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Spatio-temporal Distribution of *Aedes* Mosquitoes (Diptera: Culicidae), in Alfasher locality north Darfur State, Sudan

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Abstract
Vector- borne diseases account for significant morbidity and mortality worldwide. This is especially true for resource-poor countries and poor and marginalized populations. The current study was carried out during May 2016 – April 2017 Elfasher Locality, North Darfur State, Sudan to determine to determine seasonal and geographical distribution, and larval habitats of *Aedes* mosquitoes. A cross-sectional surveys was conducted. The sample size of households (HH) surveyed for the productivity of immature stage was calculated following WHO (2011) in four Almasani, Elsalam, Enasr and Alreef neighborhoods. Larval collection was carried out three days/ month between 07:00-01:00 hr. Larvae and pupae from containers collected identified in the laboratory. Data was analyzed using SPSS version. 20. *Ae. aegypti* was the predominant species the locality. Out of 480 HH visited, 69 were found positive for larvae (14.4%). A total of 1,724 potential breeding sites were reported and 5.5% proved to be positive. The major breeding containers were the clay jars 1,018 (59.0%), followed by barrels 768 (44.5%). The highly positive containers were the jars (33.2%). followed by the barrels (2.6%) and water tanks/others (0.3%). The highest house index (HI) was 35% during November, followed by September (27.5%). The highest Breteau indices (BI) were 45% during August, followed by September and November (25% for each). The container indices (CI) were as follows: 12% for August, and 11.5% for September. The pupal demographic-index (P/DI) was 20.5% for August and 3% foe September. Significant differences were detected between these indices in the dry- and wet- seasons, except HI. Highly significant differences between number of larvae and pupae in different seasons were detected. Therefore, community mobilization should be a priority in controlling *Ae. aegypti* populations and risk.

Keywords: *Aedes* spp., spatio-temporal distribution, darfur, Sudan

1. Introduction
Aedes mosquitoes are distributed around the world and there are over 950 *Aedes* species [1]. *Ae. aegypti* and *Ae. albopictus* [2] are vectors of several globally important arboviruses, including dengue virus (DENV) [3], yellow fever (YF) virus [4], and chikungunya virus [5]. The public health impact of *Aedes* borne-diseases (ABDs), especially the DENV has increased and widely spread worldwide. Although, mosquitoes spread several viruses and parasites between people and other animals, however, the viral disease, due to DENV has become growing public health problem in tropical and subtropical regions [6]. Currently, an estimated 2.5 billion people live in areas at risk for epidemic transmission [7] and 50-100 million new infections are estimated to occur annually in >100 endemic countries [8] (WHO, 2012). In addition, the dengue fever (DF) has spread widely than before [7], as well as dispersal of its main vectors (i.e. *Ae. aegypti* and *Ae. albopictus*) into new areas [9], resulting in 30-fold increase in incidence.
In Sudan, ABDs represent a major health problem. While, YF outbreaks have been reported in disparate regions in Darfur [8] (WHO, 2012), DF and DHF occurred in repetitive outbreak patterns in the coastal area of the Red Sea State [10] and during August and September (2018) in Kassala State (in addition to chikungunya virus). Moreover, DF and DHF have also been reported from Kassala State [11]. However, the disease might probably spread over new areas in the country since *Ae. aegypti* has been reported from Gedarif State, eastern Sudan [12]. *Ae. aegypti* was reported in different regions in eastern, central and former Southern Sudan [13]. No entomological data from western Sudan is found. Since the vaccine for DENV is still under research, prospect for successful vector control remains the only way to prevent its transmission [14, 15].

Vector control interventions are largely based on source reduction, eliminating *Ae. aegypti* larval habitats from the domestic environment, with increasing community involvement and intersectoral action in recent decades [16, 17]. Therefore, the current study was conducted to characterize larval habits, examine the larval indices for identifying risk areas of transmission at different geographic regions, as well as the seasonal and spatial distribution of *Aedes* mosquitoes in Alfasher Locality, North Darfur state, Sudan.

2. Materials and Methods

2.1 Study area

North Darfur State is located in the western part of Sudan between 8.22 °- 5.27° E and 8.9°-18.0° N (map). The State occupies an area 294,000 km² and it is bordered by Libya, and the Republic of Chad. The State contains 18 administrative localities. The population of the State is estimated at 2 million people (2009 census). Most of the inhabitants (75%) live in the rural areas and 25% in towns. The state is mainly flat, with small hills, mountains (Jebel Marrah) and seasonal streams or water courses (locally called khors). The soil in the state is mainly sand soil, especially in the northern part. Northern Darfur State is located in a desert and semi-desert regions with low rainfall and high evaporation potential and savanna. The short rainy- season is from July to September, with an average annual rain of 160 mm. The maximum temperature (39.8 °C) in May and the minimum (13.2°C) in February. The average relative humidity (RH) is 19 - 75% and reaches its maximum in August and its minimum in April. The people in the state live in different types of houses. In the rural areas, people live in typical African huts built of wood and grasses, whereas in the urban areas, they live in houses constructed of bricks and mud. Most of the people are farmers, many are herders of cows, camels, goats and sheep. The main source of larval habitats in the area is stored water for drinking and other household activities. These include different type of containers, such as Jar clay pots (locally known as zeers), barrels, jerry cans, water tanks, *etc*.

2.2 Entomological survey

Entomological surveys were carried out for one year in the two selected areas in Alfashir Locality, North Dar Fur state. In each area, blocks of houses and the surroundings were randomly selected for inspection of *Aedes* mosquitoes. Entomological surveillance data were collected monthly during the study period. The surveys were carried out by a well-trained team (3-4 health workers) and a supervisor. The team visited the selected houses (households) for the immature stages. Information on the number of inspected houses, types and positive containers (with *Aedes* pupae or larvae), and houses with positive containers were recorded. Moreover, larval surveys were also done at outdoor sites near the houses. The larval habitats were described. Locations positive to *Aedes* larvae marked using Global Positioning System (GPS):

Alslam (1336466-2521632), Alreef (1337024+2520490), Almasane (1337487+2522388), and Alnasr (1338635+2521249).

The seasonal abundance of *Aedes* mosquitoes was studied. The numbers of the larvae were recorded and counted every month from indoor and outdoor sites. Samples of 4th instar (L4) were preserved in 70% ethanol and identified to species level. Furthermore, the larval number /month were recorded.

2.3 Data analysis

The data obtained from the study was analyzed using SPSS version 20. Parametric tests were used for normally distributed data and non-parametric tests were used to analyze non-normalized data. Moreover, *Aedes* larval indices; House index (HI), container index (CI), Breteau index (BI) and Pupal/demographic (P/D) were calculated using the following formulae:

House index (HI): % of houses positive for larvae:

HI = No. of houses positive for *Aedes* larvae x 100 No. of houses inspected

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

CI = No. of positive containers x 100 No. of containers inspected

Breteau index (BI): Number of positive containers for *A. aegypti*/ 100 houses):

BI = No. of positive containers / 100 houses inspected.

Pupal/demographic (P/D):

P/D = Total no. of collected pupae of *Aedes* mosquitoes / Total no. of inhabitants in the inspected houses.

3. Results

During the survey, other species of mosquitoes were found in the old, neglected uncovered water clay jars (zeers), *viz*. *Culex* spp, The jars were heavily contaminated with algae, and the small volume of water remaining in them was sticky as a result of algal presence. The study focused only on *Aedes* spp. A total of 2,933 *Aedes* mosquito larvae collected from different receptacles were identified using pictorial morphological key. All of specimens (100%) proved to be *Ae. aegypti* (Table 1).

A total amount of 480 houses were visited during the survey. Of these, 69 were found positive for mosquito larvae (14.4%). A total of 1,792 potential breeding sites were registered, of which 358 (20.0%) were actually positive (Table 2).

The key- containers, which were found most positive and formed the major breeding sites were the jars (zeer; 1,018), which constitute 57.0%, followed by barrels. (768), which represent 43.0%. The high positivity containers were the zeers (94.4%), followed by the barrels (5.6%; Table 2).

The wet-season showed greater percentage of HI (17.8±4.0%), compared to dry -season (11.4±2.0%). The BI was higher in the wet- season (29.5±8.8%) than the dry-season (14.1±2.7%). Moreover, the CI was 9.2±2.7 % in wet-season and 3.6±0.6 % in the dry-season. However, the %P/DI was found to be 7.8±5.2 % in the wet-season and 0.1± 0.1% in the dry-season (Table 3). In summary, the mean percentage of HI was 12.9%, BI was 17.7%, CI was 4.9%, and PDI was 1.9%.

Table 1: *Aedes* Species identification using pictorial morphological key in the selected areas of Alfasher town, North Dafur State, Sudan.

Total larvae	Total identified	% *Aedes* species	
		Ae. aegypti	Others
2,933	2,933	100	0.0

Table 2: Containers and their types, categories and positivity in Alfasher town, North Darfur State, Sudan (May 2016-April 2017).

No. of containers inspected	Jars (zeers) (Total & %)	Barrels (Total & %)*	Tanks/others Total & (%)*
1,792	1,018 (57%)	768 (43%)	6 (0.3%)
Total & % (+) containers*	% (+) jars	% (+) barrels	% (+) tanks/others
358 (20%)	338 (94.4%)	20 (5.6%)	0 (0.0%)

***** From the total inspected

Table 3: Percentage (mean±SE) of house Index (HI), Breteau Index (BI), Container Index (CI) and Pupal/ Demographic Index (P/DI) during dry- and wet -seasons in Alfasher town, North Darfur State, Sudan.

Season	% HI	% BI	% CI	%PDI
Dry	11.4±2.0	14.1±2.7	3.6±.6	0.1±0.1
Wet	17.8±4.0	29.5±8.8	9.2±2.7	7.8±5.2
Total	12.9±1.8	17.7±2.9	4.9±.8	1.9±1.2

4. Discussion

Monitoring vector communities is an integral part of disease surveillance and control programs. Nearly 30% of the emerging infectious disease events are caused by vector-borne pathogens with wildlife origins [18]. The present study showed that *Ae. aegypti* was the only species present in the study area. The findings are in line with the recent studies, which indicated that *Ae. aegypti* was known in the Greater Darfur five States and an outbreak of YF was registered in all of these states. During this outbreak, the entomological indices calculated for each locality during outbreak demonstrated that the highest house index and container were recorded from Wadi Salih and Mokgar localities. All indices were high in Central Darfur state (Hose Index = 17.9% and Container Index =10.6%) [19].

The study also investigated that the key- containers, which were found most positive and formed the major breeding sites. These were the clay jars (59.0%), followed by the barrels (44.5%). The highest positivity containers were the jars (33.2%), followed by barrels (2.6%) and, finally, the water tanks and other containers.

The information about the key containers (major breeding sites) and where they are located (key premises) is very important, because it allows VC personnel to adjust their strategies and focus on these containers/premises to be their main target during their campaigns. Key premises can be eligible for repeated larval treatments, while the house owners can be subjected to awareness-raising programs. This approach already showed to be successful in VC programs in Vietnam [20].

Another important point is the fact that only breeding sites on ground levels are surveyed. *Bromelias* in trees, tree holes, and roof gutters are not inspected as potential breeding sites. However, a study recently done by Gustave *et al*. [21] showed that roof gutters are becoming the most important *Ae. Aegypti* breeding sites in Guadeloupe, an island which had a number of dengue epidemics during the past 10 yr. Therefore, systematically ignoring these potential breeding sites can have negative consequences for the success of a VC program.

Due to poor rainfall and shortage of water supply, the residents stored water in various containers for long duration and these containers constituted the major mosquito breeding sources. Similar findings were reported from Tirunelveli District, India [22]. Containers that retained water for long periods of time make good or suitable breeding habitats for mosquitoes, such as the artificial containers [23, 24]. In Dar es-Salaam, Tanzania, water- storage often occurs in the presence of piped water systems, because of intermittent water supply and, due to the necessity of collecting supplementary rain-water [24].

The present study revealed that the house index, container index, Breteau index and Pupal Demographic Index increased during August and September. This may be attributed to rainy -season (wet-season), *i.e.* Increased rainfall, humidity and temperature, which are the favorable conditions for vector breeding [25].

The highest mean of HI was observed in September 2016 and, BI during August 2016.The highest mean of CI was reported during September 2016. However, the mean PDI was registered in August 2016. This might be also attributed to the rains during these months. This is in line with findings reported from Morelos in Mexico [26]. Significance differences between *Aedes* indices during different months of the year ($p<0.05$) were detected. These variations in indices can be attributed to the seasonality and its effect on larval productivity, which varied from dry- to wet -season. Moreover, significant differences between number of *Aedes* mosquito larvae and pupae in different months were also detected ($p<0.05$). The present study emphasized that the number of *Aedes* larvae was higher in the wet-season than in the dry-seasons. Many studies have reported similar findings in many other parts of the world, e.g. Thailand [27, 28] and Côte d'Ivoire [2]. Significant differences between the numbers of positive houses, total positive containers, positive jars and positive barrels in different sites were detected. However, it can be concluded that larval indices are important for the vector control efforts; these indices help the decision-makers to understand, plan and prioritize locations, categories and habitats to be targeted during their campaigns and, simultaneously to evaluate the effectiveness of control measures adopted.

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