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Investigation of *Aedes aegypti* aquatic stages in kerenik locality, West Darfur state, Sudan

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Abstract
Darfur states have experienced several outbreaks of dengue, chikungunya, and yellow fever several times in last decades. During August 2019 - January 2020 west Darfur state has been affected by massive outbreak of dengue and chikungunya resulted in high morbidity and mortality rates particularly in Kerenik and El Geneina localities in West Darfur. This study aims to determine key productive container of *Aedes* mosquito larvae and pupae, in addition to calculate stegomyia indices. A cross-sectional entomological survey for immature stages of *Aedes* mosquitoes was carried out during the mid of the outbreaks of dengue and chikungunya which were reported from three affected administrative units of Kerenik locality in November 2019. Both *Ae. aegypti* and *Ae. vittatus* were found. In Morni neglected pots are the most productive container to *Aedes* pupae (contribution of pupae) 0.7% followed by plastic water tanks 0.3%, Zeers 0.1% and barrels 0.06% respectively. In Kerenik town barrels were the most productive container 0.7 followed by plastic tanks 0.2%, Zeers 0, 07% and lastly neglected pots 0.04%. Whereas in Um Tagok Zeers were the most productive container 0.5%, neglected pots 0.2%, barrels 0.1%, and plastic tanks 0.09% respectively. The high abundance of *Aedes* aquatic stages was recorded in Kerenik (HI= 5.8, BI= 6.6,) and the lowest recorded in Morni (HI= 0.9, BI 1.3). The role of *Ae. vittaus* on transmission of arbovirus diseases in western Darfur state needs further investigations. Vector borne disease early warning system and response are highly needed to minimize the potential risks and burden of the disease.

Key words: *Aedes aegypti*, pupal productivity, contribution of pupae, Breatu index, container index, pupae per person, house index, mornei, um tagok, and kerenik

Introduction
General background
Aedes-borne disease represents a major health problem in deferent regions of the Sudan specifically east and west states. While, yellow fever outbreaks have been reported in different regions in Darfur as well as repeated outbreaks of dengue fever / hemorrhagic in four Darfur states specially in west Darfur state with 76 cases and 12 deaths in El geneina town and reported high morbidity and mortality [1, 2, 3, 4]. Furthermore, most arboviral pathogens affecting greater Darfur in 2015 were reported from west Darfur [5]. Kerenik locality has a history of yellow fever, dengue, and chikungunya in recent decades, with high mortality and morbidity rates affecting most of the population of the locality and the nearest areas around the border with Serba, Geneina, and Habila localities, in addition to some villages of north and central Darfur in south and east sides [4, 5, 6]. Moreover, the chikungunya outbreak is also affecting west Darfur state several times. In 2019, the state reported 237 cases and 3 deaths [6]. The abundance and the transmission potential of *Aedes* mosquitoes are influenced by climatic factors such as rainfall and temperature [7, 8, 9]. The study was conducted to identify pupal productivity and measure the stegomyia indices. Moreover, calculating sample size to conduct routine entomological surveys regularly and monitor interventions was considered, along with characterizing larval habitats of *Aedes* mosquitoes, and collecting demographic information to help in planning and programmatic decision-making. Furthermore, there is a lack of reliable

published data on vector composition and virus serotypes in west Darfur state, specifically in Kerenik Locality.

Methods

Study area: West Darfur State is in the western part of Sudan between 11^0- 15^0 N and 220 - 250 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 10^0 - 40^0 C. The maximum temperature reaches 40^0 C and above in summer (March to June), with a mean monthly temperature of around 35^0 C, and the minimum temperature is 10^0 -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 (July - November).
Kerenik locality is the biggest locality in west Darfur, located in the eastern part of the state, the only locality from the rest without boundaries with the Republic of Chat. It is bordered by El Geneina locality from the west, Serba from the north and central and northern Darfur from the east, whereas central Darfur from the south. 582, 115 people inhabit the locality, most of them are farmers, pastoralists and nomads distributed in rural areas, IDPs camps in big towns such as Kerenik, and Morni. The locality consists of 5 administrative units named Kerenik, Morni, Genderni, Um tagok and Azerni.

Study sites

- Kerenik town is the capital city of the locality east of El geneina town (45 km), inhabited by 84,814 people. The town is surrounded by the Kaga valley from the east, south, and the mountains alongside the seasonal dam surrounding the town from the south.
- Morni town located at the southern site (65 km) from El geneina, Barei valley crossing the town from the east, and Morni Mountain to the south. IDP camp occupied the east and west areas of the town. 242,776 people inhabit the area.
- Um Tagok located at the eastern part of the locality 12 km east of Kerenik town, close to Kaga valley from the south and bordered by North Darfur from east. The population of the area is 131,017 persons.

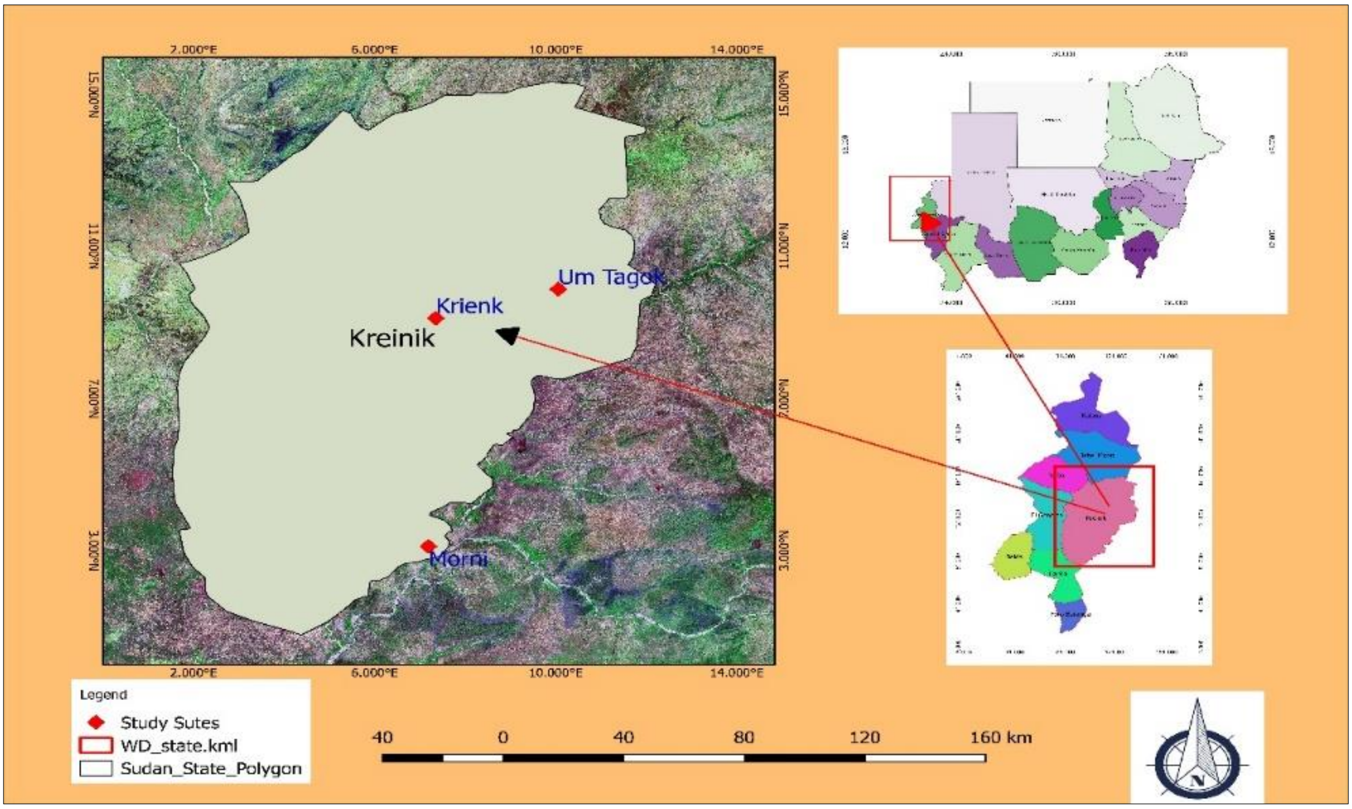

Fig 1: showed the study area

Entomological surveys: Entomological surveys were carried out during the mid of the dengue fever and chikungunya outbreak in November 2019. Entomological surveillance data were collected on a daily basis during the study period and using standard modified forms from all target premises in the three study sites. 15 Well-trained teams carried out entomological surveys. Each team comprised of two health workers and a supervisor; one of the workers checked water containers, sorting out the aquatic stages, cleaning positive containers, and the other one disseminated a health education message on water source reduction at household level. Each team visited a maximum of 20 houses a day. All positive water containers are emptied, sorted larvae and pupae, counted, and recorded in the data collection form. Then, all positive containers are cleaned, crushed from eggs, larvae and pupae of *Aedes* mosquitoes in the presence of household members. A random sample of *Aedes* larvae from each site were preserved into 80% ethanol for further morphological identification.
Sample size of *Aedes* aquatic stages for routine surveillance

was calculated using the formula Shannon-Wiener index (H') = ∑ pilog10 (pi) [10].

Morphological identification of *Aedes* larvae

Random samples of *Aedes* larvae were morphologically identified using identification key [11].

Statistical analysis

- Data was analysed using Microsoft Excel. *Aedes* indices were calculated using the following formulas according to the [10]:
- House index (HI) = No. of positive houses for *Aedes* larvae and pupae/ No. of inspected houses X100
- Container index (CI) = No. of positive containers by *Aedes* larvae and pupae /containers inspected x 100
- Breteau index (BI)= No. of positive containers/total houses inspected per 100 houses.
- Pupa per Person index (PPI): Total number of collected pupae of *Aedes* mosquitoes/Total number of inhabitants in the inspected houses.
- Pupae per House Index (PHI) = Number of pupae/Number of houses inspected

Results: The study findings revealed that both *Ae. aegypti* and *Ae. vittatus* were found in Kerenik town. In Morni, neglected pots are the most productive container for *Aedes* pupae contribution of pupae 0.7%, followed by plastic water tanks 0.3%, Zeers 0.1% and barrels 0.06% respectively. In Kerenik town barrels were the most productive container at contribution of pupae 0.7, followed by plastic tanks at 0.2%, Zeers 0, 07%, and lastly, neglected pots at 0.04%. Whereas in Um Tagok Zeers were the most productive container at 0.5%, neglected pots 0.2%, barrels 0.1%, and plastic tanks 0.09% contribution of pupae respectively (table: 4,5). The highest container positivity was Zeer 3%, followed by barrels of 2,9% in Um Tagok, whereas the lowest infested container was plastic water tanks at 0.09% recorded in Krienk (table 3). The high abundance of *Aedes* aquatic stages was recorded in Kerenik (HI= 5.8, BI= 6.6,) and the lowest recorded in Morni (HI= 0.9, BI 1.3), figure 2. The highest pupal productivity was recorded in Barrels at Kerenik town 1100 pupae out of 1277 (proportion of pupae = 0.7%), followed by zeers 124 (proportion of pupae = 0.07%) and neglected pools 53 (proportion of pupae = 0.03%) and PPI= 0.09%. In Morni, neglected pools are the most productive container for pupal stage 509 pupae out of 1070 (proportion of pupae = 0.4%, followed by water tanks 377 (proportion of pupae = 0.3%), and zeers 130 (proportion of pupae = 0.1%), and (PPI = 0.1%). In Um Tagok zeers was recorded the highest container productivity of pupae 609 out of 1158 (proportion of pupae = 0.5%) followed by neglected pools 232 (proportion of pupae = 0.2%), barrels 211 (proportion of pupae = 0.1%) and PPI = 0.03% Table 4, 5. Therefore, the sample size for routine surveillance and assessing interventions was determined by conducting the preliminary survey in three study sites before the comprehensive surveys by searching for 100 positive households (n= 10 households) to be applied to all study sites (N= less than 1) in all study sites table 2.

Discussion: The current study was carried out as cross-sectional surveys to determine baseline key productive containers and assess stegomyia indices of *Aedes* aquatic stages. In the current study, four types of water containers were reported to be positive for *Ae. aegypti* immature stages. However, zeers were the most preferable and productive container for *Aedes* aquatic stages in the surveyed premises. The study findings showed that the presence of *Aedes aegypti* and *Aedes vittatus* in the area. Previous studies on the distribution of mosquitoes have listed common species in Sudan and Ethiopia such as *Ae. aegypti, Ae. vittatus, Ae. caspius and Ae. caballas* [12, 13, 14]. The common breeding habitats of *Aedes aegypti* are jar pots (zeers), barrels, neglected pots and water tanks. These are the main source of *Aedes aegypti* and also in different areas in Darfur region and other parts of the Sudan [1, 2, 3, 4, 15, 16, 17, 18]. The highest container positivity was recorded in Zeers, followed by barrels reported in Um Tagok town. These findings, in line with the study findings from Thailand, highlighted that the Jar pot was responsible for production of ≥70% of pupae in four surveyed areas [19]. Kerenik town also recorded high container positivity in Zeers, whereas in Morni the neglected pots are the highest positivity. The findings highlighted that the high proportion of *Aedes* pupae in zieers may refer to the suitable environment (temperature and relative humidity) that provided to the aquatic stages to breed and survive. Moreover, jar pots (zeers) are used widely in the Darfur region to store water for drinking at households. Some of Zeers with lids and others without them (artificial containers), some of them under shadow like trees or human shelters built from wood and grass or without lids. However, all of these types were found positive. These findings are in agreement with the results of [20, 21], who suggested that *Ae. aegypti* often breeds in water - holding containers inside households disconnected from the water network and even in dense urban areas, because these habitats provide suitable temperature and relative humidity. Barrels also reported high infestation in Um Tagok town. On the Market, we found *Ae. aegypti* breeds in Barrels connected with generator used in cooling machines during the operation may have days off. These observations are in line with the findings of the study conducted in peru that illustrated containers located in non-residential areas neither public zones nor small buildings were responsible for a higher proportion of production at recreational sites [19]. Plastic water tanks recorded the lowest infestation with *Aedes* larvae and pupae due to the small storage capacity (20 - 60 litres) of most of them and a few with 1000 liters capacity. This may refer to the unsuitable temperature during the day that affects the development of immature stages, as well as repeated moving the water inside containers for households' use that minimizes the storing water for a long time. Although the jar pots are widely used, water tanks (plastic & concrete), barrels, and zeers recorded high infestation in the area. This result may refer to a few inspected water tanks in comparison with other containers. Similarly, an observation was found in a study conducted in Colombia revealed that the most common containers accounted for 82.2% of all water - filled containers responsible for 0.1% of *Ae. aegypti* pupae [19]. Neglected pots also recorded high positivity in Mornei. Most of these types are dishes made from jar locally called tangl (0.5-1 litre capacity) used in watering hens inside houses. These findings, in agreement with findings from Merida Mexico, revealed that small and larger removable containers contribute strongly to larval and pupal production in Mérida, and the importance of different container types can vary from area to area and seasonality [22]. The low container positivity in study sites may depend on the large community participation towards vector control interventions in Morni town, Kerenik and Um Tagok played an important role in the reduction of vector density. Moreover, the population of

Kerenik locality have a lesson learned from previous outbreaks of yellow fever, dengue fever, and chikungunya in last decades that built good knowledge and practices to conduct water source reduction.

The highest pupal productivity was recorded in Barrels in Kerenik town, followed by zeers in Um Tagok and neglected pools in Morni. These findings are in line with a recent study conducted in Venezuela, the findings highlighted that in all surveyed sites, large water barrels produced the greatest proportion of pupae [19]. In Kerenik town, the small size capacity of jar pots may reduce the storage period of water due to repeated consumption on a daily basis. Further, most barrels are in public areas such as market and grain mills during the rainy season. Most of the time they are closed, the owners go to the farms for a long time. Similarly, findings from Cuba showed that approximately 78% of production was from containers that were abandoned, i.e., were not currently in use [19]. Additionally, source reduction behavior of household members focused more on jar pots more than barrels, because the jar pots were used for storing water for drinking and barrels for other purposes such as cleaning, showering, and watering followers. Furthermore, the highest productivity in Morni was recorded in neglected pots more than in other containers, due to the location of these containers behind human dwellings (peri-domestic). Specifically, small jar dishes are used for watering hens (changing water may take one week or max depends on consumption and climatic factors. This, in line with findings from Puerto Rico, illustrates that productivity of *Ae. aegypti* pupae is affected by climate and environmental factors that influence the density of immature stages of *Aedes* mosquitoes [19]. Moreover, water tanks at common water points (economic/private) represent high pupal productivity after neglected pots. This in line with study findings from Kenya showing that in all four sites, significantly more positive containers were located outdoors than indoors [23]. Because these containers are characterized by a large capacity (commercials) to, cover most needs of the population in residential units and IDP camps in the absence of water netting. This situation made it a big challenge to keep these tanks clean due to regular operation in the absence of local regulations that can obligate water point owner to conduct regular cleaning and draining at least once - two days per week to interrupt the mosquito life cycle. In Um Tagok, Zeer (jar pots) were the highest productive container. This may refer to the movement of population between farms and towns during the rainy season. They spend a lot of time on farms and return to the town after a few weeks. In this study, entomological indices (Stegomyia and pupal productivity / demographic indices) for *Ae. aegypti* were determined at three study sites. The Stegomyia and pupal productivity / demographic indices remain central to the monitoring of dengue vector populations. The most used indices are the House (or premise) index (HI: percentage of houses infested with larvae and/or pupae) the Container index (CI: percentage of water-holding containers infested with larvae and/or pupae), and the Breteau index (BI: number of positive containers per 100 houses inspected). Variations in both these indices were observed between the sites. The variation might be due to the large number of small containers utilized for water storage. Moreover, varied numbers of pupae were recorded at different sites which caused the differences in the pupal indices between the inspected areas. Moreover, varied numbers of pupae were recorded in different surveyed areas which caused the differences in the pupal indices between the inspected areas.

Table 1: Preliminary survey of 100 households in Kerenik locality November 2019

Type of water containers	Kerenik			Morni			Um Tagok		
	Number inspected	Positive	NO of pupae	Number inspected	Positive	NO of pupae	Number inspected	Positive	NO of pupae
Zeer	1758	61	68	2504	7	130	1199	68	202
Barells	623	15	275	1572	5	55	313	27	77
P. tanks	2549	8	0	244	4	377	898	38	50
N. pools	969	42	53	1210	3	509	551	33	61
Total	5899	126	396	5530	19	1071	2961	166	390

Table 2: proportion of pupae and sample size calculation for routine surveillance in Kerenik locality November 2019

	Kerenik			Morni			Um Taok		
Type of container	Pi	Log Pi	Pi * Log Pi	Pi	Log Pi	Pi * Log Pi	Pi	Log Pi	Pi * Log Pi
Zeer	0.07	- 1.1	- 0.8	0.1	- 1	- .1	0.5	- 0.3	- 0.1
Barells	0.7	- 0.1	- 0.07	0.06	- 1.2	- 0.07	0.2	- 0.6	- 0.1
Plastic tanks	0.2	- 0.6	- 0.1	0.3	- 0.5	- 0.1	0.09	- 1	- 0.09
Neglected pools	0.03	- 1.5	- 0.4	0.4	- .4	- 0.1	0.2	- 0.6	- 0.1
Total	1	-3.3	H= -1.37	0.86	-3.1	H= -0.37	0.97	-2.5	H= -0.39

$N=10^H$ $N_1=0.04$ $N_2= 0.4$ $N_3 = 0.4$

Table 3: number and percentage of water containers positivity in Kerenik locality November 2019

Area	Type of water container									
	Zierrs		Barrels		Water tanks		Neglected pools		Total containers	
	No. inspected	No. positive (%)	No inspected	No. positive (%)	No inspected	No. positive (%)	No inspected	No. positive (%)	No inspected	No. positive (%)
Kerenik	4024	102 (2.5)	1375	18 (1.3)	8040	8(0.09)	2157	43(1.9)	15596	171(1)
Morni	2504	7(0.2)	1572	5(0.3)	244	4(1.6)	1210	3(0.2)	5530	19(0.3)
Um tagok	8880	271(3)	2396	71(2.9)	11614	96(0.8)	5337	96(1.7)	28227	534(1.8)
Total	15408	380(2.4)	5343	94 (1.7)	19898	108(0.5)	8704	142(1.6)	49353	724(1.4)

Table 4: Relative importance of breeding containers on production of pupae in Kerienk locality November 2019

Morni				Kerenik			Um Tagok		
Type of container	Total inspected	No. pupae	Contribution to pupae (%)	Total container inspected	No. pupae in each container	Contribution to pupae (%)	Total container inspected	No. pupae	Contribution to pupae (%)
Zeer	2504	130	0.1	4024	124	0.07	8880	609	0.5
Barrel	1572	65	0.06	1375	1100	0.7	2396	211	0.18
Water tanks	244	377	0.3	8040	377	0.2	11614	106	0.09
Neglected pools	1210	509	0.4	2157	53	0.03	5337	232	0.2
Total	5530	1081	0.86	15596	1654	1	28227	1158	0.97

Table 5: inspected houses and containers positivity in Kerenik town November 2019

Site	No. inspected houses	No. positive house	No. inspected container	No. psitive container	No, larvae	No. pupae	Population	PPI	PHI
Kerenik	2570	141	15596	171	1166	1277	13900	0.09	0.5
Morni	1400	13	5530	19	4342	1071	8961	0.1	0.8
Um Tagok	5847	187	28227	534	2256	1158	32689	0.03	0.2
Total	9817	341	49353	724	7764	3506	55550	0.22	1.5

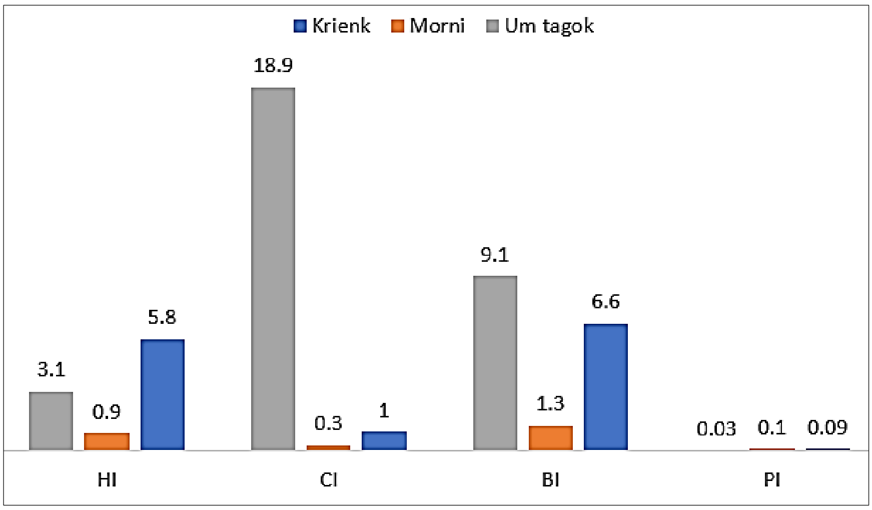

Fig 2: stegomyia indices of *Aedes* aquatic stages in Kerenik locality November 2019.

Conclusion

The surveys were not involved sylvatic areas especially rocks, banana, and mango gardens as well as Kaga valley belt due to un accessible roads. We think these areas represent a major breeding habitat of *Aedes* sylvatic species like *Ae. vittatus* that was recorded in concrete basins close to Kaga valley. So, especial concern is highly needed to study bionomic and species composition of *Aedes* mosquitoes in these areas, although the stegomyia indices was very low (at the prophylactic level) in Um Tagok and Kerenik but chikungunya incidence rate are very high.

Recommendations

The authors recommend that comprehensive study (aquatic stages and adult) should be conducted, tree holes (mango and banana guardians, rock, mountain, and Kaga valley belt to be involved). The role of *Ae. vittatus* in transmitting viruses should be studied, according to the similar distribution of the two species and history of dengue, chikungunya, and yellow fever in the study area.

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