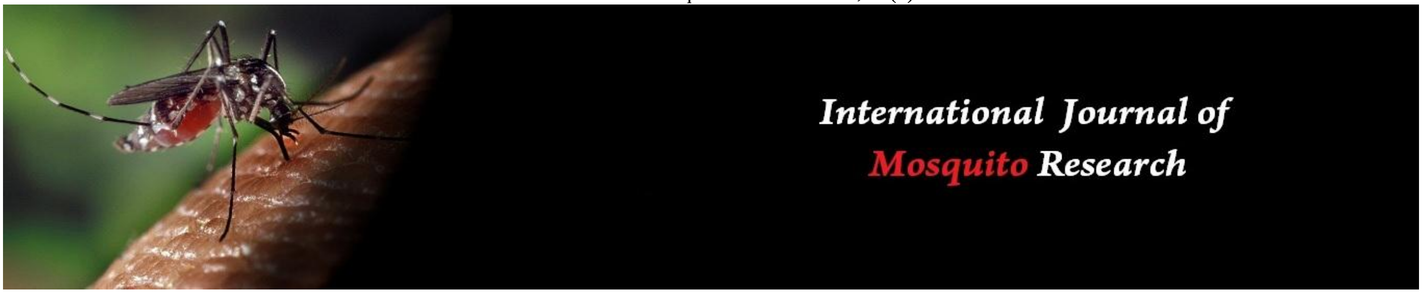

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Aedes aegypti preference habitat and baseline pupal survey in Serba and Habila Localities, West Darfur, Sudan

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Abstract
A cross-sectional survey was carried out during the period (August – November 2019) to identify baseline key containers and pupal productivity in Habila and Serba localities west Darfur, Sudan when dengue fever and chikungunya outbreak spread. The main objective of this study is to provide evidence - based data that can help in planning, implementation, and programmatic decision-making. The sample size of households (HH) for the productivity of immature stage survey was calculated following (operation1al guide for assessing the productivity of *Aedes aegypti* breeding sites, WHO 2011) in Serba and Habila. A preliminary survey and inspection of all households in the two areas was conducted on a daily basis between 07:00-01:00 hr. Data was analysed using MS (excel) and the parameters (HI, CI, BI, and PI) were calculated. Out of 14100 containers inspected, 1023 (7.8%) were found infested with larvae and pupa of the *Aedes* mosquito. The high container positivity was recorded in Serba: 646 (8.7%) out of 7354, and 306 (4.5%) out of 6746 in Habila. Major breeding containers were the clay jars (zeer) 572 (88.5%) out of 646 positive containers in Serba and 150 (49%) out of 306 positive containers in Habila. Jar clay pot (Zeer) is the major pupal productive container (95.4%) in Serba, followed by neglected pots 4 %. Whereas barrels are the major pupal productive container in Habila 42.5%, followed by zeers 35%, neglected pots 27.8% and lastly plastic water container 1.9%. Water source reduction coupled with community awareness raising should be implemented to minimize dengue and chikungunya risk as well as reduce *Aedes aegypti* indices to prophylactic levels.

Keywords: Cross-sectional survey, *Aedes aegypti*, dengue fever, chikungunya, pupal productivity, container positivity, clay jars (zeer)

Introduction
Aedes-borne diseases such as dengue, chikungunya, Zika, yellow fever, West Nile, and Japanese encephalitis, have increased and spread widely, causing serious public health problems globally [1, 2, 3]. Annually, one billion people become infected by malaria, dengue, chikungunya, Zika, yellow fever, lymphatic filariasis, and onchocerciasis. In addition, one million die due to these diseases. [4]. The major health problem in the west and eastern parts of Sudan is *Aedes*-born disease that caused several outbreaks of yellow fever, dengue, and chikungunya in the Darfur region [5, 6] as well as repeated outbreaks of dengue/dengue hemorrhagic fever in the coastal area of the Red Sea state [7]. Moreover, Sudan has experienced several outbreaks of chikungunya, dengue fever, malaria, and rift valley fever, whereas west states are the most affected parts of the country in 2019 [8]. Most chikungunya cases were reported from west Darfur state (71%). In August 2019 – March 2020, west Darfur State reported 135 positive cases of dengue fever and 247 positive cases of chikungunya from five localities, including El geneina, Bieda, Habila, Krienk and Serba [9]. However, spread of the disease might probably extend to new areas in the country [10] other states across the country, such as the Red Sea, South Darfur, Gadarif, and North Kordofan have also been affected by a dengue outbreak [11]. The common species of *Aedes* mosquitoes responsible for

causing dengue/dengue hemorrhagic fever and chikungunya among human being are *Ae. aegypti* and *Ae. Albopictus* [12]. The abundance of *Aedes aegypti* in urban areas is usually breeds in indoor and outdoor settings, including a variety of natural and artificial water-holding containers such as plastic tanks, leaves, water storage jars, cement tanks, flower vases, curing tanks, glass, rubber tires, and plastic bottles. However, in urban areas, the breeding habitats of *Aedes aegypti* aquatic stages arise commonly in neglected pots and construction sites in addition to stagnant water that can provide optimal conditions and breeding habitats. [13,14]. The climatic factors such as rainfall and temperature are influencing the abundance and the transmission potential of *Aedes* mosquitoes [15, 16, 17]. Intensified surveillance and control of mosquito during the period with heavy rainfall is recommended [15]. For designing early warning systems for the prevention and control of dengue epidemics in our communities, seasonality could be a useful tool to guide us on it [15]. Despite all these outbreaks, there is a lack of efficient published reliable data on the ecology and bionomics of *Aedes* mosquitoes, particularly in west Darfur.

2. Methods

2.1 Study area: West Darfur State is in the western part of Sudan, between 11^0 – 15^0N and 22^0 – 25^0 E. It is located across two different climatic zones, savannah with high rainfall in the southern side and the semi desert in the north. The state is mainly flat but interrupted in some areas by small hills and seasonal valleys. Different soil types in different parts occupy the state; however, most areas are dominated by sandy soil. The climate prevailing in the state is tropical continental; temperatures range in the area between 10 – 40 ^{0}C. The maximum temperature reaches 40^0 C and above in summer (March to June), with a mean monthly temperature of around 35 ^{o}C, and the minimum temperature is 10-25 ^{0}C in winter (December to February). The rainfall varied between 200 – 700 mm in the area according to the different climatic zones prevailing in the state.

2.2 Study sites

Entomological surveys were conducted in two areas: Serba and Habila both are the capital cities of the localities figure 1.

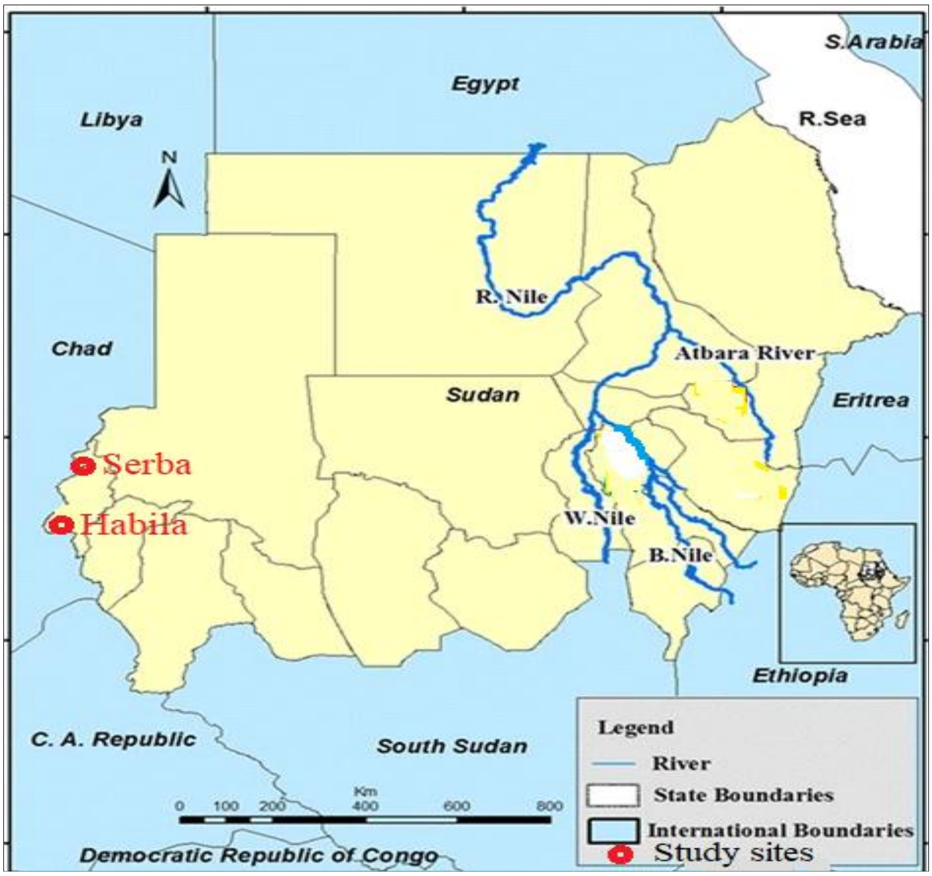

Fig 1: Showed the study sites.

2.3 Entomological survey

A well-trained volunteer and three levels of supervision carried out entomological surveys. A pre-survey conducted in Serba and Habila according to WHO 2003 [18] to provide bassline key container and pupal productivity. Further, comprehensive surveys and sampling were conducted following WHO 2011 [19]. All houses and potential habitats were inspected. Information on the number of inspected houses, types, and positive containers (with *Aedes* mosquito pupae or larvae), and houses with positive containers were recorded. Moreover, larval surveys were also done at outdoor sites close to the houses.

2.4 Data analysis

The data obtained from this study was entered onto a computer and analysed using Excel. Data from the entomological surveillance was analysed. Parametric tests were used for normally distributed data and non-parametric tests were used to analyse non-normalized data. Moreover, *Aedes* mosquito larval indices; container index (CI), Breteau index and Pupal/demographic (P/D) were calculated using the following formulas:

Container index (CI; Percentage of water-holding containers positive for *Aedes* larvae**)**
CI = No. of positive containers x 100 \total container inspected

Pupal/demographic (P/D)
P/D = Total number of collected pupae of *Aedes* mosquitoes/Total number of inhabitants in the inspected houses.
Calculation of routine sample size was done using formula Shannon-Wiener index (H') = ∑ pilog10 (pi) [10].

3. Results

100 positive houses were surveyed in each locality (Habila and Serba) during the preliminary survey to identify key containers, most productive containers, count pupae and calculate sample size to conduct regular entomological surveillance as well as to measure the impact of interventions (Table 1). Moreover, a sample size was calculated: 10 houses at each site for Habila when n= 1, and 25 houses at each site for Serba, n= 1.6 (table 2, table 3). Further, a comprehensive survey was carried out following a preliminary survey of all houses in Habila and Serba. 2,258 houses were visited in Habila 209 (9.2%) were found positive with *Aedes* larvae and pupae and 1783 houses were surveyed in Serba 466 (26.1%) were found positive with *Aedes* larvae and pupae.
The study findings revealed that four types of water containers (zeers, barrels, plastic water tanks, and neglected pots) are the most common breeding habitats of *Ae. aegypti* aquatic stages in both localities. Moreover, zeers (clay pots) are the most preferable container to be infested by *Aedes aegypti* larvae and pupae (75.8%), followed by neglected pots, barrels, and plastic water tanks, (13.7%), (8.8%), and (1.3%) respectively. In Habila locality, the highest positivity was recorded in Jar clay pots (zeers), 49%, followed by barrels and neglected pots (23.5%) for both. Whereas in the Serba locality, also zeer (Jar clay pots) was the highest container infested by *Aedes* larvae and pupae (88.5), followed by neglected pots, barrels, water tanks, 9.1%, 1.8%, and 0.1% respectively (Table 4). In contrast, the highest infestations by *Aedes* larvae and pupae in Habila was recorded in barrels, 6.2%, followed by neglected pots, zeers and water tanks, 4.8%, 4.5% and 1.5% respectively. Nevertheless, in Serba we found that zeers represents a high positivity at 21.7%, followed by barrels of 14.4%, plastic tanks12.5% and 1.4 in neglected pots (Table 6).

4. Discussion

We conducted preliminary surveys in Habila and Serba localities for the purposes of providing baseline data on key productive containers, vector preference habitat and pupal productivity, to describe the outbreak threshold as an early warning system, in addition to calculating sample size of routine entomological surveillance and measuring vector control interventions impact. For a control operation, it is recommended to have an initial pupal survey associated with 100 or more houses to be searched, due to low pupal mortality and the proximity of the pupal stage to the adult stage [20]. Because the traditional Stegomyia indices do not correlate with adult female abundance and dengue risk, they improve sampling and risk assessment methods by taking productivity into account [20, 21, 22]. Further, this is the first time to conduct pupal survey in Serba and Habila localities. Moreover, this study will help in planning by providing evidence-based data for programmatic decision making to review the local entomological stratification that applied before to these areas and classified as low transmission zone. Thus, we can assess the importance of breeding sites, establish risk thresholds and focus control operations toward the most productive containers to have the greatest impact on the adult *Ae. aegypti* mosquito populations [23].
Further, the study findings showed that Jar clay pots are the key container which represent a major positive breeding habitat for *Aedes* larvae and pupae 88.5% in Serba and 49% in Habila, followed by barrels 23.5%, neglected pots 23.5% and lastly, plastic water tanks 3.9% in Habila locality. These findings are in line with a recent study conducted in El Fasher City, north Darfur, which indicated that clay jars were found most positive and major breeding site 59%, followed by barrels 44.5%, and finally the water tanks [24]. Moreover, the same observations were reported from Kassala state [10]. Where the authors conducted a cross-sectional entomological survey in Kassala and Gedaref states and reported infestation of zeers, barrels and water-tanks in the former area. In another study conducted in El Fasher town in North Darfur state, *Ae. aegypti* was reported to thrive primarily in water storage containers, especially water jars (zeers) and barrels [25]. The Jar pots (zeers) are commonly used for storing drinking water at households in the study area (peri domestic and intra domestic). Most of the population in both localities during rainy season are spending a lot of time outside their households (1-2 months taking overnight in their farms), and these houses will provide suitable condition for *Aedes* mosquitoes to breed due to un changed water for the containers inside these houses. The type of shelter ‘’Gotia’’ (African hut built of woods and grasses), in absence of other sources of shallow may affects the behaviour of the vector to select the areas that will provide suitable condition for breeding and feeding habitat inside this type of building. Notably, the most zeers in the households were found inside the Gotias. In Serba, zeers were the highest container infested with *Aedes* larvae and pupae 88.5%, followed by neglected pots 9.1%, barrels 1.8%, and plastic water tanks 0.1%. These findings may refer to the small number of barrels used in storing drinking water inside houses due to availability of ground wells in seasonal valley close to the premises that can minimize storing water behaviour inside houses because the inhabitants bring fresh water regularly from these sources based on need during the day. Furthermore, neglected pots around or close to the human dwelling represents as second majority breeding habitat due to rainfall rate during the rainy season.
Zeers also represents the most productive container by *Aedes* pupae in Habila locality 990 pupae out of 2325 (42.5%), followed by barrels 822 (35.3%), neglected pots 468 (27.8%) and lastly, plastic water tanks 45 (1.9%). Whereas in Serba, Zeers also represents the most productive container of *Aedes* pupae 2013 (95%) out of 2108, followed by neglected pots 85(4%) and barrels 10 (0.5%). The most frequent positive containers produce a small proportion of pupae and low frequent positivity produce a huge proportion of pupae; this may vary from container-to-container. This observation is in line with studies conducted in the Bolivarian Republic of Venezuela [26]. The most productive container of *Aedes* pupae in Serba was zeer (95.4%), followed by neglected pools (4%); the lowest containers were barrels (0.5%), and plastic tanks

(0). Furthermore, in Habila, barrels were found as the most productive containers (42.5%), Zeers (35.3%), neglected pots (27.8%) and the lowest (1.9%) were recorded in plastic tanks. Developing practical and sustainable early warning systems (EWS) for infectious diseases, particularly for vector-borne diseases concluded that such systems could significantly improve control where mitigation methods were available [27].

Table1: Number of inspected containers in preliminary survey, Serba, West Darfur, Sudan August – November 2019

Type of container	Serba				Habila			
	Number inspected	Positive	NO of larvae	NO of pupae	Number inspected	Positive	NO of larvae	NO of pupae
Zeer	143	11	137	39	173	62	1083	411
Barrels	4	1	7	1	115	36	568	28
Plastic tanks	0	0	0	0	78	4	37	11
Neglected pools	0	0	0	0	208	5	23	14
Total	147	12	144	40	574	107	1711	464

Table 2: Proportion of pupae in preliminary survey, Serba, West Darfur, Sudan August – November 2019

Type of container	Pupae	Pi	Log Pi	Pi * Log Pi
Zeer	39	0.9	0.04	0.036
Barrels	1	0.02	1.7	0.034
Plastic tanks	0	0	0	0
Neglected pools	0	0	0	0
Total	40	0.92	1.74	0.07

H= 0.07 N= 10^H N=1

Table 3: Proportion of pupae in preliminary survey, Habila, West Darfur, Sudan August – November 2019

Type of container	Pupae	Pi	Log Pi	Pi * Log Pi
Zeer	411	0.88	0.06	0.0528
Barells	28	0.06	1.2	0.072
Plastic tanks	11	0.024	1.6	0.0384
Neglected pools	14	0.03	1.5	0.045
Total	464	0.994	4.36	0.2082

H= 0.2 N= 10^H N= 1.6

***** A dispersion index (N1) = $10^{H'}$ and Shannon-Wiener index (H') = $\sum p_i \log_{10} (p_i)$

Table 4: Container positivity of *Aedes aegypti* larvae and pupae, west Darfur, Sudan August – November 2019

Area	NO of Positive Zeers (%)	NO of Positive. Barrels (%)	NO of Positive Plastic tanks (%)	Positive Neglected pots (%)	Total container inspected	NO of Positive containers & %
Habila	150(49%)	72(23.5%)	12(3.9%)	72 (23.5%)	6746	306 (4.5%)
Serba	572 (88.5%)	12(1.8%)	1(0.1%)	59 (9.1%)	7354	646 (8.7%)
Total	722 (75.8)	84(8.8)	13(1.3)	131(13.7)	14100	(952 (6.7)

Table 5: Number and percentage of container infested by *Aedes aegypti* larvae and pupae, west Darfur, Sudan August – November 2019

Area	NO of inspected zeers	NO of Positive Zeers (%)	NO of inspected Barrels	NO of Positive. Barrels (%)	NO of inspected Plastic tanks	NO of Positive Plastic tanks (%)	NO of inspected Neglected pots	Positive Neglected pools (%)	Total container inspected	NO of Positive containers & %
Habila	3316	150(4.5)	1152	72(6.2%)	778	12(1.5%)	1500	72 (4.8%)	6746	306 (4.5%)
Serba	2625	572 (21.7%)	83	12(14.4%)	8	1(12.5%)	4638	59 (1.2%)	7354	646 (8.7%)
Total	5941	622 (10.4)	1235	84(6.8)	786	13(1.6)	6138	131(2.1)	14100	(1023(7.2)

Table 6: Number and percentage of *Aedes aegypti* pupae per container, west Darfur, Sudan August – November 2019

Area	Zeer	Barrels	Plastic tanks	Neglected pots	Total NO of pupae	Population	Demographic pupae
Habila	822 (35.3%)	990 (42.5%)	45 (1.9 %)	468 (27.8 %)	2325	10722	0.2
Serba	2013 (95.4%)	10 (0.5 %)	0 (0)	85 (4 %)	2108	9458	0.3
Total	2885(65)	1000 (22.5)	45(1)	553(12.4)	4433	47351	0.09

Table 7: proportion of pupae in Habila and Serba localities west Darfur, Sudan August – November 2019

Type of container	Habila		Serba	
	No of pupae	Proportion of pupae	No of pupae	Proportion of pupae
Zeer	822	0.12	2013	0.9
Barrels	990	0.15	10	0.004
Plastic tanks	45	0.006	0	0
Neglected pots	4678	0.7	85	0.04
Total	6535	0.976	2108	0.944

5. Conclusion

The most productive container of *Aedes* pupae in Serba was zeer (95.4%), followed by neglected pots (4%); the lowest containers were barrels (0.5%), and plastic tanks (0). Furthermore, in Habila, barrels were found as the most productive containers (42.5%), Zeers (35.3%), neglected pots (27.8%) and the lowest (1.9%) were recorded in plastic tanks. Developing practical and sustainable early warning systems (EWS) for infectious diseases, particularly for vector-borne diseases concluded that such systems could significantly improve control where mitigation methods were available.

6. Recommendation

The study recommend programs to assess the routine vector surveillance sampling based on evidence-based findings, importance of breeding sites, establish risk thresholds, and focus control operations toward the most productive containers to have the greatest impact on the adult *Ae. aegypti* mosquito populations.

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