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Presence, characterization and productivity profiles of *aedes* mosquitoes (Diptera: Culicidae), in Barentu, Gash Barka Zone, Eritrea

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Abstract
In Eritrea epidemics of dengue fever (DF) appeared in different places with different temperatures and altitudes. Studies on *Aedes* spp. mosquito (Diptera: Culicidae) composition, distribution and their habitats are lacking. A cross sectional survey was conducted in Barentu town, Gash Barka Zone (GBZ), during the period Dec. 2017-May 2018, to investigate the existence, characterization and productivity profiles of *Aedes* mosquito using the standard WHO techniques. A total of 413 households (HHs) from three villages, *viz.* Selam, Fithi and Biyara, in Barentu, were selected. All water-holding containers in and around the HHs were inspected for *Aedes* larva and pupa. Adults were also collected from indoor and outdoor resting sites. Adults and larvae were morphologically identified to their species level. The collections were made from a total of 2,057 water containers inspected from the three villages. Of these, 668 (32%) containers were found to be positive. A total of 59,368 larvae and 5,085 pupae were collected from 11 types of containers. The most important habitat types/containers were barrels, cement water basins, water storage tanks, water clay-pots and others; all these account for >90% of the larvae and the pupae collected. The house, container, Breteau and pupal/demographic indices were calculated. These larval indices, adult biting index (ABI) or human landing rate (HLR) were high. The vector identification results revealed that *Aedes aegypti* (Linnaeus) is the only *Aedes* species found in the area. The larval habitats were abundant both outdoor and indoor. The actual resting places were also found both indoor and outdoor. It is concluded that the area is at risk for DF. Some recommendation were suggested.

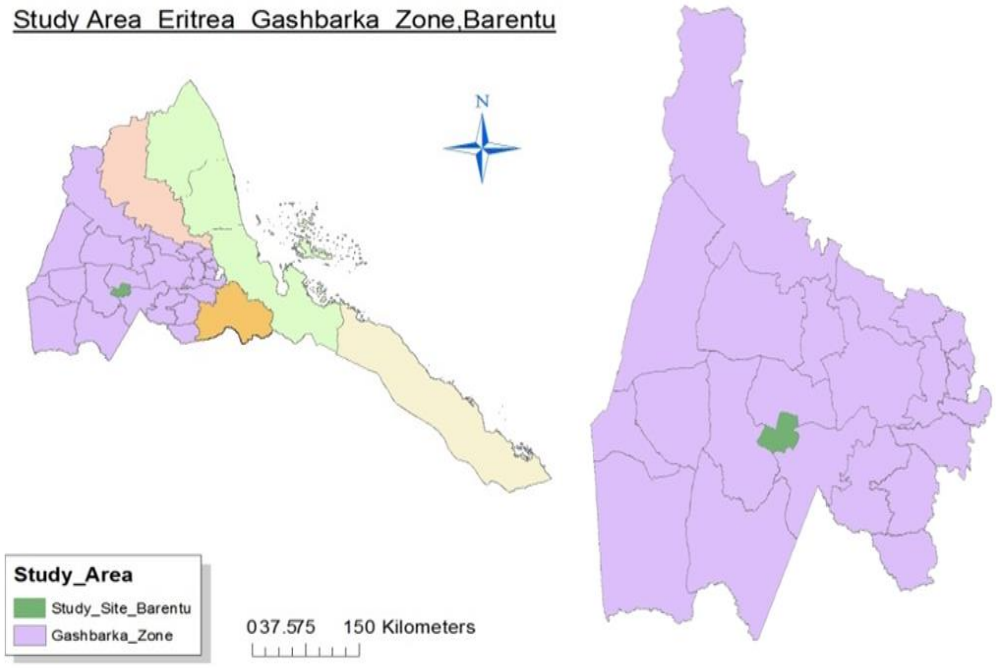
Keywords: *Aedes aegypti*, yellow fever, larval indices, habitats, eritrea

1. Introduction
Aedes aegypti and *Ae. albopictus* transmit important viral infections, e.g. Yellow fever (YF), dengue (DF), chikungunya (CHIKV), and Zika virus (ZV). YF is endemic in 47 countries, responsible for 29,000 to 60,000 deaths annually [1]. DF-related deaths apparent cases globally was 58.4 million in 2013. Whereas in 1990 estimate was 8.3 million [2]. The total number of disability-adjusted life years (DALYs) lost due to DF was 1.14 million in 2013 alone. CHIKV and ZV infections usually occur in localized outbreaks. However, with increased international travel, they also can be pandemic [3, 4, 5]. The full spectrum of disease manifestations in people who are infected with ZV is not yet known has recently raised concerns [6]. CHIKV, though rarely fatal, causes significant long-term morbidity (debilitating arthralgia) [5]. *Aedes* mosquito species are also the vectors of several other endemic viral infections, *e.g*. West Nile fever, Mayaro virus infection, and Eastern equine encephalitis virus [7, 8].
The actual numbers of DF cases are under reported and many cases are misclassified. One recent [9] estimate indicates that 390 million DF infections occur every year of which 96 million (67–136 million) manifest clinically (with any severity of disease). In 2012, a study on the prevalence of DF, estimated that 3.9 billion people in 128 countries are at risk of infection with DFV [10]. Likewise available data suggested that DF is endemic to 34 African countries [11]. In Eritrea, epidemics of DF appeared at different places with different temperatures and altitudes [12]. The present work aims at identifying the *Aedes* spp in the study zone, i.e. Gash Baraka Zone (GBZ), their habitats and their productivity.

2. Materials and Methods
2.1 Study Design
Cross sectional study was followed.

2.2 Study Area

GBZ (fig.1), 33,450km^2, is located in the western Eritrea (15^{0}06'40.54"N, 37^{0}35'25.1"E). GBZ has a total of 14 subzones, 182 'administrative areas, with a total population of 912,026 (farmer and traders) evenly distributed. Climate is tropical. Temperatures (19 - 40 ^{o}C); altitude (450-1800m) above the sea level (asl); humidity between 35-77%. The zone has 1 Referral Hospital, 2 hospitals, 16 Health centers and 53 health stations; it is a malaria endemic area. Barentu town is located about 250 km to the west of the capital city of Eritrea (Asmara); population is 71,185. Barentu sub-zone has one referral hospital, one health center and 5 health stations.



Study area: Barentu site, Gash Barka Zone, Eritrea, Africa

2.3 Sample size

The sample size was determined using the following formula:
n = 400 HH Where: n = sample size
z = the critical value for achieving (1-α) % confidence level, here, z = 1.96. For 95% confidence interval.
p = the anticipated proportion.
Here p=0.5 since there are no previous studies.
q = 1-p; d = the desired margin of error, thus d=5%
deff = the design effect. Since cluster sampling was used, deff was taken as 2, based on previous similar HH surveys.
The entomological indices: House Index (HI), Container Index (CI), and Breteau Index (BI), were used for measuring the larval population. These indices were calculated as follows: HI (No. of HHs positive/ No. of HHs inspected x 100); CI (No. of positive containers / No. of containers inspected x 100); Breteau Index (No. of positive containers / No. of HHs inspected x 100); PI (No. of pupae / No. of HHs inspected x 100) and PDI (No. of pupae / No. of population x 100) [13].

2.4 Sampling Techniques

Cluster sampling was used by dividing the area into 3 clusters, based on the Administrative Areas (AA). HHs (408) were randomly selected from these clusters. First the town was divided in to 3 AAs (villages), namely Selam, Biyara and Fithi; the starting point was in the middle of every AA. A pen was rolled to indicate the starting HH, and a HH nearest to the sharp- end was taken as a direction. Then moving to the right and counting according to the sample interval (no. of HH in the AA/ no. of HHs to be surveyed in that AA. If the selected HH was not accessible, the next HH was chosen.

2.5 Data Collection

In each HH, water containers in indoor and larval habitats (*i.e*. barrels, tires, *etc*.) at outdoor sites, close to the human dwellings, were surveyed for larvae and pupae of *Aedes* mosquitoes 3 days/month (07:00am-01:00pm) from Dec. 2017-May 2018. The total number of larvae and pupae found in each container was recorded / HH. HI, CI and BI were calculated. Larvae and pupae from different habitats were also sampled, preserved in 70% ethanol in Eppendorff tubes and identified at species level [14, 15, 16]. In addition, more samples of larvae were taken to the insectary and reared to adult to verify the identification. Furthermore, adults were collected from indoor and outdoor resting sites using mouth aspirator, preserved on silica gel in Eppendorff tubes and identified to their species level. Positive containers were filtered using a sieve by pouring on clean water to the white pans. Occurrence of *Aedes* in containers with little amount of water (wet containers) were checked using Pasteur pipettes, kept in a specimen tube and labeled with location. The number of positive containers and the number of water-filled containers inspected was recorded. Total larvae in positive containers were counted to give approximate density /larval habitat.

2.6 Mosquito Rearing, Processing and Identification

The field-collected immature stages of *Aedes* mosquitoes, kept in well-labeled vials, transferred to paper cups covered with a netting material and fixed with a rubber band. They were reared in the laboratory to the adult stage. After emergence, adults, as well as specimens of larvae (3rd and 4th instars) and pupae were transferred into 70% ethanol in Eppendorff tubes. Moreover, the wild collected adults were

killed by chloroform and preserved as described above. The preserved specimens of mosquitoes were examined under dissecting microscope and the species were determined morphologically [14, 15, 16].

2.7 Data Analysis
The data were entered and analyzed using the statistical package for social sciences (SPSS), where suitable statistical test was performed.

3. Results
3.1 *Aedes* prevalence
A total of 413 HHs and their surrounding areas were inspected for *Aedes* mosquitoes (larvae and pupae) breeding places, *viz.* Containers, in the three villages: Selam 179, Fithi 144 and Biyara 90 HH. Table (1) shows that 278 (67.3%) of the HHs were found to be positive for both stages,; 65 HHs were positive for both indoor and outdoor. Overall 2,057 wet-containers (1,882 indoor and 175 outdoor) were inspected. Among these, 566 indoor and 102 outdoor containers proved to be positive. The study showed no differences between the months and study villages, but there were significant differences on the infestation level between the containers inspected (Tables 2 and 3). Indoor containers, *e.g*. barrels and cement water basins, showed 42.8 and 31.4%, respectively. Regarding the outdoor sites, metal drums and soft drinks boxes represented 48.5 and 19.5%, following the same order. Fig. (1), shows the relationship between temperature, R.H. and rainfall during the study period.

Table 1: Total households (HHs) and total containers inspected indoors (in) and outdoors (out) in the three villages

Characteristics		Total HH's	(+) HH's		Total containers		(+) containers	
			In	Out	In	Out	In*	Out**
Month	Dec.	69	57 (83%)	7	332	13	105(32%)	7
	Jan.	69	58 (84%)	4	368	5	98 (27%)	4
	Feb.	69	38 (55%)	5	335	9	78 (23%)	5
	March	69	53 (77%)	5	356	8	153 (43%)	5
	April	69	39 (57%)	4	228	4	78 (34%)	4
	May	68	33 (48%)	40	263	136	54 (21%)	77
Villages	Selam	179	118 (66%)	26	763	96	239 (31%)	53
	Fithi	144	101 (70%)	27	633	50	204 (32%)	33
	Biyara	90	59 (66%)	12	486	29	123 (25%)	16
Sub- total		413	278 (67%)	65	1882	175	566 (30%)	102 (58.3%)
Grand total			343		2,057		668 (33.4%)	

***** Indoor containers: Clay pots, barrels, jerry- cans, cement water basins, water storage tanks, tires and flowers vases

****** Outdoor containers: Tree-holes, discarded tires, discarded tins, metal drums, cement water tanks, coconut shells and shower water tanks

Table 2: Distribution of larvae and pupae by container type (Indoor).

Container Categories		No. containers visited	(+) containers	No. of larvae	% larvae	No. pupae	% pupae
Indoor container type	Clay pots	201	85 (42%)	3,446	6.6	377	8.4
	Barrels*	891	311 (35%)	22,254	42.8	2,040	45.4
	Jerry cans	431	44 (10)	1,826	3.5	148	3.3
	Cement water basin	71	55 (77%)	16,338	31.4	1,401	31.2
	Water tanks	44	27 (61%)	5,898	11.3	384	8.6
	Tires	32	8 (255)	218	0.4	12	0.3
	Flower vase	84	16 (19)	196	0.4	8	0.2
	Soft drinks bottles	49	0	0	0.0	0	0.0
	Soft drink boxes	22	0	0	0.0	0	0.0
	Others	57	20 (35%)	1,808	3.5	119	2.7
Total		1,882	566(30%)	51,984	100.0	4,489	100.0

* Barrels: plastic containers of 200L and 100L

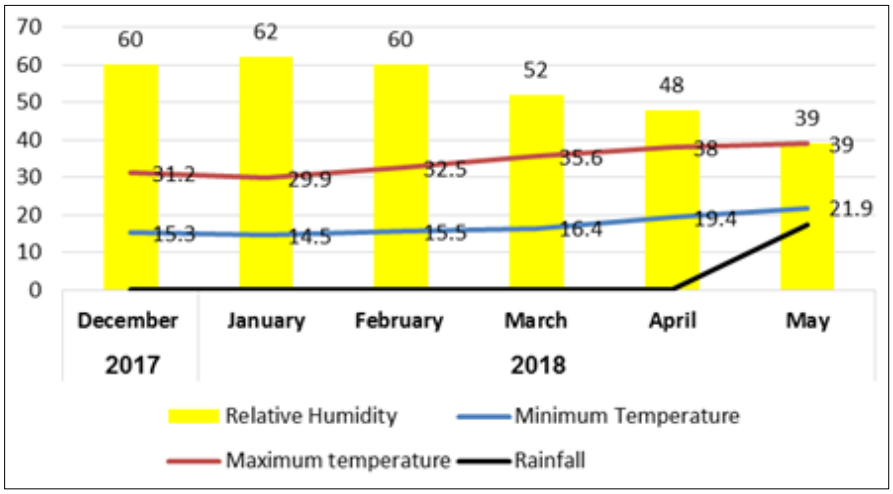


Fig 1: Monthly relationship between temperature, R.H. and Rainfall (Source: Asmara International airport and Zonal administration office) (Y axis = %; X axis = month)

Table 3: Distribution of larvae and pupae by container (Cont.) type (Outdoor).

Container categories		No. cont.	(+) Cont.	No. of larvae	% larvae	No. of pupae	No. of pupae / cont. (Mean ± SD)	% pupae
Outdoor container types	Tree-holes	12	8 (67%)	50	0.7	4	0.33±0.89	0.7
	Tires	49	32 (65%)	750	10.2	72	3.60±5.42	12.0
	Tins	38	16 (42%)	172	2.3	12	0.75±1.18	2.0
	Metal drums*	21	19 (90%)	3,583	48.5	240	11.43±15.71	40.1
	Cement water tanks	7	5 (71%)	1,215	16.5	115	16.43±18.89	19.2
	Coconut shells	15	8 (53%)	69	0.9	4	0.44±0.73	0.7
	Shower water tanks	10	1 (10%)	3	0.04	0	0	0.0
	Soft drink boxes	13	8 (62%)	1410	19.1	144	11.08±13.18	24.0
	Others	12	5 (42%)	127	1.7	8	1.14±1.46	1.3
Total		177	102 (58%)	7382	100	599	5.40±10.89	100

* Metal drums: 200L barrels.

3.2 Water Container Preference

3.2.1 Indoors

Indoor containers preference of *Aedes* mosquito fig. (2), indicated that the most preferred breeding containers were cement water tanks (CWT) and water storage tanks (WST). The survey revealed that 78% and 61.4% respectively of these tanks, were found to be positive for larvae and/or pupa. These two were followed by other types of containers, e.g. poultry drinking vessels (59%), barrels (35%), and clay pots (42%).

4.2.2 Outdoors

Among the 177 examined containers, 102 were positive fig. (3). Remarkable dominance of metal drums was seen; >90% of the wet metal drums (MD) were found positive for either larvae or pupa. CWT, tree-holes and discarded tires (DT) also account for 77.5, 66.7 and 65.3%, respectively.

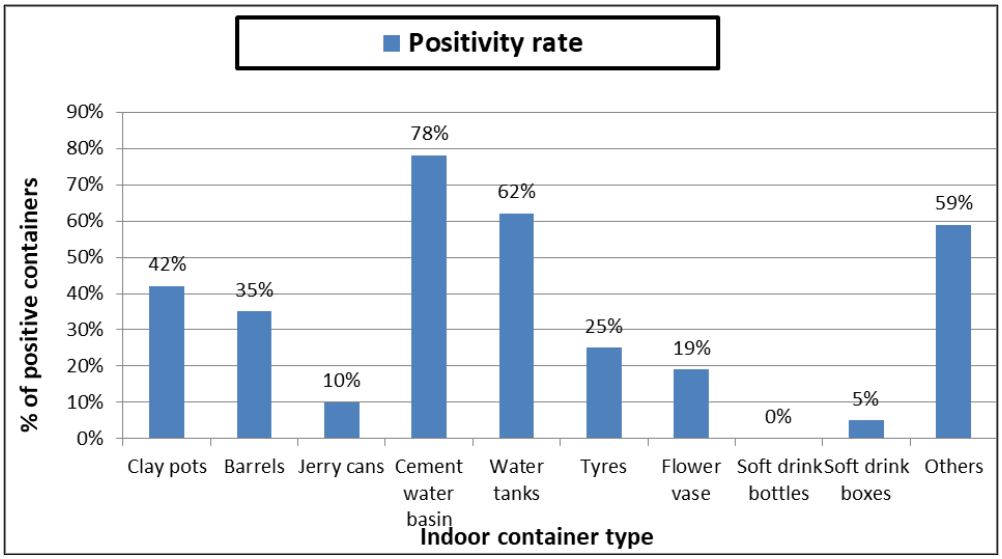


Fig 2: Containers positivity rate (Indoors).

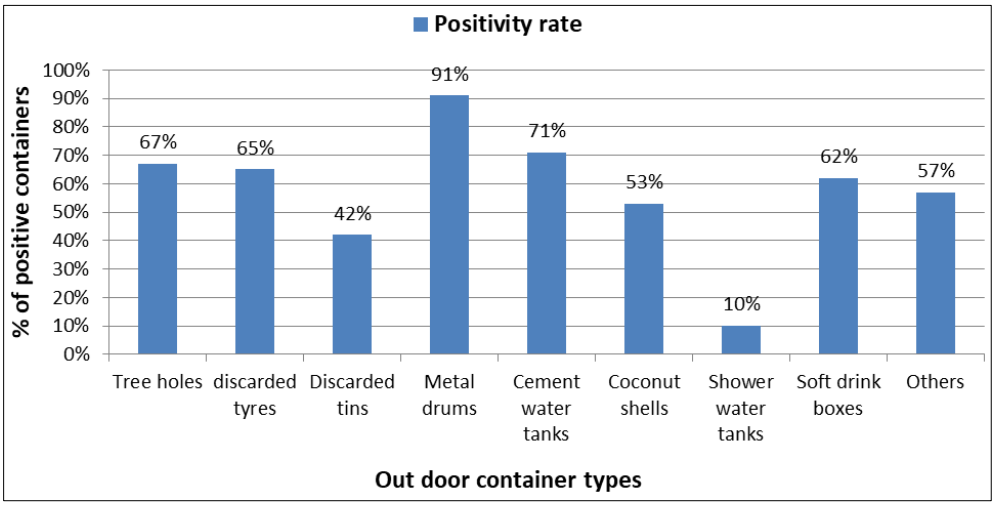


Fig 3: Containers positivity rate (Outdoor).

4.2.1 Monthly Distribution of breeding habitats and neighborhood

The results in fig. (4) Indicated that, during the dry-season (Dec. to April), very few outdoor breeding containers were found to be positive for larvae or pupa. Only 22% of the total positive containers were found in the first 5 months of the study. The rest (78%) wet- containers were found on May after a single rainfall during that month. Generally, among the 175 outdoor breeding places, 58.3% were found to be positive. On the other hand, containers visited at indoor sites showed no difference between the months. The numbers of wet-containers inspected were between 228 (April) to 368 (January).

Fig. (5) Shows the distribution of HH and containers between the study sites. At Selam, 66% of the houses and 34% of containers were positive, in Fithi (70% and 35%) and in Biyara it was 65% and 27% respectively.

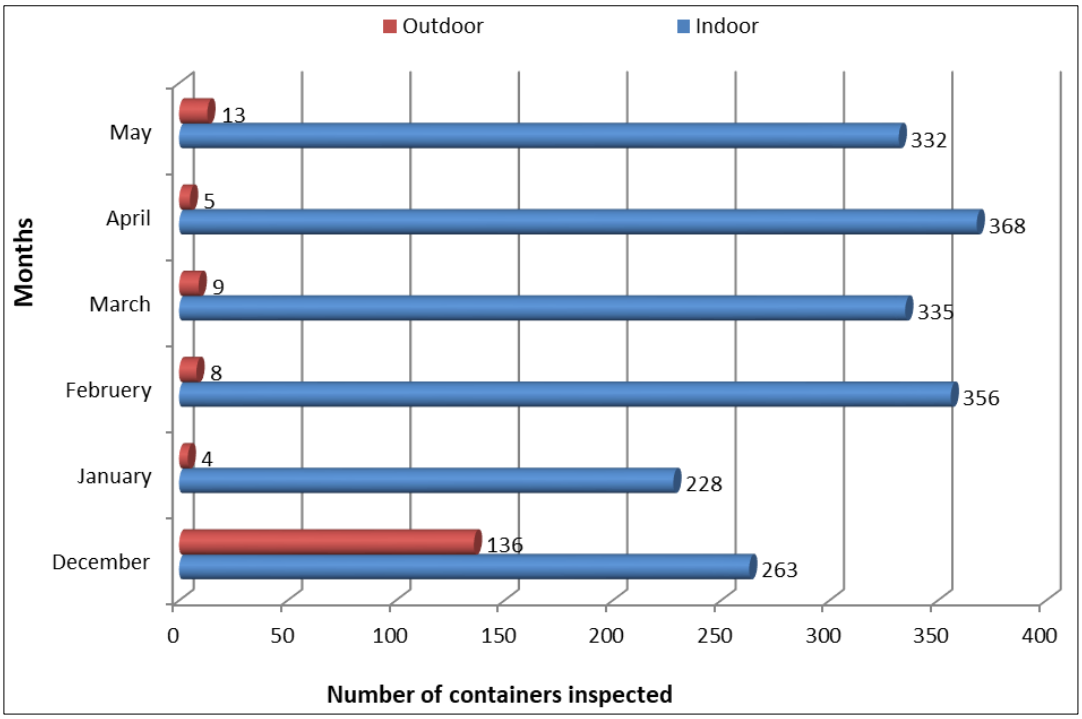


Fig 4: Monthly distribution of *Aedes* mosquito breeding habitats at indoor and outdoor

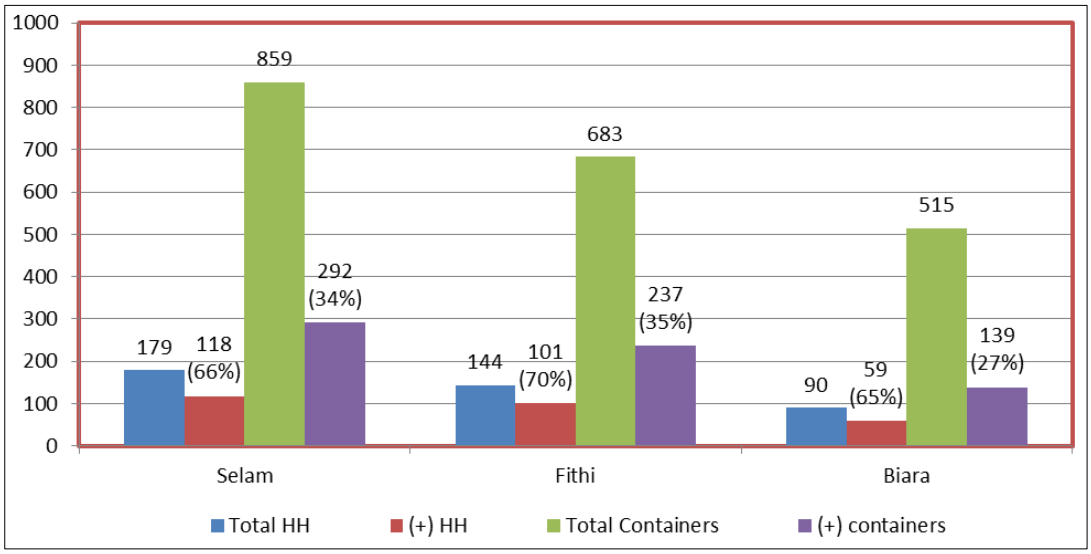


Fig 5: Distribution of total and positive, households and containers by village in Barentu town. (Y axis = number; X axis = villages)

4.3 Larval productivity profiles of breeding containers

4.3.1 Indoor

A total of 51,984 immatures were collected from the 3 villages. The analysis of larval productivity by different container categories (Fig. 6) showed that barrels and CWT were the most productive indoor containers; together they are responsible for the production of 74% of all the larvae collected (barrels 42.8% and CWT 31.4%). Other types of container, *e.g.* WST, clay pots and jerry- cans produced the rest of the larva. They accounts for 11, 7 and 4%, respectively.

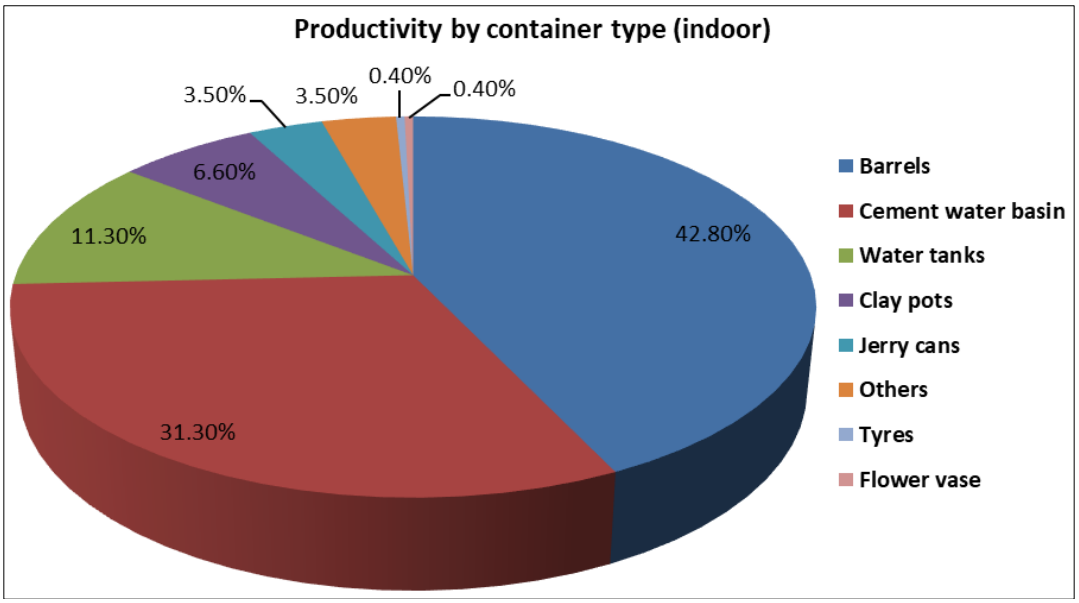


Fig 6: Percent larval productivity profile by different indoor container types

4.3.2 Outdoor

As shown in fig. (7), nearly half of the mosquito larva collected from outdoor mosquito breeding habitats were found from metal drums (48.5%), soft drink boxes (19.1%) and ranked second. The percent of larva produced in the other outdoor water- holding container were as follows: 16.5% from CWT, 10.2% from discarded tires, 2.3% from discarded tins and small number from coconut shells, tree-holes and shower water tanks.

Generally, analysis of larval productivity by different container category showed that barrels and CWT were the most productive containers indoors, while in the outdoors metal drums, CWT and soft drink boxes were the most productive.

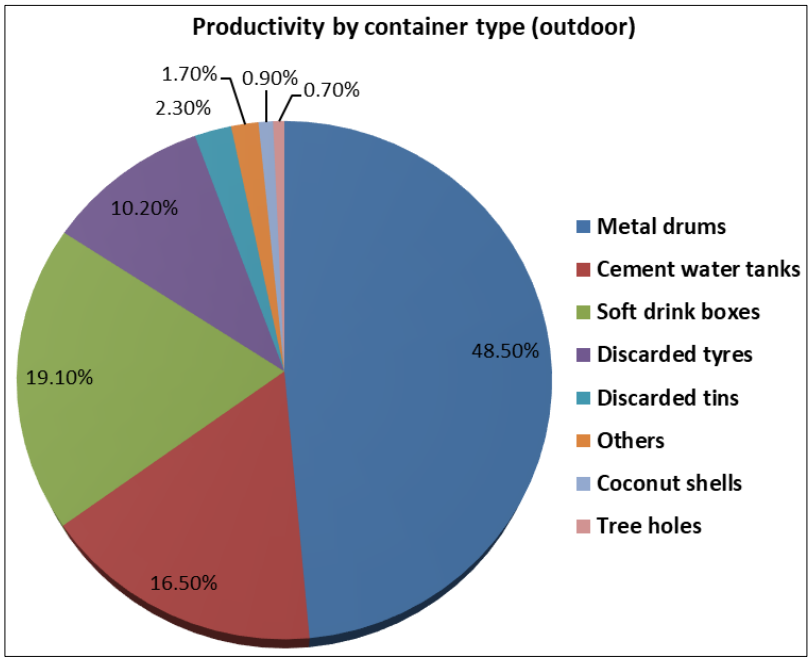


Fig 7: Percent larval productivity profile by different outdoor container types.

4.4. Larval Indices

The result of HI, CI, BI, and MBR are in tables (4 and 5). Of the 413 HHs surveyed, 67.3% proved to have *Aedes* mosquito breeding habitats. Overall, 31% of the containers had larva, pupa or both. HI ranged between 48.5% (May) and 84.1% (Jan.); CI: 20.5% (May) to 43.0% (March); and BI between 79.4% (May) and 221.7% (March), for all sites. Moreover, the MBR, for those collected outdoors by the human biting technique, the mosquito bite/person/hr ranged from 1.75 (April; Biyara) to 3.25 (Feb.; Fithi, Tables 5 and 6).

4.5. Species identification

From the total immature mosquitoes collected from wet-containers, 540 were identified to their species level (Table 6). Among these, 453 (83.9%) were *Aedes* mosquito, *viz*. *A. aegypti*, and the remaining were *Culex* (7.8%) and *Anopheles* (7.2%).

Table 4: Monthly larval indices and villages (neighborhood)

Characteristics		Total HH's	(+) HH's	Total container	(+) container	No. of larvae	HI	CI	BI
Month	Dec.	69	57	332	105	13575	82.6	31.6	152.2
	Jan.	69	58	368	98	8127	84.1	26.6	142.0
	Feb.	69	38	335	78	7503	55.1	23.3	113.0
	March	69	53	356	153	10240	76.8	43.0	221.7
	April	69	39	228	78	7633	56.5	34.2	113.0
	May	68	33	263	54	4906	48.5	20.5	79.4
Village	Selam	179	118	763	239	23590	65.9	31.3	133.5
	Fithi	144	101	633	204	13267	70.1	32.2	141.7
	Biyara	90	59	486	123	15127	65.6	25.3	136.7
Total		413	278	1,882	566	51,984	67.3	30.1	1,37.0

Table 5: Monthly Adult Biting Index (ABI) or Human Landing Rate (HLD)

Characteristics		Bite/Person/hr.
Month of collection	Dec.	2.42
	Feb.	3.25
	April	1.75
Villages	Selam	2.42
	Fithi	3.25
	Biyara	1.75

Table 6: Monthly Number of mosquitoes identified to their species level and villages

Characteristics		Total sam-ples	*Aedes* spp.	(%) *Aedes*	*Culex*	(%) *Culex*	*Anoph.*	% *Anoph*	Mean±SD	S.E.	C.V.%
Month	Dec.	90	69	76.7	16	17.8	5.6	5	23±1.19	0.13	5.0
	Jan.	90	85	94.4	4	4.4	1.1	1	25±0.69	0.07	2.0
	Feb.	90	64	71.1	14	15.6	13.3	12	21.3±0.38	0.04	1.0
	Mar.	90	90	100.0	0	0.0	0.0	0	30±0.00	0.00	0.0
	Apr.	90	80	88.9	4	4.4	6.7	6	26.7±0.22	0.02	1.0

	May	90	65	72.2	4	4.4	16.7	15	21.7±0.37	0.04	2.0
Village	Selam	180	160	88.9	9	5.0	6.1	11	26.7±0.7	0.05	2.6
	Fithi	180	153	85.0	13	7.2	7.8	14	25.5±0.66	0.05	2.6
	Biyara	180	140	77.8	20	11.1	7.8	14	23.3±0.97	0.07	4.1
Total		540	453	83.9	42	7.8	39	7.2			

4. Discussion

The common breeding habitats found in the study area were similar in the three villages, viz. barrels, clay pots, cement water tanks, water storage tanks and jerry cans. The majority of the residents in Barentu town store their water in containers, tanks or basins for domestic use. Stored water for many days was very common, due to irregular supply of water by the municipality. The municipality supply water to the town using tankers (trucks; 89%) and very few HHs (11%) have tap-water, but the supply is irregular. Same was reported from Pakistan due to insufficient water supply and inefficient removal of urban trash. These two factors resulted in increased number of containers around human dwellings, thereby creating ideal breeding habitats for *A. aegypti* [17]. Similarly, in some villages in India [18], residents store water in various containers for long periods of time; these constituted the major breeding sources.

In the present work, larvae and/or pupae were found in all the containers, except in soft drink bottles; however, mosquitoes preferred to breed in cement water tanks (77.5% infestation), water -storage tanks (61.4%), clay pots (42.3%), barrels (34.9%). In Kenya [19], outdoor containers were buckets, drums and tires, the indoors are the drums, buckets and pots. Jerry-cans were found in large numbers in the HHs, of the villages of Barentu, but their importance as breeding sites was limited and not productive. Some studies have identified jerry- cans as preferred outdoor breeding habitats [20]. The low productivity can be attributed to the short-term storage in jerry-cans [20]. Containers that retained water for long period of time make good or suitable breeding habitats like barrels, cement water basins, water - storage tanks and metal drums [21, 22].

In Barentu town, *A. aegypti* and *Culex* were found breeding together in different water- holding containers, especially in tires and containers that contain leaf-litter, which are found under the shade, *viz*. under trees. However, there were more *A. aegypti* breeding in areas with high vegetation cover [23]. This co-existence in HHs was also reported in Kenya [19]. Their co-existence was attributable to the abundance of suitable containers, the availability of shade and sufficient organic matter for larval feeding [24]. *A. aegypti*, *A. albopictus*, and *Culex pipiens* complex are known to breed in containers [25]. However, in present study, *A. aegypti* was found to be the most dominant species breeding in domestic previously reported containers abundantly found close to human habitation. The identified *Aedes* was *A. aegypti*. In Central Africa [26] and Laos [27] *A. aegypti* was strongly associated with urban environments.

Altitude is an important factor in limiting the distribution of *Anopheles* and *Aedes*. According WHO [28], in India, *A. aegypti* ranges from sea level approx. 1,200m asl. However, in Columbia, *Aedes* was found up to 2,200m asl. Theoretically, it was believed that, elevation <500m asl has moderate to heavy mosquito population; elevation > 500m asl has low mosquito population. The limit for *Aedes* mosquito distribution is 1000-1500m asl. GBZ study area has an altitude of 1,005m asl; it is a suitable elevation for *A. aegypti* distribution. But according the study conducted in Eritrea [12], DF epidemics occurred in different latitudes, *e.g.* Massawa (20m asl) and Mendefera (1954m asl).

A. aegypti can tolerate a wide-range of temperature than *A. albopictus*, even though the latter species is expected to be live longer than *A. aegypti* [29]. In Madagascar, *A. albopictus* occurred in regions with 0-6 dry months/yr, whereas *A. aegypti* can endure up to 9 dry months/yr [30].

Seasonality, especially rainy-season, is one of the important elements for the breeding and development of mosquitoes and significantly influences them [31]. Several studies have shown that rains plays an important role in DF epidemiology [32]. In Jeddah, KSA, mosquito population increases after rain, which creates suitable habitats [33]. Increasing rains may increase suitable larval habitats and vector population size by creating new breeding sites [32]. The present study, was conducted on the dry-season (Dec. 2017- May 2018) and the preferred breeding habitats were indoor containers, and on the 6th month of the study (May), after a single rainfall, the breeding habitats were extended from indoor to outdoor containers, *e.g.* discarded tires and discarded tins. A recent study from the neighbor Ethiopia [34] emphasized that mosquito preferred to breed in tires and plastic drums very dominantly and some other containers used for domestic use. In Kenya [20], four sites, revealed that more containers were located outdoors than indoors; buckets, drums, tires and pots produced >75% of the pupa.

The larval indices (CI, BI and HI), are generally used to calculate the presence, distribution and densities of *Aedes* population in an area. HI was (67.3%), BI (137.0%) and CI (30.1%). This indicates that the town has a very high arboviral diseases transmission possibility (*i.e*. at risk). CDC [35], established the level of DF transmission in relation to the larval indices. HI<1% and BI<5% are expected to be low risk of DF transmission. Whereas HI >10% and BI >50, indicate high disease transmission. Generally, BI>20 is an alarming situation for diseases transmission. Regarding ABI/HLR <0.2/human/ hr is a sign of low disease transmission, and ABI/HLR > 2/human/ hr is high risk area.

Temperature changes, among other internal and external factors, can impact the development- time and the vectorial-capacity of *Aedes* mosquitoes [29]. A review of entomological sampling methods done by both UNICEF/UNDP/World Bank/WHO, noted that the best hatching success of *Aedes* mosquitoes is at 24-25^{o}C.; approaches 98% in 48hr. As the temperature increases, the hatching- rate decreases. At 34-35^{o}C, the hatching- rate is only 1.6% in 48hr. *A. aegypti* egg-hatching is usually stimulated by three factors: ambient oxygen concentration in the water, conditioning of eggs at 26.6-27^{o}C and a R.H. of 50-80%. Based on the above, the conditions of the GBZ is favorable for *Aedes* mosquitoes.

5. Conclusions

a) The community in the study area store water in different containers for long periods of time for the domestic use. In addition, different discarded tins and tires hold rain-water during summer time. All these proved to be breeding habitats for *A. aegypti*.

b) The larval indices are high in the study area.
c) The climate of the study area is suitable for the survival and oviposition of the vector.
d) Chances of *Aedes*- borne diseases transmission to occur are high, because of the high mosquitoes' density in the study area.

6. Recommendations

Generally, for the control of container breeding mosquitoes, it is possible to use IVC/IVM. Viz.

1. Covering water -holding containers,
2. Public health education, and awareness-raising
3. Eliminating water-filled unused containers and emptying containers at least once a week,
4. Proper waste management system, and
5. Proper water-supply system

7. References

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