kruskal-Wallis test was employed to determine differences among monthly *Ae. aegypti* density indices, such as *Ae. aegypti* females per surveyed house (FSH), *Ae. aegypti* females per *Aedes* positive house (FPH) (Rodrigues *et al*., 2015), Adult house index (AHI), and Adult Density (AD). The numbers of *Ae. aegypti* per day, numbers of unfed, fed and gravid with respect to their resting habitats (tyres, vegetation, rooms) were compared using the Chi-square test. Effect of study towns, season, time of collection, and place of collection (indoor and outdoor) on the number of female *Ae. aegypti* was analyzed using a generalized linear model (GLM) with negative binomial distribution. The incidence risk ratio (IRR), 95% confidence intervals (95% CI), and p-value were determined. The human blood index (HBI) and bovine blood index (BBI) of *Ae. aegypti* were calculated as the proportion of *Ae. aegypti* positive for human blood and the proportion positive for bovine blood out of the total blood meals tested, respectively. The presence of differences between HBI and BBI and indoor and outdoor collections was analyzed using Chi-square test. Values with $p < 0.05$ were considered statistically significant. Species diversity of mosquitoes collected among the study towns were determined using the Shannon (H') index as $H' = -\Sigma[(pi).\ln(pi)]$ where 'pi' is proportion of total number of samples represented by species 'i' out of the total number of samples.

***Aedes aegypti* susceptibility to insecticides:** Phenotypic resistance/susceptibility of female *Ae. aegypti* to public insecticides were interpreted following WHO criteria. Susceptible were those with mortality in the range of 98-100%, possibly or suspected for resistance with mortality 90-97%, and resistant with mortality <90% (WHO, 2016b). Probit analysis was used to determine the estimated duration at which 50% (KDT_{50}) and 95% (KDT_{95}) of the exposed *Ae. aegypti* were knocked-down.

Knowledge, attitudes and practices: Data on respondents' knowledge, attitude and practice were summarized using frequencies and percentages in tables. Correct responses of the respondents to knowledge (*Aedes* larval habitats, biting time), attitudes (perception on *Aedes* species, effect of *Aedes* on the respondents daily life), and practices (protective and avoidance measures taken) were scored using alternatives that ranged from high (maximum ≥ the mean) and low (minimum < the mean). Scoring for each response was employed following a previous study (Mbanzulu *et al*., 2022b). The logistic regression analysis was employed for identifying the factors that contributed to correct knowledge, attitudes and practices towards *Aedes* mosquitoes and their role in transmitting dengue and chickungunya.

All explanatory variables significantly ($p<0.05$) associated in the bivariate analyses were entered into the multivariate analysis. All values with $p < 0.05$ were considered significant. The focus group discussion questions were analyzed using content analysis as previously described (Mayring, 2014).

CHAPTER FOUR: RESULTS

4.1. Spatio-temporal occurrence and habitat characteristics of *Ae. aegypti* larvae/pupae

4.1.1. Occurrence of *Ae. aegypti* larvae/pupae

Overall, 11440 larvae/pupae were collected of which 9099 (79.5%) were *Aedes* larvae/pupae (Table 1). All the *Aedes* larvae/pupae that emerged to adults were *Ae. aegypti*, and hence all the *Aedes* larvae and pupae collected are hereafter considered to be *Ae. aegypti*. Among the 9099 *Ae. aegypti* larvae/pupae, 4875 (53.6%) were collected from Awash Sebat, 2687 (29.5%) from Awash Arba and 1537 (16.9%) from Werer. Furthermore, 1544 *Culex* larvae/pupae were collected among which 741 (47.99%) were from Werer, 643 (41.6%) from Awash Sebat and 160 (10.4%) from Awash Arba. Similarly, 797 *Anopheles* larvae/pupae were collected, of which 606 (76%) were from Awash Sebat town, 162 (20.3%) from Awash Arba, and 29 (3.6%) from Werer.

Table 1. Distribution of mosquitos larvae/pupae collected from water-holding containers in Awash Sebat, Awash Arba and Werer towns, Afar Regional State, Ethiopia, May 2022-April 2023

Study sites	TCI(+ve)	*Ae. aegypti* n (%)	*Anopheles* n (%)	*Culex* n (%)	Total N (%)
Awash Sebat	379 (276)	4875 (53.56)	606 (76.04)	643 (41.67)	6124 (53.54)
Awash Arab	142 (121)	2687 (29.54)	162 (20.32)	160 (10.37)	3009 (26.30)
Werer	82 (62)	1537 (16.9)	29 (3.64)	741 (47.96)	2306 (20.16)
Total	603 (459)	9099 (100)	797 (100)	1544 (100)	11440 (100)

TCI, Total containers inspected; +ve, Positive container

4.1.2. Larval/pupal habitat types and level of *Ae. aegypti* infestation

A total of 603 potential mosquito larvae/pupae habitats were inspected of which 409 were from domestic and 194 from peri-domestic sites. Among these, 459 (76.1%) were positive for at least one immature mosquito (Table 2). In Awash Arba town, 2610/2687 (97.1%) of the *Ae. aegypti* larvae/pupae were collected from domestic sites and 77/2687 (2.9%) from the peri-domestic sites. In Werer, 1411/1537 (91.8%) of the total *Ae. aegypti* larvae/pupae collections were made from domestic sites and 126/1537 (8.2%) from the peri-domestic. Likewise, in Awash Sebat town, 3533/4875 (72.5%) of the total *Ae. aegypti* catches were made from domestic and 1342/4875 (27.5%) from peri-domestic sites. Tyres were the most productive *Ae. aegypti* larvae/pupae habitats in all the towns. In Werer, tyres in domestic sites

harbored 1351/1537 (87.9%) of *Ae. aegypti* larvae/pupae collections and 1974/2687 (73.5%) in Awash Arba. Similarly, in Awash Sebat 1488/4875 (30.5%) of the *Ae. aegypti* were collected from tyres 1011/4875 (20.7%) from water-storage drums, and 764/4875 (15.8%) from water tanks made of cement. Discarded plastics contributed 106/4875 (2.2%) of the *Ae. aegypti* larvae/pupae in Awash Sebat and 44/4875 (1.6%) in Awash Arba, whereas flowerpots had 164/4875 (3.3%) in the domestic areas of Awash Sebat.

Table 2. Container types, location and level of *Ae. aegypti* infestation in Awash Sebat, Awash Arba and Werer towns, Afar Regional State of Ethiopia, May 2022-April 2023

Study sites	Container location	Container types	Containers inspected (+ve)	*Ae. aegypti* n (%)
Awash Sebat	Domestic	Tyres	101 (88)	1488 (30.5)
		Drums	31 (22)	1011 (20.7)
		Flower pots	14 (10)	164 (3.4)
		Discarded plastics	9 (6)	106 (2.2)
		Others (cement-water tank, bowl, jerrycan)	47 (37)	764 (15.7)
	Peri-domestic	Tyres	127 (89)	992 (20.3)
		Drums	8 (4)	83 (1.7)
		Flower pots	0 (0)	0 (0)
		Discarded plastics	0 (0)	0 (0)
		Others	42 (20)	267 (5.5)
		Sub-total	379 (276)	4875 (100)
Awash Arba	Domestic	Tyres	95 (84)	1974 (73.5)
		Drums	33 (27)	572 (21.3)
		Flower pots	0 (0)	0 (0)
		Discarded plastics	2 (1)	44 (1.6)
		Others	3 (1)	20 (0.7)
	Peri-domestic	Tyres	9 (8)	77 (0.8)
		Drums	0 (0)	0 (0)
		Flower pots	0 (0)	0 (0)

		Discarded plastics	0 (0)	0 (0)
		Others	0 (0)	0 (0)
		Sub-total	142 (121)	2687 (100)
Werer	Domestic	Tyres	53 (46)	1351 (87.9)
		Drums	3 (2)	0 (0)
		Flower pots	0 (0)	0 (0)
		Discarded plastics	0 (0)	0 (0)
		Others	18 (8)	60 (3.9)
	Peri-domestic	Tyres	8 (6)	126 (8.2)
		Drums	0 (0)	0 (0)
		Flower pots	0 (0)	0 (0)
		Discarded plastics	0 (0)	0 (0)
		Others	0 (0)	0 (0)
		Sub-total	82 (62)	1537 (100)
	Overall total	Domestic	409 (332)	7554/9099
		Peri-domestic	194 (127)	1545/9099

4.1.3. Larval/pupal indices

Overall, 720 house surveys were made during the course of the study in which case 123 (17.1%) were associated with larvae/pupae in domestic and peri-domestic sites (Table 3). The container indices (CIs) ranged from 25 to 94.7% in Awash Sebat, 0 to 95.9% in Awash Arba, and 0 to 84.4% in Werer. The House Indices (HIs) ranged from 5 to 80% in Awash Sebat, 5 t0 75% in Awash Arba and 0 to 75 in Werer. Breteau Indices (BIs) ranged from 5 to 450 in Awash Sebat, 0 to 235 in Awash Arba, and 0 to 190 in Werer. The highest CIs of Awash Sebat (94.7%) and Awash Arba (95.9%) were observed in September 2022, and that of Werer (84.4%) in August 2022. In addition, a relatively higher House Index (80%) was observed in Awash Sebat during August, as compared to Awash Arba (55%) and Werer (35%) sites. Moreover, higher Breteau Index (450) and Pupal Index (1320) were in Awash Sebat.

Table 3. *Aedes aegypti* larval/pupal indices in Awash Sebat, Awash Arba and Werer towns, Afar Regional State of Ethiopia, May 2022-April 2023

Study sites	Study Period	Positive houses	Total houses	Total containers	Positive container	No. pupae	Indices			
							CI (%)	HI (%)	BI	PI
Awash Sebat	May 2022	1	20	4	1	0	25	5	5	0
	Jun 2022	2	20	18	4	33	22.2	10	20	165
	Jul 2022	6	20	32	9	71	28.1	30	45	355
	Aug 2022	16	20	116	86	170	74.1	80	430	850
	Sept 2022	15	20	95	90	264	94.7	75	450	1320
	Oct 2022	14	20	85	75	131	88.2	70	375	655
	Nov 2022	3	20	8	4	9	50	15	20	45
	Dec 2022	2	20	12	3	6	25	10	15	30
	Jan 2023	2	20	3	2	1	66.6	10	10	5
	Feb 2023	1	20	2	1	2	50	5	5	10
	Mar 2023	1	20	2	1	3	50	5	5	15
	Apr 2023	1	20	2	1	7	50	5	5	35
	Total	64	240	379	276	699	51.9	26.6	115.4	290.4
Awash Arab	May 2022	0	20	0	0	0	0	0	0	0
	June 2022	0	20	0	0	0	0	0	0	0
	Jul 2022	6	20	15	12	0	80	30	60	0
	Aug 2022	11	20	39	32	89	82	55	160	445
	Sept 2022	15	20	49	47	89	95.9	75	235	445
	Oct 2022	8	20	30	25	83	83.3	40	125	415
	Nov 2022	1	20	2	1	4	50	5	5	20
	Dec 2022	2	20	5	3	0	60	10	15	0
	Jan 2023	1	20	2	1	0	50	5	5	25
	Feb 2023	0	20	0	0	0	0	0	0	0
	Mar 2023	0	20	0	0	0	0	0	0	0
	Apr 2023	0	20	0	0	0	0	0	0	0
	Total	44	240	142	121	265	41.7	18.3	50.4	112.5
Werer	May 2022	0	20	0	0	0	0	0	0	0
	Jun 2022	0	20	0	0	0	0	0	0	0

Jul 2022	0	20	0	0	0	0	0	0	0
Aug 2022	7	20	45	38	123	84.4	35	190	615
Sept 2022	6	20	27	20	136	74.1	30	100	680
Oct 2022	1	20	6	2	5	33.3	5	10	25
Nov 2022	0	20	0	0	0	0	0	0	0
Dec 2022	1	20	4	2	5	50	5	10	25
Jan 2023	0	20	0	0	0	0	0	0	0
Feb 2023	0	20	0	0	0	0	0	0	0
Mar 2023	0	20	0	0	0	0	0	0	0
Apr 2023	0	20	0	0	0	0	0	0	0
Total	15	240	82	62	269	20.2	6.25	25.8	112.1
Overall total	123	720	603	459	1233	37.9	17.1	63.9	171.7

4.1.4. Distribution of *Ae. aegypti* larvae/pupae across months and season

The distribution of *Ae. aegypti* larvae/pupae varied between the dry and wet seasons and among the towns (Figure 19). Peak numbers of *Ae. aegypti* larvae/pupae collections in Awash Sebat and in Awash Arba towns were made in September 2022 and in Werer town during August 2022.

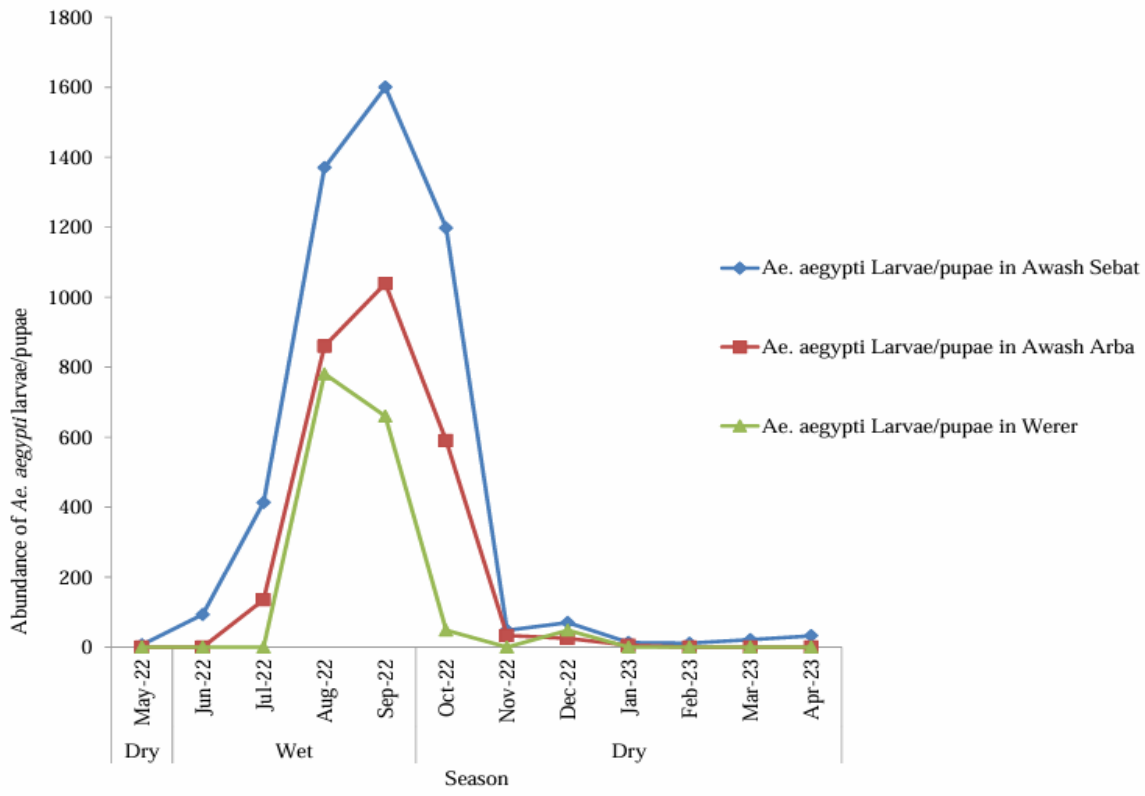

Figure 19: Monthly distribution of *Ae. aegypti* larvae/pupae in Awash Sebat, Awash Arba and Werer towns of Afar Regional State, Ethiopia, May 2022-April 2023.

4.1.5. *Aedes aegypti* larvae/pupae occurrence and associated habitat physical characteristics

The results from one-way analysis of variance (ANOVA) and mean comparisons of the physical characteristics and densities of the *Ae. aegypti* larvae/pupae are depicted in Table 4. Significantly higher mean density of *Ae. aegypti* larvae/pupae was collected during the wet season ($F = 15.075$, $p < 0.001$), from discarded tyres ($F = 4.775$, $p = 0.001$), from habitats with mud and habitats with gravel and soil substrate ($F = 7.882$, $p < 0.001$) and habitats originated from rain water ($F = 6.020$, $p = 0.003$). Significant differences were observed in mean *Ae. aegypti* larval density with substrate types. Tukey's post-hoc test indicated that habitats with mud and with gravel and soil substrates contained significantly higher *Ae. aegypti* larvae/pupae densities compared to habitats with cement substrates ($p < 0.001$). In addition, mean density of *Ae. aegypti* larvae was much higher in habitats of rain water than in the habitats of tap water ($p = 0.002$).

Table 4. Physical characteristics of habitats and mean density of *Ae. aegypti* larvae/pupae/dips in Awash Sebat, Awash Arba and Werer towns, Afar Regional State of Ethiopia, May 2022-April 2023

Characteristics	Variables	Mean ± SE	F	P-value
Season	Dry	1.47 ± 0.07	15.075	<0.001
	Wet	1.84 ± 0.03		
Substrate types	Mud	1.81 ± 0.05	7.882	<0.001
	Sand	1.59 ± 0.13		
	Gravel and soil	1.71 ± 0.06		
	Cements	1.09 ± 0.12		
Sun light Exposure	Shaded	1.75 ± 0.11	0.125	0.883
	Partially shaded	1.71 ± 0.04		
	Open	1.67 ± 0.07		
Habitat permanency	Semi-permanent	1.67 ± 0.09	2.493	0.462
	Temporary	1.74 ± 0.04		
Habitat type	Flower pots	1.42 ± 0.25	4.775	0.001

	Drums	1.56 ± 0.08		
	Discarded plastics	1.43 ± 0.11		
	Tyres	1.84 ± 0.03		
	Others (Cement tank)	1.56 ± 0.14		
Turbidity	Clean	1.60 ± 0.07	8.299	0.043
	Turbid	1.77 ± 0.03		
Water usage	Sometimes	1.65 ± 0.05	5.311	0.136
	Not used	1.77 ± 0.04		
Water source	Tap water	1.52 ± 0.08	6.020	0.003
	Rain water	1.82 ± 0.03		
	Mixed water	1.75 ± 0.09		
Habitat location	Domestic	1.86 ± 0.08	0.338	0.126
	Peri-domestic	1.70 ± 0.03		
Presence of vegetation	Present	1.72 ± 0.06	0.006	0.823
	Absent	1.70 ± 0.04		

According to the bivariate regression analysis, a containers located at a domestic site was 2.63 times more likely to harbor *Ae. aegypti* larvae compared to the one located at semi-domestic sites (COR = 2.63, CI = 1.84-377, p <0.001). A habitat with rain water source was 4.87 times more likely to harbor *Ae. aegypti* than a habitat of different water source (COR = 4.87, CI = 2.79-8.48, p <0.001). A habitat in shaded site was 4 times likely to harbor *Ae. aegypti* larvae compared to the one exposed to direct sunlight (COR = 4.00, CI = 1.13-14.17, p = 0.032). A water-holding tyre was 7.0 times more likely to harbor *Ae. aegypti* larvae (COR = 7.10, CI= 3.66-13.76, p <0.001) and a water storage drum 7.0 times more likely (COR= 7.0, CI= 2.45-19.95, p <0.001) both compared to water tank made of cement (Table 5).

Table 5. A bivariate analysis of relationships between *Ae. aegypti* larvae/pupae occurrence (presence/absence) and physical characteristic of habitats in Awash Sebat, Awash Arba and Werer towns, Afar Regional State of Ethiopia, May 2022-April 2023

Habitat characteristics	Variables	COR (95% CI)	p-value
Season	Wet	1	
	Dry	0.41 (0.28-0.58)	<0.001
Water source	Mixed	1	

	Tap	0.82 (0.53-1.27)	0.372
	Rain	4.87 (2.79-8.48)	<0.001
Substrate types	Cement	1	
	Mud	1.9 (1.14-3.19)	0.014
	Sand	1.4 (0.44-4.41)	0.566
	Gravel with soil	1.8 (1.21-2.71)	0.004
Sun light exposure	Exposed fully	1	
	Shaded	4.00 (1.13-14.17)	0.032
	Partially shaded	1.86 (1.32-2.62)	<0.001
Habitat types	water tank made of cement	1	
	Drum	7.0 (2.45-19.95)	<0.001
	Discarded plastics	2.50 (0.48-12.88)	0.273
	Tyres	7.10 (3.66-13.76)	<0.001
Habitat location	Peri-domestic	1	
	Domestic	2.63 (1.84-377)	<0.001
Habitat permanency	Semi-permanent	1	
	Temporary	2.44 (1.13-5.31)	0.124
Water turbidity	Clear	1	
	Turbid	1.53 (.98-2.39)	0.061
Water usage	Sometimes	1	
	Not used	1.14 (0.78-163)	0.469

COR, Crude odds ratio; CI, Confidence interval

On the basis of multiple regression analysis, a discarded tyre was 15.89 times likely to harbor *Ae. aegypti* larvae/pupae compared to a water tank made of cement(AOR = 15.89, CI = 3.55-71.09, p <0.00) and a water storage drum was 19.0 times likely to support *Ae. aegypti* larvae compared to a water tank made of cement (AOR = 19.84, CI = 4.64-84.89, p <0.001) (Table 6). In addition, a container located in domestic sites was 3.76 times more likely to harbor *Ae. aegypti* larvae/pupae as compared to a habitat in peri-domestic sites (AOR = 3.76, CI = 1.27-11.12, p = 0.017).

Table 6. A multivariate analysis of relationship between *Ae. aegypti* larvae/pupae occurrence and predictors (physical characteristics) of habitats in Awash Sebat, Awash Arba and Werer towns, Afar Regional State, Ethiopia, May 2022-April 2023

Habitat characteristics	Variables	AOR (95% CI)	p-value
Season	Wet	1	
	Dry	0.59 (0.24-1.46)	0.260
Water source	Mixed	1	
	Tap	0.08 (0.02-0.31)	<0.001
	Rain	1.48 (0.10-2.27)	0.353
Substrate types	Cement	1	
	Mud	0.86 (0.19-3.72)	0.837
	Sand	0.70 (0.08-6.29)	0.752
	Gravel with soil	1.03 (0.32-3.34)	0.961
Sun light exposure	Exposed fully	1	
	Shaded	5.33 (0.57-49.47)	0.141
	Partially shaded	0.47 (0.13-1.61)	0.227
Habitat types	water tank made of cement	1	
	Drum	19.84 (4.64-84.89)	<0.001
	Discarded plastics	2.65 (0.18-38.23)	0.474
	Tyres	15.89 (3.55-71.09)	<0.001
Habitat location	Peri-domestic	1	
	Domestic	3.76 (1.27-11.12)	0.017

AOR, Adjusted odds ratio; CI, Confidence interval

4.1.6. Association between *Ae. aegypti* larvae/pupae density and habitat chemical characteristics

Ae. aegypti larvae/pupae density showed significant positive relation with dissolved oxygen ($\beta = 0.523$, $p < 0.001$) and total water hardness ($\beta = 0.475$, $p = 0.034$). Water temperature positively associated with density of *Ae. aegypti* larvae/pupae although not statistically significant ($p = 0.135$) (Table 7). On the other hand, habitat water salinity was negatively related to larval density even though not statistically significant.

Table 7. Relationship between habitat chemical characteristics and Ae. aegypti larval/pupae density in Awash Sebat, Awash Arba and Werer towns, Afar Region, Ethiopia, May 2022 to April 2023

Variables	Standard error	Beta (β)	t	p-value
Water temperature (°C)	0.017	0.130	1.506	0.135
Total hardness (mg/l)	0.002	0.475	1.890	0.034
Dissolved oxygen (mg/l)	0.028	0.523	4.077	<0.001
Salinity (%)	0.675	-0.494	-1.714	0.089

4.2. Species composition, resting habitats and blood meal sources of adult *Ae. aegypti*

4.2.1. Species composition and abundance of adult mosquitoes

A total of 2,745 adult mosquitoes comprising the genus *Aedes* 1433 (52.2%), *Culex* 47.1% (1292) and *Anopheles* 20 (0.7%) were collected (Table 8). The highest number was collected from Awash Sebat 61.8% (1698) followed by Werer 538 (19.6%), and Awash Arba 509 (18.5%). *Ae. aegypti* was dominant in Awash Sebat town 1202 (70.8%) and *Culex* spp. in Werer 54.1% (291) and in Awash Arba 145 (48.5%) towns. The Shannon diversity index ranged from 12.19-14.33.

Table 8. Composition and abundance of mosquito species in Awash Sebat, Awash Arba and Werer towns, Afar Regional State of Ethiopia, May 2022-April 2023

Genus /Species	Sex	Awash Arba n (%)	Werer n (%)	Awash Seba n (%)	Total N (%)
Ae. aegypti	Female	172 (33.8)	59 (11.0)	611 (36)	842 (30.7)
	Male	71 (13.9)	44 (8.2)	476 (28.0)	591 (21.5)
An. gambiae s.l	Female	3 (0.6)	6 (1.1)	0(0)	9 (0.3)
An. stephensi	Female	1 (0.2)	0(0)	5 (0.3)	6 (0.2)
	Male	1 (0)	2(0.3)	2 (0.1)	5 (0.2)
Culex spp.	Female	145 (28.5)	291 (54.1)	368 (21.7)	804 (29.3)
	Male	116 (22.8)	136 (25.3)	236 (13.9)	488 (17.8)
	Total	509 (100)	538 (100)	1698 (100)	2745 (100)
Shannon diversity (H')		13.89	12.19	14.33	

4.2.2. Resting behavior and abdominal status of *Ae. aegypti*

Among the 842 female *Ae. aegypti*, 556 (66%) were caught from outdoors and 286 (34.%) from indoors (Table 9). The indoor to outdoor ratio of female *Ae. aegypti* was 1:2. Females of *Ae. aegypti* were collected more frequently from tyres 314 (37.3%) followed by rooms 201

(23.9%) in Awash Sebat town, and from tyres 87 (10.3%) followed by vegetation 70 (8.3%) in Awash Arba. Abundance of female *Ae. aegypti* in tyres was significantly the highest compared to other collection places in the three towns 314 (37.29%) (X^2, 27.374, df = 12; p = 0.007). The indoor collection in Awash Sebat 280 (33.2%) was much higher than in Awash Arba 0.4% (3) or in Werer 3 (0.4%). Higher numbers of unfed *Ae. aegypti* were collected from tyres 250 (37.9%) and rooms 170 (25.8%) in Awash Sebat, from tyres 73 (11.1%) and vegetation 44 (6.7%) in Awash Arba, and from tyres 11 (1.7%) and others 25 (3.8%) in Werer town. Moreover, higher number of freshly-fed *Ae. aegypti* was collected from tyres 48 (34.04%) followed by rooms 24 (17.02%) in Awash Sebat, from tyres 13 (9.3%) followed by vegetation 9 (6.5%) in Awash Arba, and from tyres 10 (7.2%) and vegetation 5 (3.6%) in Werer. On the other hand, higher number of half-gravid *Ae. aegypti* were collected from vegetation and tyres whereas gravid *Ae. aegypti* from tyres in Awash Sebat town.

Table 9. Distribution and abdominal status of *Ae. aegypti* in Awash Sebat, Awash Arba and Werer towns, Afar Regional State of Ethiopia, May 2022-April 2023

Study sites	Collection habitats	Abdominal status of *Ae. aegypti*					
		Fed	Unfed	Gravid	Half-gravid	Total	p-value
Awash Sebat	Rooms n (%)	24 (17.3)	170 (25.8)	7 (38.9)	0 (0)	201 (23.9)	0.002
	Kitchen n (%)	7 (5.0)	69 (10.5)	3 (16.7)	0 (0)	79 (9.4)	-
	Tyres n (%)	48 (34.5)	250 (37.9)	8 (44.4)	8 (30.8)	314 (37.3)	0.007
	Vegetation n (%)	9 (6.5)	8 (1.2)	0 (0)	0 (0)	17 (2.0)	<0.001
	Others (bricks, scrap metals) n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0.0)	0.005
Awash Arba	Rooms n (%)	3 (2.2)	0 (0)	0 (0)	0 (0)	3 (0.4)	0.002
	Kitchen n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	-
	Tyres n (%)	13 (9.3)	73 (11.1)	0 (0)	1 (3.8)	87 (10.3)	0.007
	Vegetation n (%)	9 (6.5)	44 (6.7)	0 (0)	17 (65.4)	70 (8.3)	<0.001
	Others n (%)	5 (3.6)	7 (1.1)	0 (0)	0 (0)	12 (1.4)	0.005
Werer	Rooms n (%)	3 (2.2)	0 (0)	0 (0)	0 (0)	3 (0.4)	0.002

Kitchen n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	-
Tyres n (%)	10 (7.2)	11 (1.7)	0 (0)	0 (0)	21 (2.5)	0.007
Vegetation n (%)	5 (3.6)	2 (0.3)	0 (0)	0 (0)	7 (0.8)	<0.001
Others n (%)	3 (2.2)	25 (3.8)	0 (0)	0 (0)	28 (3.3)	0.005

4.2.3. Average catches of *Aedes aegypti* across seasons and collection times

Highest number of female *Ae. aegypti* was collected in September 2022, 383 (45.5%) followed by October 2022, 198 (23.5%) and August 2022, 119 (14.1%) (Table 10). There was significant monthly variation in the number of female *Ae. aegypti* collected per surveyed house (Kruskal-Wallis test, $\chi2 = 31.737$, df = 11, p = 0.001), per positive house (Kruskal-Wallis test, $\chi2 = 21.878$, df = 11, p = 0.025) and adult house indices (Kruskal-Wallis test, $\chi2 = 107.000$, df = 11, p <0.001) and *Ae. aegypti* adult density (Kruskal-Wallis test, $\chi2 = 30.272$, df = 11, p = 0.001) across the months. Highest female *Ae. aegypti* densities per surveyed house, per positive house and adult *Ae. aegypti* density were observed during September 2022.

Table 10. Number of *Ae. aegypti*, adult density/house, Adult House Index (AHI), Adult Density (AD), during May 2022-April 2023 in Awash Sebat, Awash Arba and Werer towns, Afar Regional State of Ethiopia, collected by Prokopack aspirator

Collection month	Surveyed Houses	Positive houses	Females *Ae. aegypti*	Males *Ae. aegypti*	Female density FSH	FPH	AHI	AD
May 2022	60	0	0	0	0	0	0	0
Jun 2022	60	0	0	0	0	0	0	0
Jul 2022	60	13	57	30	0.9	4.4	21.7	1.5
Aug 2022	60	19	119	98	1.9	6.3	31.7	3.6
Sept 2022	60	35	383	271	6.4	10.9	58.3	10.9
Oct 2022	60	21	198	145	3.3	9.4	35.0	5.7
Nov 2022	60	9	38	18	0.6	4.2	15	0.9
Dec 2022	60	3	15	11	0.3	5	5.0	0.4
Jan 2023	60	5	10	4	0.1	2	8.3	0.2
Feb 2023	60	3	4	2	0.03	1.3	5.0	0.1
Mar 2023	60	4	6	3	0.1	1.5	6.7	0.2

Apr 2023	60	5	12	9	0.2	2.4	8.3	0.3
Total	720	117	842	591	1.2	5.1	16.1	1.9

AHI= Adult house index, AD=Adult Density, FSH = *Ae. aegypti* females per surveyed house, FPH = *Ae. aegypti* females per *Aedes* positive house

Significantly higher number of female *Ae. aegypti* collection was made during the wet season than during the dry (p <0.001) (Figure 20). On the other hand, no significant difference was observed in the number of *Ae. aegypti* among the collection times (p = 0.362).

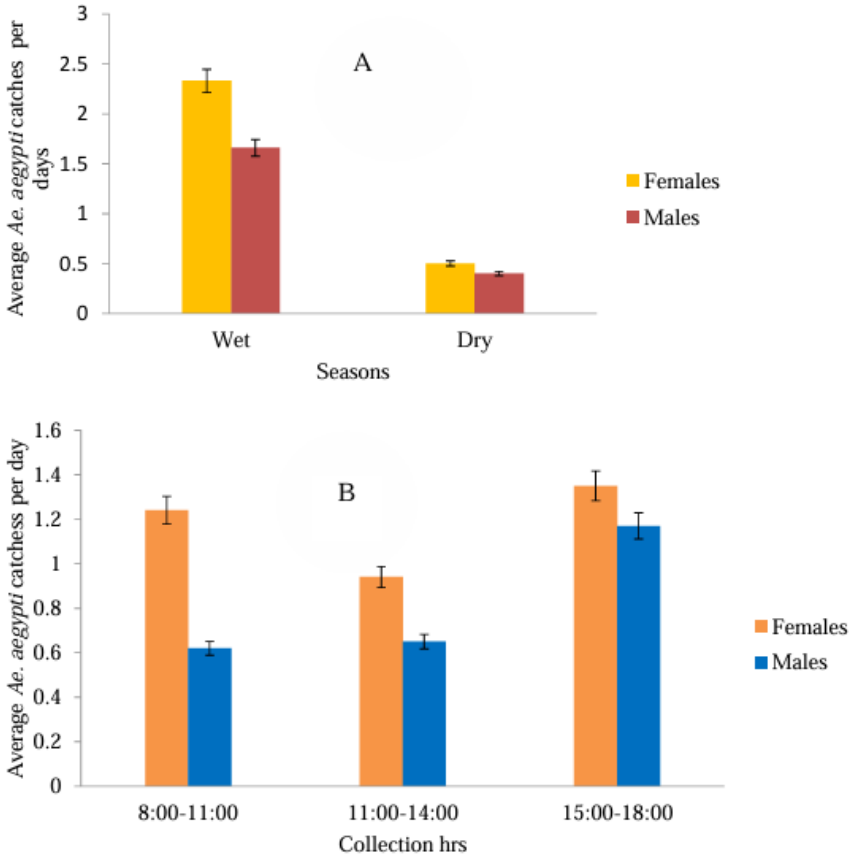

Figure 20: Number of female *Ae. aegypti* collected per season (A) and per collection time (B) in in Awash Sebat, Awash Arba and Werer towns, Afar Regional State of Ethiopia, May 2022-April 2023.The error bars represent 95% confidence intervals.

4.2.4. Effect of environment and season on the abundance of female *Ae. aegypti*

The number of *Ae. aegypti* in Awash Sebat town was about 16 times compared to the number in Werer town (IRR = 16.36, p < 0.001) and the number in Awash Arba was about 3 times than that of Werer (IRR = 2.82, p <0.001) (Table 11). Similarly, more *Ae. aegypti* females were collected during the wet season compared to the dry (IRR = 6.29, p<0.001). A tyre was

about 9 times more likely to harbor adult female *Ae. aegypti*. Moreover vegetation was 5 times more likely to harbor female *Ae. aegypti* compared to others (open barrels, bricks). On the other hand, *Ae. aegypti* was less likely collected during 11:00-14:00 collection hours (IRR = 0.71, p = 0.022) compared to 15:00-18:00 hrs.

Table 11. Effect of study sites, season, collection time and resting habitat on the number of female *Ae. aegypti* in Awash Sebat, Awash Arba and Werer towns, Afar Regional State of Ethiopia, May 2022-April 2023

Predictors		IRR	95% CI	p-value
Intercept		0.02	0.01-004	<0.001
Study sites	Werer	1	1	
	Awash Sebat	16.36	10.13-26.43	<0.001
	Awash Arba	2.82	1.77-4.51	<0.001
Season	Dry	1	1	
	Wet	6.29	4.80-8.25	<0.001
Collection time	15:00-18:00	1	1	
	8:00-11:00	1.21	0.82-1.77	0.06
	11:00-14:00	0.71	0.52-0.95	0.022
Collection places	Others (barrels, bricks)	1	1	
	Tyres	9.32	3.71-23.42	<0.001
	Rooms	4.26	1.81-10.0	0.001
	Kitchen	2.59	0.99-6.75	0.094
	Vegetation	5.74	2.38-13.81	0.001

IRR: Incidence risk ratio

4.2.5. Blood meal sources of *Ae. aegypti*

A total of 145 *Ae. aegypti* were tested for their blood meal sources. The human blood indices (HBI) of outdoor collected *Ae. aegypti* ranged from 2.8% in Werer town to 15.9% in Awash Sebat (Table 12). In addition, HBI of indoor collected *Ae. aegypti* ranged from 0% in Awash Arba to 7.6% in the Awash Sebat. Furthermore, the bovine blood indices (BBI) of *Ae. aegypti* collected from outdoors ranged from 0% in Werer town to 7.6% in the Awash Sebat. For the indoor collected BBI ranged from 0% in Werer and Awash Arba towns to 7.6% in Awash Sebat town. The overall HBI of *Ae. aegypti* in study area was 53/145 (36.7%). The BBI was 18/145 (12.4%), whereas the mixed human and bovine blood meal index was 15/145 (10.3%)

in the towns. There was no statistical difference between the human fed and animal fed *Ae. aegypti* and also between indoor and outdoor collections.

Table 12. Blood meal sources of *Ae. aegypti* in Awash Sebat, Awash Arba, and Werer towns, Southern Afar Regional State of Ethiopia, May 2022-April 2023

Study towns	Origin	HBI +ve (%)	BBI +ve (%)	Mix +ve (%)	Number of Un (%)	Total N (%)
Awash Sebat	Indoor	11 (7.6)	5 (3.4)	2 (1.4)	13 (8.9)	31 (21.3)
	Outdoor	23 (15.9)	11 (7.6)	4 (2.7)	25 (17.2)	63 (43.5)
Awash	Indoor	0 (0.0)	0 (0.0)	2 (1.4)	1 (0.7)	3 (2.1)
Arba	Outdoor	14 (9.7)	2 (1.4)	4 (2.7)	7 (4.8)	27 (18.6)
Werer	Indoor	1 (0.7)	0 (0.0)	1 (0.7)	2 (1.4)	4 (2.7)
	Outdoor	4 (2.8)	0 (0.0)	2 (1.4)	11 (7.6)	17 (11.6)
	Total	53 (36.7)	18 (12.4)	15 (10.3)	59 (40.6)	145 (100)

Note: N = number of tested; +ve= number of positive *Ae. aegypti* for human, bovine and human and bovine blood meals; HBI = human blood index in %; BBI = bovine blood index in %; Mix = human and bovine mixed blood index (%); Un = Unidentified blood meals.

4.3. Detection of dengue and chikungunya viruses among *Ae. aegypti*

A total of 1166 *Ae. aegypti* were tested for dengue and chikungunya viruses using RT-qPCR (Figure 21). However, dengue and chikungunya viruses were not detected among the *Ae. aegypti* tested.

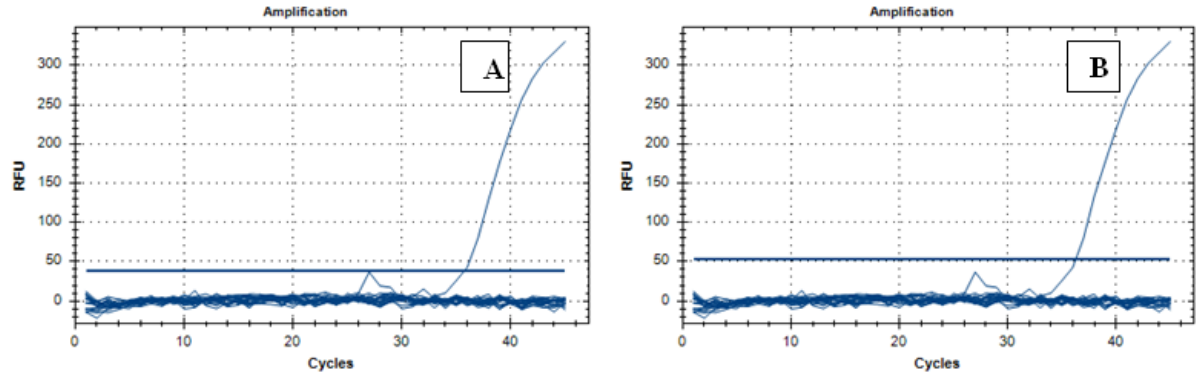

Figure 21: RT-qPCR based detection results of dengue and chikungunya viruses in *Ae. aegypti***.** Dengue positive control (A) and chikungunya positive control (B).

4.4. Susceptibility status of *Ae. aegypti* to public health insecticides

4.4.1. Knockdown Times of *Ae. aegypti* exposed to pyrethroids

The knockdown time of 50% (KDT_{50}) of *Ae. aegypti* exposed to deltamethrin ranged from 12.15 to 17.06 minutes and the corresponding KDT_{95} values ranged from 18.74 to 34.01 minutes (Table 13). The KDT_{95} of *Ae. aegypti* from Awash Sebat exposed to deltamethrin (34.01 minutes) was longer than the value of the other towns (Table 13). *Ae. aegypti* exposed to permethrin showed the lowest KDT_{50} values in Awash Arba town (KDT_{50}, 5.11). Overall, the KDT_{50} and KDT_{95} values of pyrethrods on *Ae. aegypti* varied across the study localities.

Table 13. The time (in minute) to knockdown, 50% and 95% of *Ae. aegypti* exposed to pyrethroid insecticides in Awash Sebat, Awash Arba and Werer towns of Afar Regional State of Ethiopia, July-October 2022

Insecticides	Localities	KDT_{50} [95% CI]	KDT_{95} [95% CI]
Deltamethrin	Awash sebat	17.06 [15.81-18.28]	34.01 [31.02-38.12]
	Awash Arba	12.15 [11.41-12.97]	18.74 [16.98-21.56]
	Werer	16.53 [15.37-17.67]	30.92 [28.24-34.62]
Permethrin	Awash Sebat	8.92 [7.34-10.26]	24.21 [21.23-28.93]
	Awash Arba	5.11 [2.91-6.94]	19.23 [16.23-24.09]
	Werer	15.12 [12.45-17.66]	29.89 [24.64-41.53]
Alpha-cypermethrin	Awash Sebat	14.35 [13.35-15.35]	24.93 [22.77-27.97]
	Awash Arba	13.47 [12.64-14.36]	20.78 [19.04-23.32]
	Werer	16.71 [13.85-19.46]	32.90 [27.26-44.98]

CI, Confidence interval; KDT_{50}, time taken for 50% of the tested *Ae. aegypti* to be knocked-down; KDT_{95} time taken for 95% of the tested *Ae. aegypti* to be knocked-down

4.4.2. Mortality rates of *Ae. aegypti* to insecticides

The percentage mortalities of *Ae. aegypyi* exposed to insecticides are presented in Figures 22-24. *Ae. aegypti* from Awash Sebat town showed phenotypic resistance to bendiocarb (88% mortality rate) and propoxur (87%). Deltamethrin, permethrin, alpha-cypermethrin, and pirimiphos-methyl induced 100% mortality against *Ae. aegypti* in Awash Sebat town (Figure 22). No mortality was recorded among *Ae. aegypti* in the control group in all study sites.

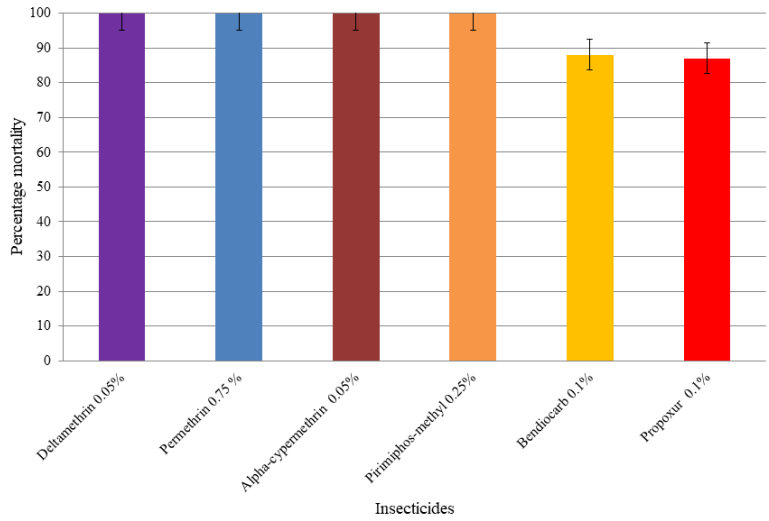

Figure 22: Susceptibility status of *Ae. aegypti* to insecticides in Awash Sebat, Afar Regional State of Ethiopia, July-October 2022. Error bars represent 95% confidence intervals.

Aedes aegypti in the Awash Arba were resistant to bendiocarb and propoxur, and possibly resistant to alpha-cypermethrin (95% mortality) (Figure 23). But, they were fully susceptible to deltamethrin, permethrin, and pirimiphos-methyl.

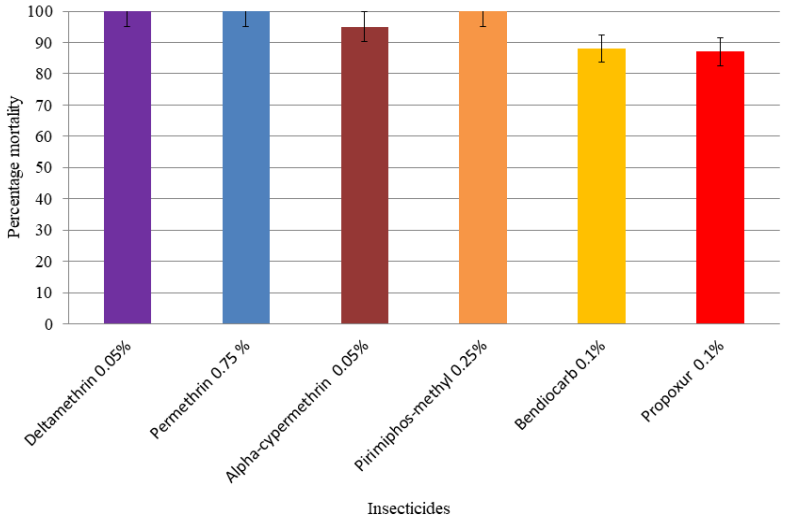

Figure 23: Susceptibility status of *Ae. aegypti* to insecticides in Awash Arba town, Afar Regional State of Ethiopia, July-October 2022.

In Werer town, mortality of *Ae. aegypti* exposed to propoxur was 87% and to alpha-cypermethrin 99% (Figure 24). However, it was susceptible to deltamethrin, permethrin, and pirimiphos-methyl.

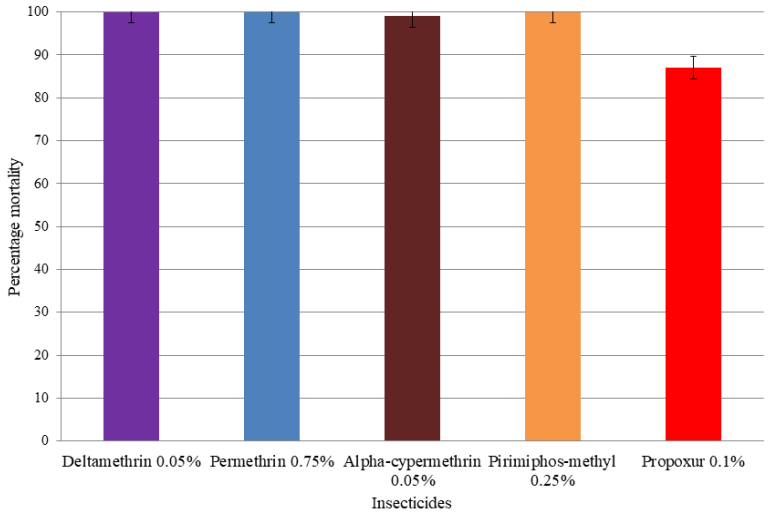

Figure 24: Susceptibility status of *Ae. aegypti* to insecticides in Werer town, Afar Regional State of Ethiopia, July-October 2022.

4.5. Knowledge, attitude and practice of communities towards *Aedes* mosquitoes and chikungunya and dengue fever

4.5.1. Demographic characteristics of the respondents

A total of 384 individuals participated in the study making a 100% response rate. Among the participants, 231 (60.2%) were males (Table 14). Close to 138 (36%) aged 29-38 years, 109 (28.4%) had no formal education, 325 (84.6%) were married, and 91 (23.7%) were merchants. About 149 (38.8%) of the houses were located near bushy or grassy areas and 148 (38.5%) near water. Furthermore, 380 (98.8%) of the community used tap water. About 276 (71.8%) of the respondents stored water for various purpose including for washing 190 (49.5%), for drinking 118 (30.7%) and washing and drinking 64 (16.7%).

Table 14. Socio-demographic characteristics of Awash Sebat, Awash Arba and Werer towns community members, Afar Regional State of Ethiopia, February, 2023

Socio-demographic variables		Awash Sebat n (%)	Awash Arba n (%)	Werer n (%)	Total N (%)
Age	18-28	51 (13.3)	32 (8.3)	46 (11.9)	129 (33.6)
	29-38	40 (10.4)	58 (15.1)	40 (10.4)	138 (35.9)
	39-48	19 (4.9)	27 (7.0)	7 (1.8)	53 (13.8)
	49-58	20 (5.2)	10 (2.6)	7 (1.8)	37 (9.6)
	>58	14 (3.6)	11 (2.9)	2 (0.5)	27 (7.0)
Sex	Male	78 (20.3)	83 (21.6)	70 (16.2)	231 (60.2)
	Female	66 (17.2)	55 (14.3)	32 (8.3)	153 (39.8)
Education	University	33 (8.6)	24 (6.3)	20 (5.2)	77 (20.1)
level	Secondary	41 (10.7)	26 (6.8)	19 (4.9)	86 (22.4)
	Primary	34 (8.8)	48 (12.5)	29 (7.5)	110 (28.6)
	No-formal education	36 (9.4)	40 (10.2)	34 (8.8)	109 (28.4)
Marital status	Married	126 (32.8)	119 (30.9)	80 (20.8)	325 (84.6)
	Single	18 (4.7)	16 (4.2)	20 (5.2)	54 (14.1)
	Widowed	0 (0)	3 (0.8)	2 (0.5)	5 (1.3)
Occupation	Housewife	30 (7.8)	29 (7.5)	16 (4.2)	75 (19.5)
	Merchant	33 (8.6)	41(10.6)	17 (4.4)	91 (23.7)
	Daily laborer	24 (6.2)	15 (3.9)	15 (3.9)	54 (14.1)
	Health professio nal	19 (4.9)	7 (1.8)	10 (2.6)	36 (9.4)
	Unemployed	13 (3.4)	3 (0.7)	8 (2.1)	24 (6.3)
	Student	9 (2.3)	11 (2.8)	5 (1.3)	25 (6.5)
	Farmer	6 (1.6)	10 (2.6)	17 (4.4)	33 (8.5)
	Government emp loye	10 (2.6)	20 (5.2)	16 (4.2)	46 (11.9)
House types	Public	0 (0)	3 (0.8)	34 (8.8)	37 (9.6)
	Private	144 (37.5)	135 (35.1)	46 (11.9)	325 (84.6)
	Temporary	0 (0)	0 (0)	22 (5.7)	22 (5.7)
House	Near water	30 (7.8)	36 (9.4)	82 (21.4)	148 (38.5)
location	Near bushy/grass	53 (13.8)	89 (23.2)	7 (1.8)	149 (38.8)
	Near construction	67 (17.4)	13 (3.4)	11 (2.8)	91 (23.7)

	Other	0 (0)	0 (0)	2 (0.5)	2 (0.5)
Water source	Tap	144 (37.5)	138 (35.9)	98 (25.5)	380 (98.9)
	Stream/river	0 (0)	0 (0)	4 (1.0)	4 (1.0)
Water stored	Yes	109 (28.4)	130 (33.8)	37 (9.6)	276 (71.8)
at home	No	35 (9.1)	8 (2.1)	65 (16.9)	108 (28.1)
Purpose of	Drinking	46 (11.7)	13 (3.4)	59 (15.4)	118 (30.7)
stored water	Washing	59 (15.4)	125 (32.5)	6 (1.5)	190 (49.5)
	Wash & drink	28 (7.3)	0 (0)	36 (9.4)	64 (16.7)
	Other	11 (2.8)	0 (0)	1 (0.3)	12 (3.1)

4.5.2. Knowledge about *Aedes* mosquitoes and their role as vectors of chikungunya and dengue fever

Majority of the respondents, 346 (90%) perceived mosquitoes in their locality. However, only 114 (32.9%) reported the color or morphological feature of *Aedes* mosquitoes (Table 15). About 87 (76.3%) stated that containers, which store water for long period, serve for *Aedes* breeding. About 41 (35.9%) pointed out that *Aedes* mosquitoes breed in used tyres, 26 (22.8%) in water holding construction tanks and 16 (14.3%) in drums. Majority of respondents, 71 (62.3%) reported that *Aedes* mosquito occur abundantly during the wet season in relation to availability of rainfall, poor waste disposal, and increasing house construction. About 73 (64%) perceived that *Aedes* mosquitoes bite during night-time, whereas 30 (26.3%) during daytime. Half 57 (50%) perceived human legs most bitten by *Aedes* followed by hands, 28 (24.6%). About 43 (37.7%) perceived that they used to be bitten by *Aedes* mosquitoes in grassy areas and 23 (20.2%) in construction sites. Only 39 (34.2%) of the respondents reported that *Aedes* mosquitoes transmit disease to human.

Table 15. Knowledge of communities about *Aedes* mosquitoes and their role as disease vectors in Awash Sebat, Awash Arba and Werer towns, Afar Regional State, Ethiopia, February 2023

Variables	Categories	Awash Sebat n (%)	Awash Arba n (%)	Werer n(%)	Total N (%)
Do you know mosquitoes in your area?	Yes	132 (91.7)	126 (91.3)	88 (86.3)	346 (90.1)
	No	12 (8.3)	12 (8.7)	14 (13.7)	38 (9.9)
What is the color of mosquitoes you know?	Small black and white strips on legs	50 (14.5)	39 (11.3)	25 (7.2)	114 (32.9)

	Grey	75 (21.7)	67 (13.6)	60 (18.8)	202 (58.4)
	I don't know	7 (2)	20 (5.8)	3 (0.9)	30 (8.7)
Where do *Aedes* breed?	Drums	8 (7.0)	6 (5.3)	2 (1.7)	16 (14.3)
	Used tyres	17 (14.9)	15 (13.2)	9 (7.9)	41 (35.9)
	Discarded plastic/metals	3 (2.6)	1 (0.9)	0 (0)	4 (3.5)
	Flower pots	0 (0)	0 (0)	0 (0)	0 (0)
	Construction tanks	12 (10.5)	9 (7.9)	5 (4.4)	26 (22.8)
	I don't know	10 (8.8)	8 (7.0)	9 (7.9)	27 (23.7)
What is season for abundance of *Aedes* species*?*	Dry	9 (7.9)	3 (2.6)	6 (5.3)	18 (15.9)
	Wet	31 (27.2)	21 (18.4)	19 (16.7)	71 (62.3)
	I don't know	10 (8.8)	15 (13.2)	10 (8.8)	35 (30.7)
Reasons for *Aedes* abundance	Rainfall availability	24 (21.1)	18 (15.8)	13 (11.4)	55 (48.2)
	Poor waste disposal	7 (6.1)	8 (7.0)	5 (4.4)	20 (17.5)
	House construction	10 (8.8)	7 (6.1)	0 (0)	17 (14.9)
	I don't know	9 (7.9)	6 (5.3)	7 (6.1)	22 (19.3)
When do you encounter biting of *Aedes* most frequently?	Night time	34 (29.8)	22 (19.3)	17 (14.9)	73 (64.0)
	Day time (morning-after noon)	13 (11.4)	11 (9.6)	6 (5.3)	30 (26.3)
	Anytime	3 (2.6)	6 (5.3)	2 (1.7)	11 (9.6)
Which parts of your body is bitten most frequently by *Aedes*?	Legs	20 (17.5)	22 (19.3)	15 (13.2)	57 (50.0)
	Hands	14 (12.3)	9 (8.8)	5 (4.5)	28 (24.6)
	Faces	7 (6.1)	4 (2.6)	4 (3.5)	15 (13.2)
	I don't know	9 (7.9)	4 (3.5)	1 (0.9)	14 (12.3)
Where do *Aedes* bite frequently?	At home	10 (8.9)	6 (5.3)	7 (6.1)	23 (20.2)
	Grassy area	13 (11.4)	17 (14.9)	13 (11.4)	43 (37.7)
	Away from home	2 (1.7)	1 (0.9)	0 (0)	3 (2.6)
	Construction sites	12 (10.5)	9 (7.9)	2 (1.7)	23 (20.2)
	At work places	4 (3.5)	2 (1.7)	0 (0)	6 (5.3)
	I don't know	9 (7.9)	4 (3.5)	3 (2.6)	16 (14.0)
Where do *Aedes* rests frequently?	Wall surface	12 (10.5)	6 (5.3)	9 (7.9)	27 (23.7)
	Underneath furniture	5 (4.5)	11 (9.7)	3 (2.6)	19 (16.7)
	Tyres	22 (19.3)	15 (13.2)	11 (9.7)	48 (42.1)

	Vegetation	4 (3.5)	3 (2.6)	0 (0)	7 (6.1)
	I don't know	7 (6.1)	4 (3.5)	2 (1.8)	13 (11.4)
Do you think *Aedes* transmit	Yes	17 (14.9)	15 (13.2)	7 (6.1)	39 (34.2)
diseases to human?	No	33 (28.9)	24 (21.1)	18 (15.8)	75 (65.8)

4.5.3. Knowledge related to chikungunya and dengue fever transmission

Majority of the respondents, 284 (74%), heard about chikungunya disease in their locality (Table 16). More specifically, 126 (91.3%) of Awash Arba town, 121 (84%) Awash Sebat and 37 (36.3%) of Werer respondents reported chikungunya. Their primary source of information was health professionals 170 (59.9%) followed by television 29 (10.2%). A total of 50 (17.6%) respondents in Awash Sebat, 48 (16.9%) in Awash Arba, and 17 (6.0%) in Werer reported fever as the major sign and symptom of chikungunya. In addition, 30 (10.6%) among the Awash Sebat respondents, 27 (9.5%) from the Awash Arba and 9 (32%) from Werer reported headache as a sign and symptom of the disease. Majority of the respondents, 260 (91.5%) did not perceive the transmission route of chikungunya. Nearly half of the respondents, 128 (45.1%) perceived that chikungunya is transmitted to human through physical contact with infected person. Only 24 (8.5%) responded that chikungunya is transmitted by the bite of *Aedes* mosquitoes.

Table 16. Community knowledge related to chikungunya diseases transmission, in Awash Sebat, Awash Arba and Werer towns, Afar Regional State of Ethiopia, February 2023

Variables	Categories	Study towns			
		Awash Sebat n (%)	Awash Arba n (%)	Werer n (%)	Total N (%)
Have you been heard of chikungunya disease in your area?	Yes	121 (84)	126 (91.3)	37 (36.3)	284 (74)
	No	23 (16)	12 (8.7)	65 (63.7)	100 (26)
What is (are) your source of information?	Television	8 (2.8)	12 (4.2)	9 (3.2)	29 (10.2)
	Radio	2 (0.7)	11 (3.9)	4 (1.4)	17 (6.0)
	Health professionals	81 (28.5)	68 (23.9)	21 (7.4)	170 (59.9)
	School	2 (0.7)	10 (3.5)	0 (0)	12 (4.2)
	Television and Health professionals	6 (2.1)	12 (4.2)	3 (1.1)	21 (7.4)
	Others (friends)	19 (6.7)	9 (3.2)	0 (0)	28 (9.8)
	I don't know	3 (1.1)	4 (3.2)	0 (0)	7 (2.5)
What is (are) sign	Nausea	11 (3.9)	9 (3.2)	7 (2.5)	27 (9.5)

and symptoms of chikungunya disease?	Joint pain	13 (4.6)	23 (8.1)	6 (2.1)	42 (14.2)
	Headache	30 (10.6)	27 (9.5)	9 (3.2)	66 (23.2)
	Vomiting	12 (4.2)	18 (6.3)	5 (1.8)	35 (12.3)
	Fever	50 (17.6)	48 (16.9)	17 (6.0)	115 (40.5)
	Pain behind the eyeball	0 (0)	0 (0)	0 (0)	0 (0)
	Bleeding	0 (0)	0 (0)	0 (0)	0 (0)
	I don't know	5 (1.8)	1 (0.4)	0 (0)	6 (2.1)
Do you know chikungunya diseases mode of transmission?	*Aedes* bite	13 (4.6)	6 (2.1)	5 (1.8)	24 (8.5)
	Anopheles bite	29 (10.2)	33 (11.6)	16 (5.6)	78 (27.5)
	Body contact	39 (13.7)	80 (28.2)	9 (3.2)	128 (45.1)
	I don't know	40 (14.1)	7 (2.5)	7 (2.5)	54 (19.0)
What is the season of frequent outbreak to disease?	Dry	39 (13.7)	11 (3.9)	13 (4.6)	63 (22.2)
	Wet	57 (20.1)	108 (38.0)	17 (6.0)	182 (64.1)
	I don't know	25 (8.8)	7 (2.5)	7 (2.5)	39 (13.7)
What do you take as a pain relief to CHIV disease?	Chemotherapy	57 (20.1)	17 (6.0)	20 (7.0)	94 (33.1)
	Traditional medicine	62 (21.8)	109 (37.4)	17 (6.0)	188 (66.2)
	Religious solution	2 (0.7)	0 (0)	0 (0)	2 (0.7)

The great majority of respondents, 356 (92.7%) did not perceive dengue fever (Table 17). Only a few 28 (7.3%) heard about dengue fever in their locality among which 50% (14) heard from health professionals, 8 (28.6%) from television and 4 (14.3%) from both television and health professionals. Fever was mentioned as a major sign and symptom of dengue fever by 10 (35.7%), bleeding by 6 (21%) and pain behind the eyeball by 3 (10.7%). 10 (35.7%) of these respondents perceived that dengue fever is transmitted to human with body contacts with infected person. However, 4 (14.3%) knew that dengue was transmitted by *Aedes* mosquitoes.

Table 17. Community knowledge related to dengue fever transmission in Awash Sebat, Awash Arba and Werer towns, Afar Regional State of Ethiopia, February, 2023

		Study towns			
Variables	Categories	Awash Sebat n (%)	Awash Arba n (%)	Werer n (%)	Total N (%)
Heard of dengue fever?	Yes	15 (10.4)	7 (5.1)	6 (5.9)	28 (7.3)
	No	129 (89.6)	131 (94.9)	96 (94.1)	356 (92.7)
What is (are) your sources of information?	Television	4 (14.3)	3 (10.7)	1 (3.6)	8 (28.6)
	Radio	2 (7.1)	0 (0)	0 (0)	2 (7.1)
	Health professionals	8 (28.6)	4 (14.3)	2 (7.1)	14 (50)
	School	0 (0)	0 (0)	0 (0)	0 (0)
	Television Heath professionals	1 (3.6)	0 (0)	3 (10.7)	4 (14.3)
	Others (from friends)	0 (0)	0 (0)	0 (0)	0 (0)
	I don't know	0 (0)	0 (0)	0 (0)	0 (0)
What is (are) sign and symptoms of dengue fever?	Nausea	3 (10.7)	0 (0)	0 (0)	3 (10.7)
	Joint pain	2 (7.1)	1 (3.6)	2 (7.1)	5 (17.9)
	Headache	2 (7.1)	0 (0)	0 (0)	2 (7.1)
	Vomiting	1 (3.6)	0 (0)	0 (0)	1 (3.6)
	Fever	4 (14.3)	3 (10.7)	3 (10.7)	10 (35.7)
	Pain behind the eyeball	2 (7.1)	1(3.6)	0(0)	3 (10.7)
	Bleeding	3 (10.7)	2 (7.1)	1(3.6)	6 (21.4)
	I don't know	0 (0)	1 (3.6)	0 (0)	1 (3.6)
Do you know dengue fever mode of transmission?	*Aedes* bite	3 (10.7)	1 (3.6)	0 (0)	4 (14.3)
	Anopheles bite	2 (7.1)	3 (10.7)	2 (7.1)	7 (25)
	Body contact with patients	5 (17.9)	1 (3.6)	4 (14.3)	10 (35.7)
	I don't know	3 (10.7)	0 (0)	0 (0)	3 (10.7)
What is the season of outbreak to dengue?	Dry	5 (17.9)	0 (0)	2 (7.1)	7 (25)
	Wet	10 (2.6)	7 (25)	4 (14.3)	21 (75)
	I don't know	0 (0)	0 (0)	0 (0)	0 (0)
Means of treatment for dengue?	Chemotherapy	8 (28.6)	5 (17.9)	4 (14.3)	17 (60.7)
	Traditional medicine	7 (25)	2 (7.1)	2 (7.1)	11 (39.3)
	Religious solution	0 (0)	0 (0)	0 (0)	0 (0)

4.5.4. Community attitude regarding *Aedes* mosquitoes and their association with chikungunya and dengue fever

Majority of the respondents, 234 (82.4%), considered chikungunya a public health problem in their localities (Table 18). Chikungunya was a major public health concern for 94 (31.1%) of Awash Sebat residents, 111 (39.1%) of Awash Arba, and 29 (10.2%) of Werer. Majority of the respondents, 20/28 (71.4%), did not considered dengue fever as a public health problem in their localities. Furthermore, majority of the respondents, 75 (65.8%) perceived that *Aedes* mosquitoes affect their life, as they are nuisances and inflict painful bite and 39 (34.2%) as disease vectors.

Table 18. Community attitude regarding Aedes mosquitoes and their associated risks to chikungunya/dengue diseases, in Awash Sebat, Awash Arba and Werer towns of Afar Regional State of Ethiopia, February 2023

Variables	Categories	Awash Sebat n (%)	Awash Arba n (%)	Werer n (%)	Total N (%)
Do you think CHIK is a public health risk in your area?	Yes	94 (33.1)	111 (39.1)	29 (10.2)	234 (82.4)
	No	27 (9.5)	15 (5.3)	8 (2.8)	50 (17.6)
Do you think DEN is a public health risk in your area?	Yes	4 (14.3)	2 (7.1)	2 (7.1)	8 (28.6)
	No	11 (39.3)	5 (17.9)	4 (14.3)	20 (71.4)
How do you feel *Aedes* mosquitos' effect in your area?	Nuisance (Low)	33 (28.9)	24 (21.1)	18 (15.8)	75 (65.8)
	Disease (High)	17 (14.9)	15 (13.2)	7 (6.1)	39 (34.2)

4.5.5. Community practices in the control of *Aedes* mosquitoes

Close to 30% of the respondents 29 (25.4%) in Awash Arba, 23 (20.2%) in Awash Sebat, and 12 (9.6%) in Werer respondents used insecticide treated bed nets to prevent *Aedes* mosquitoes (Fig 25). Moreover, 6 (5.3%) in Awash Sebat, 6 (5.3%) in Werer and 5 (4.4%) in Awash Arba pointed out that removing containers as strategy to control *Aedes* mosquitoes. In addition, 5 (4.4%) in Werer, 4 (3.5%) in Awash Sebat and 3 (2.6%) in Awash Arba of the respondents did not know *Aedes* control strategies in their localities.

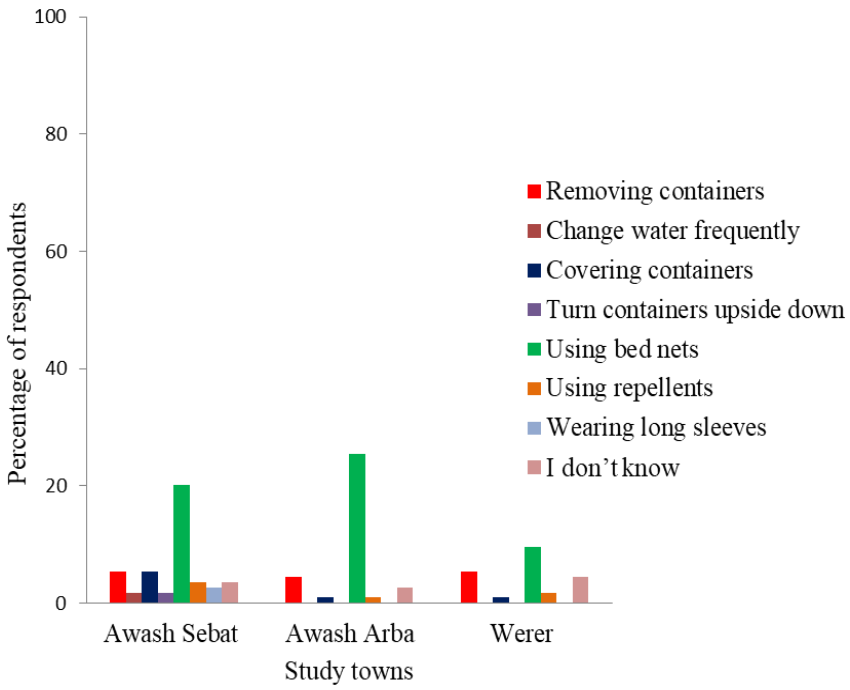

Figure 25: Practices of communities to control *Aedes* mosquitoes in Awash Sebat, Awash Arba and Werer towns of Afar Regional State of Ethiopia, February, 2023.

4.5.6. Knowledge, attitude, and practices scores

Majority of the respondents, 89 (78.1%) had low knowledge related to *Aedes* mosquito larval habitats (Table 19). Similarly, 81 (71.1%) had low level of knowledge about *Aedes* mosquitoes biting times, and 87 (76.3%) had low level of perception about *Aedes* mosquitoes as vector of disease transmission in their surroundings. They associated *Aedes* mosquitoes mainly with the nuisances and painful biting rather than diseases transmission. In addition, 84 (73.7%) of the respondents revealed low level of practices in the control of *Aedes* mosquitoes in their localities.

Table 19. Knowledge, attitudes, and practices scores of communities, in Awash Sebat, Awash Arba and Werer towns, Afar Regional State of Ethiopia, February, 2023

Variables	Variable Category	Awash Sebat n (%)	Awash Arba n (%)	Werer n (%)	Total N (%)
Knowledge on *Aedes*	High	11 (9.6)	9 (7.9)	5 (4.5)	25 (21.9)
Larval habitats	Low	39 (34.2)	30 (26.3)	20 (17.5)	89 (78.1)
Knowledge on *Aedes*	High	12 (10.5)	13 (11.4)	8 (7.0)	33 (28.9)
biting time	Low	38 (33.3)	26 (22.8)	17 (14.9)	81 (71.1)
Attitudes on effect of *Aedes* on	High	15 (13.2)	8 (7.0)	4 (3.5)	27 (23.7)

daily life	Low	35 (30.7)	31 (27.2)	21 (18.4)	87 (76.3)
Practices to control *Aedes*	High	12 (10.5)	10 (8.8)	8 (7.0)	30 (26.3)
	Low	38 (33.3)	29 (225.4)	17 (14.9)	84 (73.7)

4.5.7. Factors associated with communities' knowledge, attitudes and control practices of *Aedes* mosquitoes

A respondent from Awash Arba (COR, 2.49, p = 0.016) was 3 times more likely to know *Aedes* larval habitat compared to Awash Sebat town (Table 20). Similarly, a health professional (COR = 6.63, p= 0.001), a student (COR= 4.91, p= 0.016), a merchant (COR = 3.51, p= 0.020), and a government employee (COR = 3.27, p= 0.042) had greater knowledge on *Aedes* larvae habitats compared to housewife. A respondent from Awash Arba (COR, 0.28, p < 0.001) and Werer (COR = 0.08, p <0.001) towns had lower perception of *Aedes* mosquitoes in transmitting diseases to humans compared to the one from Awash Sebat. A university graduate (COR= 1.89, p = 0.038) had higher perception of the effect of *Aedes* mosquitoes in transmitting diseases to human compared to respondents with no formal education. Male respondents (COR= 1.86, p = 0.015), respondents with age group 29-38 (COR= 3.92, p 0.004), health professional (COR= 3.86, p= 0.047) and respondents with university education background (COR= 2.74, p=0.008) were more likely involved in the control of *Aedes* mosquito bites. Furthermore, respondents from Awash Arba (COR=0.01, p<0.001) and Werer (COR = 0.03 p= 0.003) were less likely involved in the control of *Aedes* mosquito bites.

Table 20. Bivariate analysis of factors associated with communities' knowledge, attitudes and practices towards *Aedes* mosquitoes in Awash Sebat, Awash Arba and Werer towns of Afar Regional State of Ethiopia, February 2023

Predictors		Knowledge of *Aedes* larval habitats		Attitudes on the effect of *Aedes* on daily life		Practice to control *Aedes* bite	
		COR (95% CI)	p-value	COR (95% CI)	p-value	COR (95% CI)	p-value
Gender	Female	1		1		1	
	Male	1.06 (0.64-1.79)	0.808	1.1 (0.7-1.6)	0.679	1.86 (1.1-3.1)	0.015
Age	>58	1		1		1	
	18-28	1.94 (0.76-4.97)	0.167	1.01 (0.4-2.4)	0.974	1.78 (0.7-4.3)	0.196
	29-38	2.37 (0.78-7.24)	0.130	1.22 (0.5-2.8)	0.639	3.92 (1.6-9.9)	0.004
	39-48	1.80 (0.56-5.79)	0.321	0.69 (0.3-1.8)	0.443	2.01 (0.7-5.5)	0.176

	49-58	1.73 (0.68-4.37)	0.246	0.88 (0.3-2.4)	0.814	2.52 (0.8-7.8)	0.110
Study	Awash Sebat	1		1		1	
towns	Awash Arba	2.49 (1.2-5.26)	0.016	0.28 (0.2-0.5)	<0.001	0.01 (0-0.01)	<0.001
	Werer	0.70 (0.4-1.28)	0.244	0.08 (0-0.1)	<0.001	0.03 (0-0.3)	0.003
Occupa	House wife	1				1	
tion	Merchant	3.51 (1.22-10.1)	0.020	1.50 (0.5-4.3)	0.452	1.55 (0.5-4.7)	0.435
	Daily laborer	2.62 (0.7-9.96)	0.158	1.59 (0.6-4.5)	0.378	0.96 (0.3-2.9)	0.940
	Farmer	2.90 (0.9-8.77)	0.059	1.68 (0.6-5.0)	0.355	1.05 (0.3-3.4)	0.933
	Student	4.91 (1.3-17.9)	0.016	1.36 (0.4-4.3)	0.603	2.17 (0.7-6.6)	0.169
	Health professional	6.63 (2.2-19.89)	0.001	2.74 (0.9-8.3)	0.074	3.86 (1.0-14.6)	0.047
	Gov't employ	3.27 (1.0-10.28)	0.042	1.92 (0.6-6.3)	0.282	3.14 (0.7-14.6)	0.145
	Unemployee	3.11 (1.0-9.56)	0.048	1.09 (0.3-3.9)	0.886	1.79 (0.5-6.6)	0.385
Educati	No-formal	1		1		1	
on	education						
level	University	1.79 (0.9-3.7)	0.118	1.89 (1.0-3.4)	0.038	2.74 (1.3-5.8)	0.008
	Secondary	1.49 (0.7-3.0)	0.270	1.15 (0.6-2.2)	0.662	1.45 (0.7-2.8)	0.277
	Primary	1.33 (0.6-2.8)	0.452	1.14 (0.6-2.1)	0.664	1.41 (0.7-2.9)	0.342

COR, Crude odds ratio

Multiple logistic regression analysis indicated that a health professional (AOR = 7.73, p = 0.020), a student (AOR = 5.21, p = 0.004) and a merchant (AOR = 2.98, p = 0.048) were more knowledgeable compared to a housewife (Table 21). On the other hand, respondents from Werer town (AOR = 0.48, p = 0.033) had lower *Aedes* larval habitat knowledge as compared to Awash Sebat. Moreover, respondents from Awash Arba (AOR = 0.26, p <0.001) and Werer (AOR = 0.08, p < 0.001) were lower perceptions on the effect of *Aedes* mosquitoes on their daily life. Respondents with age group 29-38 (AOR = 3.74, p = 0.027), university (AOR = 4.64, p < 0.009) educated respondents had involved the practice to control *Aedes* mosquito bites.

Table 21. Multi-variate analysis of potential determining factors associated with communities' knowledge, attitudes and practices towards *Aedes* mosquitoes in Awash Sebat, Awash Arba and Werer towns, Afar Regional State of Ethiopia, February, 2023

Variables	Category	Knowledge of *Aedes* larval habitats		Attitudes on the effect of *Aedes* species		Practice to control *Aedes* species bite	
		AOR (95% CI)	p-value	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Gender	Female	-	-	-	-	1	
	Male	-	-	-	-	1.38 (0.7-2.6)	0.318
Age	>58	-		-	-	1	
	18-28	-	-	-	-	1.79 (0.6-5.4)	0.302
	29-38	-	-	-	-	3.74 (1.2-12.0)	0.027
	39-48	-	-	-	-	2.14 (0.6-7.4)	0.321
	49-58	-	-	-	-	3.50 (0.9-14-1)	0.077
Study towns	Awash Sebat	1		1		1	1
	Awash Arba	2.06 (0.9-4.5)	0.068	0.26 (0.2-0.5)	<0.001	0.01 (0-0.1)	<0.001
	Werer	0.48 (0.2-0.9)	0.033	0.08 (0-0.1)	<0.001	0.03 (0-0.3)	0.003
Occupation	House wife	1				1	
	Merchant	2.98 (1-8.8)	0.048	-	-	0.70 (0.2-2.8)	0.614
	Daily laborer	1.83 (0.5-6.2)	0.337	-	-	0.83 (0.2-3.5)	0.797
	Farmer	1.53 (0.5-4.9)	0.594	-	-	0.09 (0-0.8)	0.132
	Student	5.21 (1.7-16.1)	0.004	-	-	1.20 (0.3-4.7)	0.788
	Health professional	7.73 (1.4-43.0)	0.020	-	-	1.70 (0.3-8.6)	0.521
	Gov't employ	3.22 (0.9-10.6)	0.056	-	-	0.30 (0.1-1.4)	0.124
	Unemployee	2.48 (0.8-7.8)	0.120	-	-	1.55 (0.3-6.9)	0.568
Education level	No-formal education	1		-	-	1	
	University	-	-	1.87 (0.9-3.7)	0.066	4.64 (1.6-14.6)	0.009
	Secondary	-	-	1.36 (0.7-2.8)	0.400	1.46 (0.5-3.9)	0.453
	Primary	-	-	0.96 (0.5-1.9)	0.914	1.61 (0.6-4.1)	0.317

4.5.8 Results of focus group discussion

1. **Are there fever-inducing illnesses in your area?**

A group of 10 individuals participated in a focus group discussion (FGD) in Awash Sebat town, 12 in Awash Arba and 15 in Werer. All the respondents pointed out that their localities are at risk of fever inducing illnesses such as malaria, typhus, typhoid, chikungunya (Tilobign local Amharic name). Recently, there is an increased risk of chikungunya (tilobign) outbreak in their localities.

2. **What are the signs and symptoms of chikungunya?**

About three forth of the FGD participants in Awash Sebat and Awash Arba perceived that fever, headache and joint pain, are the major symptoms of CHIKV disease. "I was tested positive for chikungunya disease in the health center of Awash Arba, in 2020 G.C. when there was chikungunya outbreak and I experienced fever and jointed pain which was unforgettable" a 42 years old FGD- male participant, at Awash Arba town. Similarly, a 37 years old male FGD participant from the Awash Sebat town said, that I was one of the people who suffered from chikungunya outbreak during 2020 G.C, I encountered difficulties to stand due to the joint pain as a result of CHIK disease. On the other hand, majority of the FDG members from Werer town were unaware of CHIK sign and symptoms.

3. **What do you think is the mode of chikunguyna transmission in your locality? Do you think mosquitoes transmit chikungunya?**

Almost half of the members of FGD of all towns pointed out that chikungunya is transmitted through body contact with patients. However, a 25 years male from Awash Sebat FGD said that, "I heard from the social media that mosquitoes, "keyneta" in Afarigna, are responsible for chikungunya and malaria". In addition, one participant from Awash Arba said that mosquitoes are responsible for chikungunya transmission are zebra mosquitoes, which have white and black stripes on their bodies like zebra". Three forth of the FGD participants from the Werer town were not aware about the chikungunya disease and its route of transmission. A 23 years old female FGD participant from Werer said that it is the first time to hear that the mosquitoes bite can cause diseases other than malaria. Most participants in each FGD group reported that they do not know the exact transmitting agent of chikungunya virus in their locality. However, a participant from Awash Arba told that "I heard from the television that small black mosquitoes, which are seeking hosts during the daytime especially during morning and afternoon, are responsible to the chikungunya diseases transmission".

4. What do you know about the *Aedes* mosquito lifecycle

We communicated with the FGD participants with the local term Keyneta (local name of mosquitoes in Afar Region) or (Binbi in Amharic) because majority of them were not aware about the scientific name of *Aedes* mosquitoes responsible for chikungunya disease. Some of the participants reported that keyneta breed in water but they did not know the transformation of larvae/pupae from the water into the terrestrial lands as adult mosquitoes. A 45 years old female FGD participant from Awash Arba, argued during the discussion, she said, "they are just water warms not connected to the emergence of adult mosquitoes". Majority of the discussant said that mosquitoes usually rest in shady places both inside and outside the houses. A question raised for the discussant, what is the frequent biting time of the mosquitoes they replayed that mosquitoes bite during the night time and sometimes during the day from 1:00-11:00 in the afternoon from 3:00-18:00, a 38 years old male participants in Awash Arba town). Majority of the discussant responded that their body parts frequently bitten by mosquitoes include feet, hands and faces.

5. What is the medication to CHKV?

Majority of the participants from each FGD reported that medication is not common in chikungunya disease except in some occasions as a pain relief. "We did not receive any medications from health institutions during the CHIK outbreak in 2020 G.C. in Awash Sebat towns. Rather, we use locally available traditional medicines such as garlic, ginger and their combination as a food" (a 24 years old male participant at Awash Sebat town).

6. What do you think is the controlling practice of *Aedes* mosquitoes in your localities?

All the participants said that, insecticide treated bed nets was distributed to prevent malaria and any mosquito bites during the night-time in all the three towns. On the other hand, a respondent from Awash Sebat town also indicated "We have to clean our surroundings and manage the stagnant water so that we can prevent the breeding of any mosquitoes from our localities" (a 32 years old male FGD participant in Awash Sebat town).

CHAPTER FIVE: DISCUSSION

This study identified and characterized *Ae. aegypti* larval/pupal habitats. The study also described resting behavior, blood meal sources, insecticide susceptibility status, and viral infection rates. Furthermore, a community perception towards *Aedes* mosquitoes and risks to chikungunya and dengue fever was assessed. Used tyres were the most common larvae/pupae habitats and adult resting places of *Ae. aegypti*. Water storage drums were also the second most common larvae/pupae habitats. Density of *Ae. aegypti* larvae/pupae was positively related to used tyres, water-storage drums, and domestic containers but negatively related to tap water sources. Total hardness and dissolved oxygen of habitat water were positively related to the mean density of larvae/pupae of *Ae. aegypti*. The human blood-fed *Ae. aegypti* were relatively higher than the bovine blood-fed. *Ae. aegypti* females exhibited phenotypic resistance to bendiocarb and propoxur, suspected resistance to alpha-cypermethrin and susceptible to deltamethrin, permethrin and pirimiphos-methyl. Health professionals, students, and merchants had a sound knowledge regarding *Aedes* species larval habitats. In addition, respondents with age group 29-38 and university graduate respondents were more involved in controlling *Aedes* mosquito bites.

Discarded tyres at domestic sites harbored the highest numbers of *Ae. aegypti* larva/pupae, followed by water-storage drums, water tanks made of cement, flowerpots, and discarded plastics in Awash Sebat, Awash Arba, and Werer towns, Gabi-Rasu Zone, southern Afar Regional State, Ethiopia. Tyres were also preferred habitats of *Ae. aegypti* larvae/pupae in Dire Dawa City administration (Getachew *et al*., 2015; Waldetensai *et al*., 2021b), in Metema and Humera districts of northwest Ethiopia (Ferede *et al*., 2018), and in Kebridehar town of Somali Regional State (Yared *et al*., 2024). Similar observations were also reported from Cameroon (Tedjou *et al*., 2020) and Malaysia (Nordin *et al*., 2017). The high abundance of *Ae. aegypti* larvae/pupae in the tyres in comparison to other habitat types could result from a relatively low level of disturbance, providing shade and protection (Egid *et al*., 2022). Shaded habitats were reported as most preferred for *Ae. aegypti* larvae/pupae in a previous report (Yee *et al*., 2012). According to the present data and also based on previous reports, it remains central to train and engage communities of the southern parts of the Afar Region including the study towns in the effort to control *Ae. aegypti* populations and hence transmission of chikungunya and dengue fever.

Water-storage drums were observed to be the second most inhabited habitats to harbor the number of *Ae. aegypti* larvae/pupae. This was in agreement with the reports from different parts of the world, including Mozambique and Nicaragua (Hammond *et al*., 2007; Abilio *et al*., 2018). In Awash Sebat, Awash Arba, and Werer, households store water in drums for drinking, domestic use, or construction purposes. Even so, the drums are not properly covered. Such inadequate handling of water stored in domestic containers like drums may serve as prolific breeding grounds for *Ae. aegypti*, as previously observed (Kudom, 2020). Thus, community members should be trained adequately so that they can keep female mosquitoes from laying eggs in water-storage drums.

The majority of the *Ae. aegypti* larvae/pupae habitats were located closer to the residential houses, which is in agreement with a previous report from Malaysia (Saifur *et al*., 2012). One reason for this might be the higher prevalence of water-holding containers for domestic use, for drinking and washing. Thus, emphasis should be given to domestic sites in surveying and managing *Ae. aegypti* larvae/pupae in the towns. No *Ae. aegypti* larva/pupa was collected from inside the residential houses. This was contrary to previous research findings from Sudan and Western Kenya, where *Ae. aegypti* larvae and pupae occurred inside residential rooms (Ahmed *et al*., 2019; Ngugi *et al*., 2017). The variations could result from the differences in the frequency of water use, coverage of water containers, and room access for mosquitoes. Furthermore, the frequency of container cleaning inside the house rooms affects the occurrence of immature *Aedes* mosquitoes, as previously reported (Anitha and Jasper, 2013).

The CI, HI, and BI values observed during the months of July 2022 to October 2022 exceeded 5% in the towns. The BI and HI values were also higher than the previous studies in Tanzania (Kahamba *et al*., 2020) and Cameroon (Kamgang *et al*., 2010). However, the average CI, HI, and BI indices in the towns were relatively lower than the indices previously reported from the Dire Dawa City administration (Getachew *et al*., 2015). The index values of *Ae. aegypti* were high compared to the World Health Organization epidemic thresholds of transmission risk for yellow fever, dengue fever, and other arboviruses (WHO, 1971; WHO, 2016c). These high larval/pupal infestation indices of *Ae. aegypti* suggest a high risk of arbovirus infections such as dengue fever, yellow fever, and chikungunya in the southern parts of Afar Region, Ethiopia. Thus, *Ae. aegypti* larvae/pupae habitat management practices should be implemented in the towns to reduce the viral disease outbreaks.

The average pupae index (PI), 171.7, was lower than the previous study in Dire Dawa City administration, Ethiopia (Waldetensai *et al*., 2021b) but higher compared to the reports from Bacongo and M'flou areas of the Republic of Congo (Wilson-Bahun *et al*., 2020). The pupal index is important to quantify *Aedes* species infestation and predict epidemiological risks, as it gives numeric figures by dividing the number of pupae in each site per hectare, houses, and per person (Bowman *et al*., 2014). In addition, the pupal index is vital; it is directly proportional to the adult population. Furthermore, pupae mortality is minimal as compared to larvae (Barrera *et al*., 2006; Garelli *et al*., 2009).

The number/occurrence of *Ae. aegypti* larvae/pupae increased from August 2022 to October 2022, then declined towards April 2023 in all the towns. The wet season contributed to increased larvae/pupae density. A similar finding was reported from Tanzania, where more *Ae. aegypti* larvae collection was undertaken during the wet season (Kahamba *et al*., 2020). The high *Ae. aegypti* larvae during the wet season in the present study in the towns could result from an increased number of water-holding containers due to the availability of rainfall, temperature, and humidity in the months of June to early October (Balehegn *et al*., 2019). The diapause nature of *Ae. aegypti* eggs during the dry season may also contribute to increased abundance of the *Ae. aegypti* larvae/pupae during the wet season (Mayilsamy, 2019). Thus, emphasis should be given to applying larval source management during the wet season in the towns. Furthermore, potential water container management during the dry season is required, since the habitats during the dry season serve as reservoirs for increased density of *Ae. aegypti* during the wet season (Charlwood *et al*., 2000).

The highest *Ae. aegypti* larvae/pupae density was observed in habitats with mud and gravel with soil substrates. This might result from differences in organic content across the substrates in breeding containers. Higher density of *Ae. aegypti* larvae/pupae was also associated with turbid water. This is in agreement with the study conducted in the Central African Republic, where *Ae. aegypti* larvae/pupae were observed in turbid water (Kamgang *et al*., 2013). However, in Tanzania, a higher density of *Ae. aegypti* immature stages was collected from containers with clean water (Kahamba *et al*., 2020). The high density of *Ae. aegypti* larvae in turbid water in the present study might be attributed to the associated detritus, which serves as food to the larvae, or reduced predators (Kamgang *et al*., 2013).

The key predictors identified for the occurrence (presence/absence) of *Ae. aegypti* larvae/pupae were habitat type, habitat location, and water source. The occurrence of *Ae.*

aegypti larvae/pupae was highly observed in tyres and water-storage drums as compared to others water containers (cement tanks, buckets) water containers. Similar findings were reported in Zanzibar, where tyres were major sources for the occurrence of *Ae. aegypti* larvae/pupae habitat (Kampango *et al*., 2021). A habitat with a tap water source was negatively associated with *Ae. aegypti* larvae/pupae. This was in agreement with the study in Brazzaville, Congo (Wilson-Bahun *et al*., 2020). The occurrence of *Ae. aegypti* larvae/pupae was significantly more associated with containers located in the domestic sites than in the peri-domestic. This could be due to the availability of containers and blood meal sources in the domestic sites than in the peri-domestic.

There was a significant positive relation between *Ae. aegypti* larvae/pupae density and water dissolved oxygen. Similar findings were reported from the Kinshasa area of the Democratic Republic of Congo previously (Mbanzulu *et al*., 2022a). *Ae. aegypti* larvae/pupae density was also positively related to total water hardness. It was contrary to a study in West Bengal, India, where total hardness was negatively related with the density of *Ae. agypti* larvae (Sarkar *et al*., 2024). These differences might arise from differences in the concentrations of minerals such as calcium and magnesium ions present in the water. Thus, there is a need to understand the correlations among habitat characteristics and *Ae. aegypti* larval/pupal densities, as they possibly suggest an effect on the productivity of *Ae. aegypti* and hence future control and management strategies.

Besides to identifying and characterizing *Ae. aegypti* larval habitats, knowledge of adult resting habitats, blood meal sources, and viral infection status has paramount epidemiological importance to reduce the spread of *Aedes*-borne diseases. This is peculiar to a country like Ethiopia, where there are likely real social and logistical impediments in the country. Thus, the insecticide-free removal of mosquito habitat should at least be identified as an intervention that could reduce the abundance of outdoor *Ae. aegypti* as well as the risk of infection. Apart from resource constraints in getting and utilizing public health insecticides, the identification and control of the local outdoor resting and breeding habitats of *Ae. aegypti* is an eco-friendly approach that reduces the risk of insecticide resistance emergence in *Aedes* mosquitoes (Diallo and Diallo, 2020).

Aedes aegypti were collected in higher numbers from outdoor resting sites, such as from tyres (422; 50%) and vegetation (94; 11%) than indoors. *Ae. aegypti* might prefer to rest outdoors close to human habitations. A relatively lesser proportion (207; 24%) was collected from

indoors. This indicates that *Ae. aegypti* could rest both outdoors and indoors, preferably outdoors. Similar findings were reported from Dire Dawa (Waldetensai *et al*., 2021a), Kenya (Lutomiah *et al*., 2016), and Malaysia (Saifur *et al*., 2012), in which case more *Ae. aegypti* were collected from outdoors than indoors. Contrary to this observation, reports from Dire Dawa and Somali Regions of Ethiopia (Waldetensai *et al*., 2023; Yared *et al*., 2024), South-eastern Senegal (Diallo and Diallo, 2020), and Colombo, Sri Lanka (Janaki *et al*., 2022) indicated more *Ae. aegypti* indoors than outdoors. Thus, the outdoor and indoor resting behavior of *Ae. aegypti* facilitates the rational use of financial allocation, technical and human resources for *Ae. aegypti* control in the Afar Regional State and other areas of Ethiopia. Relatively, a higher Shannon diversity index of mosquitoes was observed in Awash Sebat town as compared to Werer and Awash Arba. This revealed that *Ae. aegypti*, *An. gambiae*, *An. stephensi* and *Culex* species found in Awash Sebat are relatively evenly distributed in the Awash Sebat town.

Used tyres were the most common outdoor resting habitats of *Ae. aegypti*. This finding was contrary to the study observed in Dire Dawa, where outdoor collection of *Ae. aegypti* was made from open barrels (Waldetensai *et al*., 2021b). The outdoor resting behavior of *Ae. aegypti*, such as in tyres and vegetation, has important implications for control strategies, particularly during outbreaks of *Aedes*-transmitted viral diseases. This will help to apply public health insecticides to prevent *Ae. aegypti* resting in tyres as previously observed in Darwin, Australia (Pettit *et al*., 2010). In addition, identifying tyres as preferred resting habitats helps inform outdoor *Ae. aegypti* habitat management, especially, tyres removal by local residents, and reduce the chance of *Ae. aegypti* resting and breeding in tyres and hence prevents the risk of *Aedes*-borne disease transmission.

Entomological surveillance uses different types of indices to identify the risk levels of *Ae. aegypti* for *Aedes*-borne disease transmission. Indices based on actual counts of the number of adult females are more accurate (Cromwell *et al*., 2017). The *Ae. aegypti* adult density values are associated with the risk of *Aedes*-borne disease transmission. In the present study, the adult house index (AHI), female *Ae. aegypti* per surveyed house (FSH), and female *Ae. aegypti* per positive house (FPH) showed that the mosquito was present during the majority of the months. The differences in *Ae. aegypti* adult indices could be attributed to its aggregated distribution pattern in the areas. However, determining adult *Ae. aegypti* density helps for early warning of potential vector population build-up and associated viral disease outbreaks. This strengthens the implementation of proactive vector and disease control

strategies. Removing the tyres or other potential outdoor and indoor resting habitats could support management of *Ae. aegypti*.

Understanding the extent of temporal variability is an important factor in vector control. *Ae. aegypti* was dominant in the study areas from July to October 2022. A similar finding was reported in Congo Kinshasa (Manzambi *et al*., 2023). This could result from the favorable environmental factors such as rainfall, temperature, and biotic factors during the wet season (Rodrigues *et al*., 2015; Valdez *et al*., 2018; Seid *et al*., 2024). Thus, characterizing mosquito density and abundance in different seasons plays a crucial role in integrated vector control management (Fillinger *et al*., 2004). The number of *Ae. aegypti* caught varied significantly with collection hours. The highest *Ae. aegypti* collection was made during 15:00-18:00 hrs, whereas the fewest collections were made in 11:00-14:00 hrs. Similar findings were reported in Kinshasa, Congo, where the majority of *Ae. aegypti* collections were made between 15:00-18:00 hrs (Manzambi *et al*., 2023). This has implications in the control of *Ae. aegypti*, where the times of target vector control tools would be applicable in a specific resting site.

Relatively, a higher number of *Ae. aegypti* females, 53 (36%) were fed on humans, 18 (12.4%) on bovine, and 15 (10.3%) on both humans and bovine (mixed). There were no statistical differences among the human and bovine-fed *Ae. aegypti*. This indicates that *Ae. aegypti* did not have a preferred host in the presence of both human and cattle in the towns. In other words, *Ae. aegypti* was an opportunistic feeder in the study towns. Similar findings were reported in Kenya (Kamau *et al*., 2023). However, the HBI in this study was lower compared to previous values from Thailand (92%) and Kenya (51%) (Ponlawat and Harrington, 2005; Kamau *et al*., 2023). These variations could result from differences in the relative distance and accessibility of hosts and variation in the blood antibody/antigen detection methods (Kamau et al., 2023). The blood meal source of 59/145 (40.6%) of the fed *Ae. aegypti* was not identified. These could most likely feed on other hosts, such as cats, donkeys, chickens, and dogs.

None of the 1166 (24 pools) *Ae. aegypti* diagnosed for dengue and chikungunya viruses was positive. The absence of virus-infected *Ae. aegypti* is not uncommon in Ethiopia (Waldetensai *et al*., 2023), Tanzania (Ngingo *et al*., 2022), and Kenya (Iwashita *et al*., 2018). The lack of dengue and chikungunya viruses among the tested *Ae. aegypti* might be due to their low number, less probability of getting infected with the viruses, or very low number of positive human hosts to CHIKV and DENV in the towns. Thus, this might not show the

absence of dengue fever, chikungunya, and other viral diseases in the towns. Although the probability of getting positive *Ae. aegypti* to chikungunya and dengue viruses is low with small sample sizes, it seems difficult to assert that the risk is high without a circulating virus. However, a study conducted in Singapore indicated dengue epidemics in areas with even adult *Ae. aegypti* density per premise of less than 1% (Chen *et al*., 1994). In the present study, the high density of *Ae. aegypti* collected per house from August 2022 to October 2022 could trigger arboviral disease transmission. Thus, the risk of dengue fever, chikungunya, and other viral disease transmissions could be high in the towns.

Insecticide application is a quick and effective response against *Ae. aegypti* during the arboviral disease outbreak period. However, there is a scarcity of evidence on the susceptibility of *Ae. aegypti* against to the available insecticides in Ethiopia in general and in the Afar Region in particular (Waldetensai *et al*., 2021b). This study evaluated the susceptibility status of *Ae. aegypti* to public health insecticides in the Southern Afar Regional State.

The pyrethroids caused higher KDT_{50} and KDT_{95} against *Ae. aegypti* in Awash Sebat and Werer towns compared to the result of a similar study in Sudan (Husham *et al*., 2010). However, the KDT_{50} value of deltamethrin in Nigeria (Ukpai and Ekedo, 2018) was almost twice the KDT_{50} observed in Awash Sebat. The KDT_{50} value of permethrin in the Awash Sebat town was similar to a value in Mlabani, Tanzania. However, the KDT_{95} values of deltamethrin and permethrin in Mlabani towns (Kahamba *et al*., 2020) were higher than the values of the present study. Overall, the tube test results showed that *Ae. aegypti* was susceptible to deltamethrin in Sudan and Tanzania but possibly resistant in Nigeria (Husham *et al*., 2010; Kahamba *et al*., 2020; Ukpai and Ekedo, 2018).

Aedes aegypti was observed to be phenotypically resistant to bendiocarb with < 90% mortality. *Ae. aegypti* resistance to bendiocarb was also reported in Tanzania (Kahamba *et al*., 2020). However, the current observation was contrary to the report from Sudan, Nigeria, and Cambodia (Husham *et al*., 2010; Ukpai and Ekedo, 2018; Boyer *et al*., 2022). *Ae. aegypti* was also resistant to propoxur in all the towns. Similarly, phenotypic resistant of *Ae. aegypti* to propoxur was reported in Thailand (Pethuan *et al*., 2007).

Phenotypic resistance of *Ae. aegypti* could result from frequent use of insecticide-treated bed nets and indoor residual insecticide spraying to control mosquito-borne diseases, as

previously indicated (Barbosa *et al*., 2018). Moreover, Ethiopia has had a long history of pesticide use to increase agricultural production since 1964 (Mengistie, 2016; Negatu *et al*., 2021). Agricultural pesticides induce insecticide resistance because of similar mode of action and bioactive ingredients (Nkya *et al*., 2013). Similarly, agro-pesticides may increase insecticide tolerance as the pesticides are washed into mosquito breeding habitats, which confer resistance mechanisms at the adult stage (Poupardin *et al*., 2008). Thus, resistance of *Ae. aegypti* to bendiocarb and propoxur in the present study could potentially arise from their frequent exposure to carbamate insecticides in agriculture. In addition, *Ae. aegypti* resistance to propoxur and bendiocarb may occur as a result of mutations in the insecticide target site enzyme Acetyl-cholinesterase (AChE) as observed in Cuba (Bisset *et al*., 2006).

The pyrethroid alpha-cypermethrin caused less than 97% mortality against adult *Ae. aegypti* in the Awash Arba town, which revealed emergence of possible resistance. This might be due to the frequent use of pyrethroids including alpha-cypermethrin in the insecticide-treated nets and agricultural pesticides. This may lead to failure of vector control programs in the study area and elsewhere in the country if resistance management is not undertaken. Similar findings were reported in Ghana (Owusu-Asenso *et al*., 2022).

Aedes aegypti populations were susceptible to pirimiphos-methyl in all the towns. This result is in agreement with the study conducted in Cambodia (Boyer *et al*., 2022). However, this result was contrary to the observation in Nigeria (Ukpai and Ekedo, 2018). *Ae. aegypti* was susceptible to deltamethrin and permethrin in the towns. This is similar to the reports in Tanzania (Kahamba *et al*., 2020) and in Peru (Pinto *et al*., 2019), where deltamethrin showed greater than 99% mortality in adult *Ae. aegypti*. These findings were contrary to those in Malaysia (Ishak *et al*., 2015) where deltamethrin and permethrin-resistant phenotypes were observed. Thus, deltamethrin, permethrin, and pirimiphos-methyl can be used to control *Ae. aegypti* and hence viral disease outbreaks in the towns and elsewhere in the country.

In addition to *Aedes* mosquito surveillance, effective vector control strategies need the local community's participation and integrating the entomological data to community's knowledge, attitude and prevention practices. In Ethiopia, there is no well-established community-based *Aedes* mosquito control and management strategy and control measure are taken during outbreaks (Waldetensai *et al*., 2021b). Thus, exploring community knowledge, attitudes, and control practices of *Aedes* mosquitoes associated with chikungunya and dengue fever is important to design *Aedes* control strategy.

A small proportion of Awash Arba, Werer, and Awash Sebat community members who participated in the questionnaire perceived that *Aedes* mosquitoes breed in tyres and construction tanks. Similar perceptions were reported in Colombia (Jaramillo-Ramírez and Álvarez, 2017) and in Kinshasa, Congo (Mbanzulu *et al*., 2022b) where tyres were reported as a habitat of *Aedes* larvae. The majority of the respondents had low knowledge scores (78.1%) about the larval habitats of *Aedes* mosquitoes. Similarly, the majority of the respondents (76.3%) associated *Aedes* mosquitos' effect with nuisance and biting pain, while 23.7% associated it with disease risks. Furthermore, a larger proportion of the respondents (73.7%) had low practice scores to avoid *Aedes* bites. Thus, these findings revealed that residents in the study towns had no adequate knowledge of *Aedes* mosquito larval habitats and were not completely adopting the preventive practices in order to reduce larval habitats of the mosquitoes. This low of knowledge, attitude, and practice behavior calls for alarm for massive *Aedes* mosquito larval habitats and *Aedes*-associated disease awareness campaigns in the study towns.

The majority of the respondents (about 64%) outlined that *Aedes* mosquitoes bite during the night-time while about 30% of the respondents considered biting of *Aedes* mosquitoes during the daytime. This may show knowledge gaps exist in the community regarding the biting time of *Aedes* mosquitoes in the study towns, as literature showed that *Aedes* mosquitoes are known to bite mostly during the day-times (Captain-Esoah *et al*., 2020). On the other hand, respondents pointed out that *Aedes* mosquitoes near grassy areas frequently bite them. This may be due to mosquitoes preferring grassy areas for resting and breeding environments, as previously described (Mendenhall *et al*., 2017). Thus, grassy areas should be targeted during the intervention strategies (Nagoor *et al*., 2017).

During the household survey, the majority of the respondents (74%) were aware of CHIK in their localities. Similarly, all the FGD participants from Awash Arba, Awash Sebat, and close to 30% in Werer were aware of chikungunya. Although, respondents in the household survey knew the high risk, sign, and symptoms of the chikungunya in their localities, they were largely unaware of its routes of transmission, in which majority of them responding that CHIK could be transmitted through body contact with patients with CHIKV. Similarly, almost half of the FGD participants pointed out that chikungunya is transmitted through body contact with patients with CHIKV. Even a few participants were told that sharing clothes and utensils with individuals infected with CHIKV is responsible for CHIK transmission. This is

an indication of the knowledge gaps among the community, particularly in the route of transmission of CHIK and other *Aedes*-transmitted viral diseases. However, few FGD participants from Awash Sebat and Awash Arba pointed out that mosquitoes responsible for chikungunya are zebra mosquitoes, which have white and black stripes on their bodies like zebras. Thus, it is very imperative to recommend to the regional government and other stakeholders to facilitate training platforms and provide regular training to the communities to increase the knowledge of the communities, particularly the route of transitions of CHIK and other *Aedes*-transmitted viral diseases in the study towns. The majority of the respondents were less informed (less than 8%) about DEN in their localities. They were largely unaware of its routes of transmission in which majority of them responded that DEN could be transmitted through body contact with infected patients.

Respondents in Awash Sebat and Awash Arba heard and knew about chikungunya and a few about the dengue fever outbreak in their localities through health professionals and television channels. A similar finding was observed in Dire Dawa, where respondents gained information about dengue fever via health professionals (Kebede *et al*., 2023). Thus, health professionals and television channels played a role in providing the sources of information to the individuals about the outbreaks of CHIK in their localities. However, knowledge about a disease outbreak itself is not necessarily enough to bring about the communities' behavioral change to get rid of disease occurrence and incidence as previously indicated in (Koenraadt *et al*., 2006; Shuaib *et al*., 2010; Paz-Soldán *et al*., 2015). Thus, the study conducted in Martinique revealed that integrating regular surveillance of epidemiological and entomological data and digitizing them, especially when connected to social media, has been used to quantify transmission dynamics and bring human behavioral change towards mosquito-borne diseases (Roche et al., 2017). Thus, it is vital to include the overall outbreaks and associated risks of CHIK in the television programs, particularly in the regional television channel.

In the present study, very few respondents (4.2%) had gained CHIK information, and no respondents gained DEN information through schools, indicating the lack of an updated health education in the educational institutions in the country. In contrast, a study in Brazil revealed that school-based interventions can promote change in attitudes and behaviors in the population, which could lead to a reduction in infestation and a lower risk of illness and death from *Aedes*-transmitted viral diseases (Santos *et al*., 2022). Thus, the development of school-

based *Aedes*-transmitted disease control interventions and incorporating them in educational programs is very important in order to enhance the knowledge of CHIK for both teachers and students; this could motivate all actors towards CHIK prevention and control. Furthermore, school-based CHIK prevention and control programs could provide a sustainable practice for community awareness through the involvement of teachers and students.

The majority of respondents in the household survey were unaware of the vector role of *Aedes* mosquitoes in spreading CHIK to humans. Similarly, three-fourths of the FGD participants from the study towns did not know that the virus is transmitted to humans by the bite of infected *Aedes* mosquitoes. Similar observations have been reported in Jamaica, where the population had poor knowledge of mosquito-borne diseases (Alobuia *et al*., 2015). On the other hand, the findings from Belize revealed that more than 85% of the respondents confirmed that DEN and CHIK are viruses transmitted by *Aedes* mosquitoes.

The common mosquito control practices taken to reduce *Aedes* abundance and bites by the respondents in their localities include using insecticides-treated bed nets, removing water-holding containers, covering the containers, and wearing long-sleeved clothes during night time. However, the majority of the respondents had a low level of practice in the control of *Aedes* mosquitoes. This finding was contrary to China (Liu *et al*., 2014), the United States of America (Morse *et al*., 2019), and Colombia (Ramírez and Alvarez, 2017), observations have been reported in which the majority of the population was positively involved in source reduction preventive practices.

Occupation was significantly associated with the knowledge of breeding sites of *Aedes* mosquitoes. For instance, health professionals, students, and merchants had higher knowledge of *Aedes* larval habitats. Moreover, the respondents aged 29-38, had a higher level of control practice of *Aedes* mosquitoes, as adult workers who stay outdoors were more likely to get bitten. University-educated respondents also had a high level of control practice of *Aedes* mosquitoes. A similar finding was observed in France; higher educational levels were associated with the increased number of control measures (Raude *et al*., 2012).

In conclusion, the study documented fundamental aspects of *Ae. aegypti* bionomics including the preferred larvae/pupae habitats with their physico-chemical characteristics, resting sites, blood meal sources, susceptibility to public health insecticides. In addition, the study assessed local community's knowledge, attitude and practices towards *Aedes* mosquitoes and the

associated risks to chikungunya/dengue diseases. Thus, control strategies of *Aedes* mosquitoes should target the most prolific larval/pupal habitats such as used tyres and water-storage drums. Future studies should be inspired to investigate the susceptibility of *Aedes* mosquitoes to public health insecticides including molecular resistant gene detection to determine the resistance pattern in the study area.

Strengths and limitations of the study

This study investigated *Ae. aegypti* bionomics, including type and physicochemical characteristics of larvae/pupae habitats, resting behavior, blood meal sources, insecticide susceptibility status, and community awareness about *Aedes* species and their associated risks to chikungunya and dengue fever, which could be used as national and regional baseline data for the control of *Aedes* species and *Aedes*-borne diseases in Ethiopia.

In spite of providing important data about *Ae. aegypti* in Ethiopia, such as its first insecticide susceptibility status report, the study has some limitations. For instance, it was not able to characterize the biological entities that potentially influence the occurrence and density of *Ae. aegypti* larvae/pupae habitats. Similarly, confirmation of potential sub-species was not diagnosed using molecular tools due to an absence of primers and reagents. In addition, the blood meal sources of *Ae. aegypti* was detected by ELISA not by PCR due to a lack of primers and reagents. Moreover, except for alpha-cypermethrin (0.05%) and propoxur (0.1%) insecticides, the susceptibility status of *Ae. aegypti* to insecticides was evaluated according to the discriminating doses of the insecticides recommended for *Anopheles* species. This was due to the unavailability of insecticide-impregnated papers with corresponding discriminating doses for *Ae. aegypti* at the time. We were unable to detect the resistant genes of *Ae. aegypti* for bendiocarb and propoxur due to lack of reagents. Finally, due to incomplete daily minimum and maximum temperature, rainfall, and humidity data obtained from the National Metrology Agency of Ethiopia for the study towns, we were unable to correlate the climatic condition with the density of *Ae. aegypti*.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

This study identified diverse artificial water-holding containers, including tyres, water storage drums, flowerpots, discarded plastic, and other water-holding containers such as water-stored cement tanks, jerrycans, buckets, and polythene sheets. The larval/pupal productivity of *Ae. aegypti* was higher in water-holding containers, which were located at domestic sites. The values of larval indices (Container index, House index and Breteaux index) observed were highly indicative of potential outbreaks of *Aedes*-borne diseases. This could put the residents of the towns at high risk of infections with *Aedes*-borne viral diseases such as chikungunya and dengue fever. Moreover, adult *Ae. aegypti* was the most abundant species found particularly in Awash Sebat town. Significantly higher numbers and proportions of *Ae. aegypti* were collected from outdoors. A higher densities of female *Ae. aegypti* occurred during the wet season. Therefore, based on the findings, our point-by-point concluding remarks are:

- *Aedes aegypti* larval density was higher in tyre and drum habitats, which were located at domestic sites.
- Habitat types (tyres and drums), habitat locations (domestic sites) positively contribute for the occurrence of *Ae. aegypti* larvae/pupae, while water sources (tap water) was negatively influence larvae/pupae occurrence.
- Dissolved oxygen and total hardness of the containers positively influence *Ae. aegypti* larval/pupal productivity.
- The threshold limit of larval indices (Container index, House index, and Breteaux index) observed were higher during the wet season and triggers *Aedes* control campaign in the study towns.
- Significantly, higher number and proportion of females of *Ae. aegypti* collections were made from tyres followed by rooms.
- Highest female *Ae. aegypti* per surveyed house and per positive houses occurred during the month of September, 2022.
- The wet season and collection places (tyres, rooms and vegetation) were the determinants for the higher abundance of adult *Ae. aegypti*.
- *Ae. aegypti* could prefer to fed on humans followed by bovines. Mixed blood meals sources were also detected.

- *Ae. aegypti* showed resistant to bendiocarb and propoxur and possibly resistant to alpha-cypermethrin, it was susceptible to deltamethrin, permethrin, and pirimiphos-methyl insecticides.
- Health professionals, students and merchants had a sound knowledge, regarding *Aedes* species larval habitats. In addition, respondents with age group 29-38, university graduate respondents were more involved to control *Aedes* mosquito bites.

6.2. Recommendations

Based on the findings of the study, the following points would be forwarded.

For *Aedes* mosquitoes control practices (Operational bodies)

- Since used tyres were the hotspots for the density, occurrence of *Ae. aegypti* larvae/pupae and resting adults, tyre removal (dysfunction not to hold water) of mosquito habitat should be implemented as an intervention in th. Aerial insecticide spray would also targeted outdoor resting sites such as tyre, vegetation.
- The threshold limit of larval indices observed were higher during the wet season. Thus, regular surveillance and monitoring of *Aedes* mosquitoes should be carried out to reduce the *Ae. aegypti* density and hence prevent *Aedes*-borne diseases transmission.
- Behavior and vectorial role of *Ae. aegypti* should be regularly evaluated in Awash Arba, Awash Sebat, and Werer towns and other similar settings to enhance *Aedes* control interventions.
- Proper use of existing insecticides is mandatory to prevent *Ae. aegypti* resistance to insecticides in the study areas.

For policy makers (MoH, EPHI and Research Institutions)

- Policy makers should consider the potential risk of urban development with increasing house construction which produce new habitat for *Aedes* mosquitoes and facilitate *Aedes*-borne disease transmission.
- *Aedes aegypti* monitoring and surveillance systems should be designed with considerations of urban development and environmental management options particularly management of solid waste disposals.

- Insecticide resistance management strategies should be implemented to delay resistance development and prolong functional lifespan of insecticides and supplementary intervention tools should be incorporated.
- *Aedes* mosquito control strategies should be designed based on regular mosquito surveillances with the involvement of the local communities about *Aedes* mosquitoes and their associated risks towards local viral disease outbreaks by targeting on people with lower education background.

For further studies

- Further studies in different zones of the Afar Regional State and Ethiopia should be inspired with a special focus on the biotic (algae, micro-organisms and aquatic mosquito predators) in both productive and non-productive larval habitats, towards a sound understanding of *Ae. aegypti* larval ecology and application of appropriate larval control measures.
- Research is also required to investigate the role of used tyre in the ecology and management in *Aedes* mosquito control strategies in the study towns.
- Regular monitoring of insecticide resistance should be carried out in the study areas and elsewhere in Ethiopia, including molecular aspects, to mark and evaluate resistance patterns

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Appendices

Appendix I: Households' Information Sheet

Research Title: Bionomics, Insecticide Susceptibility Status and Community Perception of *Aedes eagypti* in Afar Region, Ethiopia

Funded by: Addis Ababa University, the Vice president for Research and Technology Transfer Office through its thematic research grant through, Aklilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University, Addis Ababa Ethiopia, P.O. Box, 1176, Tel, 251 11 276 30 91.

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Study Households Consent Form

Name of the Household: _____Age___ Sex___ Code _____Study town/district_____

I am Mohammed Seid Legas, currently attending my PhD in Tropical and Infectious Diseases program at Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Ethiopia. I am conducting research on the Bionomic, insecticide susceptibility status and perceptions of communities *Aedes aegypti* that is the primary vector of many infectious diseases such as yellow fever, dengue fever and chikungunya fever diseases, which are very common diseases in the eastern and northeastern parts of Ethiopia including Afar Regional State. Now I kindly invite you to be part of this research. I need your participation in this research only to allow us collect adult and immature mosquitoes from your home (indoor/outdoor). During data collection, please ask me for further clarification if you are not clear the process. If you have

questions later, you can ask me (Mohammed Seid, the principal investigator) or my supervisor/s (Dr. Abebe Animut and Dr. Esayas Aklilu).

Aedes mosquitoes especially *Aedes aegypti* is responsible for the transmission of many viral diseases of human including dengue fever and chikungunya. Knowledge of *Aedes* mosquitoes Bionomics (Breeding and resting sites, blood meal sources, insecticide susceptibility status and community perception towards *Aedes aegypti* will help to improve mosquito control in the area. In this study, we are going to determine the spatio-temporal occurrence of *Aedes* larvae/pupae, resting behavior, blood meal sources, insecticide susceptibility status, viral infection rates and community perception of *Aedes aegypti* in the selected towns of Afar Regional State. The findings (results) from this study will help inform effective *Aedes* control strategies in the study areas and other arboviral diseases endemic setting in the country.

Appendix II: Immature mosquito collection format

SN	Items	Description	Remark
1	Geographic location, GPS coordinates in decimal degrees	1. Latitude_____3. Longitude_____ 2. Elevation_____4. GPS Accuracy_____	
2	Ecology/habitats	3. Domestic 2. Peri-domestic 3. Sylvatic	
3	Collection Place	1. Indoor 2. Outdoor	
4	Sources of water for habitats	1. Tap water 2. Rain 3.River 4. Pond/lake 5. Other(specify)_____	
5	Collection types	1. Dipping 2. Pipetting 3. Netting and sieving 4. Other_____	
6	Breeding habitat type	1. Flower pots 2. Water storage drum 3. Discarded plastics or metal containers 4. Tyres 5. Buckets 6. Other (Specify)_____	
7	Water movement	1. Stagnant 2. Slow 3. Fast	
8	Usage of the water from the habitats	1. Frequently 2. Sometimes 3. Not used	
9	Water turbidity	1. Clear 2. Turbid 3.Other_____	
10	Presence of mosquito predators	1. Present 2. Absent	
11	Habitat volume	1. Depth____ 2.Length __ 3. Width_____ Litter_____	
12	Habitat substrates	1. Mud 2. Sand 3. Gravel with soil 4. Cements 5. Other __	
13	Exposure to sun light	1. Shaded 2. Partially shaded 3. Sunlight	
14	Presence of vegetation	1. Present 2. Absent	
15	Nature of the adjacent land surface	1. Cultivated 2. Grazing 3. Vegetation 4. Forest	
16	Habitat distance from nearby inhabited houses	.....	
17	Habitat distance from	(0–50m, 50-100m, >100m).....	

	the nearest plant	
18	Habitat climatic condition	1. Temperature_____ 2. Relative humidity_____3. pH_____ 5.Other_____
19	Chemical features of the habitats	1. Dissolved Oxygen_____2. Conductivity_____ 3.Total Alkalinity____4. Total dissolved solids_____ 5. Total hardness___
20	Permanency of breeding sites	1. permanent 2.semi-permanent 3.Temporary
21	Did intervention applied to this mosquito breeding container?	1=Yes; 2=No
22	Total number of inhabitants living in the household	_____
	Is the habitats positive for mosquito larvae	1. Yes 2. No

23	If habitats positive for mosquito larval count per dip	No.	*Anopheles*					*Aedes*					*Culex*					
			I	II	III	IV	Pup	I	II	III	IV	pup	I	II	III	IV	pup	
		1																
		2																
		3																
		4																
		5																
		6																
		7																
		8																
		9																
		10																
		11																

		12																
		13																
		14																
		15																
24	Adult mosquito species reared and their numbers	*Anopheles gambiae s.l* Total females_____ Total males_____																
		*Anopheles*_____ Total females_____ Total males_____																
		Anopheles _____ Total females_____ Total males_____																
		Aedes aegypti Total males, _____ Total females_____																
		*Aedes*_____ total males, _____ Total females_____																
		Culex, Total males_____ Total females_____																

Appendix III: Adult mosquito collection form

SN	Items	Description	Remark
1	Geographic location; GPS coordinates in decimal degrees	1. Latitude_____3.Longitude_____ 2. Elevation_____4.GPS Accuracy_____	
2	Ecology/habitats	1. Domestic 2. Peri-domestic 3. Sylvatic	
3	Collection place (choose one)	1. Indoor 2. Outdoor	
4	Collection method(Choose one)	1.Propopack 2.Pyrethrum spray 3.Mouth aspiration 4.Sweeping net 5.other_____	
5	If outdoor collection, specify types of shelter	1.Tree 2.Vegetation 3.Fence 4.Ground hole 5. Pit shelter 6. Animal shelter 7 Other_____	
6	If indoor collection, collection area	Bedroom 2. Dining room 3. Kitchen 4. Bathroom 5.Store room 6. Other_____	
7	Types of Object/Surface adult mosquito collected	1.Cardboard 2.Rubber 3.Cement 4.Leather 5.Wood 6.Metal 7. Stone 8. Plastic 9.Plant 10.Other_____	
8	If indoor collection, types of roof	1.Metal 2.Thatch 3. Other specify	
9	If indoor collection, presence of hole on roof	1.Present 2. Absent	
10	If indoor collection, specify type of ceiling	1. Fully closed ceiling 2. Partially closed ceiling 3. No ceiling	
11	If indoor collection, status of eaves	1. Fully open 2. Open on 1 to 4 sides 3. No eaves	
12	If indoor collection, type of wall surface (choose one)	1.Mud 2. Cement 3. Wood 4. Painted mud 5. Painted cement 6. Thatch 7. Brick 8. Other (specify): _____	
13	Was the house sprayed within last year?	1. sprayed 2. Not sprayed 3. Unknown 4. If sprayed, date: _____	
14	House distance from the nearest breeding site	_____	
15	Total number of mosquito collected	1. *Anopheles*:Female_____;Male:_____ 2.*Aedes*: Female: _____Male: _____ 3. *Culex:* Female:_____ ;Male: _____	

16	No. of *Aedes*_____by abdominal status	1)Unfed_____ 2) Fresh fed_____ 3)Semi-gavid_____ 4)Gravid_____	
	No. of *Aedes*_____by abdominal status	1)Unfed_____ 2) Fresh fed_____ 3)Semi-gavid_____ 4)Gravid_____	
	No. *Anopheles*_____ by abdominal status	1)Unfed_____ 2) Fresh fed_____ 3)Semi-gavid_____ 4)Gravid_____	
	No. *Anopheles*_____ by abdominal status	1)Unfed_____ 2) Fresh fed_____ 3)Semi-gavid_____ 4)Gravid_____	
	No. *Culex*_____ by abdominal status	1)Unfed_____ 2) Fresh fed_____ 3)Semi-gavid_____ 4)Gravid_____	
	No. *Culex*_____ by abdominal status	1)Unfed_____ 2) Fresh fed_____ 3)Semi-gavid_____ 4)Gravid_____	

Sampling description

1. Sampling hours and minutes_____
2. Name of the collector_____
3. Signature of the collector _____
4. Additional Notes_____
   _____
   _____
   _____
   _____

Appendix IV: Questionnaires

Addis Ababa University, Aklilu Lemma Institute of Pathobiology

My name is Mohammed Seid Legas; I am a Ph.D student at Addis Abba University, Aklilu Lemma Institute of Pathobiology. I am working a Ph.D research titled "**Bionomics, Insecticide Susceptibility Status and Community Perception of *Aedes aegypti* in Afar Region**, Ethiopia" which can help in designing effective *Aedes*-transmitted viral diseases control strategies. As a part of the research, I am assessing factors, which contributed for the outbreak of Aedes-transmitted viruses such as dengue fever and chikungunya in the Awash Sebat, Awash Arab, and Werer towns of Afar Region. Therefore, I kindly request you to fill this questionnaire. Thank you!!!

Questionnaire to access the community's awareness about *Aedes* mosquitoes and *Aedes*-transmitted diseases

I. Demographic characteristics of study participants

Questionnaire to access the communities' Knowledge, Attitude and Practices about Chikungunya and its mosquito vectors

Part I Demographic characteristics of study participants

Household name_____Code_____Date /_____/_____/_____District___

S. No.	Variables	Remark
1	Age _____years	
2	Sex: Male 2. Female	
3	Educational level 1. No formal education 2. Primary 3. Secondary 4. University	
4	Occupation 1. Housewife 2. Merchant 3. Daily laborer 4. Heath professional 5.Unemployed 6. Student 7. Farmer 8. Government employee 9. Other (specify)_____	
5	Marital Status 1. Married 2. Single 3. Divorced 4. Widowed	
6	Family size _____	
7	Family income _____in ETB	
8	House types: 1. Public housing 2. Private housing 3. Temporary housing	
9	House location: 1. Near water source 2. Near bushy and grassy area 3. Near construction site 4. Other _____	
10	Sources of drinking water: 1. Tap 2. River 3. Stream 4. Other_____	

11	How many year do you live in your village: 1. 1-2yrs 2. 3-5years 3. >5yrs	
12	Have you traveled out of your village in the last six months? 1. Yes 2. No	
13	Do you have Mosquito nets in your house? 1. Yes 2. No	

Part II Communities' Knowledge and attitude about chikungunya diseases

S.No.	Variables	Remark
1	Have you ever heard chikungunya diseases in your area? 1. Yes 2. No If Yes by what means? 1. On television 2. Radio 3.Health professionals 4. School 5.Other (specify)_____	If no, skip the remaining Q.
2	Have you been tested for chikungunia diseases before? 1.Yes 2.No	
3	Do you think Chikungunya virus is serious in your village 1. Yes 2. No	
4	Do you know the signs and symptoms of chikungunya? 1. Yes 2. No	
5	If your answer is yes for question 4 above signs and symptoms of chikungunya? 1. Prolong high fever 2. Muscular pain 3. Bleeding from nose and other areas 4.Rashes 5. Headaches 6. nausea and vomiting 7.Other (specify)_____	
5	What do you think the mode of transmission of Chikungunya? 1. By *Aedes* mosquito bite 2. By *Anopheles* mosquito bites 3. Body contacts with patients 4. I don't know 5. Other (specify)_____	
6	In what season is CKV is more common? 1. Dry 2. Wet 3. Other(specify)_____	
7	Do you think chikungunya be prevented? 1. Agree 2. Disagree	
8	Can chikungunya be treated? 1. Yes 2. No If Yes by what means? By chemotherapy 2. Herbal medicine 3. Religious solution 4. Other specify_____	
9	1. What is the most effective preventive measure for controlling CKV? 1. Use of mosquito nets 2. Traditional medication 3.Use of mosquito repellants 4. Use of mosquito window door screen 5. Other_____	
10	If you or your family has a fever what do you do at fist? 1. Go to health facility 2. Go to private provider 3. Get medication from the pharmacy 4.Wait for the fever to go away . Other (Specify)_____	
11	Do you think you are at risk of CKV? 1. Yes 2. No	

Part III Knowledge and attitudes on mosquito ecology and behaviors

S.No.	Variables	Remark
1	Have you ever seen any mosquito in your area? 1.Yes 2. No	
2	If your answer is yes for question no.1 above, do you like the presences of mosquitoes in your area? 1. Yes 2. No	
3	If your answer is No for question 2 above why? 1. Nuisance/pain due to bite 2. Transmit diseases 3. Other (specify)_____	
4	If your answer is Yes for question no. 1, how mosquitoes do looks like? 1. Small black mosquito with white strips on legs 2. Grey 4. Other_____	
5	How do you call the mosquito species found in your locality? *1. Anopheles 2. Aedes 3. Culex* 4. Other (specify)_____	
6	Do you think mosquitoes transmit diseases to human such as chikunginya? 1. Yes 2. No	
7	If your answer is yes for question 6 above, what are the mosquitoes responsible to chikungunya diseases? *1. Anopheles 2. Aedes 3. Culex* 4. I don't know 6. Other _____	
9	When do you think *Aedes* mosquitoes increase in number? 1. Wet 2. Dry 3. I don't know	
	What do you think the factor for their increase? 1. Rainfall availability 2. House construction 3. Poor waste disposal 4. I don't know	
8	Do you store water in your home? 1. Yes 2. No	
9	If Yes for question Q.8, why? 1. For drinking 2. For wash 3. For construction 4. Other_____	
10	What is the frequency of changing the stored water in your home? 1. Daily 2.weekly 3. twice a week 4. monthly 5. other_____	
11	Do you have other natural stagnant water in locality? 1. Yes 2 No	
12	If your answer is Yes in the question 11 above what are these? 1. Rivers 2. Streams 3. Ponds 4. Lakes 5. Other_____	
13	Do you know mosquitoes breed in aquatic habitats? 1. Yes 2. No	
14	If your answer is Yes for question 13 above, what are these potential breeding habitats? 1. Don't know 2. Water storage drum 3. Tyres 4. Discarded plastics 5. Used jerrycans 6. Cement genda 7. Other specify)_____	
15	If your answer is Yes for question 13 above, do you see movable worm like creature in stored water in your locality? 1. Seen 2. Not seen	

15	If your answer is Seen in question 14, above do you know that moveable worm like creature is a mosquito immature later changed to adult mosquitoes? 1. Yes 2.No	
16	Where does adult chikungunya mosquito prefer rest? 1. Underneath furniture 2. tyres 3.wall surface 4. Vegetation 5 Other_____	
17	Where do *Aedes* mosquitoes frequently bite 1. At home 2. Away from home 3. Near grassy areas 4.At work Near water sources 5. Near construction areas 6. During transportation 7. Other (specify)_____	
18	What is the most frequent mosquito bite time? 1. Any time 2. Daytime 3. Nighttime 4. Other (specify)_____	
19	Which parts of your body frequently bitten? 1. Face 2. Legs 3. Hands 4. Other_____	
19	Do you remain at home in the morning from 8 to 10 am? 1. Yes 2. No	
20	Do you remain at home in the afternoon 4–6 pm? 1.Yes 2. No	
21	If your answer is Yes for question 20 above what type of cloths do you wear? 1. Short sleeves 2. Long Sleeves 3. Other (specify)_____	
22	Do you use skin repellents 1.Yes 2. No	
23	Do you use mosquito repellent in the house? 1. Yes 2. No	
24	What is your appropriate practice to mosquito reduction and avoid biting in your area 1. Don't know 2. Removing water-holding containes 3. Changing stored water frequently 4. Turn containers upside down 5. Covering water containers tightly 6.Using bed nets. 7. Reducing stagnant water around 8. Using mosquito repellent 9. Other (specify)_____	
25	Who is responsible for controlling *Aedes* mosquitoes the diseas cause? 1. Government 2. Health workers 3. Local communities 4. Others_____	

Communities' Knowledge, Attitude and Practices about Dengue fever and its mosquito vectors

S.No.	Variables	Remark
1	Have you ever heard Dengue fever diseases in your area? 1. Yes 2. No If Yes by what means? 1. On television 2. Radio 3.Health professionals 4. School 5.Other (specify)_____	
2	Have you been tested for Dengue fever diseases before? 1.Yes 2.No	
3	Do you think Dengue fever virus is serious in your village 1. Yes 2. No	
4	Do you know the signs and symptoms of dengue fever? 1. Yes 2. No	
5	If your answer is yes for question 4 above signs and symptoms of Dengue fever? 2. Prolong high fever 2. Muscular pain 3. Bleeding from nose and other areas 4. Rashes 5. Headaches, nausea and vomiting 6.Other (specify)_____	
6	What do you think the mode of transmission of Dengue fever? 2. By *Aedes* mosquito bite 2. By Anopheles mosquito bites 3. Body contacts with patients 4. I don't know 5. Other (specify)_____	
7	In what season is dengue fever is more common? 1. Wet 2. Dry 3. I don't know 4. Other(specify)___	
8	Do you think Dengue fever be prevented? 1. Agree 2. Disagree	
9	Can dengue fever be treated? 1. Yes 2. No If Yes by what means? By chemotherapy 2. Herbal medicine 3. Religious solution 4. Other specify_____	
10	2. What is the most effective preventive measure for controlling Dengue fever? 2. Use of mosquito nets 2. Traditional medication 3.Use of mosquito repellants 4. Use of mosquito window door screen 5. Other_____	
11	If you or your family has a fever what do you do at fist? 2. Go to health facility 2. Go to private provider 3. Get medication from the pharmacy 4.Wait for the fever to go away . Other (Specify)_____	
12	Do you think you are at risk of Dengue fever in your area? 1. Yes 2. No	

II. Knowledge and Attitude on dengue fever transmitting mosquitoes

S.No.	Variables	Remark
1	Have you ever seen any mosquito in your area? 1.Yes 2. No	
2	If your answer is yes for question no.1 above, do you like the presences of mosquitoes in your area? 1Yes 2. No	
3	If your answer is No for question 2 above why? 2. Nuisance/pain due to bite 2. Transmit diseases 3. 4.Other (specify)_____	
4	If your answer is Yes for question no. 1, how mosquitoes do looks like? 1.Small dark mosquito with white strips on legs 2. Grey 3. Other_____	
5	How do you call the mosquito species found in your locality? *2. Anopheles* 2. *Aedes* 3. *Culex* 4. Other (specify)_____	
6	Do you think mosquitoes transmit viral diseases such as dengue and chikunginya? 1. Yes 2. No	
7	If your answer is yes for question 6 above, what are the mosquitoes responsible to dengue diseases? *1. Anopheles 2. Aedes 3. Culex* 4. I don't know 5. Write local mosquito name_____	
8	Do you store water in your home? 1. Yes 2. No	
9	If your answer is Yes for question 8 above, why? 1. For drinking 2. For wash 3. For construction 4. Other_____	
10	What is the frequency of changing the stored water in your home? 2. Daily 2.weekly 3. twice a week 4. monthly 5. other_____	
11	Do you have other natural stagnant water in locality? 1. Yes 2 No	
12	If your answer is Yes in the question 11 above what are these? 2. Rivers 2. Streams 3. Ponds 4. Lakes 5. Other_____	
13	Do you know mosquitoes breed in aquatic habitats? 1. Yes 2. No	
14	If your answer is Yes for question 13 above, what are these potential breeding habitats? 2. Don't know 2. Water storage drum 3. Tyres 4. Discarded plastics 5. Used jerrycans 6. Cement genda 7. Other specify)_____	
15	If your answer is Yes for question 13 above, do you see movable worm like creature in stored water in your locality? 1. Seen 2. Not seen	
15	If your answer is Seen in question 14, above do you know that moveable worm like creature is a mosquito immature later changed to adult mosquitoes? 1. Yes 2.No	

16	Where does adult dengue fever mosquito prefer living? 2. Underneath furniture 2. tyres 3. Wall surface 4. vegetation 5. Other_____	
17	Where are mosquitoes frequently bite 2. At home 2. Away from home 3. Near grassy areas 4.At work Near water sources 5. Near construction areas 6. During transportation 7. Other (specify)_____	
18	What is the most frequent mosquito bite time? 1. Any time 2. Day time 3. Night 4. Other (specify)_____	
19	Do you remain at home in the morning from 8 to 10 am? 1. Yes 2. No	
20	Do you remain at home in the afternoon 4–6 pm? 1.Yes 2. No	
21	If your answer is Yes for question 20 above what type of cloths do you wear? 2. Short sleeves 2. Long Sleeves 3. Other (specify)_____	
22	Do you use skin repellents 1.Yes 2. No	
23	Do you use mosquito repellent in the house? 1. Yes 2. No	
24	What is your appropriate practice for mosquito reduction and avoid biting in your area 1. Clean the house 2. Removing water-holding containers 3. Changing stored water frequently 4. Turn containers upside down 5. Covering water containers tightly 6. Fumigating house 7. Usingbed nets 8. Reducing stagnant water around 8. Using mosq uito repellent 9. Don't know 10. Other (specify)_____	
25	Who is responsible for controlling *Aedes* mosquitoes the diseas cause? 5. Government 6. Health workers 7. Local communities 8. Others_____	

Tropical Medicine and Health

RESEARCH

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Spatio-temporal occurrence and habitat characteristics of *Aedes aegypti* (Diptera: Culicidae) larvae in Southern Afar region, Ethiopia

Mohammed Seid[1,2*], Esayas Aklilu[1] and Abebe Animut[1]

Abstract

Background Describing spatio-temporal occurrence and habitat characteristics of *Aedes* mosquito larvae is crucial for the control of *Aedes* borne viral diseases. This study assessed spatio-temporal abundance and habitat characteristics of *Aedes* larvae in the Southern Afar Region, Ethiopia.

Methods Immature mosquitoes were surveyed in Awash Sebat, Awash Arba, and Werer towns of the Southern Afar Region once per month from May 2022 to April 2023. Larvae and pupae surveys were carried out along the available water-holding containers. The collected larvae/pupae were reared to adults and identified by species/genus morphologically. The physical and chemical properties of the habitats were also characterized.

Results A total of 9099 *Aedes* larvae/pupae were collected, of which 53.6% (4875) were from Awash Sebat, 29.5% (2687) from Awash Arba and 16.9% (1537) from Werer. Water-holding tyres harboured the highest number of *Aedes* larvae/pupae followed by water-storage drums. All the *Aedes* larvae/pupae reared to adults were morphologically identified as *Aedes aegypti*. The overall Container Index was 47.28%, House Index 18.19%, Breteau Index 59.94% and Pupal Index 171.94. Significant positive relations were observed in the occurrences of *Ae. aegypti* larvae/pupae with water-holding tyre (AOR = 15.89, CI = 3.55–71.09, $p < 0.001$), water storage drums (AOR = 19.84, CI = 4.64–84.89, $p < 0.001$), domestic habitat (AOR = 3.76, CI = 1.27–11.12, $p = 0.017$), and significant negative relations were observed with *Ae. aegypti* larvae/pupae occurrence and tap water source (AOR = 0.08, CI = 0.02–0.31, $p = 0.001$). *Ae. aegypti* larvae/pupae densities showed positive relations with dissolved oxygen ($\beta = 0.523$, $p < 0.001$) and total hardness ($\beta = 0.475$, $p = 0.034$) of water.

Conclusions Diverse types of artificial water-holding containers were positive for *Ae. aegypti* larvae/pupae. *Ae. aegypti* larvae/pupae were abundant in used water-holding tyres, water storage drums, and cement tanks in Awash Sebat, Awash Arba, and Werer towns. This could put the residents of the towns at high risk of infections with *Ae. aegypti* transmitted viral diseases such as chikungunya and dengue outbreaks. Thus, we recommend artificial water-holding container management as a strategy to control *Ae. aegypti* and hence the arboviral diseases transmission.

Keywords *Aedes aegypti*, Afar Region, Ethiopia, Habitat characteristics, Occurrence

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Introduction

Human infections associated with *Aedes* mosquito-transmitted viruses such as dengue virus (DNV), yellow fever virus (YFV) chikungunaya (CHIKV), and zika virus (ZIKV) are spreading at an alarming rate. They exert a huge burden on populations, health systems, and economies in tropical countries [1]. Dengue fever alone is endemic in more than 125 countries, and the number and geographic distribution of the cases have increased significantly in recent years [2]. Africa is considered as an epicentre for the emergence and re-emergence of life threatening arboviruses such as DNV and CHIKV particularly in East African countries [3]. They have dramatically increased over the last two decades [4]. Several arboviral infection outbreaks have been reported from various parts of Ethiopia since the 1960s. These include dengue fever outbreaks in Gewane district of Afar Region [5], yellow fever in South Omo [6], dengue and chikungunya in Dire Dawa city administration and Somali Region [6–8].

Determinants of the increasing trend of the pathogenic arboviruses and their mosquito vectors are thought to be unprecedented urbanizations combined with inadequate solid waste management, the global movement of people and goods, and most importantly the continuing global climate change [3, 9]. Furthermore, it is also suggested that the global temperature increase may increase the environmental suitability for dengue and other vector-borne arboviral diseases [10].

Aedes aegypti is an efficient vector for a number of arboviral diseases [11]. In Ethiopia, *Ae. aegypti* is considered to be a major vector of viral diseases in humans [7, 8]. The species breeds in a variety of man-made water-holding containers such as discarded tyres, mud pots, discarded sinks, polythene sheet, plastic bowl, and buckets [12], sometimes in indoor water storage containers [13, 14] but mainly outdoors [15]. It breeds abundantly during wet seasons and is strongly associated with climatic factors such as rainfall, humidity, and temperature [16]. Abundance of *Aedes* larvae and associated viral diseases transmission risk can be described as house index (HI), breteau index (BI) and container index (CI) [17, 18].

In the absence of effective therapeutic drugs and vaccines against many of the *Aedes*-borne viral diseases, surveillance and control of *Aedes* larvae remains a top priority [19]. Larval control of mosquitoes is advantageous as adult mosquitoes can fly relatively long distances and survive in a wide range of microhabitats [20]. To implement an effective *Aedes* larvae control strategy, there should be adequate knowledge of its spatio-temporal distribution including habitat location and productivity, seasonal occurrence, habitat type, exposure to sun light, habitat cover and shade, distance from the nearest house, temperature and rainfall [21–24]. In addition, knowledge of chemical characteristics of larval habitats such as pH, conductivity, total alkalinity, hardness, total dissolved solids, dissolved oxygen, and ammonia are also central in prioritizing the larval control and prevention strategies. Although, there have been repeated reports of mosquito-borne viral disease outbreaks in the southern part of the Afar Regional State of Ethiopia, spatio-temporal distribution, habitat characteristics and species composition of *Aedes* larvae remain poorly understood. Thus, this study aimed to assess the spatio-temporal occurrence, habitat characteristics and the species composition of *Aedes* larvae in selected towns of the Afar Regional State, Ethiopia.

Materials and methods

Study areas

Larvae and pupae of *Aedes* species were surveyed in Awash Arba, Werer, and Awash Sebat towns, Gabi-Rasu Zone, Afar Regional State, Ethiopia from May 2022-April 2023. Awash Arba town is located at about 226 kilo metres (k.m.s) at 9.141111°N, 40.15889°E and 720–1100 above sea level (m.a.s.l), Werer at 256 kms at 9.33453°N, 40.181385°E and 720–1100 m.a.s.l and Awash Sebat at 214 km at 8.98810°N, 40.163936°E and 820–1120 m.a.s.l all to the Northeast of Addis Ababa (Fig. 1). Awash Arba and Werer towns are found in the Amibara district while Awash Sebat town is found in the Awash Sebat administration. Awash Arba and Werer are semi-arid with temperature of 25–35 °C and an average annual rainfall of 530 mm. Awash Sebat experiences 22.6 to 30.6 °C of annual temperature and 606.6 mm of mean annual rainfall [25]. More than 75% of the inhabitants in the towns are pastoralists and agro-pastoralists.

The climate in Afar Regional State is broadly divided into wet and dry seasons. The wet season (locally referred to as Hagaya) the main rainy season of the region spans from June to September while the long dry season (locally known as Gilal) covers the months from October to May [26]. The residents of the three towns store water using man-made containers such as drums, jerrycans and cement tanks at their domestic and peri-domestic areas due to the scarcity of water. Water in the containers was mainly harvested from rainwater and piped water.

Study design

The three towns were selected purposively in consultation with the local Health Bureaus and on the basis of the recent repeated dengue fever and chikungunya reports. There were 2238 houses in Awash Arba, 3033 in Werer and 3149 in Awash Sebat (Amibara District and Awash Sebat Town Health Bureau, 2022, unpublished). Longitudinal prospective study design was used to collect *Aedes* larvae/pupae. A total of 240 surveys were undertaken

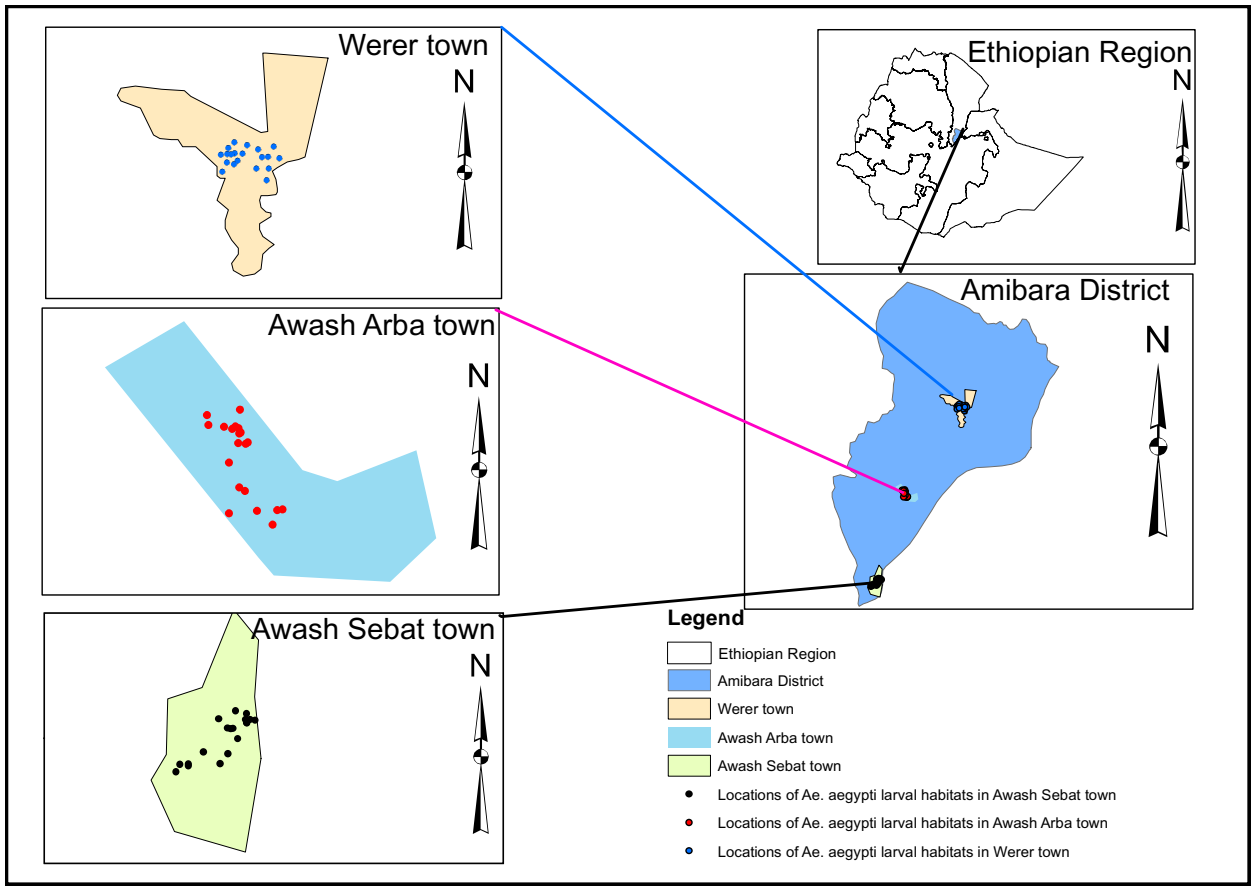

Fig. 1 Map of Study areas: Awash Arba, Awash Sebat and Werer Towns, Southern Afar Region, Ethiopia (Source: Ethio_GIS, 2023)

in each town (20 houses and their environs per month) over a year. To determine the house interval, the total residential houses of each town were divided by 240 total houses to be surveyed throughout the sampling period. For instance, 2238/240 for Awash Arba, 3033/240 for Werer and 3149/240 for Awash Sebat and gave 9, 12 and 13 intervals for each town respectively. The residential houses were categorized into blocks with the minimum distance between blocks being about 300 meter (m). The blocking of the residential houses was started from the downtown to the peripheral areas to make larval/pupal collection suitable. Thus, the first house was selected randomly from the total of five randomly selected residential houses. The next house was selected systematically using the calculated house interval for each town. To avoid overlapping of the breeding sites among the selected houses and to increase the chances of getting larvae/pupae of *Aedes* species (considering the flight ranges of *Aedes* species); about 300 m distance was added before counting the house interval. *Aedes* larvae/pupae surveys were carried out in relation to each selected house in the following manner. (1) domestic area (inside the house rooms) and outside the house within a radius of about 50 m; (2) Outside the house compound between 50 and 300 m (peri-domestic area) with modification of Minakawa et al. [27].

Aedes larvae/pupae collection and rearing

All available water-holding containers such as used tyres, water storage drums, cement tanks, and flower pots were inspected [28] for the presence of mosquito larvae/pupae and their coordinate readings were recorded using a hand-held global positioning system unit (GPS) (Garmin GPS 60, Garmin international) (Fig. 2). Physical and chemical characteristics of the habitats were recorded during larval collections using a form prepared for the purpose.

A minimum of 10 dips of water samples were collected for larvae/pupae from relatively large water-holding containers using standard dipper (350 ml). Ladles and pipets were used to collect mosquito larvae/pupae from relatively smaller habitats. During the surveys, each water-holding container was classified as positive (if containers harboured at least one mosquito larvae/pupae)

Fig. 2 Selected artificial water-holding containers surveyed for *Aedes* larvae/pupae in Awash Arba, Awash Sebat and Werer towns, Ethiopia from May 2022 to April 2023: (**A**) Tyres, (**B**) Water storage drum, (**C**) Water tank made of cement (**D**) Flower pots, (**E**) Plastic bowl (**F**) Discarded plastic, (**G**) Plastic drum, (**H**) Jerrycan, (**I**) Polythene sheet

or negative (if it did not harbour any mosquito larvae/pupae). Larvae and pupae collected from each positive container were categorized to their respective genus, counted, transferred to a plastic-jar labelled with the date of collection and town, transported to the field insectary in Awash Sebat town where larvae and pupae were transferred to white enamel trays and reared to adults. In the field insectary, larvae in the trays were given powdered fish food (®/TM/©2019 Germany) and covered with netting until they changed to pupae. Then, pupae were transferred to beakers and placed in netting cages (30 × 30 × 30 cm3) with a 10% sugar solution, reared to adults and identified to species morphologically under a standard dissecting microscope following taxonomic keys [29–31].

Larval and pupal habitat characterisation

Physical habitat characteristics measured larval/pupal habitat location (domestic and peri-domestic), water volume, presence/absence of emerging vegetation, source of water, usage of water, turbidity of water, substrate type, and habitat permanence were recorded. Substrate was categorized as mud, sand, gravel with soil

and cement. A larval habitat was considered permanent if it harboured water throughout the year and semi-permanent contained water for approximately 2 to 3 months. Similarly, temporary habitats those which stored water for a short period of time up to 2 to 3 weeks [32]. Distance between water-holding container (habitat) and the nearest house, and between habitat and nearest plant (tree or shrub) were recorded [33]. Habitat temperature was recorded at the time of collection using ordinary thermometer. The turbidity of water was determined as clean or turbid after taking water samples in glass test tubes and holding them against a white background [27].

Habitat exposure to sunlight was observed visually and recorded as shaded, partially shaded or exposed fully. The presence of aquatic vegetation was observed and recorded as present or absent. Distance of larval/pupal habitat to the nearest house was measured using a tape (meter). About 250 ml water was collected from larvae/pupae positive habitats using polyethylene bottles, transported with cold boxes and analysed for habitat chemical characteristics such as alkalinity, salinity, conductivity, total dissolved solids, dissolved oxygen and total hardness at the Chemistry Department, Addis Ababa University with the permission of the Africa Center of Excellence for Water Management, using a standard method of water examination [34].

Statistical analysis

Data were analysed using SPSS version 20. Mean larval/pupal density of *Ae. aegypti* for each container was calculated by dividing the total number of larvae or pupae to the number of dips. Prior to data analysis, data were log-transformed [log (n+1)] to fit the normal distribution curve and checked for normality by the Shapiro–Wilk test. Independent T test was used to determine the mean density of *Ae. aegypti* between two groups of physical characteristics of larval habitats and One-way analysis of variance (ANOVA) was used to compare mean larvae/pupae densities of *Ae. aegypti* of more than two groups of physical characteristics among the habitats. When significant differences were observed in using ANOVA, Tukey's post-hoc test was used for pairwise comparisons of the means [35]. Pearson correlation was used to assess the correlations of *Ae. aegypti* larvae/pupae and habitat chemical characteristics. Bivariate analysis was performed to assess associations between habitat positivity for *Ae. aegypti* larvae/pupae and physical characteristics of larval habitats. Then, multiple logistic regression was carried out to determine key predictors. The odds ratio (OR), 95% confidence intervals (95% CI) and p value were determined. In addition, multiple linear regression was used to assess the relations between *Ae. aegypti* larvae/pupae density and the habitat chemistry of water-holding containers. All p values < 0.05 were considered statistically significant. The larval/pupal infestation level was computed using indices namely, House index (HI), Container index (CI), Breteau index (BI) and Pupal index (PI) [36].

$$\text{House index} = \frac{\text{Number of positive house}}{\text{Number of house inspected}} \times 100$$

$$\text{Container index} = \frac{\text{Number of positive containers}}{\text{Number of containers inspected}} \times 100$$

$$\text{Breteau index} = \frac{\text{Number of positive containers}}{\text{Number of house inspected}} \times 100$$

$$\text{Pupal index} = \frac{\text{Number of pupae}}{\text{Number of house inspected}} \times 100$$

Results

Occurrence of *Aedes aeygpti* larvae/pupae

All of the *Aedes* larvae/pupae that emerged to adults were morphologically identified as *Aedes aegypti* and hence all *Aedes* larvae and pupae collections are hereafter considered to be *Ae. aegypti.* A total of 11,440 larvae/pupae were collected, of which 79.5% (n = 9099) were *Ae. aegypti*. Among the 9099 larvae/pupae, 53.6% (n = 4875)

Table 1 Mosquito larvae/pupae collected from water-holding containers from Awash Sebat, Awash Arba and Werer towns of Afar Regional States, Ethiopia, May 2022- April 2023

Study sites	THS (+ve)	TCI(+ve)	Mosquito genera/species			Total *n* (%)
			Ae. aegypti n(%)	*Anopheles n*(%)	*Culex n* (%)	
Awash Sebat	240 (93)	375 (275)	4875 (53.56)	606 (76.04)	643 (41.65)	6124 (53.53)
Awash Arba	240 (45)	142 (121)	2687 (29.54)	162 (20.32)	160 (10.36)	3009 (26.30)
Werer	240 (21)	82 (62)	1537 (16.9)	29 (3.64)	741 (47.99)	2307 (20.17)
Total	720 (159)	599 (459)	9099 (100)	797 (100)	1544 (100)	11,440 (100)

TCI, Total containers Inspected THS, Total Houses Surveyed; + ve, Positive houses and containers

were collected from Awash Sebat, 29.5% ($n=2687$) from Awash Arba, and 16.9% ($n=1537$) from Werer (Table 1). A total of 1544 *Culex* larvae/pupae were collected, among which 47.99% ($n=741$) were from Werer, 41.6% ($n=643$) from Awash Sebat, and 10.36% ($n=160$) from Awash Arba. Moreover, 797 *Anopheles* larvae/pupae were collected, of which 76% ($n=606$) were from Awash Sebat, 20.3% ($n=162$) from Awash Arba, and 3.6% (n = 29) from Werer towns (Table 1).

Productivity of *Ae. aegypti* larvae/pupae with container types and location

In Awash Arba town, 97.1% (2610/2687) of the *Ae. aegypti* larvae/pupae were collected from domestic sites and 2.9% (77/2687) from the peri-domestic sites. Similarly, in Werer, 91.8% (1411/1537) of the total *Ae. aegypti* larvae/pupae collections were from domestic sites and 8.2%(126/1537) from the peri-domestic sites. Likewise, in Awash Sebat town, 72.5% (3533/4875) of the total *Ae. aegypti* catches were made from domestic and 27.5% (1342/4875) from peri-domestic sites (Table 2).

The highest *Ae. aegypti* larvae and pupae collections in all the towns was made from water-holding tyres. In Werer, domestic water-holding tyres harboured 87.9% (1351/1537) of *Ae. aegypti* larvae/pupae collections and 73.5% (1974/2687) in Awash Arba. In the domestic sites of Awash Sebat town, 30.5% (1488/4875) of the *Ae. aegypti* were collected from water-holding tyres, 20.7% (1011/4875) from water-storage drums, and 15.8% (764/4875) from water tanks made of cement. Discarded plastic contributed 2.2% (106/4875) of the *Ae. aegypti* larvae/pupae in Awash Sebat and 1.6% (44/4875) in Awash Arba whereas flowerpots had 3.3% (164/4875) in the domestic areas of Awash Sebat (Table 2).

Larval/pupal indices

Overall, 720 surveys were made during the 12 months, of which 460 resulted in at least one positive water-holding container for *Ae. aegypt, Anopheles* or *Culex* larvae/pupae (Table 3). 15.8% ($n=114$) of the surveys were associated with *Ae. aegypti* larvae/pupae in domestic and peri-domestic sites. The container indices (CIs) ranged from 8.3 to 85.3% in Awash Sebat, 5 to 91.8% in Awash Arba and 33.3 to 88.4% in Werer. The House Indices (HIs) were also from 5–80% in Awash Sebat, 5–75% in Awash Arba and 5–35 in Werer. Similarly, Breteau Indices (BIs) were 5–405 in Awash Sebat, 5–225 in Awash Arba and 10–190 in Werer. The highest CIs of Awash Sebat (85.3%) and Awash Arba (75%) were observed in September 2022 and that of Werer (35%) in August 2022. In addition, relatively higher House Index (80%) was observed in Awash Sebat during August, as compared to Awash Arba (55%) and Werer (35%) sites. Moreover, higher Breteau Index (405) and Pupal Index (1320) were recorded in Awash Sebat in September than Werer and Awash Arba areas.

Spatial and temporal distribution of *Ae. aegypti* larvae/pupae

The spatio-temporal distribution of *Ae. aegypti* larvae/pupae varied between the dry and wet season in the study towns. The peak *Ae.aegypti* larvae/pupae collections in Awash Sebat and in Awash Arba towns were made during September 2022 and in Werer town during August 2022 (Fig. 3).

Association of *Ae. aegypti* larval/pupal density and occurrence with physical characteristics

The results of mean comparisons of the physical characteristics and densities of the *Ae. aegypti* larvae/pupae were depicted in Table 4. Significantly higher mean densities of *Ae. aegypti* larvae/pupae were collected during the wet season ($F=15.075$, $p<0.001$), from tyre habitat types ($F=4.775$, $p=0.001$), habitats with gravel with soil substrate ($F=7.085$, $p<0.001$) and rain water sources ($F=6.020$, $p=0.003$). Significant differences were observed in *Ae. aegypti* mean larval density with substrate types. Further, Tukey's post-hoc test indicated that water-containers with mud and gravel with soil substrates had significantly higher *Ae. aegypti* larval/pupal densities compared to cement substrates ($p<0.001$). In addition, significant mean differences were observed between tap and rain water sources ($p=0.002$).

The bivariate analysis revealed that containers located at domestic sites, rain water source, shaded and partially shaded habitats, water-holding tyres, water storage drums, mud and gravel with soil substrates were significantly associated with the occurrences of *Ae. aegypti* larvae/pupae. On the other hand, dry season was less likely to harbour *Ae. aegypti* (Additional file 1). Moreover, the multiple logistic regression analysis showed that occurrences of *Ae. aegypti* larvae/pupae were more likely found in tyres (AOR = 15.89, CI = 3.55–71.09, $p<0.001$) and water storage drums (AOR = 19.84, CI = 4.64–84.89, $p<0.001$) as compared to other habitats. In addition, containers located in domestic sites were 3.76 times more likely to harbour *Ae. aegypti* larvae/pupae as compared to peri-domestic sites (AOR = 3.76, CI = 1.27–11.12, $p=0.017$) and habitats with tap water source were 0.08 times less likely harboured *Ae. aegypti* larvae/pupae as compared to mixed water source (AOR = 0.08, CI = 0.02–0.31, $p=0.001$) (Table 5).

Table 2 Container types, location and level of *Ae. aegypti* infestation in Awash Sebat, Awash Arba and Werer towns of Afar regional states of Ethiopia, May 2022 to April 2023

Study sites	Container location	Container types	Containers inspected (positive)	*Ae. aegypti n* (%)
Awash Sebat	Domestic	Tyres	98 (83)	1488 (30.5)
		Drums	31 (22)	1011 (20.7)
		Flower pots	14 (10)	164 (3.4)
		Discarded plastics	9 (6)	106 (2.2)
		Others(cement–water tank, bowl, jerrycan)	46 (36)	764 (15.7)
	Peri-domestic	Tyres	127 (79)	992 (20.3)
		Drums	8 (4)	83 (1.7)
		Flower pots	0 (0)	0 (0)
		Discarded plastics	0 (0)	0 (0)
		Others	42 (20)	267 (5.5)
		Sub-total	375 (260)	4875 (100)
Awash Arba	Domestic	Tyres	95 (69)	1974 (73.5)
		Drums	33 (18)	572 (21.3)
		Flower pots	0 (0)	0 (0)
		Discarded plastics	2 (1)	44 (1.6)
		Others	3 (1)	20 (0.7)
	Peri-domestic	Tyres	9 (8)	77 (0.8)
		Drums	0 (0)	0 (0)
		Flower pots	0 (0)	0 (0)
		Discarded plastics	0 (0)	0 (0)
		Others	0 (0)	0 (0)
		Sub-total	142 (97)	2687 (100)
Werer	Domestic	Tyres	53 (37)	1351 (87.9)
		Drums	3 (2)	0 (0)
		Flower pots	0 (0)	0 (0)
		Discarded plastics	0 (0)	0 (0)
		Others	18 (8)	60 (3.9)
	Peri-domestic	Tyres	8 (6)	126 (8.2)
		Drums	0 (0)	0 (0)
		Flower pots	0 (0)	0 (0)
		Discarded plastics	0 (0)	0 (0)
		Others	0 (0)	0 (0)
		Sub-total	82 (5)	1537 (100)
	Overall total	Domestic	405 (294)	7554/9099
		Peri-domestic	194 (116)	1545/9099

Relation of *Aedes* aegypti larval/pupae density with larval habitat chemistry

Positive correlations were observed between *Ae. aegypti* larvae/pupae densities and habitat chemistries including water temperature, total alkalinity, total hardness, electrical conductivity, total dissolved solids, dissolved oxygen and salinity (Additional file 2). Moreover, further multiple linear regression revealed that *Ae. aegypti* larvae/pupae densities showed positive relations with dissolved oxygen ($\beta = 0.523$, $p < 0.001$) and total hardness ($\beta = 0.475$, $p = 0.034$) of the water (Table 6). On the other hand, water temperature seemed to positively influence density of *Ae. aegypti* larvae/pupae but the relation was not statistically significant. Similarly, salinity of water-holding containers was negatively related even if its effect was not statistically significant.

Table 3 *Aedes aegypti* larval/pupal indices in Awash Sebat, Awash Arba and Werer towns of Afar regional state of Ethiopia May 2022 to April 2023

Study sites	Study period	Positive houses	Total houses	Total containers	Positive containers	No. pupae	Indexes			
							CI (%)	HI (%)	BI	PI
Awash Sebat	Jun2022	2	20	18	4	33	22.22	10	20	165
	Jul 2022	6	20	32	9	71	28.1	30	45	355
	Aug2022	16	20	116	69	170	59.5	80	345	850
	Sept2022	14	20	95	81	264	85.3	70	405	1320
	Oct 2022	14	20	86	64	131	74.4	70	320	655
	Nov2022	3	20	8	4	9	50	15	20	45
	Dec2022	2	20	12	3	6	25	10	15	30
	Jan 2023	2	20	3	2	1	66.6	10	10	5
	Feb 2023	1	20	2	1	2	50	5	5	10
	Mar2023	1	20	2	1	3	50	5	5	15
	Apr 2023	1	20	2	1	7	50	5	5	35
	Total	63	240	375	240	699	87.6	26.25	100	291.25
Awash Arba	May2022	0	20	0	0	0	0	0	0	0
	June2022	0	20	0	0	0	0	0	0	0
	Jul 2022	6	20	15	10	0	66.6	30	50	0
	Aug2022	11	20	39	30	89	76.9	55	150	445
	Sept2022	15	20	49	45	89	91.8	75	225	445
	Oct2022	8	20	30	24	83	80	40	120	415
	Nov2022	1	20	2	1	4	50	5	5	20
	Dec2022	2	20	6	3	0	50	10	15	0
	Jan 2023	1	20	2	1	0	5	50	5	25
	Feb 2023	0	20	0	0	0	0	0	0	0
	Mar2023	0	20	0	0	0	0	0	0	0
	Apr 2023	0	20	0	0	0	0	0	0	0
	Total	44	140	143	114	265	35.03	22.08	47.5	112.5
Werer	May2022	0	20	0	0	0	0	0	0	0
	Jun2 022	0	20	0	0	0	0	0	0	0
	Jul 2022	0	20	0	0	0	0	0	0	0
	Aug2022	7	20	43	38	123	88.4	35	190	615
	Sept2022	6	20	24	14	136	58.3	30	70	680
	Oct 2022	1	20	6	2	5	33.3	5	10	25
	Nov2022	0	20	0	0	0	0	0	0	0
	Dec2022	1	20	4	2	5	50	5	10	25
	Jan 2023	0	20	0	0	0	0	0	0	0
	Feb 2023	0	20	0	0	0	0	0	0	0
	Mar2023	0	20	0	0	0	0	0	0	0
	Apr 2023	0	20	0	0	0	0	0	0	0
	Total	15	80	77	56	269	19.2	6.25	23.33	112.08
Overall total		114	460	599	410	1233	47.28	18.19	59.94	171.94

HI, House Index; CI, Container index; BI, Breteau Index, PI, Pupal Index

Discussion

The recent outbreaks of dengue fever and chikungunya virus in the Eastern and North-eastern parts of Ethiopia including the Afar Region became a public health concern [5, 7, 8]. As the majority of such arboviral diseases lack effective therapeutic treatment and vaccines, managing *Aedes* species that transmit the diseases is the preferred controlling strategy. In Ethiopia, limited studies were conducted on larval habitats of *Aedes* mosquitoes with a few months of collections which could overlook the most prolific breeding time [12, 37]. Thus, longitudinal studies to understand the distribution and

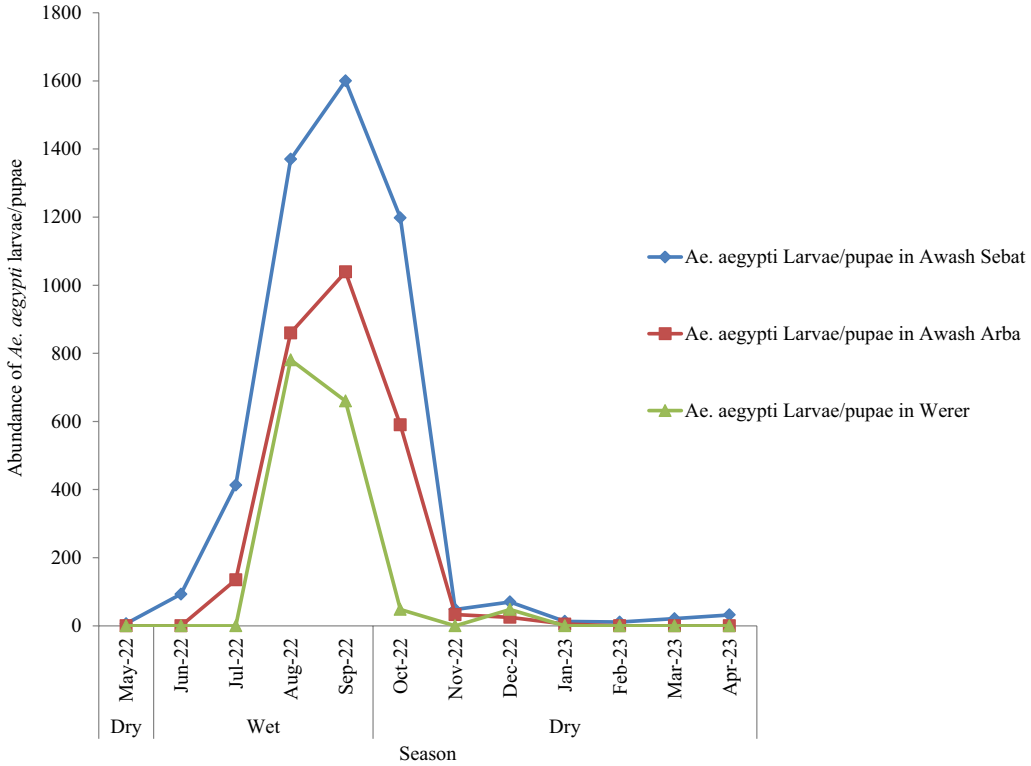

Fig. 3 Spatial and temporal distribution of *Ae. aegypti* larvae/pupae in Awash Sebat, Awash Arba and Werer towns of Afar regional state of Ethiopia from May 2022 to April 2023

habitat characterization of the *Aedes* mosquito remain poorly investigated in the country. This necessitated a longitudinal study of *Aedes* species larval habitats in time and space in the Southern part of Afar region. The study explored diverse *Ae. aegypti* larvae/pupae habitats and characterized them. Thus, the findings serve as a baseline data to the targeted *Aedes* mosquito control interventions and to minimize the risks of *Aedes*-transmitted viral diseases.

Aedes aegypti larvae/pupae were found most abundantly in water-holding tyres in Awash Sebat, Awash Arba and Werer towns of Southern Afar. This was in agreement with previous studies in Dire Dawa City administration [12, 38], in Metema and Humera areas of Northwest Ethiopia [37], and in Kebridehar town of Somalia Regional State [39]. Similar results were also reported from Malaysia [40]. However, the study conducted in Zanzibar city of Tanzania, showed discarded plastics and metals as preferred habitats for *Ae. aegypti* larvae [41]. Tyres as a major *Ae. aegypti* larval habitat in the present study towns could be due to their low level of disturbance, they provide shade and protection for both larvae and adults [42]. For instance, in the current study, *Ae. aegypti* larvae/pupae densities were highest in shaded larval habitats, and shade of breeding habitats was positively correlated to mosquito larvae/pupae as previously indicated [43]. Thus, proper management of water-holding tyres including other potential habitats should be implemented to reduce the breeding of *Ae. aegypti*.

Besides to tyres, water storage drums were also observed to be the second *Ae. aegypti* larval/pupal habitats. Our finding of water storage drums as a breeding habitat of *Ae. aegypti* was comparable with the reports from different parts of the world including Mozambique and Nicaragua of America [44, 45]. In the study towns, the households store water with drums for drinking and domestic use or for house construction purposes. Improper storages of water with domestic containers like drums may serve as the breeding ground for a possible infestation with *Ae. aegypti* larvae/pupae as previously reported [46]. Thus, adequate use of water storage drums could be a strategy to control *Ae. agypti* larvae/pupae.

About 83% (7554/9099) of *Ae. aegypti* larvae/pupae collections were made from the domestic sites of residential houses while the 17% (1545/9099) were from the peri-domestic sites. The abundance of *Ae. aegypti* larvae/pupae mostly close to human habitations in domestic and peri-domestic breeding habitats were also reported previously [47]. However, no *Ae. aegypti* larvae/pupae were

Table 4 Physical characteristics of water-holding containers and mean density of *Ae. aegypti* larvae/pupae in Awash Sebat, Awash Arba and Werer towns, Afar regional state, Ethiopia, May 2022 to April 2023

Characteristics	Variables	Mean ± SE	F	*P*-value
Season	Dry	1.47 ± 0.07	15.075	< 0.001
	Wet	1.84 ± 0.03		
Substrate types	Mud	1.75 ± 0.05	7.085	< 0.001
	Sand	1.59 ± 0.13		
	Gravel with soil	1.76 ± 0.05		
	Cements	1.09 ± 0.12		
Sun light Exposure	Shaded	1.75 ± 0.11	0.125	0.883
	Partially shaded	1.71 ± 0.04		
	Open	1.67 ± 0.07		
Habitat permanency	Semi-permanent	1.67 ± 0.09	2.493	0.462
	Temporary	1.74 ± 0.04		
Habitat type	Flower pots	1.42 ± 0.25	4.775	0.001
	Drums	1.56 ± 0.08		
	Discarded plastics	1.43 ± 0.11		
	Tyres	1.84 ± 0.03		
	Others(Cement tank)	1.56 ± 0.14		
Turbidity	Clean	1.60 ± 0.07	8.299	0.043
	Turbid	1.77 ± 0.03		
Water usage	Sometimes	1.65 ± 0.05	5.311	0.136
	Not used	1.77 ± 0.04		
Water source	Tap water	1.52 ± 0.08	6.020	0.003
	Rain water	1.82 ± 0.03		
	Mixed water	1.75 ± 0.09		
Habitat location	Domestic	1.86 ± 0.08	0.338	0.126
	Peri-domestic	1.70 ± 0.03		
Presence of vegetation	Present	1.72 ± 0.06	0.006	0.823
	Absent	1.70 ± 0.04		

SE, Standard error

collected from inside the house rooms. This result was contrary to previous research findings from Sudan and in Western and coastal Kenya in that they reported *Ae. aegypti* larvae and pupae from inside residential rooms [19, 48]. The absence of *Ae. aegypti* larvae/pupae inside the house rooms in the present survey could be due to the fact that the hygienic condition of water-holding containers found inside the house rooms which were cleaned by the households frequently. They were also covered which make the containers being unproductive to *Aedes* mosquito immature as previously reported [49]. Overall, the collection of *Ae. aegypti* larvae/pupae in higher number and proportions were from the domestic sites followed by peri-domestic sites. Thus, emphasis should be given to domestic sites followed by the peri-domestic sites in surveying and managing *Ae. aegypti* larvae/pupae in the study areas.

Table 5 Relationship between *Ae. aegypti* larvae/pupae occurrences and larval habitat physical characteristics in Awash Sebat, Awash Arba and Werer towns of Afar regional states of Ethiopia, May 2022 to April 2023

Habitat characteristics	Variables	AOR (95% CI)	*p*-value
Season	Wet	1	
	Dry	0.60 (0.24–1.46)	0.260
Water source	Mixed	1	
	Tap	0.08 (0.02–0.31)	< 0.001
	Rain	1.48 (0.10–2.27)	0.353
Substrate types	Cement	1	
	Mud	0.86 (0.19–3.72)	0.837
	Sand	0.70 (0.08–6.29)	0.752
	Gravel with soil	1.03 (0.32–3.34)	0.961
Sun light exposure	Exposed fully	1	
	Shaded	5.33 (0.57–49.47)	0.141
	Partially shaded	0.47 (0.13–1.61)	0.227
Habitat types	Other (water tank made of cement)	1	
	Drum	19.84 (4.64–84.89)	< 0.001
	Discarded plastics	2.65 (0.18–38.23)	0.474
	Tyres	15.89 (3.55–71.09)	< 0.001
Habitat location	Peri-domestic	1	
	Domestic	3.76 (1.27–11.12)	0.017

AOR, Adjusted odds ratio; CI, Confidence interval

Table 6 Relationship between habitat chemistry and *Ae. aegypti* larval/puae density in Awash Sebat, Awash Arba and Werer towns of Afar regional states of Ethiopia, May 2022 to April 2023

Variables	Standard error	Beta	t	*p*-value
Water temperature(°C)	0.017	0.130	1.506	0.135
Total hardness(mg/l)	0.002	0.475	1.890	0.034
Dissolved oxygen(mg/l)	0.028	0.523	4.077	< 0.001
Salinity (%)	0.675	− 0.494	− 1.714	0.089

R2 = 0.289; Adjusted R2 = 0.262; F (10.583)

Estimating *Aedes* species infestation status using indices like CI, HI, and BI is important to measure the success of vector control strategies [50]. The CI, HI, and BI values observed during the wet season, especially in the months of July 2022 to October 2022 were exceeded 5% in the study towns. The BI and HI values observed were also higher than the findings from the previous studies from Tanzania [51] and Cameroon [52]. However, the average CI, HI, and BI reported in the present study were relatively lower than indices previously reported from Dire Dawa City administration [12]. Thus, index values of *Ae. aegypti* species estimated, were high compared to the

World Health Organization epidemic thresholds of transmission risk established for yellow fever [53], for dengue and other arboviruses [54]. The high infestation indices of *Ae. aegypti* suggest a risk for large outbreaks of arbovirus infections such as dengue, yellow fever and chikungunya in the Southern Afar Region of Ethiopia. Thus, *Aedes* species habitat management practices should be implemented in the study area to reduce the *Aedes* mosquito density and prevention of arboviral diseases outbreaks.

The average pupae indices (PIs) observed in the present study were higher compared to the Bacongo and the M'flou areas of Republic of Congo [50]. However, lower than from previous study in Dire Dawa City administration, Ethiopia [38]. The pupal index was included in the study as it has more epidemiological significance [28]. Including pupal indices in the *Aedes* species breeding sites preference is important to quantify *Aedes* species infestation and predicts epidemiological risks as it gives numeric figures by dividing number of pupae in each site per hectar, per houses and per person [55]. In addition, pupal indices are also vital since the relationship between pupal densities and adult densities are usually directly proportional [56].

The results from the analysis of variance (ANOVA) indicated that the mean density of *Ae. aegypti* larvae/pupae were influenced by the physical characteristics such as wet season, turbid water, gravel with soil substrate type, rain water source, and tyre habitat type. Similarly, the abundances of *Ae. aegypti* larvae/pupae were increased from August 2022 to October 2022 then declined towards April 2023 in all the study towns. The results suggest that the wet season contributed to the increased in the larvae/pupae density and abundance. Similar findings were observed in Tanzania, where more *Ae. aegypti* larvae collections were undertaken during the wet season [51]. Wet season as major *Ae. aegypti* larvae collection in the present study areas could be due to the fact that the increase in the number of water-holding containers as a result of the availability of local rainfall, temperature, and humidity of the study sites. For instance, the Afar Regional State received a large amount of rainfall during the months of June to early October and the rest of the months are dry [26]. Thus, particular emphasis should be given to apply the larval source management especially during the wet season in the study areas [57]. However, the *Ae. aegypti* larvae/pupae collected during the dry season were not undermine since breeding sites present during dry season serve as a reservoir which may have an impact on the spread of mosquito-borne diseases during the wet season [58].

The types of substrate present in the water-holding containers was also the determinant factor to the density of *Ae. aegypti* larvae/pupae. The highest *Ae. aegypti* larvae/pupae density was observed in gravel with soil substrate than other water-holding containers. This may be due to differences in the organic content of the substrates of breeding containers. In the current study, higher density of *Ae. aegypti* larvae/pupae were also associated with turbid water than the clean water. Inline with this, the study conducted in the Central African Republic observed that *Ae. aegypti* larvae/pupae were observed in turbid water [33]. However, contrary to the present finding, the study conducted in Tanzania reported that higher *Ae. aegypti* immatures were collected from containers with clean water than turbid water[51]. The existence of high density of *Ae. aegypti* larvae in turbid water in the present study may be due to turbid water may contained detritus which serve as food to the larvae or it also aid to prevent the larvae from aquatic predators by hiding them [33].

Further, the multiple logistic regression analysis identified key predictors including tyres and water storage drums, domestic sites and tap water source for the occurrences of *Ae. aegypti* larvae/pupae. For instance, water-holding tyres and water storage drums were significantly associated with *Ae. aegypti* larvae/pupae occurrence. Similar findings were reported in Zanzibar, tyre as a major risk factor for the occurrence of *Ae. aegypti* larvae/pupae habitat [59]. Tap water source of the habitats was negatively associated to the occurrences of *Ae. aegypti* larvae/pupae. This was in agreement with the study conducted in Brazzaville Congo [50]. In addition, the occurrence of *Ae. aegypti* larvae/pupae was more significantly associated with containers located in domestic sites than in the peri-domestic sites. This could be due to the existence of domestic containers for longer period in the study areas.

The multiple linear regression result revealed that significantly positive relations were observed between *Ae. aegypti* larvae/pupae density and dissolved oxygen in water-holding containers. Similar previous findings were observed in the Kinshasa area of Democratic Republic Congo [60]. Moreover, *Ae. aegypti* larvae/pupae densities were also positively related with total hardness of water-holding containers. This result was in agreement with the study conducted in Northern Iran, where total hardness of the breeding containers had a positive relations on increase larval density [61]. On the other hand, water temperature seemes to positively influence density of *Ae. aegypti* larvae/pupae but the relation was not statistically significant. Similarly, salinity of water-holding containers was negatively related even if its effect was not statistically significant. Thus, understanding the correlations among larval habitat characteristics and *Ae. aegypti* larval/pupal densities suggest the effect of these factors on the productivity of *Ae. aegypti* and which in turn

can be used to proposed future control and management strategies for *Aedes*-borne diseases.

Limitations of the study

The study mainly considered characterizing the abiotic factors (physicochemical characteristics) of *Ae. aegypti* larvae. Thus, further research should be inspired to include the biological entities influencing the density of *Ae. aegypti* larvae/pupae in their breeding habitats. In addition, research should also extend to investigate the physicochemical parameters since we measured these parameters during the wet season. Molecular identification of *Ae. aegypti* species and sub-species were not performed due to logistics constraints such as the unavailability of primers and reagents.

Conclusion

Aedes aegypti larvae and pupae occurred in a wide range of water-holding containers in domestic and peri-domestic areas. The major *Ae. aegypti* larvae/pupae habitats were water-holding used tyre, water storage drums, water tanks made of cement, flowerpots, and discarded plastics. The productivity of *Ae. aegypti* larval habitats were higher in tyres and drums habitats, which were located at domestic sites. In addition, dissolved oxygen and total hardness of the containers influence *Ae. aegypti* larval/pupal productivity. The values of larval indices (Container index, House index and Breteaux index) observed were highly indicators to undergo vector control campaigns to control *Aedes*-borne diseases and their potential outbreaks in the study areas. Thus, data recorded in the present could serve as baseline to implement vector control programmes, especially in designing of larval sources management in the study areas. Further studies in different zones of the Afar region and Ethiopia should be inspired, where arboviral diseases outbreak experienced with a special focus on the biotic and other abiotic factors in both productive and non-productive larval habitats towards a sound understanding of *Ae. aegypti* larval ecology and application of appropriate larval control measures.

Supplementary Information

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Additional file 1: Table S1. Bivariate analysis of the relationship between *Ae. aegypti* larvae/pupae occurrences and larval habitat physical characteristics in Awash Sebat, Awash Arba and Werer towns of Afar regional states of Ethiopia, May 2022 to April 2023.

Additional file 2: Table S2. Correlation coefficients between habitat chemistry and *Ae. aegypti* larvae/pupae density in Awash Arba, Awash Sebat and Werer towns of Afar Region, Ethiopia, May 2022 to April 2023.

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Author contributions

MS and AA designed the study. MS conducted data collection, analysis and wrote the first draft of the manuscript. EA and AA revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed to support the findings in present study are included in the manuscript and its additional files.

Declarations

Ethics approval and consent to participate

The study was ethically approved by the institutional review board (IRB) of Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Ethiopia with the reference number ALIPB IRB/80/2022. Written permission letters were obtained from the district health bureaus and health centers. The selected households were informed about the objective of the study and their agreements were sought prior to the larvae/pupae surveys and collections.

Consent for publication

Not applicable.

Competing interests

The authors declared that they have no competing interests.

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Publisher's Note

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Resting habitat, blood meal source and viral infection rate of *Aedes aegypti* (Diptera: Culicidae) in the Southern Afar Region of Ethiopia

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Abstract

Background Knowledge of *Aedes* species distribution, preference to feed on humans, and susceptibility to viruses is crucial in preventing transmission of *Aedes*-transmitted viruses. This study aimed to determine resting behavior, blood sources, and viral infection status of *Aedes aegypti* in Awash Sebat, Awash Arba, and Werer towns of Afar Region.

Methods Adult mosquitoes were collected using a Prokopack aspirator between 8:00–14:00 and 15:00–18:00 h both indoor and outdoor of the house. The mosquitoes were sorted by sex, date of collection, collection places, and abdominal status and identified by species/genus using standard keys. Blood meal sources and dengue virus and chikungunya virus infection status of *Ae. aegypti* were determined using ELISA and RT-qPCR respectively.

Result A total of 2,745 adult mosquitoes comprising the genera *Aedes* (1433; 52.2%) *Culex* (1292; 47.1%) and *Anopheles* (20; 0.7%) were collected. The proportion of female *Ae. aegypti* in Awash Sebat (611; 36%) was highest as compared to females *Ae. aegypti* in Awash Arba (172; 33.8%), and in Werer (59; 11%). A higher proportion of outdoor resting of *Ae. aegypti* was caught from tyres rather than other indoor and outdoor locations (314; 37.29%) (X^2 27.374, df = 12; $p = 0.007$). Seasonal and monthly variation was observed in *Ae. aegypti* collection, where the wet season and the months of August 2022, September 2022, and October 2022 had high *Ae aegypti* density. The overall human blood and bovine blood indices of *Ae. aegypti* were 53/145 (36.6%) and 18/145 (12.4%), respectively. Furthermore, dengue and chikungunya viruses were not detected from the *Ae. aegypti* examined.

Conclusion The majority of *Ae. aegypti* collections were made during the wet season from outdoor resting sites, particularly from tyres. Thus, outdoor targeted management of *Ae. aegypti* is recommended as a strategy particularly tyre removal during the wet season, to reduce resting and proliferation of *Ae. aegypti* and hence prevent the risks of *Aedes*-borne disease transmission.

Keywords *Aedes aegypti*, Afar Region, Blood meal, Chikungunya, Dengue viruses, Ethiopia, Resting behavior

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Introduction

Mosquito-borne viral diseases threaten over a billion people and kill millions annually around the globe. They are increasing threats to human survival but are underreported in Africa [1–3]. In Ethiopia, yellow fever outbreak reports from 1960 to 1962 estimated over 100,000 infections and 30,000 deaths [4]. Recently, yellow fever outbreaks were documented in the South Omo area of the country [5]. Dengue fever outbreaks have been reported in Dire Dawa [6], Godey [7], Humera, Metema [8], Arba Minch [9], and Borena [10]. In addition, chikungunya outbreaks have been reported in Dire Dawa, Afar, and Somali regions [11, 12]. Such outbreaks could potentially cause unrecognized and/or underreported deaths owing to the highly limited testing facilities in the country.

Aedes aegypti is a primary vector of viral diseases, including dengue fever, yellow fever, and chikungunya [13]. In Ethiopia, *Ae. aegypti* has been suggested as a vector of yellow fever, dengue, and chikungunya [14, 15]. Moreover, *Aedes bromeliae, Aedes vittatus*, *Aedes hirsutus, Aedes simpsoni* complex, and *Aedes africanus* have been implicated as potential vectors [5, 6, 16, 17]. Other *Aedes* species, including the zoophilic *Aedes albopictus* [18], *Ae. africanus,* and *Aedes luteocephalus,* may also act as secondary vectors of these diseases in Africa [13].

Studies have indicated that the global distribution of *Ae. aegypti* is context-specific and varies with the local environmental and genetic factors [19, 20]. Anthropogenic landscape change (uncontrolled new habitat formation) and high host availability have allowed *Ae. aegypti* to expand beyond its sylvatic origin [21, 22]. In addition, changes in the global temperature and precipitation have also been associated with the expansion of *Aedes* mosquitoes [22, 23]. These factors can shape the behavior and adaptability of the species, which further provides potential to respond to environmental pressures and control measures and can affect disease transmission dynamics and invasion potential [24].

Aedes aegypti has shown variations in resting habitat preference. For instance, it prefers to rest indoors as previously observed in Sri Lanka [25]. On the other hand, the study conducted in Senegal revealed that *Ae. aegypti* preferred outdoor to indoor [26]. *Ae. aegypti* prefers to rest in a wide range of indoor and outdoor resting habitats, including rooms, kitchens, vegetation, used tyres, bricks, and scrap metals [26, 27]. Recent observation indicated that used tyre is reported to be the potential outdoor oviposition sites as observed in Ghana [28] and resting habitats of *Ae. aegypti* as previously reported in Senegal [27]. Thus, knowledge of the resting behavior of *Aedes* species along with the local environmental factors is the basis for designing effective control strategies [29]. However, studies on *Aedes* species resting habitat variability are limited in Ethiopia. Much of the information used to plan control strategies is extrapolated from research outside the country and limited local studies [14–16]. Similarly, such data is also lacking in the Southern Afar Region, where dengue and chikungunya outbreaks have occurred frequently.

Aedes aegypti feeds on mammalian blood for its egg development [30]. The species was also observed to feed on human blood during daylight hours [31], particularly during the early morning and early evening [32]. Knowledge of its blood-feeding preference provides insight into its role as a disease vector [33] and helps inform efficient strategies for control [34]. In Tanzania, Kenya, Senegal, and Cameroon, the species feeds on a wide range of hosts, such as humans, bovines, pigs, dogs, cats, rats, and others [26, 35–37]. The preference of *Ae. aegypti* to feed on humans can be estimated using the human blood index (HBI), which is the proportion of tested mosquitoes that have fed on a human host [36]. A similar method is used for bovine blood meal sources in which the proportion of tested mosquitoes that have fed on a bovine host. An *Ae. aegypti* blood meal sources can be detected using enzyme-linked immunosorbent assay (ELISA) [35], precipitin test [38], and polymerase chain reaction (PCR) [39] methods. While feeding on blood, the mosquito takes viruses from an infected host and transmits it to susceptible hosts, including humans [40]. Transmission of arboviral diseases depends, in part, on the occurrence and abundance of vector mosquitoes [41], mosquitoes feeding behavior, and the replication capability of the viruses in the mosquito and the human host [42]. The viral infection status of *Aedes* species can be detected by indirect immune-fluorescent assay (IIFA), antigen capture ELISA, and polymerase chain reaction [43], of which the latter being most preferred [44].

In the absence of licensed vaccines and effective drugs against most of the arboviral diseases [45], and also local scale variations in the transmission of these diseases, an effective strategy to control the abundance of vector mosquito species is a priority. In turn, a successful vector control strategy depends on knowledge of the behavior and vectorial role of the local *Aedes* species. Therefore, this study assessed resting habitats, blood meal sources, and dengue and chikungunya viruses infection status of *Ae. aegypti* in the Afar Region, Ethiopia.

Materials and methods

Study area and period

Adult mosquito collection was undertaken in Awash Arba, Werer and Awash Sebat towns, Gabi-Rasu Zone, Afar Regional State, Ethiopia once per month in May 2022-April 2023. The study area is described previously [46, 47]. A dengue fever outbreak has occurred during 2019 in Gewane and Amibara districts of the Afar Region

including, the present study towns. About 588 people were affected by the outbreak. In the case of Awash Sebat and Awash Arba, it was a mixed infection of dengue fever and chikungunya [48]. Moreover, a sero-prevalence study conducted in Awash Arba and Awash Sebat town administration in 2022 revealed that the prevalence of acute chikungunya infection was 47.8%, while the prevalence of previous exposure to the disease was 6.3% [49]. There were also unpublished reports of Awash Sebat, Awash Arba, and Werer town health bureaus during 2021, in which case the people suffered from chikungunya and dengue fever diseases (Amibara district and Awash Sebat town administration health bureaus, 2021, unpublished).

There are two seasons in the Afar Regional State of Ethiopia, namely, wet and dry seasons. The wet season (locally referred to as Hagaya) the main rainy season of the region, spans from June to September, while the long dry season (locally known as Gilal) covers the months from October to May [50].

Study design

The study towns were selected on the basis of recent dengue fever and chikungunya as reported by the Ethiopian Public Health Institute [48] and the Amibara district and Awash Sebat town administration health bureaus, 2021, unpublished. A longitudinal prospective study design was employed to collect adult *Ae. aegypti*. A total of 240 repeated house surveys were undertaken (20 houses and their environs per month) in each town over the course of the year. A systematic random sampling technique was used to determine the house interval, in which case the total households of each town were divided by 240 total house surveys during the 12 months. Accordingly, 9 (2238/240) was the interval for Awash Arba, 12 (3033/240) for Werer, and 13 (3149/240) for Awash Sebat. The first house was selected randomly among five houses from downtown, and the subsequent houses were selected at the interval for the town until the required number was achieved. In some circumstances, the houses were constructed closely, and assuming the flight range of adult *Ae. aegypti* from its place of emergence, a minimum of 300 m distance was kept between all the selected houses [51].

Adult mosquito collection, identification and processing

Mosquitoes were collected using a Prokopack aspirator (John W. Hock Co., Gainesville, FL, USA, Model: 1419) between 08:00–14:00 to 15:00–18:00 h [52, 53] from indoors and outdoors of the selected houses. Indoor collection targeted all parts of the inside of the house, including the bedroom, dining room, bathroom, and kitchen. While outdoor collections were made from the available potential resting sites, including tyres, animal shelters, and vegetation.

Collected mosquitoes were anesthetized by freezing and sorted by sex, date of collection, location, and abdominal status and identified according to their genera and species [54–56]. All the collected *Aedes* mosquitoes were morphologically identified as *Ae. aegypti*, whereas the *Anopheles* mosquitoes as *Anopheles gambiae* and *Anopheles stephensi*. While the *Culex* mosquitoes were identified to the genus level as *Culex* spp. Fresh-fed and semi-gravid female *Ae. aegypti* were preserved individually in 1.5 ml Eppendorf tubes, with silica gel for later blood meal source analysis. Male, unfed, and gravid *Ae. aegypti* were pooled, each pool containing 10 to 30 mosquitoes preserved in 1.5 ml Eppendorf tubes and stored at −80 °C for further dengue and chikungunya viruses detection.

Blood meal source identification

Blood meal source detection was performed following previously described methods [57]. The abdomen of freshly-fed or half-gravid female *Ae. aegypti* was cut and put individually into a 1.5 ml Eppendorf tube and ground with 100 µl phosphate-buffered saline (PBS) (Sigma Aldrich, Co., 3050, USA) using a pestle. The pestle was rinsed with 100 µl PBS to make a total of 200 µl final volume. Then, 100 µl homogenate was added to a 96-well U-shaped ELISA plate. 100 µl animal sera was added to the plates as a positive control. Moreover, 100 µl homogenate of unfed *Ae. aegypti* (from ALPB laboratory colony) and 100 µl PBS were added to the plate as negative controls. A similar preparation was made on a different plate for the detection of the human blood meal source of the mosquitoes. The plates were covered and incubated at room temperature for 2 h. After incubation, the well contents were discarded and tapped upside-down five times on tissue paper and washed three times with 200 µl PBS-Tween-20 (Tween 20; Sigma Aldrich USA). This was followed by the addition of 50 µl of host-specific conjugate of human peroxidase conjugate (lot no. 040831; catlog no. 474–1002) or bovine, phosphatase conjugate (lot no. 120108; catlog. No. 14–12-06) to each well and incubated for 1 h at room temperature. Plates were washed three times with 200 µl PBS-Tween 20, and 100 µl of ABTS® (Gaithersburg, MD, 20878, USA) was added to each well and incubated for 30 min. Finally, positive samples, including positive controls, were detected visually. Immediately, using an ELISA reader, the value of each plate was determined at a 405 nm wavelength. Samples were considered positive if absorbance values exceeded two times the mean of three negative controls,

unfed mosquitoes/PBS-blank solution. Human blood obtained from volunteers and cow blood from abattoirs were used as positive controls.

Dengue and chikungunya viruses detection

RNA was extracted from 24 pools of mosquito samples using the TRIzol RNA extraction method as described in [58]. The final elution volume was 100 µl. The purity and concentration of extracted RNA were assessed by using the Nano drop 2000 (Thermo Fisher Scientific). Extracted RNAs were further pooled into 7 pools in equal proportions based on collection season (dry and wet), sex (males and females), and study towns of *Ae. aegypti*. The pooled RNAs were concentrated by using RNAClean XP Beads (BECKMAN COULTER, A63987) and eluted by one-third of the pool volume. In short, 1.8 × RNAClean XP beads were added to the tubes containing the RNAs and incubated at room temperature for 15 min. Then, the tubes were put on a magnetic stand, and the supernatant was discarded after the solution cleared. The beads were washed twice with 80% ethanol, and after complete drying of the ethanol, the tubes were removed from the magnetic stand and eluted with DEPEC-treated nuclease-free water with one-third of the starting RNA volume.

Two-step RT-qPCR was used to detect dengue and chikungunya viruses nucleic acids. First, the pooled and concentrated RNAs were reverse transcribed by using the Supper Script (SSIV) RT kit (ThermoFisher Scientific, Cat. No. 18090010) following the manufacturer's protocol with the random hexamers option. Then, HotStar Taq DNA polymerase (Qiagene, cat. No. 203203) was used for the detection of dengue or chikungunya viruses from the cDNA using the primers and probes. The sequences of the primers and probes were taken from [59], with slight modification of the fluorophores and quenchers for the probes. Due to the company we used for the synthesis of the probes did not have the CAL Flour Orange and Black Hole quencher 1 (BHQ1) plus fluorophore and quencher combination. As a result, we opted for the available FAM, BHQ1 flourophore quencher combination. Since the experiment was single-plex, this change not affected the outcome of the result. Probes for dengue fever was DENV3P_probe FAM-CCCAGCGTCAATATGCTG T-MGB, DENV_3P_QF ACTAGAGGTTAGAGGAGA CCCCC, DENV_3P_QRA GGCGCTCTGTGCCTGGAT T, DENV_3P_QRB TGGCGTTCTGTGCCTGGAAT, for chikungunya, CHIK_NSP2_Probe FAM AAAAGTATC TCCAGGCGG-MGB, CHIK_NSP2_NQF CATCTG CACYCAAGTGTACCA, CHIK_NSP2_NQR GCGCAT TTTGCCTTCGTAATG. The primers and probes were at final concentration of 0.16 µM and 0.08 µM in a total of 25 µl reaction. The amplicon size of the dengue virus was 107 base pairs, while that of the chikungunya virus was 208 base pairs. A previously known clinical sample positive for the viruses and molecular grade water were included as positive and negative control respectively, in the run together with the samples. Thermocycling conditions were 45 cycles of initial denaturation and enzyme activation at 95 °C for 15 min, denaturation at 95 °C for 10 s, annealing and elongation at 60 °C for 1 min.

Data analysis

Data were entered, cleaned, and analyzed using SPSS version 20. The numbers of unfed, fed, and gravid collected with respect to their resting habitats were compared using the Chi-square test. The effect of study towns, resting places, season, and collection time on the abundance of female *Ae. aegypti* was analyzed using a generalized linear model (GLM) with a negative binomial distribution. The risk ratio (IRR), 95% confidence intervals (95% CI), and *p*-value were determined. In addition, the HBI and BBI were calculated as the proportion that fed on human and bovine blood out of the total tested for blood meal sources [23]. The presence of differences between HBI and BBI and indoor and outdoor collections was analyzed using the Chi-square test. Values with $p < 0.05$ were considered significant. The Kruskal–Wallis test was employed to determine differences among monthly *Ae. aegypti* density indices, such as *Ae. aegypti* females per surveyed house (FSH), *Ae. aegypti* females per *Aedes* positive house (FPH) [60], Adult house index (AHI), and Adult Density (AD). During the surveys, each surveyed house was classified as positive if the house and its indoor and outdoor sites harbored at least one adult *Aedes* mosquito or negative (if it did not harbor any adult *Aedes* mosquito).

Results

Mosquito species composition

A total of 2,745 adult mosquitoes comprising the genus *Aedes* (1433; 52.2%), *Culex* (1292; 47.1%) and *Anopheles* (20; 0.7%) were collected (Table 1). The highest number of mosquitoes were collected from Awash Sebat (1698; 61.8%) followed by Werer (538; 19.6%), and Awash Arba (509; 18.5%). The proportion of female *Ae. aegypti* in Awash Seabt (611; 36%) was highest as compared to females of *Ae. aegypti* in Awash Arba (172; 33.8%), and in Werer (59; 11%). *Ae. aegypti* was the most common species in Awash Sebat (1202; 70.8%), whereas *Culex* species were most common in Werer (291; 54.1%) followed by in Awash Arba (145; 48.5%) towns.

Resting habitats and abdominal status of Ae. aegypti

Among the 842 female *Ae. aegypti* (556; 66%), were collected from outdoors and 286 (34%) from indoors (Table 2). Even so, indoor collection in Awash Sebat (280;

Table 1 Mosquito species composition and abundance collected in Southern Afar Region, Ethiopia, May 2022-April 2023

Genus /Species	Sex	Awash Arba n (%)	Werer n (%)	Awash Sebat n (%)	Total N (%)
Ae. aegypti	Female	172 (33.8)	59 (11.0)	611 (36)	842 (30.7)
	Male	71 (13.9)	44 (8.2)	476 (28.0)	591 (21.5)
An. gambiae s.l	Female	3 (0.6)	6 (1.1)	0(0)	9 (0.3)
An. stephensi	Female	1 (0.2)	0(0)	5 (0.3)	6 (0.2)
	Male	1 (0)	2 (0.3)	2 (0.1)	5 (0.2)
Culex spp.	Female	145 (28.5)	291 (54.1)	368 (21.7)	804 (29.3)
	Male	116 (22.8)	136 (25.3)	236 (13.9)	488 (17.8)
Total		509 (100)	538 (100)	1698 (100)	2745 (100)

Table 2 Abdominal status of *Aedes aegypti* female collected from different sites in the Southern Afar Region, Ethiopia, May 2022-April 2023

Towns	Collection Site	Fed n(%)	Unfed n(%)	Gravid n(%)	Semi-gravid n(%)	Total n(%)	*p*-value
Awash Sebat	Rooms	24 (17.3)	170 (25.8)	7 (38.9)	0 (0)	201 (23.9)	0.002
	Kitchen	7 (5.0)	69 (10.5)	3 (16.7)	0 (0)	79 (9.4)	-
	Tyres	48 (34.5)	250 (37.9)	8 (44.4)	8 (30.8)	314 (37.3)	0.007
	Vegetation	9 (6.5)	8 (1.2)	0 (0)	0 (0)	17 (2.0)	< 0.001
	Others	0 (0)	0 (0)	0 (0)	0 (0)	0 (0.0)	0.005
Awash Arba	Rooms	3 (2.2)	0 (0)	0 (0)	0 (0)	3 (0.4)	0.002
	Kitchen	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	-
	Tyres	13 (9.3)	73 (11.1)	0 (0)	1 (3.8)	87 (10.3)	0.007
	Vegetation	9 (6.5)	44 (6.7)	0 (0)	17 (65.4)	70 (8.3)	< 0.001
	Others	5 (3.6)	7 (1.1)	0 (0)	0 (0)	12 (1.4)	0.005
Werer	Rooms	3 (2.2)	0 (0)	0 (0)	0 (0)	3 (0.4)	0.002
	Kitchen	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	-
	Tyres	10 (7.2)	11 (1.7)	0 (0)	0 (0)	21 (2.5)	0.007
	Vegetation	5 (3.6)	2 (0.3)	0 (0)	0 (0)	7 (0.8)	< 0.001
	Others	3 (2.2)	25 (3.8)	0 (0)	0 (0)	28 (3.3)	0.005
	Total	139 (100)	659 (100)	18(100)	26 (100)	842(100)	

33.2%) was much higher than from Awash Arba (3; 0.4%) or Werer (3; 0.4%). Tyres were the most preferred resting places (314; 37.3%) followed by rooms (201; 23.9%) in Awash Sebat, and tyres 87 (10.3%) followed by vegetation (70; 8.3%) in Awash Arba. The abundance of female *Ae. aegypti* in tyres (314; 37.29%) was significantly higher compared to the other collection sites in the three towns (X^2, 27.374, df = 12; $p = 0.007$). Higher proportion of fresh-fed *Ae. aegypti* (48; 34.04%) were collected from tyres than rooms (24; 17.02%). More semi-gravid *Ae. aegypti* were collected from vegetation and tyres whereas gravid *Ae. aegypti* were from tyres in Awash Sebat town.

Average catches of Aedes aegypti across seasons and collection times

Monthly, a total of 60-house surveys were undertaken (20 houses and their indoor and outdoor sites of each town) (Fig. 1). The number of positive houses for *Ae. aegypti* was increased from July 2022 to October 2022, in which the highest number of positive houses were observed during September 2022 (35 houses). The highest number of female *Ae. aegypti* ($n = 383$) was collected in September 2022, followed by October 2022 ($n = 198$) and August 2022 ($n = 119$). The number of female *Ae. aegypti* per surveyed house varied significantly across the months, per positive houses, adult house indices, *Ae. aegypti* adult density as indicated by asterisks (*) in the legend key. The highest female *Ae. aegypti* adult densities, Female *Ae. aegypti* per surveyed house, per positive house were observed during September 2022.

Effect of environment and season on the abundance of female Ae. aegypti

The numbers of *Ae. aegypti* from Awash Sebat (IRR = 16.36, $p < 0.001$) and Awash Arba (IRR = 2.82, $p < 0.001$) towns were significantly higher compared

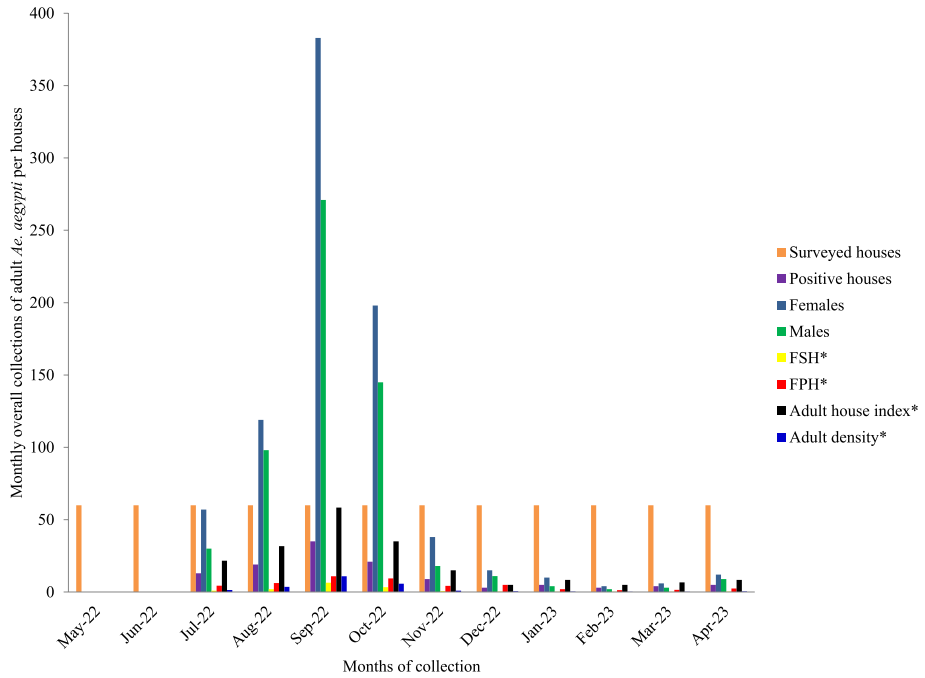

Fig. 1 Monthly overall collections of adult *Ae. aegypti* per houses in Awash Sebat, Awash Arba and Werer towns of Southern Afar, Ethiopia, May 2022-April 2023. Statistically significant changes are marked with asterisks ($p < 0.05$). FSH = *Ae. aegypti* females per surveyed house, FPH = *Ae. aegypti* females per *Aedes* positive house

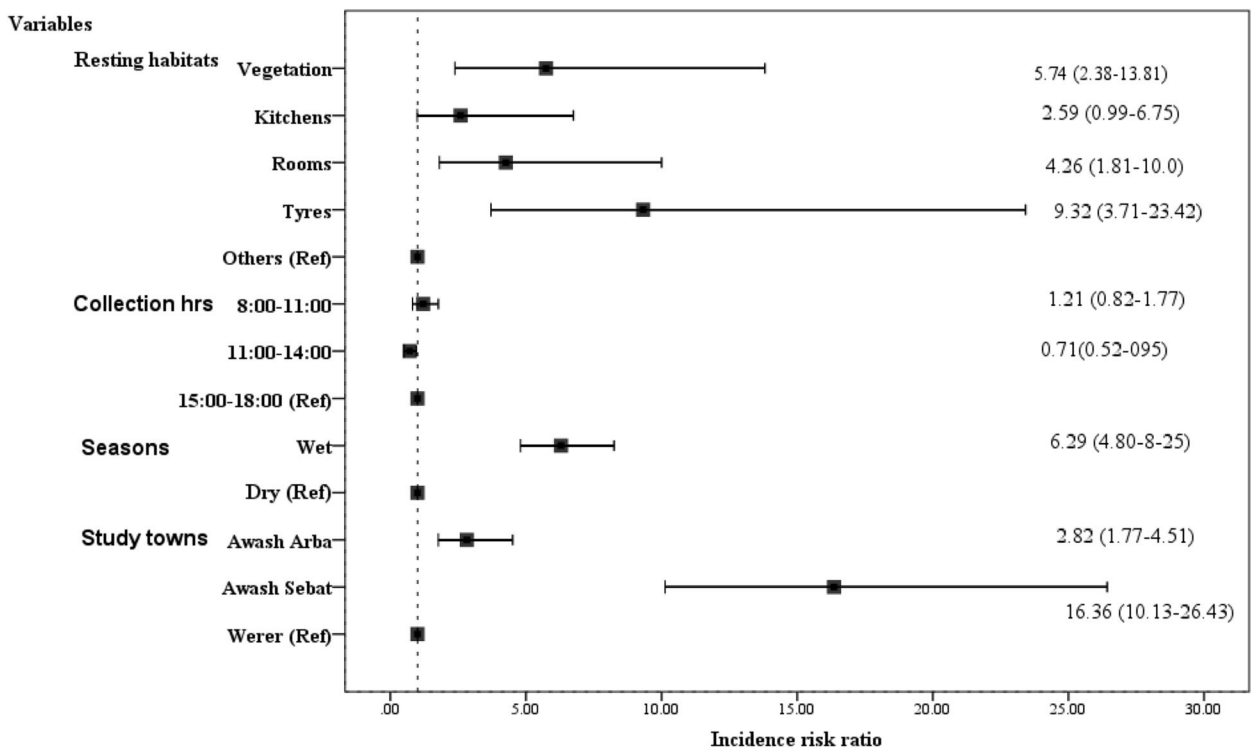

Fig. 2 Effect of environment and time on the number of female *Ae. aegypti* catches in Awash Sebat, Awash Arba, and Werer towns, Southern Afar, Ethiopia, May 2022-April 2023

to the Werer town (Fig. 2). Similarly, significantly more *Ae. aegypti* were collected during wet season compared to the dry (IRR = 6.29, $p < 0.001$), from tyres (IRR = 9.32, $p < 0.001$), rooms (IRR = 4.26, $p = 0.001$) and vegetation (IRR = 5.74, $p < 0.001$). On the other hand, *Ae. aegypti* was less likely collected during 11:00–14:00 collection hours (IRR = 0.71, $p = 0.022$).

Significantly higher females *Ae. aegypti* collections were made during the wet season ($p < 0.001$) (Fig. 3). On the other hand, there were no significant differences observed in *Ae. aegypti* abundance among the collection times ($p = 0.362$).

Blood meal sources of *Ae. aegypti*

A total of 145 freshely-fed and half-gravid *Ae. aegypti* were tested for blood meal source identification of which the overall human blood indices (HBI) were 53/145 (36.7%), while that of bovine blood indices (BBI) were 18/145 (12.4%). Moreover, 15/145 (10.3%) were mixed of human and bovine blood meal sources. The human blood index (HBI) of *Ae. aegypti* ranged from 4 (2.8%) in Werer to 23 (15.9%) in Awash Sebat town among the outdoor collections (Fig. 4). In addition, the HBI ranged from 0% in Awash Arba to 11 (7.6%) in the Awash Sebat among the indoor collections. The bovine blood index of *Ae. aegypti* ranged from 0% in Werer to 11 (7.6%) in the Awash Sebat towns from outdoor collection, and 0% in Werer and Awash Arba towns to 11 (7.6%) in Awash Sebat town from indoors. There was no statistical difference among the human feed and bovine fed between indoor collected (X^2 10.409, df = 6, $p = 0.108$) and outdoor collected *Ae. aegypti* (X^2, 10.106, df = 6, $p = 0.120$).

Detection of dengue fever and chikungunya virus from Ae. aegypti

A total of 1166 *Ae. aegypti* were tested for dengue and chikungunya viruses using RT-qPCR. However, dengue and chikungunya viruses were not detected among *Ae. aegypti* tested (Additional file 1).

Discussion

Knowledge of the resting habitats, blood meal sources, and viral infection status of *Ae. aegypti* has paramount epidemiological importance to reduce the spread of *Aedes*-borne diseases. This is peculiar to a country like Ethiopia, where there are likely real social and logistical impediments in the country. Thus, the insecticide-free removal of mosquito habitat should at least be identified as an intervention that could reduce the abundance of outdoor *Ae. aegypti* as well as the risk of infection. Apart

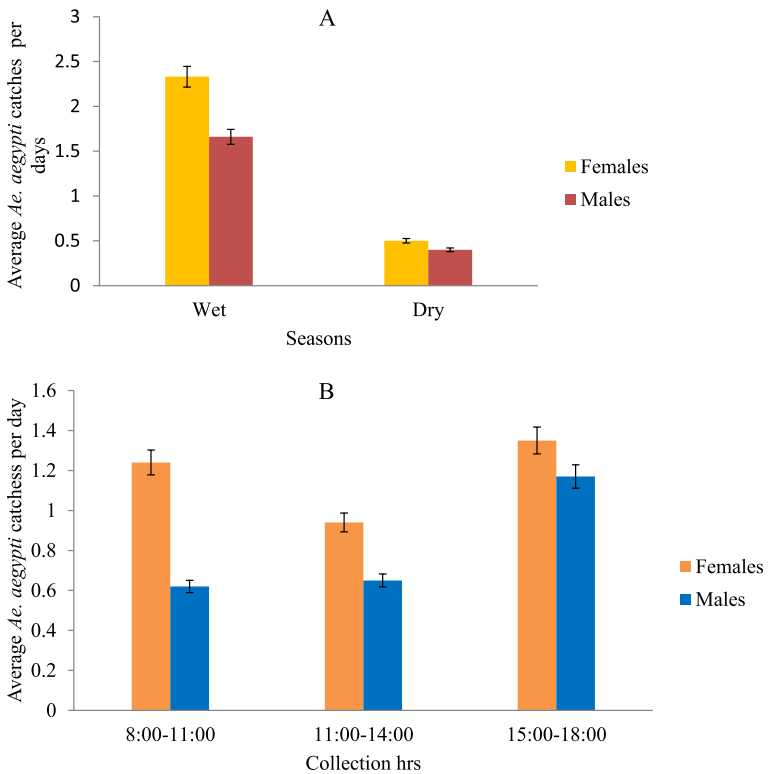

Fig. 3 Average *Ae aegypti* catches per day summarized by season (**A**) and collection times (**B**) in Southern Afar Region Ethiopia, May 2022-April 2023. The error bars represent the 95% confidence interval

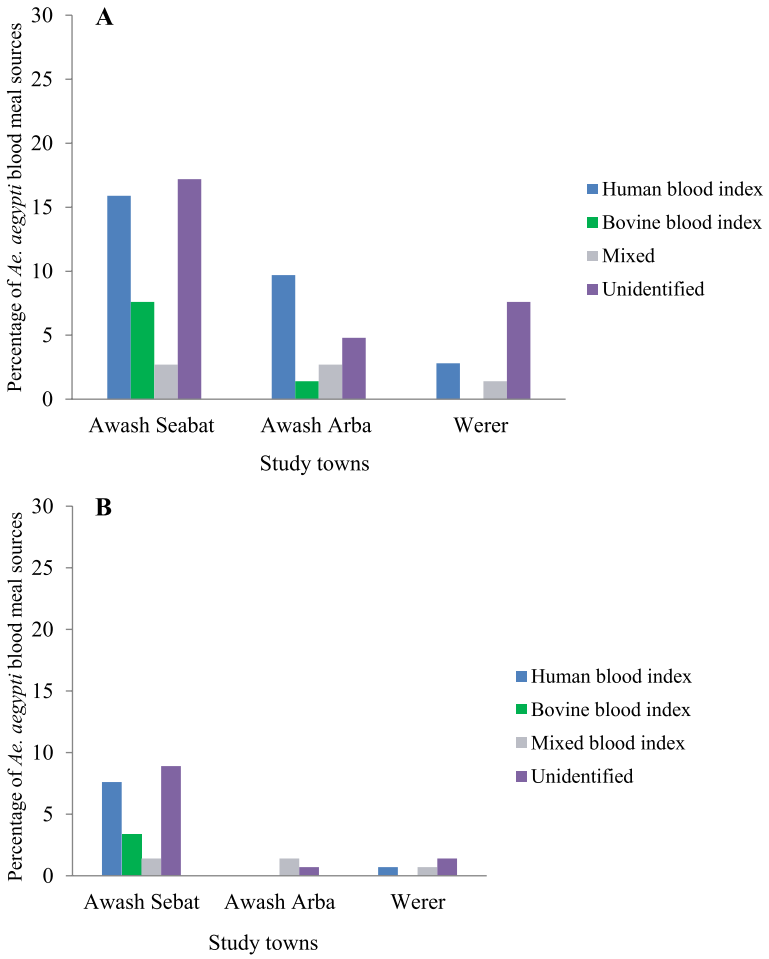

Fig. 4 Blood meal sources of *Ae. aegypti*. Outdoor (**A**) and indoor (**B**) collected *Ae. aegypti* in Awash Sebat, Awash Arba, and Werer towns, Southern Afar Region, Ethiopia, May 2022-April 2023

from resource constraints in getting and utilizing public health insecticides, the identification and control of the local outdoor resting and breeding habitats of *Ae. aegypti* is an eco-friendly approach that reduces the risk of insecticide resistance emergence in *Aedes* mosquitoes [27].

Aedes aegypti were collected in higher numbers from outdoor resting sites such as tyre (422; 50%) and vegetation (94; 11%) than indoors. This indicates that *Ae. aegypti* might prefer to rest outdoors close to human habitations. A relatively lesser proportion (207; 24%) was found resting indoors. Similar findings were reported from Dire Dawa [61], Kenya [62], and Malaysia [63], in which case more *Ae. aegypti* were collected from outdoors than indoors. However, this finding was contrary to other reports from Dire Dawa and Somali Regions of Ethiopia [14, 16], south-eastern Senegal [27], and Colombo Sri Lanka [64], where more *Ae. aegypti* were collected from indoors.

Used tyres were the most preferred outdoor resting habitats of *Ae. aegypti*. This finding was contrary to the study observed in Dire Dawa, where outdoor collection of *Ae. aegypti* was made from open barrels [61]. On the other hand, tyres were also reported as a resting habitat for *Ae. albopictus* and first detections for *Ae. aegypti* as previously observed in Canada [65]. Tyres as major *Ae. aegypti* resting places, in the present study, could be associated with their low level of disturbance, provision of shade, and protection for adults. The outdoor resting habitats of *Ae. aegypti,* such as tyres and vegetation, have important implications for control strategies, particularly during outbreaks of *Aedes*-borne viral diseases. This helps to apply cost-effective public health insecticide to prevent *Ae. aegypti* resting in tyres as previously observed in Darwin, Australia [66]. In addition, identifying tyres as preferred resting habitats helps inform outdoor *Ae. aegypti* habitat management is necessary, especially, tyres removal by local residents and reduce the chance of *Ae.*

aegypti resting and breeding in tyres and hence prevent the risk of *Aedes*-borne disease transmission.

Entomological surveillance uses different types of indices to identify the risk levels of *Ae. aegypti* for *Aedes*-borne disease transmission. Indices based on actual counts of the number of adult females are more accurate [67]. The *Ae. aegypti* adult density values are associated with the risk of *Aedes*-borne disease transmission. In the present study, the adult house index (AHI), female *Ae. aegypti* per surveyed house (FSH), and female *Ae. aegypti* per positive house (FPH) showed that the mosquito was present during the majority of the months. The differences in *Ae. aegypti* adult indices could be attributed to its aggregated distribution pattern in the areas. However, determining adult *Ae. aegypti* density helps for early warning of potential vector population build-up and associated *Aedes*-borne outbreaks. This strengthens the implementation of proactive vector and disease control strategies. This could be done by removing the tyres or other potential outdoor and indoor resting habitats of *Ae. aegypti*.

Understanding the extent of temporal variability is an important factor in vector control. *Ae. aegypti* was most commonly occurred from July to October 2022. This could result from the favorable environmental factors such as rainfall, temperature, and biotic factors during the season [60, 68]. A similar finding was reported in Kinshasa, Democratic Republic of the Congo [69]. The wet season provides ample *Ae. aegypti* breeding sites in the study localities, as previously observed [46]. Mosquito-borne diseases, including dengue fever and chikungunya, have shown seasonality in various areas [70]. Thus, characterizing the density and abundance of *Ae. aegypti* across seasons plays a crucial role in integrated vector management [71].

The number of *Ae. aegypti* caught varied significantly with collection hours. The highest *Ae. aegypti* collections were during 15:00–18:00, whereas the least collections were made in 11:00–14:00. Similar findings were reported in Kinshasa, Congo, where the majority of *Ae. aegypti* collections were made between 15:00–18:00 h [69]. This has implications for the control of *Ae. aegypti* where the times of target vector control tools would be applicable in a specific resting site.

About 53 (36%) of the fresh-fed *Ae. aegypti* fed on human blood, and 18 (12.4%) fed on bovine blood. The difference between the human-fed and the bovine-fed *Ae. aegypti* was not significant. This indicated that *Ae. aegypti* was an opportunistic feeder in the study towns. Similar findings were reported in Kenya [36]. However, the HBI observed in this study was lower compared to previous values from Thailand (92%) and Kenya (51%) [36, 72]. These variations could result from differences in the relative distance and accessibility of hosts and variation in the viral detection methods [36]. The blood meal source of 40.6% (59/145) was not identified. These could most likely feed on other hosts, such as cats, donkeys, chickens, and dogs.

Aedes aegypti was observed to be negative for dengue and chikungunya viruses. The absence of virus-infected *Ae. aegypti* is not uncommon in Ethiopia [15], Tanzania [73], and Kenya [74]. The lack of dengue fever and chikungunya viruses among the tested *Ae. aegypti* might be due to their low number, less probability of getting infected with the viruses, or very low number of positive human hosts to CHIKV and DENV in the towns. Thus, this might not show the absence of dengue fever, chikungunya, and other viral diseases in the towns. Although the probability of getting positive *Ae. aegypti* to chikungunya and dengue viruses is low with small sample sizes, it seems difficult to assert that the risk is high without a circulating virus. However, a study conducted in Singapore indicated that dengue epidemics were recorded in areas with even adult *Ae. aegypti* density per premise of less than 1% [75]. In the present study, the density of *Ae. aegypti* females collected per house especially from August 2022, to October 2022 may trigger arboviral transmission. Thus, the risk of dengue fever, chikungunya, and other viral disease transmissions could be high in the towns.

The limitation of the study is that unidentified blood meal sources of *Ae. aegypti* were observed other than human and bovine in the ELISA test. This was due to a shortage of specific conjugates (IgG) of hosts such as chickens, dogs, cats, rats, and donkeys. Thus, further study should be inspired to identify the other potential hosts of *Ae. aegypti* in the study towns.

Conclusion

Aedes aegypti was the most common species in the towns. Significantly higher numbers and proportions of *Ae. aegypti* were collected from outdoors. The largest numbers of outdoor collections were made from tyres. A higher densities of female *Ae. aegypti* occurred during the months of August 2022, September 2022, and October 2022. *Ae. aegypti* fed on both humans and bovines. We recommend implementations of *Ae. aegypti* control strategies during the months of September, October, and August to reduce the *Ae. aegypti* density and prevent potential transmission of *Aedes*-borne diseases such as dengue fever and chikungunya in the Southern Afar Region, Ethiopia. Moreover, while further targeting and control are critical, the lack of positive DENV or CHIKV in a small collection number also supports regular surveillance in the study towns.

Abbreviations

BBI	Bovine blood index
ELISA	Enzyme linked immuno-sorbent assay
FPH	*Aedes aegypti* Female per positive houses
FSH	*Aedes aegypti* Female per surveyed houses
HBI	Human blood index

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
MS, EA, and AA designed the study and carried out data collection. MS, AM, DH, KM conducted molecular screening of dengue fever and chikungunya viruses from *Ae. aegypti*. MS and YN performed the laboratory detection of blood meal sources. MS conducted data analysis and wrote the first draft of the manuscript. EA, AM, and AA revised the manuscript. All authors read and approved the final manuscript.

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Data availability
All data generated or analyzed to support the findings in present study are included in the manuscript and its additional file.

Declarations

Ethics approval and consent to participate
The study obtained ethical approval from the Institutional Review Board (IRB), Aklilu Lemma Institution of Pathobiology, Addis Ababa University, Ethiopia, with the reference number ALIPB IRB/80/22. Written permission letters were obtained from Awash Sebat, Awash Arba, and Werer towns health bureaus and health centers. The objectives of the study was explained to the households, and informed consents was obtained prior to the indoor and outdoor mosquito collections using a Prokopack aspirator. The households were informed that participation in the study was in voluntarily basis.

Consent for publication
Not applicable.

Competing of interests
The authors declare no competing interests.

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RESEARCH ARTICLE

Susceptibility status of *Aedes aegypti* (Diptera: Culicidae) to public health insecticides in Southern Afar Region, Ethiopia

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Abstract

Mosquito-borne viral diseases such as dengue fever, chikungunya, and yellow fever have been documented in Ethiopia since the 1960s. However, the efficacy of public health insecticides against *Aedes aegypti* that transmits these viruses remains poorly understood in the country, particularly in the Afar Region. Thus, the aim of the study was to assess the susceptibility status of *Ae. aegypti* to deltamethrin, permethrin, alpha-cypermethrin, pirimiphos-methyl, bendiocarb, and propoxur insecticides. Larvae and pupae of *Aedes* species were collected from Awash Arba, Awash Sebat, and Werer towns of the Afar Region of Ethiopia during July-October 2022, brought to the Aklilu Lemma Institute of Pathobiology, insectary and reared to adults. Non-blood-fed, 3–5 days-old females *Ae. aegypti* were exposed to pyrethroid, carbamate, and organophosphate insecticide impregnated papers in tube test following the standard guidelines. Knockdown rates were noted at 10 minutes interval until one hour. The mortality in mosquitoes was recorded 24 hours after 60 minutes of exposure. The mortality rates of *Ae. aegypti* exposed to propoxur were 87% in all the study towns. Similarly, 88% mortality in *Ae. aegypti* was recorded when tested with bendiocarb in Awash Sebat and Awash Arba towns. Suspected resistance of *Ae. aegypti* (95% mortality) to alpha-cypermethrin was observed in Awash Arba town. However, *Ae. aegypti* collected from all the three sites was observed to be susceptible to deltamethrin, permethrin, and pirimiphos-methyl. *Ae. aegypti* was resistant to 0.1% bendiocarb and 0.1% propoxur and possibly resistant to 0.05% alpha-cypermethrin. On the other hand, it was susceptible to 0.05% deltamethrin, 0.75% permethrin, and 0.25% pirimiphos-methyl. Thus, vector control products with deltamethrin, permethrin, and pirimiphos-methyl can be used in the control of adult *Ae. aegypti* in the Afar Region of Ethiopia. However, further studies should be carried out to evaluate the susceptibility status of *Ae. aegypti* to alpha-cypermethrin in the Awash Arba area.

Introduction

The ever-increasing outbreaks of arboviral diseases have drawn public health attention in several countries around the world [1]. East African countries, including Kenya, Somalia,

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Djibouti, Eritrea, and Ethiopia are epicenters of outbreaks of arboviral diseases such as dengue, yellow fever and chikungunya [2–4]. In Ethiopia, yellow fever caused about 30,000 deaths from over 200,000 infections during 1960 and 1962 [5]. Recently, there have been yellow fever outbreaks in South Omo [6] and dengue fever outbreaks in Dire Dawa city administration, Somali Regional State, Gewane and Amibara districts of Afar Regional State [7, 8]. In addition, chikungunya outbreaks were reported in Dire Dawa city administration, Afar and Somali Regional States [9, 10]. All these outbreaks with potentially unrecognized and/or underreported deaths could not be associated with particular virus due to the highly limited laboratory facilities in the country.

Mosquitoes transmit over 90% of the viral pathogens through their bites, among which *Ae. aegypti* is the leading vector followed by *Ae. albopictus* [11, 12]. *Ae. aegypti* is anthropophilic and occurs in close proximity to human habitations [13, 14]. *Ae. aegypti* has two morphological subspecies (ecotypes); the domestic *Ae. aegypti aegypti* and sylvan *Ae. aegypti formosus* [15, 16]. In Ethiopia, *Ae. aegypti*, *Ae. bromeliae*, *Ae. vittatus*, *Ae. hirsutus*, *Ae. simpsoni* complex, and *Ae. africanus* have been implicated as potential vectors of viral diseases [6, 8, 17–19].

In the absence of effective therapeutic drugs and vaccines to treat and control mosquito-borne viral diseases, prevention largely depends on vector control with mainly public health insecticides [13]. Pyrethroids such as deltamethrin, alpha-cypermethrin, permethrin, and organophosphates pirimiphos-methyl and fenitrothion are among the public health insecticides used against *Aedes* mosquitoes [20]. However, the potential resistance to the insecticides may adversely affect the implementation of the control strategy in endemic areas [21]. Mosquitoes could resist insecticides through their inherent ability to detoxify and avoid contact either behaviorally or physiologically [22].

Evidence from many African countries indicates that *Anopheles* and *Aedes* mosquitoes developed resistance to insecticides [23–25]. For instance, in Ethiopia, malaria-transmitting *Anopheles arabiensis* has developed resistance to insecticides in several areas of the country, including the Amibara district of Afar Regional State [25, 26]. The cause of the resistance could be frequent use of the insecticides in indoor residual spraying and long-lasting insecticidal nets for the control of mosquito-borne diseases in the area [21] or in agriculture pest control [27]. However, the susceptibility status of *Aedes* mosquitoes to currently used insecticides is lacking in the country.

Recently, the Ethiopian Public Health Institute prepared an arboviral disease vectors surveillance and control guideline in 2021, emphasizing the use of chemical and environmental methods. Vector control by chemical methods such as fumigation, repellents, and residual insecticide surface treatments were implemented in Dire Dawa city administration and Somali Regional State during dengue fever and chikungunya outbreaks (EPH, 2021, unpublished). Unfortunately, there is a scarcity of evidence on the efficacy of the insecticides for the control of virus transmitting *Aedes* species in the Southern Afar Region in the presence of increased reports of viral disease incidence and prevalence. Therefore, assessing the effectiveness of the existing public health insecticides to control *Aedes* species is crucial to strengthening the arboviral disease prevention and control programs in the country. This inspired us to generate baseline data on the susceptibility status of *Ae. aegypti* to public health insecticides in the Southern part of the Afar Regional State of Ethiopia.

Materials and methods

Study sites

The study was carried out in Awash Arba, Werer, and Awash Sebat towns, Gabi-Rasu Zone (Zone 3), of Afar Regional State, Ethiopia. The Awash Arba and Werer towns are located

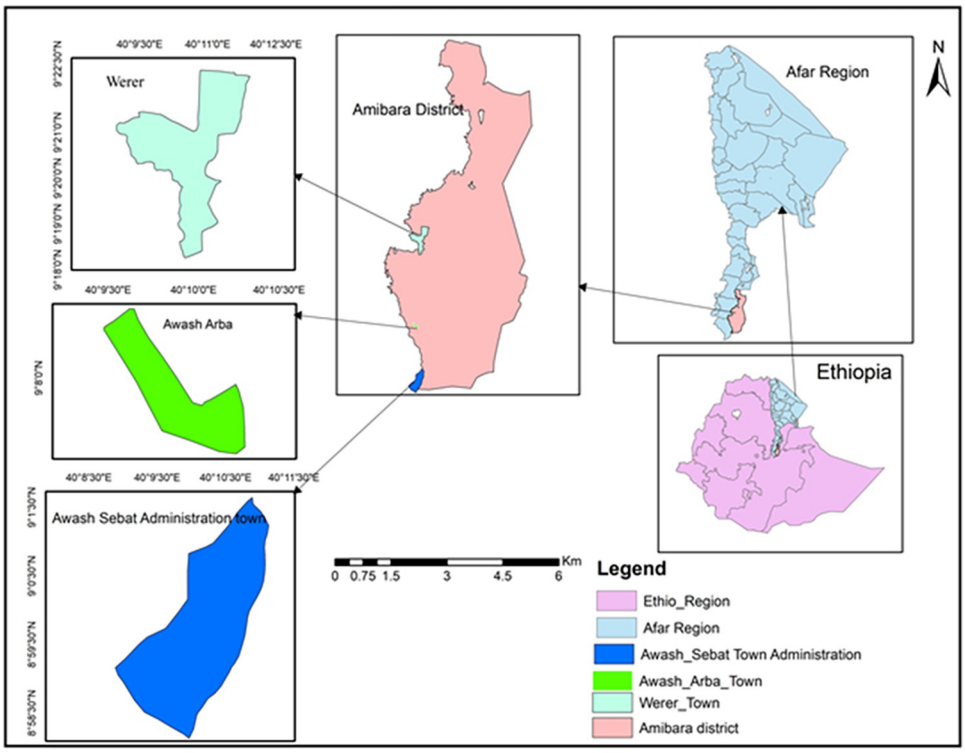

Fig 1. Map of the study areas. A map showing the locations of the three study areas: Awash Sebat, Awash Arba and Werer towns, in the Southern Afar Region, Ethiopia.

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about 250 km to the north-east of Addis Ababa at 9.33453˚N latitude, 40.181385˚E longitude, and an altitude range of 720 to 1100 meters above sea level (masl) (Central Statistics Agency, 2019, unpublished). Awash Arba and Werer sites were selected from Amibara district, which is about 30 km apart (Fig 1). Agro-ecologically, these two towns are semi-arid, with temperatures ranging from 25˚C to 35˚C and an average annual rainfall of 530 mm. The communities are pastoralist and agro-pastoralist, in which they mainly obtain income from livestock rearing and other agricultural crop production [28].

Awash Sebat town administration is located 214 km north-east of Addis Ababa at 8.98810˚N latitude and 40.163936˚E longitude (Central Statistics Agency, 2019, unpublished). Its altitude ranges from 820 masl to 1120 masl, and the temperatures vary from 22.6˚C to 30.6˚C. The town and its surroundings receive a mean annual rainfall of 606.6 mm.

The study areas are found in the middle of the Awash Valley, where infectious diseases such as malaria, chikungunya, and dengue fever are common [29]. Considerable attention has been given by the Regional government to achieve better health in the region, including the three towns [30]. The Region has hospitals, health centers, clinics, and health posts in different zones and districts. There is one hospital, one clinic, five health centers, and a few private clinics. Insecticides such as deltamethrin, permethrin, alpha-cypermethrin, pirimiphos-methyl bendiocarb, and propoxur have been used for indoor residual spraying or pyrethroids in long-lasting insecticide nets for the control of malaria and arboviral diseases in these towns [26, 31]. Similarly, since maize, sorghum, and cotton productions are common in the area, agricultural chemicals such as soil fertilizers and agricultural pesticides are being used to increase crop production [32].

Larval collection and rearing

A cross-sectional study was designed to collect larvae and pupae of *Aedes* species from July-October, 2022 in the Awash Sebat, Awash Arba, and Werer towns. The study towns were selected purposively in collaboration with the local district health professionals and on the basis of the recent outbreak reports of dengue fever and chikungunya virus (Awash Sebat town and Amibara district health bureaus, 2022, unpublished). The towns were surveyed once per month. Larvae and pupae were collected from different positive habitats, including tyres, water storage drums, discarded plastic and metallic containers, using standard dipper (350 ml), ladles, pipets, and nets. The larvae and pupae collected from each habitat were transferred into labelled plastic jars, transported to the insectary of the Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Ethiopia, and reared to adults under standard laboratory conditions. Tetra cichlid floating fish food (®/TM/©2019 Germany) was provided as food to the larvae. The emergence of larvae into pupae was checked each morning, and pupae were collected in glass flasks and transferred into (30 cm x 30 cm x 30 cm) netted cages. Emerged adult *Ae*. *aegypti* were kept at 27 ± 2˚C temperature, 75 ± 10% relative humidity, and provided access to feed on a 10% sucrose solution until the bioassay tests. Emergent adults were identified by species using morphological taxonomic keys for *Aedes* species [33].

Phenotypic susceptibility status of *Aedes aegypti*

Prior to insecticide susceptibility tests, sugar-fed females *Ae*. *aegypti* were starved for 2 hrs. Non-blood-fed, 3-5-old females *Ae*. *aegypti* mosquitoes were tested for their susceptibility to insecticides in tube test following the World Health Organization's insecticide testing procedures [20, 34]. They were exposed to pyrethroids (0.05% deltamethrin, 0.05% alpha-cypermethrin, and 0.75% permethrin), carbamates (0.1% bendiocarb and 0.1% propoxur), and organophosphate (0.25% pirimiphos-methyl) [34]. The insecticides were selected for the study because they are among the insecticide classes which have been used for the control of public health important mosquitoes in Ethiopia and the study areas. Similar concentrations of insecticides were used to test the susceptibility of *Ae*. *aegypti* in Tanzania [35]. However, we did not follow the World Health Organization's recommended discriminating concentrations for *Aedes* species due to the unavailability of insecticide-impregnated papers at the time of the experiment. This practical obstacle required a strategy that looked into the alternative doses of the insecticides. In each test, 20 females of *Ae*. *aegypti* were exposed to each of the five treatment replicates and two control test tubes, making a total of 140 mosquitoes in a single test. The exposed mosquitoes were observed for 60 minutes at an interval of 10 minutes to observe their knockdown. After an hour, the exposed females were transferred into holding tubes and kept for 24 hrs at 27.0 ± 2.0˚C temperature, 75 ± 10% relative humidity, and were offered cotton wool pads soaked in a 10% sugar solution. The mortality in mosquitoes was recorded 24 hrs after 60 minutes of exposure. *Ae*. *aegypti* susceptibility test was repeated for alpha-cypermethrin in Awash Arba town since *Ae*. *aegypti* populations appeared to be possibly phenotypic resistance to this insecticide. However, in Werer town, the susceptibility status of *Ae*. *aegypti* to bendiocarb was not conducted due to insufficient mosquito samples.

Ethical issues

We obtained ethical approval from the Institutional Review Board (IRB) of the Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Ethiopia (June 13, 2022; ALIPB IRB/80/2022). Written permission letters were obtained from the district health bureaus and health centers in the towns. The households were informed about the objective of the study, and their agreements were sought prior to the larvae and pupae collection.

Data analysis

Mortality was determined on the basis of the dead and alive females of *Ae*. *aegypti* after the 24 hours recovery period. Susceptible were those with a mortality in the range of 98–100%, possibly or suspected for resistance were with a mortality 90–97%, and resistant if mortality was <90% [20]. Probit analysis was used to determine the estimated duration at which 50% (KDT_{50}) and 95% (KDT_{95}) of the exposed *Ae*. *aegypti* mosquitoes were knocked-down. Overall adult mortalities and knockdown times were computed using SPSS Software version 20.

Results

Knockdown effect of insecticides against *Aedes aegypti*

The knockdown times of *Ae*. *aegypti* exposed to insecticides are given in Table 1. The time to knockdown 50% (KDT_{50}) of *Ae*. *aegypti* exposed to deltamethrin ranged from 12.15 to 17.06 minutes whereas KDT_{95} of the insecticide ranged from 18.74 to 34.01 minutes. The KDT_{95}, (34.01 minutes) observed for *Ae*. *aegypti* collected from Awash Sebat was found to be longer than for other towns (Table 1). On the other hand, *Ae*. *aegypti* exposed to permethrin showed the lowest KDT_{50} in Awash Arba town (KDT_{50}, 5.11). Overall, the KDT_{50} and KDT_{95} of *Ae*. *aegypti* varied among *Ae*. *aegypti* across the study localities.

Susceptibility status of *Aedes aegypti* to insecticides

The percentage mortalities of *Ae*. *aegypyi* mosquito populations against insecticides are presented in Figs 2–4. Accordingly, *Ae*. *aegypti* mosquitoes showed phenotypic resistance to bendiocarb and propoxur insecticides, with mortality rates of 88% and 87% respectively in Awash Sebat town. There was 100% mortality of *Ae*. *aegypti* to deltamethrin, permethrin, alpha-cypermethrin, and pirimiphos-methyl in Awash Sebat town (Fig 2). All *Ae*. *aegypti* in the control group showed 0% mortality in all study sites.

Ae. *aegypti* populations from Awash Arba were also observed to be resistant to bendiocarb and propoxur but were possibly resistant to alpha-cypermethrin (95% mortality) (Fig 3). *Ae*. *aegypti* populations were fully susceptible to deltamethrin, permethrin, and pirimiphos-methyl insecticides in this town.

Table 1. The times (in minute) to knockdown of 50% and 95% of *Ae*. *aegypti* exposed to commonly used pyrethroids at three localities during July-October 2022 in Southern Afar region Ethiopia.

Insecticides	Localities	KDT_{50} [95% CI]	KDT_{95} [95% CI]
Deltamethrin	Awash sebat	17.06 [15.81–18.28]	34.01 [31.02–38.12]
	Awash Arba	12.15 [11.41–12.97]	18.74 [16.98–21.56]
	Werer	16.53 [15.37–17.67]	30.92 [28.24–34.62]
Permethrin	Awash Sebat	8.92 [7.34–10.26]	24.21 [21.23–28.93]
	Awash Arba	5.11 [2.91–6.94]	19.23 [16.23–24.09]
	Werer	15.12 [12.45–17.66]	29.89 [24.64–41.53]
Alpha-cypermethrin	Awash Sebat	14.35 [13.35–15.35]	24.93 [22.77–27.97]
	Awash Arba	13.47 [12.64–14.36]	20.78 [19.04–23.32]
	Werer	16.71 [13.85–19.46]	32.90 [27.26–44.98]

CI, Confidence interval; KDT_{50}, time taken for 50% of the tested *Ae*. *aegypti* to be knocked-down; KDT_{95} time taken for 95% of the tested *Ae*. *aegypti* to be knocked-down

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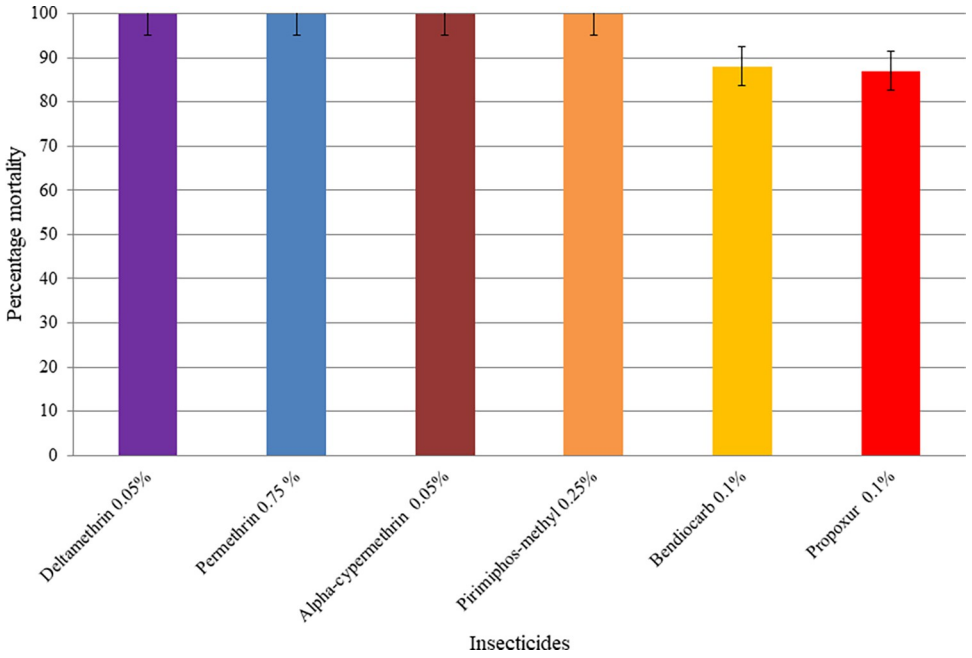

Fig 2. Insecticide susceptibility status of *Ae. aegypti* populations from Awash Sebat town, Southern Afar region, Ethiopia, July-October 2022. Error bars represent 95% confidence intervals.

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Mortality in *Ae. aegypti* from Werer town was 87% with propoxur and 99% with alpha-cypermethrin (Fig 4). However, *Ae. aegypti* from this site was found to be fully susceptible to deltamethrin, permethrin, and pirimiphos-methyl.

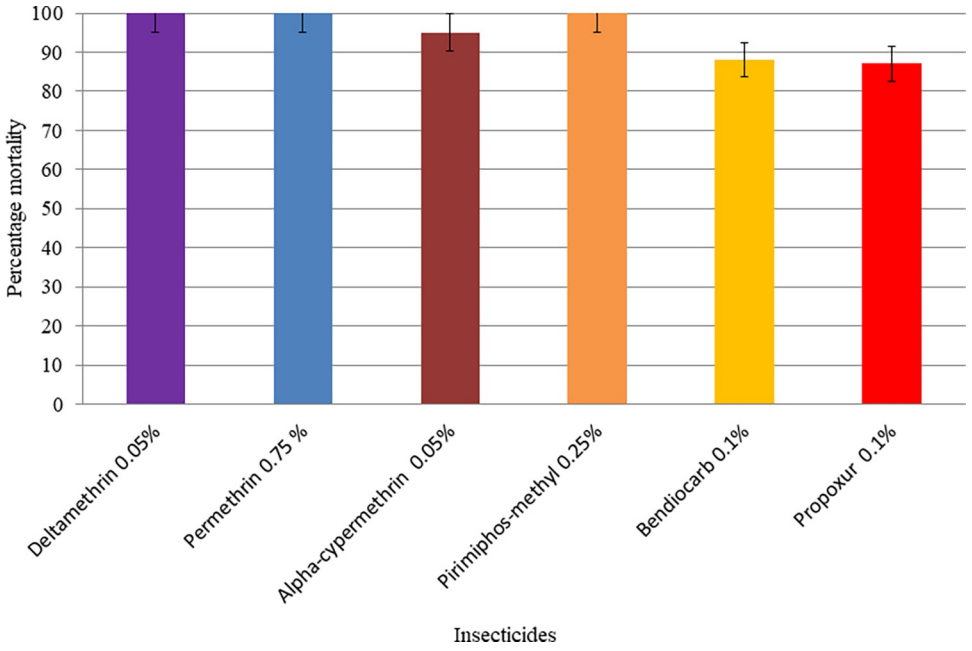

Fig 3. Insecticide susceptibility status of *Ae. aegypti* populations from Awash Arba town, Southern Afar region, Ethiopia, July-October 2022. Error bars represent 95% confidence intervals.

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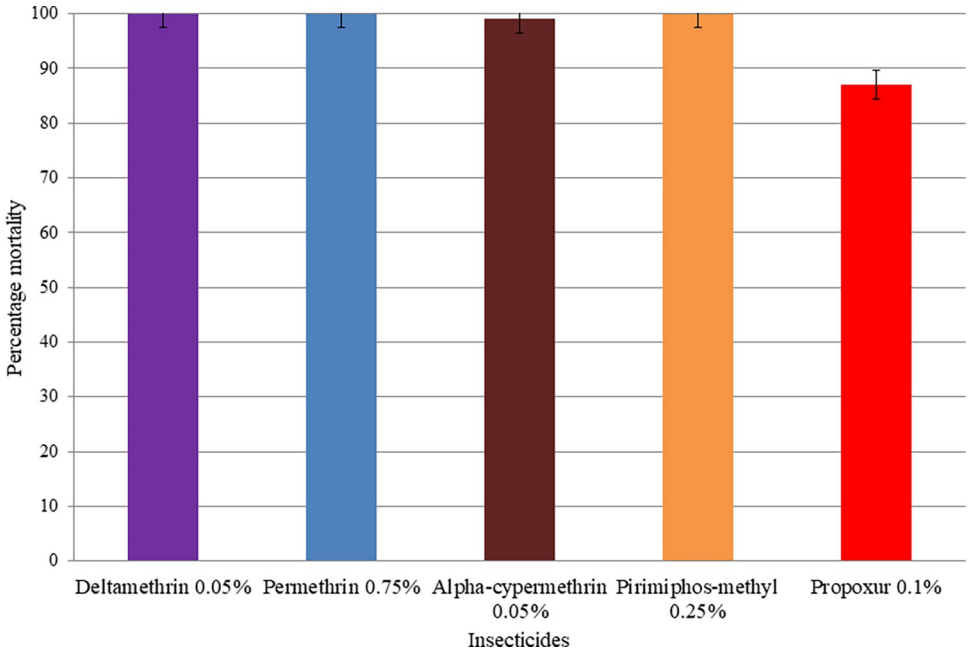

Fig 4. Insecticide susceptibility status of *Ae. aegypti* populations in Werer town, Southern Afar region, Ethiopia, July-October 2022. Error bars represent 95% confidence intervals.

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Discussion

Recent outbreaks of dengue fever and chikungunya virus fever in Eastern and the North-eastern parts of Ethiopia especially in Dire Dawa city administration, Somali Regional State, Amibara and Gewane districts of Afar Regional State require the control of the *Aedes* species that transmit the viruses. Insecticide-based mosquito control is underway in Ethiopia in general and in the Afar Regional State in particular [7, 36]. In so doing, there has been continuous evaluation of the efficacy of the insecticides against malaria-transmitting *Anopheles* species, but there is a scarcity of research findings conducted to control *Aedes* species either chemically, biologically, or by other means of vector control strategies in the country [37]. Thus, the control of the viral disease transmitting *Ae. aegypti* vector is poorly investigated and needs detailed research. As far as our knowledge is concerned, this study was the first in its kind to report the susceptibility status of *Ae. aegypti* to public health insecticides in the Southern Afar Regional State, and there are limited data to compare our findings to others in the country.

Aedes aegypti is considered by some authors to be highly exophilic [38], exophagic, and a daytime biter [39]. It usually feeds on human and other vertebrate hosts, as described in previous study [39]. However, it may tend to be endophilic and endophagic, in which case it rests and feeds indoors [40]. This makes control of *Ae. aegypti* and the *Aedes*-borne diseases it transmits more difficult [41]. As a result, different intervention tools have been adopted to control *Aedes* mosquitoes including indoor residual insecticide spraying and long-lasting insecticide treated nets in addition to managing their breeding habitats [42].

In the present study, *Ae. aegypti* showed higher KDT_{50} and KDT_{95} as compared to the study conducted in Sudan on deltamethrin insecticide. For instance, the KDT_{50} and KDT_{95} observed for deltamethrin in Sudan were 13 and 19 minutes, respectively which were lower than the current study in Awash Sebat and Werer towns [43]. However, the study conducted in Nigeria revealed that the KDT_{50} of deltamethrin was almost twice the KDT_{50} observed in Awash Sebat [44]. A relatively similar permethrin KDT_{50} was observed in Awash Sebat town

and Mlabani, Tanzania. However, higher KDT_{95} with deltamethrin and permethrin was found for Mlabani than all the present study towns [35]. Overall, the tube test revealed that *Ae. aegypti* was susceptible to deltamethrin in Sudan and Tanzania but possibly resistant in Nigeria.

Aedes aegypti was found to show varying susceptibility to different insecticides. *Ae. aegypti* populations were observed to be phenotypically resistant to bendiocarb with < 90% mortality. *Ae. aegypti* resistance to bendiocarb was also reported in the previous study in Tanzania during the rainy season [35]. However, this finding was contrary to the findings observed from Sudan, Nigeria, and Cambodia [43–45]. The World Health Organization has recommended in 2022 that the insecticide discriminating dose of bendiocarb for *Ae. aegypti* be changed from its initial 0.1% to 0.2% [34]. Thus, future efficacy studies of bendiocarb should target the 0.2% concentration [34].

In the present study, *Ae. aegypti* was found to be resistant to propoxur 0.1% concentration in all the study towns. Similar finding was reported in Thailand, where the efficacy of *Ae. aegypti* mortality to propoxur was 83%, which indicated the phenotypic resistance of *Ae. aegypti* to propoxur in this area [46].

The possible contributing factors to the emergence of phenotypic resistance of *Ae. aegypti* in the present study areas could be frequent uses of insecticide treated bed nets and indoor residual insecticide spraying to control mosquito-borne diseases as previously indicated [47]. On the other hand, Ethiopia has had a long history of utilizing pesticides to increase agricultural production and improve human health since 1964 [48, 49]. Unfortunately, however, agricultural pesticides induce insecticides resistance selection pressure in different ways. For instance, pesticides used for agriculture pest control can select vector control insecticides because of a similar mode of action, and bio-active ingredients might drive a section pressure for chemical based insecticide control tools [27]. Similarly, agro-pesticides used in agriculture may increase insecticide tolerance as the pesticides are washed into mosquito breeding habitats, which confer resistance mechanisms at the adult stage [50]. Thus, the emergence of insecticide resistance in bendiocarb and propoxur insecticides in the present study could potentially arise from the frequent exposure of the mosquito to carbamate insecticides in agriculture used as agricultural pesticides or herbicides. In addition, *Ae. aegpti* resistance to propoxur and bendiocarb may occur as a result of mutations in the insecticide target site enzyme Acetyl-cholinesterase (AChE) as observed in Cuba [51].

Pyerethroids such as deltamethrin and alpha-cypermethrin are commonly used to control malaria and other mosquito-borne diseases in Ethiopia. In the present study, the pyrethroid alpha-cypermethrin showed less than 97% of the mortality of adult *Ae. aegypti* populations in the Awash Arba town, which revealed the emergence of possibly resistance in *Ae. aegypti* in this area. This might be due to the frequent use of pyrethroids including alpha-cypermethrin in the insecticide-treated nets to control malaria and agricultural pesticides in this area as discussed above. This may lead to failures in vector control intervention programs in the study area and elsewhere in the country if resistance management is not undertaken. Similar findings were reported in Ghana [23].

Completely/fully susceptible *Ae. aegypti* populations were observed to the organophosphate, i.e., pirimiphos-methyl in all the study areas. Likewise, the study conducted in Cambodia [45] reported similar findings in that *Ae. aegypti* populations were completely susceptible to pirimiphos-methyl even in its lower concentration than the present study. However, this finding was contrary to the finding reported in Nigeria [44]. Similarly, *Ae. aegypti* was also fully susceptible to deltamethrin and permethrin in the study areas. These findings were contrary to the findings reported in Malaysia [52], where resistance phenotypes were observed in deltamethrin and permethrin. However, similar findings were reported in Tanzania [35] and

in Peru [53], where deltamethrin showed greater than 99% of mortality in adult *Ae*. *aegypti*. Thus, these findings could serve as a baseline for the Ethiopian Ministry of Health and the Afar Region Health Bureau to implement chemical interventions, particularly the pyrethroids deltamethrin, permethrin, and organophosphate pirimiphos-methyl, either in the form of aerial spray or insecticidal surface treatment, to reduce the density of *Ae*. *aegypti* and prevent the burden of arboviral diseases during disease outbreaks in different parts of the country.

Conclusion

The present study showed that *Ae*. *aegypti* populations were resistant to bendiocarb and propoxur, possibly resistant to alpha-cypermethrin, and fully susceptible to deltamethrin, permethrin, and pirimiphos-methyl insecticides. Thus, based on the findings, regular monitoring of insecticide resistance should be carried out in the study areas and elsewhere in Ethiopia, including molecular aspects, to mark and evaluate resistance patterns. Moreover, proper use of existing insecticides is mandatory to prevent *Ae*. *aegypti* resistance to insecticides in the study areas. In addition, the organophosphate pirimiphos-methyl can be used in addition to deltamethrin and permethrin in the control of adult *Ae*. *aegypti* mosquitoes in Ethiopia.

Supporting information

S1 Table. Observed knockdown times (in minutes) and number of knocked-down female *Ae*. *aegypti* exposed to insecticides during the 60 minutes in Awash Sebat, Awash Arba and Werer towns of Southern Afar, July-October 2022.
(DOCX)

S2 Table. Mortality of *Ae*. *aegypti* 24 hrs, after 1 hr exposed to insecticides Awash Sebat, Awash Arba and Werer towns, Southern Afar Regional State, Ethiopia, July-October, 2022.
(DOCX)

S3 Table. Representative GPS points of the Awash Sebat, Awash Arba and Werer towns of Southern Afar Regional State, Ethiopia recorded during *Ae*. *aegypti* larvae and pupae collection.
(DOCX)

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Author Contributions

Conceptualization: Mohammed Seid, Abebe Animut.

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Formal analysis: Mohammed Seid.

Funding acquisition: Mohammed Seid, Abebe Animut.

Investigation: Mohammed Seid, Esayas Aklilu, Abebe Animut.

Methodology: Mohammed Seid, Esayas Aklilu, Abebe Animut.

Supervision: Mohammed Seid, Esayas Aklilu, Abebe Animut.

Validation: Mohammed Seid, Esayas Aklilu, Abebe Animut.

Visualization: Mohammed Seid, Esayas Aklilu, Abebe Animut.

Writing – original draft: Mohammed Seid.

Writing – review & editing: Mohammed Seid, Esayas Aklilu, Abebe Animut.

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