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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

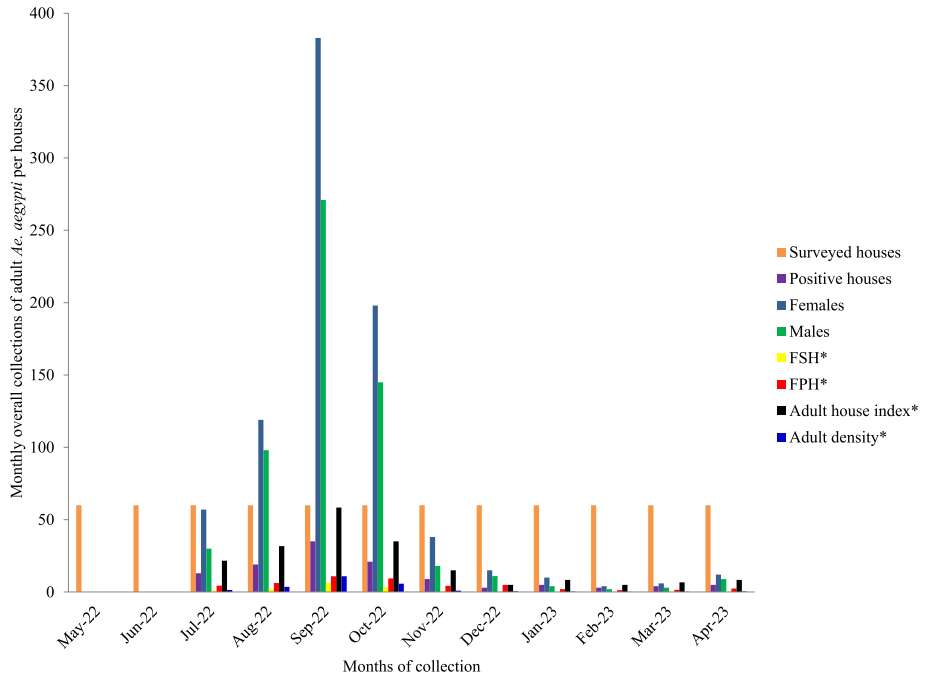

Fig. 1 Monthly overall collections of adult *Ae. aegypti* per houses in Awash Sebat, Awash Arba and Werer towns of Southern Afar, Ethiopia, May 2022-April 2023. Statistically significant changes are marked with asterisks ($p < 0.05$). FSH = *Ae. aegypti* females per surveyed house, FPH = *Ae. aegypti*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

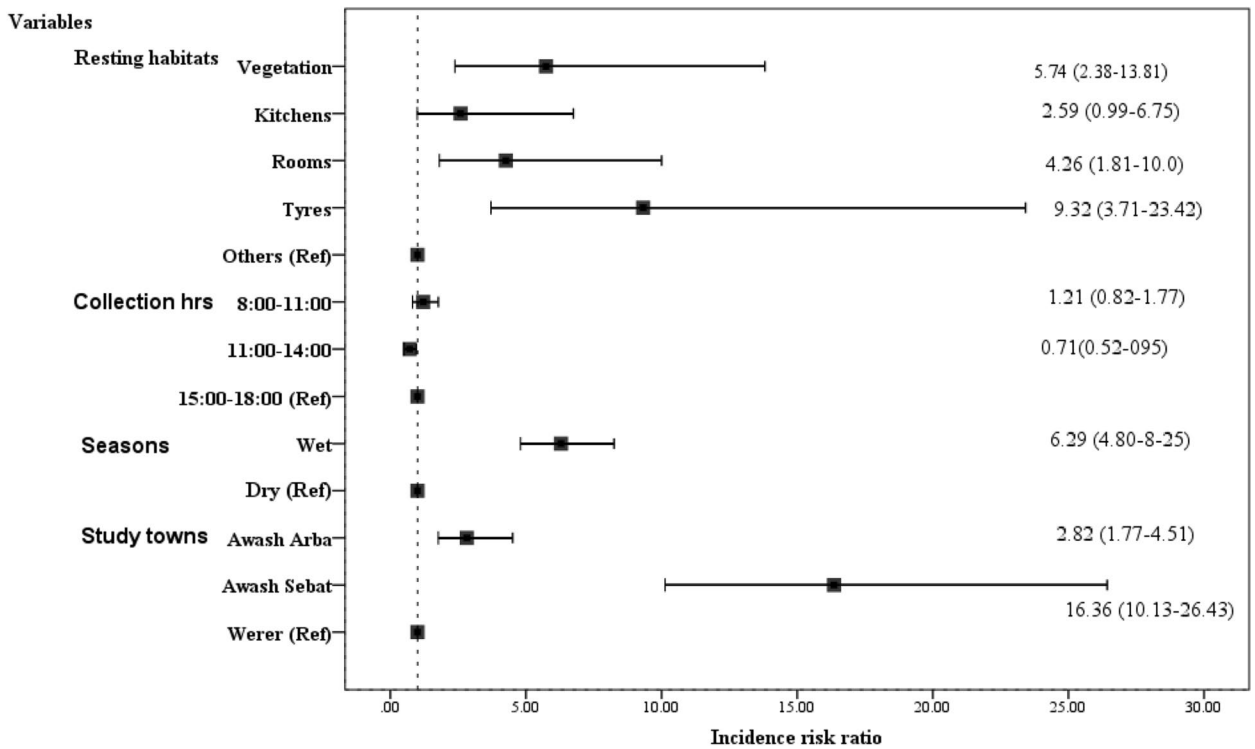

Fig. 2 Effect of environment and time on the number of female *Ae. aegypti* catches in Awash Sebat, Awash Arba, and Werer towns, Southern Afar, Ethiopia, May 2022-April 2023

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

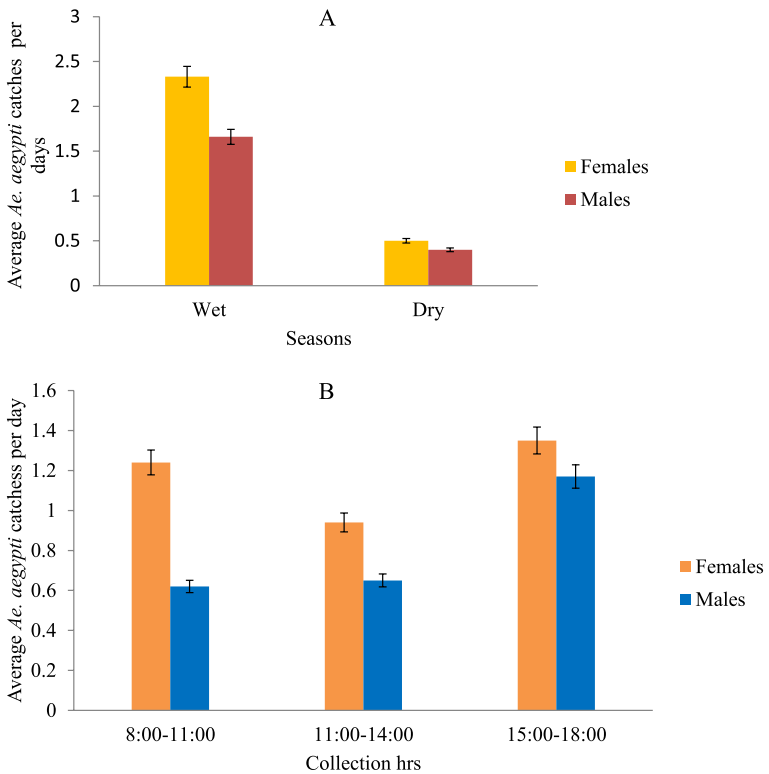

Fig. 3 Average *Ae aegypti* catches per day summarized by season (**A**) and collection times (**B**) in Southern Afar Region Ethiopia, May 2022-April 2023. The error bars represent the 95% confidence interval

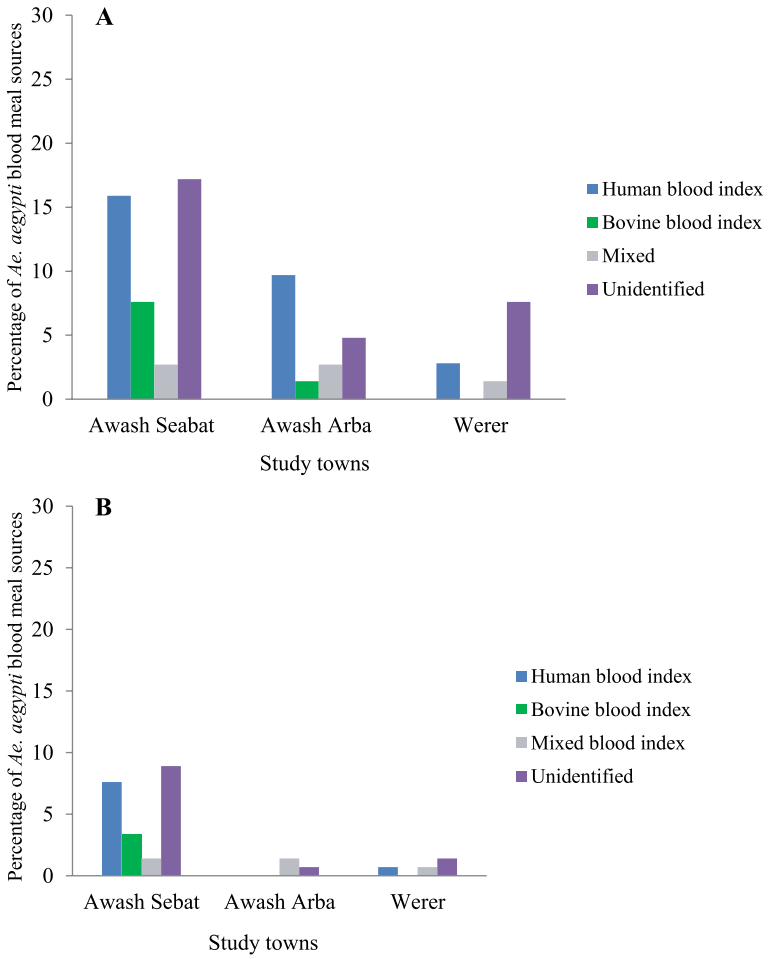

Fig. 4 Blood meal sources of *Ae. aegypti*. Outdoor (**A**) and indoor (**B**) collected *Ae. aegypti* in Awash Sebat, Awash Arba, and Werer towns, Southern Afar Region, Ethiopia, May 2022-April 2023

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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