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Abundance, characterization and productivity profiles of *Aedes* mosquitoes (Diptera: Culicidae), in Keren, Elabered and Hagas, Anseba Zone, Eritrea

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Abstract
Infections of DF was appeared in an epidemic state in different geographical part of the country including our zone, and lately CHIKV (2018) was introduced to Eritrea through the border we shared with Sudan and getting distributed to the western part mainly Gash Barka and Anseba zones. Apart from the attempts to control the spread of these diseases, there has not been any study done in respect to the *Ae.* mosquito composition, distribution or even their habitats. A survey was conducted in Keren, Hagaz and Elabered: Anseba Zone, Eritrea during the period July. - Sep. 2020, to investigate the prevalence, container positivity and productivity of *Aedes* mosquito using the standard WHO techniques. Nearly 900 HHs and their surrounding from three towns *vis*. Keren Hagaz and Elabred were included in the study. During the study, all methods of larva collections and adult collection were used. These collections were from wet water-holding containers in and around the households. Identification was made only in adult mosquitoes after rearing the immature stages in Elabered entomology laboratory, because DPX (mountant) were not available to use larva for species identification. The collections were made from a total of 3,765 water containers: *i.e.* from Keren town (1185), Hagaz town (1078) and Elabered town (1204). An average of 4 containers per HH and a positivity of 24.4% were recorded during the study period. Barrel was the most widely used container both at indoor and outdoor sites and thus it was the most productive container in both sites. Nearly 50% of pupa was collected from barrel. Cement basin, clay pot, hand washing basin and water tank (plastic or metal) were also among the most productive containers. Adult biting index (ABI) was high and *Ae. aegypti egypti* and *Ae. aegypti formosus* (both sub-species of *Ae. agypti*) were the species incriminated during the study. The resting places were in both indoor and outdoor and 72% were found to bite at outdoor site.

Keywords: Aedes mosquitoes, DF, YF, larval indices, habitats, Eritrea

1. Introduction

Mosquito-borne viruses (arboviruses) have been afflicting humans for many decades and continue to cause immense suffering [1]. *Aedes (Ae.) aegypti* and *Ae. albopictus* have received a great deal of attention worldwide, since both species are efficient vectors for human arboviral diseases [2]. A suitable habitat for both species was combined with the occurrence of five diseases, namely Zika [3, 4], dengue [5], chikungunya (CHIKNV) [6, 7], rift valley fever (RVF) [2], and yellow fever (YF) [8]. These arboviral diseases are increasingly becoming a global health concern due to their rapid geographical spread and high disease burden. Especially over the past 30 years, the distribution and public health impact of these arboviruses have increased dramatically [9-11]. At least one of the arboviral diseases was reported from 146 (58.4%) countries/territories, of which 123 (49.2%) reported multiple diseases [2].

YF is currently endemic in 47 countries and is responsible for 29,000 to 60,000 deaths annually [13]. According to WHO (2012), Outbreaks of YF have been reported in the neighboring Sudan (Darfur) in 2012 [13]. A study by Bhatt, s. *et al*., 2013 revealed that there are approximately 390 million dengue virus infections per year of which 96 million clinically (with any severity of disease) [14]. Moreover the existence of these vectors in sub Saharan Africa could alter the epidemiology of zika and other arboviral diseases [2]. The association of zika with microcephaly was the biggest concern [15]. Though the mortality rate due to CHIKV is low, it causes extended morbidity in the form debilitating arthralgia [16].

In Eritrea epidemics of DF were appeared at different places with different temperature and altitude [17]. Description of *Ae.* mosquito of the state of Eritrea is limited to a single study and it was limited to certain part. *Ae. aegypti*, was a single species isolated in the western part[18]. In the absence of vaccine, entomological surveillance and suitable vector control (VC) are the best practices to prevent DF and CHIKV. Effective strategies to control the vectors depend on a good understanding of immature and adult vector ecology. Therefore, the current study was conducted to identify the species composition, characterize larval habitats, determine the positivity and productivity profiles of containers in three towns, *viz*. Keren, Hagaz and Elabered, Anseba zone, Eritrea.

2. Materials and Methods

2.1 Study design

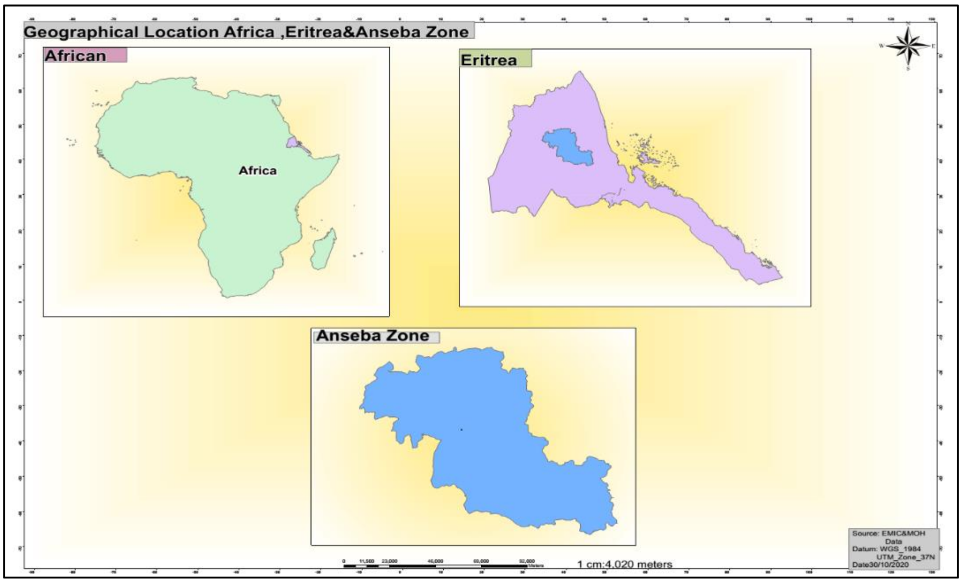
A descriptive cross-sectional study was conducted on July. 2020 – Sep. 2020, in Keren, Elabred and Hagaz, Anseba zone, Eritrea to determine the prevalence, characterization, seasonal distribution and larval habitats of *Aedes* mosquitoes.

2.2 Study Area

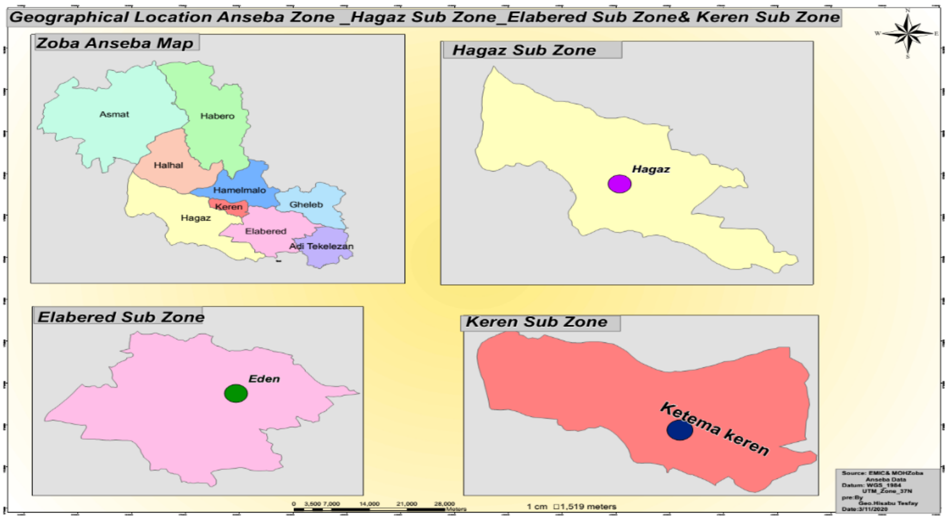
Anseba is located in the 15^0 31’ - 17^0 32’ north altitudes & 36^0 53’-38^0 54’ east longitudes. It has a total of 9 subzones, 92 'administrative areas' and 388 villages. According to zonal malaria control program (ZMCP), annual report (2018), Anseba zone has a total population of 394,419 evenly distributed throughout the zone (7074km^2). The climate prevailing in the zone is tropical. The temperatures range in the area is between 19 - 40 °C, with the altitude of 744-2404 meters above the sea level (masl). Anseba zone has humidity level between 35-77%. The inhabitants of the area are mostly traders, farmers and nomads. The zone has 33 health facilities, (1 Referral Hospital, 3 community hospitals, 5 Health centers and 24 health stations).

2.3 Study site

Samples were collected from three towns in the zone, *viz*. Keren, Hagaz and Elabered. Samples were collected (monthly) from all clusters in certain HH in each town during the study period of three months.



Study Area: Anseba Region, Eritrea, Africa



Study sites: Keren sub region, Keren Town, Elebered zub region Eden Town and Hagaz sub zone Hagaz town

2.4 Sampling Technique
In this community-based study; cluster sampling was used by dividing the areas into clusters or zones based on the administrative areas. Samples of *Aedes* larva and pupa were collected randomly every one month, i.e. 101 houses / town from July. 2020-Sep. 2020. The starting point was in the middle of every zone of respective administrative area. Pen was rolled to indicate the starting HH, and a HH nearest to the sharp end was taken as a direction and starting. Then moving to the right and counting according to the sample interval (number of houses in the administrative area divided by the number of houses to be surveyed in that administration) the next house was selected.

2.5. Data Collection
Larvae/pupae were collected between 07:00-01:00 hr during the previously mentioned study period using the Entomological surveillance kits for collection of *Aedes* mosquito.

2.5.1 Larvae and pupae collection
3.5.1.1 From small containers
Larvae and pupae were collected from barrels, tyres, cement basin, water tanks flowers vases and others. These were emptied directly into a tray or filtered through a sieve. The larvae and pupae were placed in suitable containers, and then transported to Elabered malaria entomology laboratory.

2.5.1.2 From large containers
Collection of larvae and pupae by white plastic dipper was used in large containers such as water tanks (>1000litres) and cement water basins. Lower the dipper gently into the water at an angle of about 45º, until one side is just below the surface. During our dipping care has been taken not to disturb the larva/pupa. All collected larvae and pupae were kept in water jugs (for each independent breeding-site) and transported to the laboratory. Each sample was labeled with the pertinent information (WHO, 2011).

2.6. Mosquito Rearing, Processing and Identification
The field-collected immature stages of *Aedes* mosquitoes were kept in well-labeled vials and transferred separately to paper cups covered with a netting material and fixed with a rubber band. The larvae and pupae were reared in the Elabered malaria entomology laboratory to the adult stages. When emerged, adult mosquitoes as well as specimens of larvae (3^{rd} and 4^{th} instars) and pupae were transferred into 70% ethanol in Eppendorff tubes. Moreover, the wild collected adults were killed by chloroform and then preserved as described above. The preserved specimens of mosquitoes were examined under dissecting microscope and the species were determined with the taxonomic Keys (Rueda, 2004 and Huang, 2014). Larvae were examined under a compound microscope at magnifications of 10× and 40×.

2.7. Entomological Survey
Entomological surveys were carried out in the selected area for 3 months. The entomological surveys were carried out by a well-trained team of Elabered malaria entomology laboratory, (1Entomologist, 2 public health officers and 3 insect collectors) and 3Trained health workers from each of the study sites. The teams were visited the selected houses for larval stages of *Aedes* mosquitoes. Information on the number of inspected houses, types of all wet containers, and positive containers was recorded including the productivity. Moreover, larval surveys were conducted at outdoor sites close to the house and when located, the larval habitats were described. Likewise, adults were collect from indoor and outdoor to indicate the feeding behavior and pick biting time. Locations of all the households participated in this study were marked using Global Positioning System (GPS). Samples of adult *Aedes* mosquitoes were kept in 1.5 Eppendorff tubes with silica gel for subsequent species identification.

2.8. Meteorological Data
On monthly basis temperature (minimum and maximum), R.H. and rainfall in study area were collected from the health facilities (the sentinel sites are collecting these information on regular base).

2.9. Study Instruments
The entomological surveillance kits for collection of *Aedes* mosquito included; light torch, white enamel pan, pippete and fine mesh sieve.

2.10. Data Analysis
Data analysis was performed using SPSS version 23. Positive containers were those with one or more *Ae. mosquito* larvae or pupae. Proportion of wet containers that were positive in each site was determined and a Chi- square test was used to compare the distribution of positive containers between indoor and outdoor locations. Chi- square test also used to compare the distribution of *Ae. mosquito* infestation in the three study sites, and productivity between seasons. Key larval habitats were defined as containers that are most productive for *Ae. mosquito* pupae.
Mosquito indices were calculated as follows:

House index (HI;% of houses positive for larvae of *Ae. larva/pupa***):**

Container index (CI;% of water-holding containers positive for *Aedes* larvae**):**

Breteau index (BI; Number of positive containers for *aedes mosquitoes* / 100 houses**):**

2.11. Ethical Clearance
Clearance to conduct the study was obtained from Ethical Approval Committee at the Ministry of Health, Eritrea.

3. Results
This is a result for the presence, distribution, abundance, positivity and productivity profiles of containers of *Aedes* Mosquitos in Elabered, Keren and Hagaz, Anseba zone Eritrea. A total of 902 Households (HH), 302 HH from each study site were taken. During the study, 10 Questioners (1.108%) were considered as non-responses rate because out of them, 75% of these questioners were not fulfilled.

3.1 Sessional Description
During the study period (July-September, 2020) the average temperature were __ in Elabered, ___ in Hagaz and ___ in Keren, with an average rainfall extends from 305mm in Elabered to 363mm in Hagaz. The study areas were found in the average Latitude of 38.62/15.70 in Elabered, 38.27/15.70

in Hagaz and 38.46/15.78 in keren. Meanwhile the average elevation is 1541m, 919m and 1400m in Elabered , Hagaz and keren respectively**.**

3.2. *Aedes* mosquitoes prevalence

As depicted in table 3.1 a total of 896 HHs and their surrounding from the three sites (*vis*. Elabered, keren and Hagaz) were surveyed to assess the presence of *Aedes* mosquitoes and *Aedes* mosquitoes breeding habitats. Of which more than half of the HHs (58.7%) were found to be positive for either larva, pupa or for both.

Table 3.2 shows, from these 896 HHs and their surrounding a total of 3765 wet water-holding containers were inspected during the survey. An average of 4conatiners per household and a positivity rate 24.2% were recorded during the study period of three months. With high statistically significance (x^2 =103.87, df=18, P<0.0001), the positivity of the wet water holding containers were 19.1% in Elabered, 21.9% in Hagaz and 37.7% in Keren.

The distribution of *Aedes* mosquitoes breeding places (*vis*. water holding containers) were at indoor and outdoor sites. Table 3.2 shows, in Elabered town a total of 1204 wet containers were inspected of which 717 were at indoor and the rest 487 were outdoor. At indoor sites 14.6% (105) of the containers were found to be positive for aedes mosquito's immature stages. Likewise at outdoor sites 25.7% (125) of the wet containers were positive for larva/pupae. There was a significant variation in the proportion of positive containers between in and outdoor locations within the site. (x^2 =82.59, df=9, P=0.0001).

Similarly at Hagaz town a total of 1078 wet containers indoor and 300 outdoor containers were inspected; in which 198 (18. 4%) indoor containers and 83 (27.7%) outdoor containers were positive for *Aedes* larvae and pupa. The variation between indoor and outdoor positive containers in this site were statistically significant (x^2=18.26, df=10, P<0.049).

And in Keren from the total 655 indoor containers inspected 200 (20.5%) were positive for *Aedes* larvae/ pupa and from the total 530 outdoor containers inspected 247 (46.6%) where positive for *Aedes* larvae/ pupa. But the variation between indoor and outdoor positive containers in this site were not statistically significant (X2=28.39, df=25, P=0.27). [Table 3.2]

The positivity of containers were very high at outdoor than the indoor containers. At indoor containers the positivity was 17.7%, and at the outdoor the positivity was as high as 37.7%**.**

Table 3.1: Total number of HH inspected and their positivity by site at Keren, Hagaz and Elabered towns Anseba zone Eritrea (2020)

Towns	July			Aug.			Sep.		
	Total No. of HHs	**+ve HHs**	**% of +ve HHs**	**Total No. of HHs**	**+ve HHs**	**% of +ve HHs**	**Total No. of HH**	**+ve HHs**	**% of +ve HHs**
Elabered	99	46	46.46	101	45	44.55	100	60	60.00
Hagaz	105	50	47.62	98	43	43.88	99	54	54.55
Keren	100	75	75.00	94	72	76.60	100	81	81.00
total	304	171	56.3	293	160	54.6	299	195	65.2

Table 3.2: Total number of containers inspected and their positivity by site at Keren, Hagaz and Elabered towns Anseba zone Eritrea (2020).

Town	Indoor			Outdoor			Total		
	Total inspected containers	**Total No. of +ve containers**	**% of +ve containers**	**Total inspected containers**	**Total No. of +ve containers**	**% of positive containers**	**Total contain inspected**	**+ve container**	**%**
Elabered	717	105	14.64	487	125	25.7	1204	160	13.2
Hagaz	1,078	198	18.4	300	83	27.6	1384	281	20.0
Keren	656	200	30.5	530	247	46.6	1186	447	37.7
Total	2451	433	17.7	1223	455	37.2%	3674	888	24.2

3.3 *Aedes* mosquitoes: Container preference and containers positivity rate

Indoor

This survey revealed that, among the wet containers served as *Aedes* mosquitoes breeding places, cement basin, clay-pots and soft drink boxes were found to be with the highest positivity rate, which accounts for 56.52%, 44.44% and 36.36% respectively. As depicted in Table 3.3, irrespective the positivity rate, barrel was found to be the most used water holding container by HH , and then with the highest positivity in number (410).

The preference of *Aedes* mosquitoes by town is also depicted in the same table, of which Clay-pot (30%), barrels (19.3%) and cement basins (66.66%) were the first most preferred breeding places in Elabered. In Hagaz, clay pots (45.45%) and soft drink boxes (44.44%) were the with the highest positivity and in Keren: clay-pots, barrels and jerrycans were the first three containers with high positivity accounts 66.66%, 32.23% and 27.58% respectively.

The variation in the positivity of the containers in this study was statistically significant (X2=120.97, P<0.001). September was with the highest positivity among the three months of study.

Outdoor

A total of 2452 outdoor containers were assessed for the presence of *Aedes* mosquito immature stages, among them 504 were positive. As shown in table 3.4, visible predominance of hand washing basin was seen. >66% of these hand washing basins were found positive. Discarded tins and discarded tyres were also with the highest positivity following to hand washing basin and they account for 60% and 46.11% respectively.

With a statistical significance (X2=134.85, P<0.023), the positivity of these water holding containers were different among the towns, in Elabered, barrel (32.98%), discarded tyres (30.77%) and leaky faucets were the first three, in Hagaz, hand washing basin (66.67%), discarded tyres (41.07%), and cement tank (37.84%), and in Keren , discarded tins and wood & metal drums were with the highest

positivity rate (100%) followed by hand washing containers 85.7% and cement tank 58.33%.
Similar to the indoor containers, in outdoor container, barrels were the most dominant water holding containers with the highest number of positives. A total of 247 barrels were positive for *Aedes* mosquito's larva/pupa. The rate of positivity of containers was high in September, Fig.(3.1).

Table 3.3: Container types inspected and their positivity, in Keren, Hagaz and Elabered towns, Anseba zone: Eritrea (2020) (Indoor)

Container type	Elabered			Hagaz			Keren			Total		
	Total inspected	+ve	% of +ve	Total inspected	+ve	% of +ve	Total inspected	+ve	% of +ve	Total inspected	+ve	% of +ve
Clay-Pots	10	3	30	11	5	45.45	6	4	66.67	27	12	44.44
Barrels	513	99	19.3	779	150	19.26	498	161	32.33	1790	410	22.91
Jerry Cans	80	2	2.5	192	26	13.54	58	16	27.59	330	44	13.33
Cement Basin	2	0	0	15	10	66.67	6	3	50	23	13	56.52
Water Tanks	0	0	0	4	0	0	8	2	25	12	2	16.67
Tyres	2	0	0	3	1	33.33	4	0	0	9	1	11.11
Flower Vase	81	1	1.23	56	2	3.57	61	9	14.75	198	12	6.06
Soft Drinks Bottles	2	0	0	9	4	44.44	0	0	0	11	4	36.36
Gutter	0	0	0	0	0	0	0	0	0	0	0	0
Dish	20	0	0	7	1	14.29	6	0	0	33	1	3.03
Leaky Faucet	3	0	0	1	0	0	2	2	100	6	2	33.33
Hand Washing	2	0	0	0	0	0	4	3	75	6	3	50.00
Other	2	0	0	2	0	0	3	0	0	7	0	0.00
TOTAL	717	105	14.64	1079	199	18.44	656	200	30.49	2452	504	20.55

Table 3.4: Container types inspected and their positivity, in Keren, Hagaz and Elabered towns, Anseba zone: Eritrea (2020) (outdoor)

Container type	Elabered			Hagaz			Keren			Total		
	Total inspected	+ve	% of +ve	Total inspected	+ve	% of +ve	Total inspected	+ve	% of +ve	Total inspected	+ve	% of +ve
Tree Holes	2	0	0	0	0	0.00	0	0	0	2	0	0.00
Dis. Tires	39	12	30.77	56	23	41.07	85	48	56.47	180	83	46.11
Dis. Tins	4	1	25	1	0	0.00	5	5	100	10	6	60.00
drums	0	0	0	5	0	0.00	1	1	100	6	1	16.67
Cement Tanks	7	2	28.57	37	14	37.84	100	43	43	144	59	40.97
Water Tank	1	0	0	3	0	0.00	12	7	58.33	16	7	43.75
Coconut Shell	0	0	0	0	0	0.00	0	0	0	0	0	0.00
Shower Tank	0	0	0	1	0	0.00	0	0	0	1	0	0.00
Fresh Drink	4	0	0	0	0	0.00	7	2	28.57	11	2	18.18
Leaky Faucet	7	2	28.57	4	1	25.00	2	1	50	13	4	30.77
Hand Washing	0	0	0	3	2	66.67	7	6	85.71	10	8	80.00
Flower Vas	81	11	13.58	8	2	25.00	28	4	14.29	117	17	14.53
Barrel	282	93	32.98	113	35	30.97	249	119	47.79	644	247	38.35
Jeri cans	19	2	10.53	42	5	11.90	25	6	24	86	13	15.12
Others	41	2	4.88	27	1	3.70	9	5	55.56	77	8	10.39
Total	487	125	174.8	300	83	27.67	530	247	663.7	1317	455	34.55

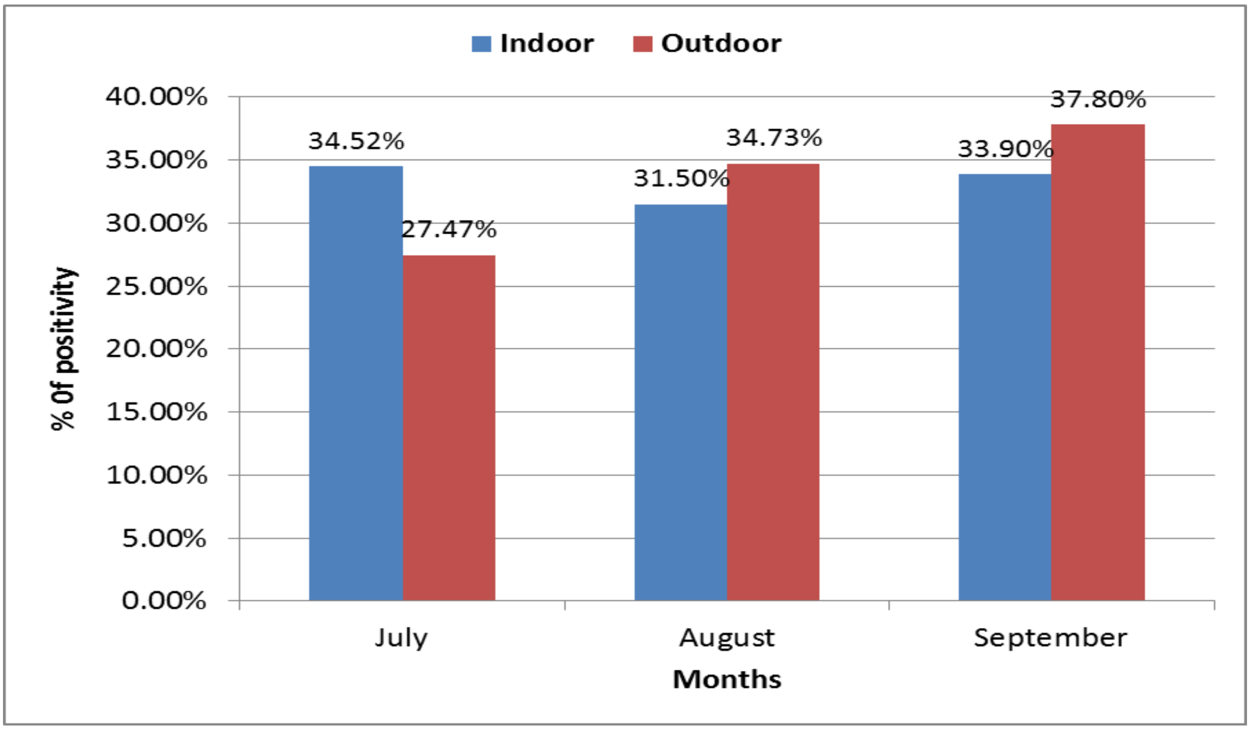


Fig 1: monthly positivity of aedes mosquito breeding containers at indoor and outdoor, Keren Hagaz and Elabered town, Anseba zone, Eritrea (2020)

3.4 Productivity Profiles of *Aedes* Mosquito Breeding Containers

Fig. (3.2), shows, among the positive containers inspected in the three study sites, infestation of *Aedes* mosquitos larvae was 58.96 larvae/container in Elabered, 60.35 larvae/container in Hagaz, and 87.93 larvae/container in Keren and the variation was statistically significant(X^2=398.35 , $p < 0.007$). The mean variation among the study areas in both indoor and outdoor containers were also significant (F=41.19, df=2, P-value=0.0001 indoor) (F=11.95, df=2, P-value=0.0001 outdoor).

3.4.1. Indoor productivity profile

The most widely used HH water container for domestic purpose in all the towns (*vis*. Elabered, Keren and Hagaz), was barrel. Jerrycans (60litres) and cement basins were also another widely used water holding containers. Obviously these three container types were the most productive containers in all the study sites. As depicted in Fig. (3.3), Barrels were the most productive which alone responsible for more than 69% of all the collected *Aedes* immature stages. With a statistical significance x^2 = 377.24 ,p=0.0001) in the productivity, cement tanks, flower vase , jerrycan and clay pot were among the most producers of the immature stages followed to barrel.

3.4.2. Outdoor productivity profile

Nearly half of the collected pupae were found from a single container. Similar to the indoor containers, in outdoor containers barrel is still the most dominant and hence it was the most productive. Fig. (3.4) shows, the productivity rate of barrel was followed by cement tanks; hand washing basin, discarded tins and discarded tyres. The Covid 19 pandemic was brought a very high practice of proper hand washing at critical times for hand washing. These hand washing basin are unfortunately used as *Aedes* mosquito breeding habitat.

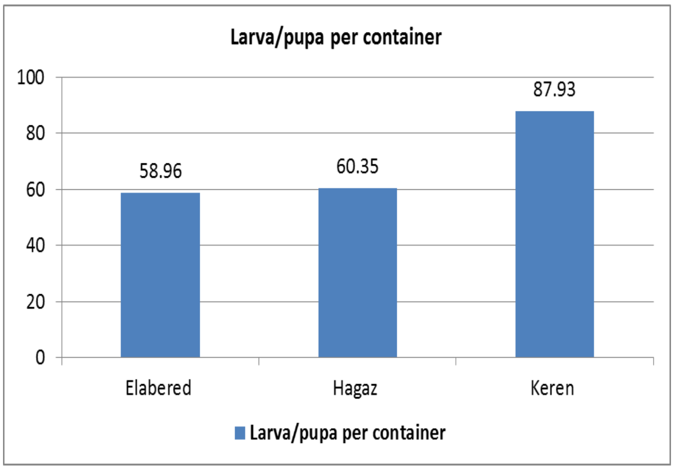


Fig 2: Number of pupa per container over the study period, Hagaz, Elabered and Keren towns, Anseba zone, Eritrea.

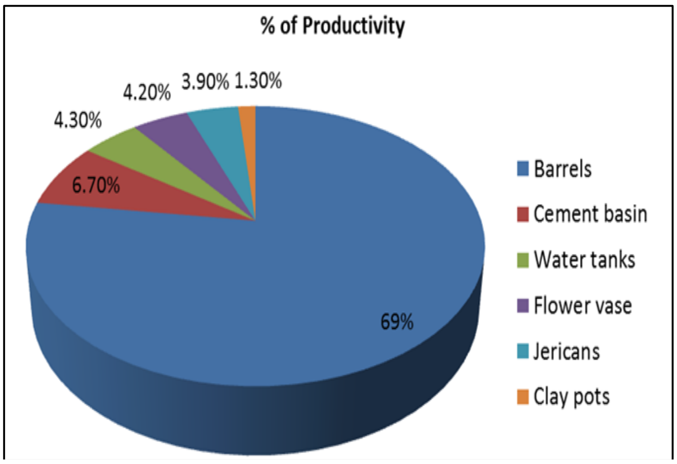


Fig 3: Percent of Pupa productivity profiles by different indoor water holding containers, Hagaz, Keren and Elabered towns, Anseba zone, Eritrea (2020)

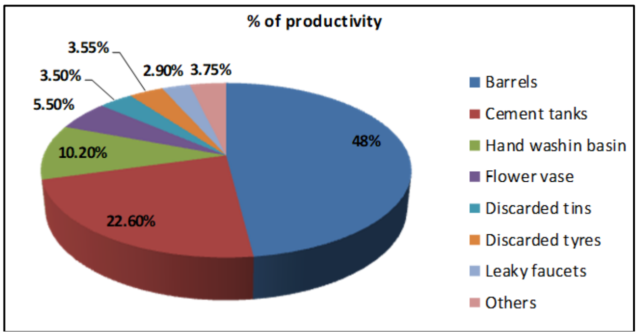


Fig 4: Percent of Pupa productivity profiles by different outdoor water holding containers, Hagaz, Keren and Elabered towns, Anseba zone, Eritrea (2020)

3.5. Larval Indices

Conducting surveillance of *Aedes* mosquito is an important factor to predict dengue/chikungunya burden in an area. The prediction of these diseases will help to warn the community before the disease spreads in their respective catchment areas. There are different indices and methods in different life cycle from egg-larva- pupa to adult available to measure *Aedes* mosquitoes inhabitation in an area, but the best of all is the larval surveillance and calculating the larval indices for different reason. These indices are House index (HI), container index (CI) and Breteau index (BI).

The BI, CI, and HI for *Aedes* mosquito immature stage of all study areas were analyzed. As depicted in fig. (3.5) of the 896 houses surveyed, 526 had *Aedes* mosquito breeding habitats. The proportion of houses infested with *Aedes* mosquito larvae can be expressed as the HI, which was 58.70 in this study. Overall, 4001 water-holding containers were identified, of which 1004 had *Aedes* mosquito breeding. The CI was 25.09. The BI, which reflects the number of positive containers per 100 houses inspected and it was found to be112.05.

3.6. Species Identification

The collected aquatic mosquitoes from different breeding habitats were reared in the lab. After a correct procedure to rear, kill and preserve these mosquitoes a total of 1113 were identified to their species level. Of these the most dominant species were *Ae. aegypti egypti* accounts for 71.9% and another sub species *Ae. aegypti formosus* accounts 17.9%, culex 8.6 and anopheles 1.5%, shown in Table 3.5.

3.7. Human landing rate (HLR) or Adult Biting Index (ABI)

HLR or ABI is another tool used to predict the appearance of dengue/yellow fever epidemics in an area. For different reasons the collection of adult mosquitoes by human landing catch was done only in Elabered (a place where the entomology laboratory is located). The collection was performed for 12hrs starting from 06:00am to 06:00pm. As depicted in table 3.6, the average HLR over the period of 3 months was 2.8.

This collection using human landing catch was done at indoor and outdoor. Table 3.6 shows, a total of 91 mosquitoes were collected, of these only 28% were from the indoor and the rest 72% were from the outdoor.

3.8. Knowledge on inhabitants on aedes mosquito immature stages

On the top of all the above findings, as depicted in table 3.7, knowledge of the inhabitants on mosquito immature stages from all the study sites was assessed. The knowledge of the community on these mosquito aquatic stages is the main domain on reduction of mosquito population at household level. However it was found to be very low that only 13.8% of the attendants know the larva/pupa. The rest 86.2% called the larva/pupa as another group of insect which is a harmless insect.

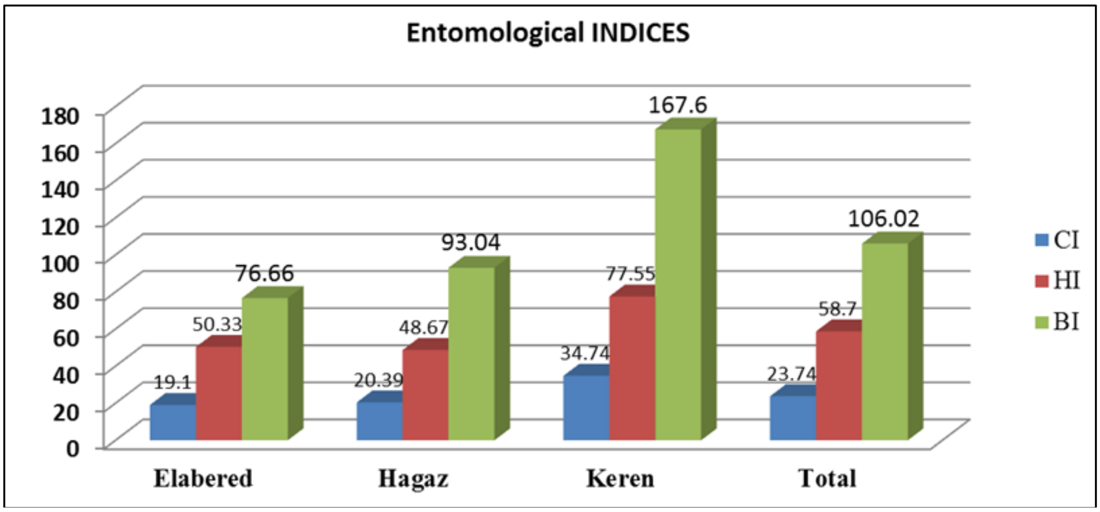


Fig 5: By sites and overall Larval induces, Keren, Hagaz and Elabered, Anseba zone, Eritrea (2020)

Table 5: Number of mosquitoes identified to their species level: Keren, Hagaz and Elabered, Anseba zone, Eritrea (2020).

Towns	Total number of mosquitoes sampled	Species			
		Ae .aegypti egypti	*Ae. aegypti formosus*	*Anopheles*	*Culex*
Elabered	332	249 (75%)	63(18.98%)		20 (6.02%)
Keren	391	281(71.87%)	70(17.9%)		40(10.2%)
Hagaz	390	270(68.2%)	67(17.18%)	17(4.3%)	36(9.2%)
Total	1113	800(71.9%)	200(17.9%)	17(1.5%)	96(8.6%)

Table 6: Monthly adult biting index (ABI) and biting behavior (indoor/outdoor): Elabered, Anseba zone, Eritrea (2020)

Elabered				
Months	Number of catch			Bite/person/hr
	Indoor	Outdoor	% of indoor	
July	5	20	25%	2.5
August	7	26	27%	3.15
September	8	25	32%	2.75
Total	20	71	28%	2.8

Table 7: Knowledge of inhabitants on mosquito immature stages in Elabered, Hagaz and Keren, Anseba zone, Eritrea.2020.

Towns	Total of people participated	Knowledge of participants of the immature stages		
		Yes	No	%
Elabered	484	116	368	23.9%
Hagaz	598	53	545	8.9%
Keren	350	29	321	8.3%
Total	1432	198	1234	13.8%

4. Discussion

There are many methods in which *Aedes* mosquitoes can be studied (*vis*. egg, larva, pupal and adult surveys). However larval survey is the most widely used methods for entomological surveillance for many practical reasons [19]. Hence the present study aimed to study larva of aedes mosquitoes in different indoor and outdoor water storage containers. Many HH had different water storage containers at indoor (any part of the house covered by a roof, also known as intra-domestic) and outdoor (any part of the house not covered by a roof, also known as peri-domestic). In all the study sites the HH were presenting with plenty of containers for water storage. ON average there were 4 water storage containers per HH. This was directly related to the limited access of the communities to piped water supply. The source of water in both sites was municipal piped water, tanker trucks and rain water. Different studies from different regions found that water supply crises push the community to own various containers and thus store water for long period of time and these domestic water storage containers serve as *Aedes* mosquitoes breeding sources [20, 21].

The most prominent and widely used breeding containers in the study sites were barrels in both the outdoor and the indoor, and thus it serves as a common *Aedes* mosquitoes breeding habitat. However the *Aedes* mosquito immature stages were found in different wet containers. At indoor sites, cement basin was the most preferred and presented with 56% positivity. Hand washing basin (50%), clay pots (44%) and leaky faucets 33.3%) were among the preferred containers followed to Cement basis. At outdoor site hand washing basin was the most preferred with a positivity of 80% despite the number of basin inspected was relatively low. It was also very high in discarded tins and discarded tyres and presented with 60% and 46.1% respectively. Water tank (40.9%), cement tanks (43.7%) and barrels were another prominent and with a high positivity in outdoor water storage containers. Container type might be different from one country to the other, but very similarly in Ethiopia, tyres, barrels, plastic drums, clay pots were among the preferred water storage containers [22] and Kenya buckets, drums, tyres and pots were the key containers [23].

Similar study from the same country (Barentu town, Gash Barka Zone, Eritrea) found that, Jerrycans were among the widely used container but with a limited presence of larva/pupa due to frequent utilization resulted to continuous cleaning and empting [18]. In the present study jerrycans, specifically 60litres was very common water storing containers especially in Hagaz town and they were found among the preferred *Aedes* breeding habitat. These jerrycans were identified as a preferred water storing containers by Ngugi *et al*., (2017) [23]. Generally HH water holding containers that are in frequent use for different purposes creates no media for *Aedes* multiplication. Whereas containers that retained water for long period were suitable breeding habitats [24, 25].

The present study found that, the breeding habitats were distributed between the indoor and the outdoor with high positive containers in the outdoor. The study from Kenya was found that >98% of the positive containers were located outside [23]. The presence of outside positive containers was a strong predictor of the abundance of female *Ae. aegypti*. Containers located outside were nearly10 times more likely to contain female pupae than those kept inside [27]. The result of the study conducted by Schneidar *et al*., (2004) found that the outdoor containers contributed disproportionately to *Ae. aegypti* abundance in Peru. The preference of these outdoor containers by *Ae.* mosquitoes as an oviposition site might be perhaps containers located outside had more nutrients necessary for larva growth and successful development thus increasing pupal abundance [27]. The present study was conducted at the rainy season from July to September but the outdoor containers were less in number than the indoor containers despite the positivity rate in the outdoor were twice than the indoor. The rainy water was specifically used for washing clothes and was poorly handled and almost without fitted covering. Therefore the containers were served as suitable breeding habitats for *Aedes* mosquitoes.

Only one species of *Aedes* was recorded in the study area. From the identified 1113 *Aedes* mosquitoes 89.8 was *Ae. aegypti*, which farther divided in to two sub species and of them, 71.9% were *Ae. aegypti egypti* and the rest 17.9% was *Ae. aegypti formosus.* And a late study by Mensur *et al.*, (2019) in the western part of the country was identified only *Ae. Aegypti* [18]. In neighbor Sudan, 33 species of aedes mosquitoes were recorded [29]. With the unlimited movement of people from and to Sudan, importation of these species may happen and continuous surveillance needs to be implementing.

The co-existence of *Aedes*, *Culex* and *anopheles* were recorded in all the study sites. Despite, *Ae. aegypti* was found to be the most dominant mosquitoes to breed in the domestic water holding containers, *Culex* and *anopheles* were also found on these containers computing with *aedes*. From the total identified mosquitoes 8.6% were *Culex* and the rest 1.5% were *Anopheles*. The co-existence of *Culex* and *Aedes* were previously proven by different studies [18, 21, 23].

It is difficult to associate between climate parameters and *Ae. aegypti* abundance. Because *Ae. aegypti* depends on humans, especially its preference for human blood and its use of artificial containers for viposision development[28,29]. Socioeconomic conditions and human behavior are the key factors on *Aedes* abundance and distribution of *Aedes* borne diseases. A study conducted in Eritrea, found that there were epidemics of dengue in the country in different altitudes with a very big geographical difference (Massawa with elevation 20masl and Mendefera (1954masl) [17]. But Altitude, temperature and rainfall are important factors in different aspect of the mosquitoes and the distribution of mosquito borne diseases. Despite an exceptions from different literatures, in which mosquitoes can be found up to 2200masl, the best elevation for heavy *Aedes* mosquito infestation is >1000masl. A study from Mexico found that *Aedes* mosquitoes are abundant at elevation up to 1,300masl, moderately abundant from 1300masl to 1,700masl, and still present but rare from 1,700 to 2150masl [30]. The present study was conducted in an area with elevation extends from approximately 900masl to 1500masl. This elevation was found very suitable that almost all the positive wet containers were found to be very productive.

A study from Eritrea found, during the dry season the *Aedes* mosquito breeding habitats were only limited to the indoor containers but after a single rainfall, the breeding habitats were visibly extended to the outdoor containers and discarded tyres and discarded tins were among the most preferred outdoor breeding habitats [19]. WHO were also noticed that rain fall increases mosquito density [31]. Yusof, was also found rainfall increase mosquitoes density by increasing availability of potential breeding places [32]. The present study was conducted during the rainy season and on the contrary it was found that the indoor breeding sites were very high in number but the positivity was high in the outdoor. The month of September was the most infested with a very high density compared to July and August. This was because during that month of September the rain was slightly reduced and thus the disturbance and exchange of water was consequently decreases and resulted to high infestation and increased density.

The distribution and the risk of epidemics from arboviruses diseases (*Vis*. dengue fever, yellow fever, chikungunya and others) can be easily predicted by calculating the larval indices (CI, BI and HI). These indices used to calculate the presence, distribution and densities of *Aedes* populations in an area. According WHO there is an epidemic risk of yellow fever, if the indices are above the threshold of 5% for Breteau index, 3% for Container index and 4% for house index [34]. CDC also noticed that, there is high dengue fever transmission if the BI >50 and HI >10. The present study was found that 58.7% HI, 23.7% CI and 106.0 for BI[34]. This finding was very high for the occurrence of the arbovirus diseases in an epidemic state in all the sites.

Constant temperature is the most important environmental criterion. Immature mosquitoes are typically best at a water temperature of 26-27 ^{O}C [35]. The development times of aedes mosquitoes always vary with temperature [36, 37], food supply [37, 38] and density or crowding of breeding sites [39]. The present study was conducted at a suitable period of time. The average temperature at both sites was 24 ^{0}c to 30 ^{0}c. The development from egg to adult for *Ae. aegypti* is 7–10 days at 25 ◦C and 7–9 days at 30°C, However, at 35 ◦C the development time (from first instar to adult stage) was 6–7 days showing that as temperatures increase, the mosquito development time is reduced [40].

5. Conclusion

- *Ae. aegypti* the main vector of the arboviruses worldwide is inhibiting in the study sites. The larval indices were found very high.
- Water scarcity (insufficient water supply from the municipalities) force HH to own plenty of water storage containers, thus serve as Aedes mosquitoes breeding habitats. These containers are almost equally distributed to indoor and the outdoor sites. The most key-containers which were found positive were barrels, clay-pots, cement basin, discarded tins and tyres, hand washing basin and leaky faucets.
- Discarded tyres on streets, inside houses used for different purposes, garages and establishments were also found to create a suitable environment for mosquito breeding.
- Different aedes suitable infrastructures are also another challenge in creating a good media for aedes infestations.
- The knowledge of the inhabitants on the mosquito immature stages, found to be very low.

6. Recommendation

- To control aedes mosquitoes, community based intervention has to be applied in all the sites. The package of these community based interventions is aimed at HH.
- Households need to be trained on water containers management in their homes and then they will correctly manage water containers consequently in order to have no aedes breeding site in their homes.
- Other integrated vector control management like tomophose application to large water holding containers has to be strengthened.
- Penalties of individuals who are not sustain larva/pupa free water holding containers have to be introduced by the municipality.
- Integration has to be made within the ministry of health programs and other sectors or line ministries to strengthen the community based aedes control interventions.

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