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Household survey of container-breeding mosquitoes and climatic factors influencing the prevalence of *Aedes aegypti* (Diptera: Culicidae) in Makkah City, Saudi Arabia

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ABSTRACT

Objective: To investigate the prevalence of container breeding mosquitoes with emphasis on the seasonality and larval habitats of *Aedes aegypti* (*Ae. aegypti*) in Makkah City, adjoining an environmental monitoring and dengue incidence. **Methods:** Monthly visits were performed between April 2008 and March 2009 to randomly selected houses. During each visit, mosquito larvae were collected from indoors and outdoors containers by either dipping or pipetting. Mosquitoes were morphologically identified. Data on temperature, relative humidity, rain/precipitations during the survey period was retrieved from governmental sources and analyzed. **Results:** The city was warmer in dry season (DS) than wet season (WS). No rain occurred at all during DS and even precipitations did fall, wetting events were much greater during WS. Larval survey revealed the co-breeding of *Aedes*, *Culex* and *Anopheles* in a variety of artificial containers in and around homes. 32 109 larvae representing 1st , 2nd, 3rd, and 4th stages were collected from 22 618 container habitats. Culicines was far the commonest and *Aedes* genus was as numerous as the *Culex* population. *Ae. aegypti* larval abundance exhibited marked temporal variations, overall, being usually more abundant during WS. Ten types of artificial containers were found with developing larvae. 70% of these habitats were located indoors. 71.42% of indoor containers were permanent and 28.58% was semi-permanent during WS. Cement tanks was the only container type permanent during DS. *Ae. aegypti* larval indices (CI, HI, BI) recorded were greater during WS. **Conclusions:** Taken together, these results indicate a high risk of dengue transmission in the holy city.

1. Introduction

Contemporaneous arthropod-borne diseases are still chief concerns for global public health[1]. Potential insect-based bio-terrorism[2] and recurrent outbreaks of mosquito-borne diseases such as Chikungunya infection[3-5] are indications of the continuous threat of diseases transmitted by insects, as does the rise in dengue virus infections during the last decade, worldwide[6,7].

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In dengue endemic regions such as Southeast Asia, even though *Aedes albopictus* (*Ae. albopictus*) is incriminated in dengue transmission[8,9], *Aedes aegypti* (*Ae. aegypti*) remains so far the major vector[10]. In this region, female *Ae. aegypti* prefers to lay their eggs in domestic containers[11]. These include a variety of containers with a majority of discarded receptacles, but also water storage containers and tires[12], wells, cement tanks and sinks[13]. In addressing this issue, Harrington *et al*[14] have argued that it is people rather than mosquitoes that contribute much more to the dissemination of dengue virus.

Concomitant with the increased development of transportation has come an increased in mass migrations of refugees and workers[10]. These increased movements of humans are expected to increase in the future and concerns have been recently formulated regarding their eventual impacts on the incidence of dengue[15,16]. Regions most likely to experience incursions of dengue serotypes and related dengue problem include the Kingdom of Saudi Arabia (KSA). A particular characteristic of this country is the scarcity of natural water resources. This difficulty of regular water supply has led many people to store water inside homes, thus creating conditions conducive to the breeding of dengue vectors. The habit of storing water, and consequently the existence of breeding opportunities for dengue vector mosquitoes, are both expected to increase with the growing need for water to supply the expanding populations.

In dengue endemic regions, the dynamics of dengue vectors and dengue occurrence is generally seasonal[17]. Evidence also exist that the seasonal abundance of dengue vectors are positively correlated with the seasonality of dengue fever activity[18,19]. Besides temperature and humidity[20], precipitations have been reported influential to dengue vectors' distribution and abundance[21,22]. KSA is inhabited by very special climatic conditions; it is very hot and dry, with precipitation generally scarce[23]. Surprisingly, the role of climate factors as they influence population dynamics of dengue vectors remains largely un-investigated.

In Saudi Arabia, three serotypes of dengue (DEN-1, DEN-2, DEN-3) were first detected in Jeddah in 1994[24] and *Aedes* mosquitoes have been implicated in many arboviral infection epidemics[25,26], including outbreaks of dengue[27]. In this country, there has been an increase in the distribution of *Ae. aegypti*; recently[28]. This mosquito has recently been incriminated in dengue epidemics in some areas, including Makkah. Fifty-five cases of dengue were reported in this city in 2008[29], with a marked increase in the incidence of the disease thereafter[30]. This study was set to investigate the abundance of container breeding mosquitoes with preeminence on *Ae. aegypti*, container habitats indoors and nearby homes, some environmental factors and dengue occurrence in Makkah City.

2. Materials and methods

2.1. City profile

Makkah is located in the Makkah Province, which is situated in the Western part of KSA (Figure 1). The city is the provincial capital and is approximately seventy three miles away from Jeddah, another city of the province. Makkah City lies at an elevation of 277 m above sea and about 50 miles inland from the Red Sea[31]. Its geographic coordinates are 21°25′36″ N and 39°49′34″ E. level.

The climate of Makkah City is arid and temperatures are high throughout the year[32]. It is very hot from May to October with the daily temperatures varying between 30 ℃ and 40 ℃. The city is quite warm even during the winter period (November to April). Natural water resources are scarce. There are about 130 mm of rainfall during the year, mainly in the winter months and due to its low-lying location, Makkah has recently experienced many flash food events[33,34].

The urban and metropolitan areas of Makkah City have a total area of 850 km^2 and 1 200 km^2, respectively[32]. The total population was 1 294 167 persons in 2004[35] and about 1 484 000 in 2009[36]. There are hundred twenty two approved residential areas, 201 716 residential blocks and more than 7 800 blocks for repair shops and warehouses[37]. Due to Hajj, Makkah has a highly fluctuating human population. From 2000 to 2009, the population size of foreign pilgrims has increased from 1.26 to 1.61 million. In addition, there are millions of local people and foreign Muslims who come for Umrah at other times of the year[38]. It is expected that the city will receive an estimated 20 million pilgrims by 2030.

This potential influx has led the local government to plan building new houses and to increase water supply[38]. The city's population inhabits the old city, but also in modern residences. Slum conditions can still be observed in various parts of the city and the slum inhabitants are mainly poor pilgrims who, could not return home[32].

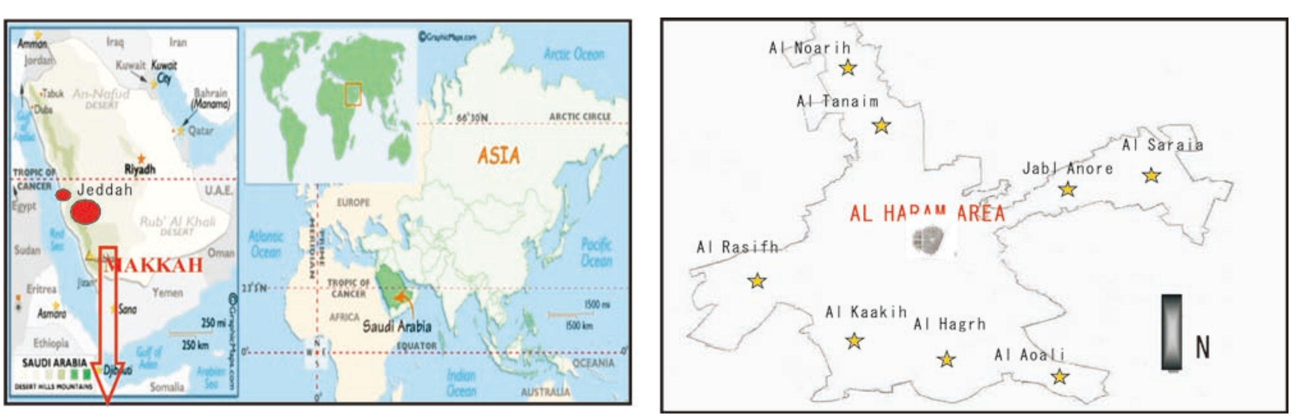

Figure 1. Location of the Kingdom of Saudi Arabia and study area in Makkah City (☆ stands for residential areas used to survey.

2.2. Study area and survey

The study was conducted in randomly selected houses in a way to cover residential areas in the north, the south, the west, the east and the center of the city. A formal entomological inspection request was addressed to householders. In total, 9 329 landlords gave agreement.

2.3. Larval sampling procedure

A survey on container breeding sites of mosquitoes was carried out from April 2008 to March 2009. Sampling was carried out on a weekly basis (four times per each month) and at each occasion, any accessible containers inside or nearby homes were checked for the presence of any larval stage. Samples were performed by dipping adopting others[39] or pipetting (following Knox and colleagues)[40] depending on the size and types of container.

In case of large containers, we used a 350 mL plastic dipper (opening area: 8 cm and depth: 9 cm) attached to an extensible aluminum handler. At each dipping occasion, 3 samples were quickly taken. In case of small containers, larvae were collected with the aid of plastic pipettes (3 mL Pasteur Pipette, Nantong Derui Trade Co., Ltd. Jiangsu, China). Sampled larvae were kept in labelled WHO standard plastic vials (location, sample number and date of sampling), placed in a humidified cooler and transported to the laboratory for further assort and identification. At the end of each collection events, the location, type and number of containers encountered were recorded.

2.4. Environmental data

Daily meteorological data for the period April 2008-March 2009 was obtained from the surface annual climatological report of the National Meteorology and Environmental center of the Ministry of Defence and Aviation Presidency of Meteorology and Environment of KSA. Data on relative humidity (RH) and ambient temperature was given as minimum and maximum values and expressed in percent (%) and degrees celsius (℃), respectively. Rainfall and precipitation data was supplied as daily values in mm.

2.5. Data collection and analysis

Field-collected larvae were separated into developmental stages under a dissecting microscope (Olympus CX41; Olympus, Tokyo, Japan) following WHO[41]. Early instars (first-instar or L1 and second-instar or L2) were allowed to develop and killed with warm water (50-55 ℃), once they reached late instars (3rd and 4th instars). Larvae were mounted on microscope slides with Canada Balsam (Sigma-Aldrich) using appropriate taxonomic keys[42-44]. Identified *Ae. aegypti*, *Culex* sp. and *Anopheles* sp. were counted, taking into account their stage of developmental, season and month of collection. Resulting numbers were used to calculate relative abundance as follows: (number of specimens belonging to a given taxonomic group)/(total number of specimens collected)×100. Abundance patterns were determined based on total numbers or percentages.

To determine *Ae. aegypti* larval indices, larval abundance data was related to house and container surveyed as per WHO guidelines[45]. Briefly, we calculated house index (HI) as the total number of infested houses divided by the total number of surveyed houses×100. Container index (CI) was considered as the total number of containers found with larvae divided by the total number of surveyed containers×100 following the authors cited above. HI and CI data were used to calculate Breteau Index (BI), which informs on the number of positive containers per 100 houses surveyed. All larval indices were related to season and month of collection. To assess temporal variations of abundance, numbers or percentages (for all taxonomic groups), HI, CI and BI (*Ae. aegypti* only) were related to month and season.

The characteristics of container habitats used by *Ae. aegypti* as breeding sites and their temporal variations in frequency larval presence were determined. We applied the term "indoors" to any container found within the wall of houses. We referred to any container location outside homes within less than 15 m around the peri-domestic area as "outdoors". Characterization of encountered container habitats was seasonally done based on the frequencies at which *Ae. aegypti* was present. A given container habitat was defined as semi-occasional, occasional, semi-permanent, permanent when the frequency of larval presence was within [0 - ⩽ 25], [<25 - ⩽ 50], [< 50 - ⩽ 75] and [< 75 - ⩽ 100], respectively. Daily minimum and maximum values of relative humidity and temperature were used to calculate monthly and seasonal means (period from May to October: DS and the period November - March: WS). Daily values of rainfall and precipitation amounts were aggregated to obtain seasonal cumulative (total) volumes of water. The numbers of days of rain and those with precipitation for each season were derived from daily rain and precipitation data.

The differences in abundance patterns of different developmental stages (L1 and L2, L3 and L4) within and between taxonomic groups were compared by analysis of variance using the statistical software package Systat v.11[46]. Mean (SE) abundances were separated using Tukey's honestly significant difference (HSD) tests when necessary. These similar statistical procedures were also used to analyze the seasonal and monthly differences in the occurrence of different taxonomic groups and larval stages. The differences in mean RH and average ambient temperature between seasons were compared statistically using one sample t-test between means from the statistical software package StatPac[47]. In all statistical analyses $P < 0.05$ was taken to express statistical significance.

3. Results

3.1. Environmental data of Makkah City in 2008-2009

No rain occurrence was reported between May 2008 and October 2008. In contrast, there were several rainy days from November 2008 to March 2009. Precipitations occurred during both periods (DS,WS), but the number of days with precipitations and the resulting cumulative rainfall amount were high during November 2008 - March 2009 compared to the period between May 2008 - October 2008. Based on this evidence, we considered the period May - October 2008 as "Dry season (DS)" and November 2008- March 2009 as "Wet season (WS)". The mean relative humidity was significantly higher during WS where the maximum value of this parameter was recorded than during DS, which recorded the lowest RH. Also the average ambient temperature was significantly lower during the rainy season, which recorded the lowest value of this parameter than during DS, which record the highest ambient temperature value (Table 1).

3.2. Age structure of larval populations

A total of 32 109 larvae from four developmental stages were collected, of which, 31.58% were first/second instars (L1/L2) and more than 68.42%, third or forth instars (L3/L4). 40.33% of L1/L2 was *Aedes*, whereas 53.81% and 5.86% were *Culex* and *Anopheles*, accordingly. The number of L1/L2 varied significantly with genus (F = 15.33, df = 2, P< 0.001), with that of *Aedes* similar to that of *Culex* (Matrix of pairwise mean difference = 28.47, P= 0.287), which, in turn, was much high than that of *Anopheles* (Matrix of pairwise mean difference = 101.35, P< 0.001). The number of L3/L4 showed significant variations between genera (F = 18.17, df = 2, P< 0.001). The number of *Aedes* L3/L4 did not differed with that of *Culex* (Matrix of pairwise mean difference = 26.95, P= 0.738), but was greater than that of *Anopheles* (Matrix of pairwise mean difference = 201.64, P< 0.001).There was significantly more L3/L4 than L1/L2 for *Aedes* (F = 11.63, df = 1, P= 0.001), *Culex* (F = 10.20, df = 1, P= 0.002) and *Anopheles* (F = 4.56, df = 1, P= 0.035) (Table 2).

Table 2
Occurrence of different larval stages of different mosquito genera in Makkah City.

Developmental stage	*Aedes*	*Culex*	*Anopheles*	Total
L1 and L2	4 091$^{a, 1}$	5 458$^{a, 1}$	593$^{b, 1}$	10 142
L3 and L4	9 686$^{a, 2}$	10 980$^{a, 2}$	1 301$^{b, 2}$	21 967
Total	13 777	16 438	1 894	32 109

L1: First instars larvae; L2: Second instars larvae; L3: Third instars larvae; L4: Forth instars larvae. Mean values in the same column or row with the same letter or number are not significantly different (Tukey's statistic, P < 0.05) for comparison of means.

3.3. Seasonal variations of larval populations

15.57% and 84.43% of the total larvae were collected during DS and WS, respectively. 35.54% of DS-collected larvae belonged to the genus *Aedes*, 49.96% were *Culex* and 14.5% were *Anopheles*. The number of larvae collected during DS differed significantly between genus (F = 3.79, df = 2, P = 0.025). The population size of *Culex larvae* in DS was analogous to that of *Aedes* (Matrix of pairwise mean difference = 13, P = 0.50), which was higher than that of *Anopheles* (Matrix of pairwise mean difference = 31.97, P= 0.017).The number of larvae collected during WS exhibited significant variations between genera (F = 45.51, df = 2, P< 0.001). The number of *Culex* larvae collected during WS was similar to that of *Aedes* (Matrix of pairwise mean difference = 51.58, P= 0.198), which was far greater than that of *Anopheles* (Matrix of pairwise mean difference = 269.80, P< 0.001). For both *Aedes* (F = 63.41, df = 1, P< 0.001) and *Culex* (F = 83.74, df = 1, P< 0.001), the number of larvae collected during DS

Table 1.
Seasonal variations of meteorological and physical parameters in Makkah City in 2008 and the beginning of 2009.

Parameters		Period		Independent group t-test	
		Dry season	Wet season	t-statistic	P-value
Meteorological parameters					
No. days of rain		0	4	-	-
Total rainfall volume (mm)		0	8.6	-	-
No. days with precipitation		7	126	-	-
Total precipitation volume (mm)		0.18	12.76	-	-
Physical parameters		-	-	-	-
Relative humidity (RH) (%)	Mean±SE	49.87±2.47^{a}	71.04±1.90^{b}	16.64	< 0.001
	Max	74	82	-	-
	Min	32	47	-	-
Ambient temperature (℃)	Mean±SE	42.5±0.39^{a}	35.12±0.53^{b}	27.47	< 0.001
	Max	46	42	-	-
	Min	36	31	-	-

Dry season: May-October 2008; Wet season: November 2008- March 2009. Mean values in the same row and with the same number are not significantly different (Independent group t-test statistic, P < 0.05).

was significantly lower the those obtained during WS. For *Anopheles*, the larval population size in WS tended to be increased when compared to that of DS, but there was no a significant difference between the two population sizes (F = 3.70, df = 1, P= 0.057) (Table 3, 4).

Table 3
Occurrence of different larval stages of different mosquito genera in Makkah City during DS and WS.

Season	*Aedes*	*Culex*	*Anopheles*	Total
DS	1 538[a,1]	2 162[ab,1]	627[c,1]	4 327
WS	12 239[a,2]	14 276[a,2]	1 267[b,1]	27 782
Total	13 777	16 438	1 894	32 109

DS: Dry season (May–October); WS: Wet season (November–April). Mean values in the same column or row with the same letter or number are not significantly different (Tukey's statistic, P < 0.05) for comparison of means.

Table 4
Seasonal house, container and Breteau indices for *Ae. aegypti* in Makkah City during 2008 and the beginning of 2009.

Season	House index (HI)			Container index (CI)			Breteau index (BI)
	THS	THP	%	TCS	TCP	%	
DS	4 255	189	4.44	8 866	182	2.05	1.66
WS	5 074	646	12.73	13 752	844	6.13	4.83

THS: Total houses surveyed; THP: Total houses found positive; TCS: Total containers surveyed; TCP: Total containers found positive; DS: Dry season; WS: Wet season.

3.4. Monthly variations of larval populations

The number of larvae of *Aedes* (F = 15.07, df = 11, P< 0.001), showed significant variations between months. The size of *Aedes* larval population gradually decreased from April to September. It gradually increased thereafter to attain a peak in March. From a mean of (576.25±67.85) individuals in April (Figure 2).

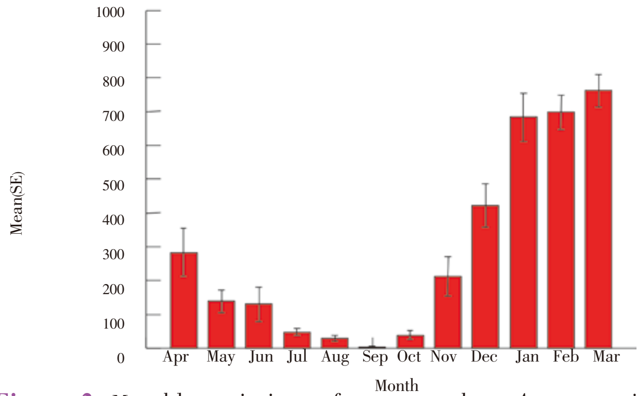

Figure 2. Monthly variations of mean numbers *Ae. aegypti* populations in Makkah City between April 2008 to March 2009.

3.5. Characteristics and variations of container habitats of *Ae. aegypti*

Table 5.
Charateristics of *Ae. aegytpi* larval collections from containers inside and around homes throughout Makkah City in 2008 and the beginning of 2009.

Container type	Number of months surveyed	Location	Number of times found positive	
			DS	WS
Cement basin	12	Indoors	May to Oct	Nov to Apr
Animal basin	12	Indoors	Jun	Nov to Apr
Troughs conditioner	12	Indoors	May, Jul	Nov, Jan, Feb, Mar, Apr
Swimming pool	12	Indoors	–	Dec to Apr
Roof tap water	12	Indoors	Oct	Nov to Dec
Water storage drum	12	Indoors	May, Jun, Oct	Nov to Apr
House water storage tank	12	Indoors	May, Jun, Aug, Oct	Nov, Jan, Feb Mar, Apr
Rubber tire	12	In/Outdoors	–	Jan to Mar
Cool water dispenser	12	In/Outdoors	–	Jan to Apr
Plant watering storage basin	12	In/Outdoors	–	Jan to Mar

Table 6.
Frequency of *Ae. aegytpi* larval presence in containers inside and around homes throughout Makkah City in 2008 and at the beginning of 2009.

Container type	No. months positive		Frequency (%) of larval presence		Characterization	
	DS	WS	DS	WS	DS	WS
Cement basin	6	6	100	100	Permanent	Permanent
Animal basin	1	6	16.6	100	Semi–occasional	Permanent
Troughs conditioner	2	5	33.3	83.3	Occasional	Semi–permanent
Swimming pool	0	6	0	100	–	Permanent
Roof tap water	1	6	16.6	100	Semi–occasional	Permanent
Water storage drum	3	6	50	100	Occasional	Permanent
House water storage tank	4	5	66.6	83	Semi–permanent	Permanent
Rubber tire	0	3	0	50	–	Occasional
Cool water dispenser	0	4	0	66.6	–	Semi–permanent
Plant watering storage basin	0	3	0	50	–	Occasional

The list of encountered containers during the survey (Table 5) shows a total of 10 types of container habitats. These containers did not have the same location. In this survey, we applied the term "indoors" to any container found within the wall of houses. We referred to any container location outside homes within less than 15 m around the peri-domestic area as "outdoors". A set (cement basin, animal basin, troughs conditioner, swimming pool, roof tap water, water storage drum and house water storage tank) was found inside homes and a second set (plant watering storage basin, rubber tire and cool water dispenser) was located in the peri-domestic environment. In some cases, rubber tires and cool water dispensers were also found indoors.

3.6. Seasonal larval prevalence and characterization of container habitats

All container types were found with immature larval stage of *Ae. aegypti*, but their prevalence differed from one container to another and relatively to season. During DS, cement basin recorded the highest number of times of prevalence (6 times), with *Ae. aegypti* being found at each monthly visit occasion. It was followed by house water storage tank (4 times), water storage drum (3 times), trough conditioner (twice), roof top water tank (once) and animal basin (once). No larvae were found in swimming pool, rubber tire, cool water dispenser and plant watering storage basin during the dry season. During WS, cement basin, animal basin, swimming pool, roof tap water, water storage drum recorded the highest number of times found with *Ae. aegypti* (6 times). They were followed by trough conditioner and house water storage tank (5 times), cool water dispenser (4 times), rubber tire and plant watering storage basin (3 times).

Based on this evidence, we considered the cement basin as "permanent breeding site" during both seasons. Other container habitats that could be considered as permanent, but only during WS include animal basin, swimming pool, roof tap water, water storage drum. Container habitats such as trough conditioner, water cooler house water storage tank could be referred to as semi-permanent during the rainy season. Animal basin, trough conditioner, roof tap water can be referred to as occasional developing sites for *Ae. aegypti* during DS. Rubber tire and plant watering storage basin seem to be not attractive to *Ae. aegypti* during WS (Table 6).

4. Discussion

A total of 32,109 larvae were collected during one year survey in Makkah City. The genera recognized in this survey (*Anopheles, Culex* and *Aedes*), all have species that have been described earlier in Saudi Arabia. Many *Anopheles* species have been described [(*An. cinereus, An. multicolor*, *An. stephensi*[48], *An. flaviatilis, An. sergentii, An. tenebrosus*[49], *An. arabiensis*[50], *An. dthali, An. rupicolus, An. turkhudi* and *An. pretoriensis*[51]. More than ten *Culex* species have been described in the Kingdom (*Cx. laticinctus*[48], *Cx. molestus*[52], *Cx. tritaeniorhynchus*, *Cx. univittatus, Cx. ochracea annulata*[49], *Cx. pipiens molestus, Cx. pipiens autogenicus. Cx. pipiens fatigans, Cx. quinquefasciatus*) and *Cx. theileri*[50].

In the current entomological survey, *Culex* was most frequently encountered genus. Larvae belonging to this genus were present in quite high numbers throughout the survey period. *Culex* spp. mosquitoes are known to be the most important vectors of West Nile virus (WNV)[53] of which infections are increasingly occurring in many parts of the world[54,55] including the Arabian Peninsula[56]. In nature, this flavivirus is maintained by a bird-mosquito transmission cycle[57,58]. Many people rear birds inside homes (Aziz, pers. com); thus the observed indoor-breeding of *Culex* mosquitoes combined with the intimate and extended human-bird interactions warrants special attention.

As the survey was focused on water-filled artificial containers, this increased indoor/peri-domestic breeding is likely related to how works domestic water management in Saudi Arabia homes. Due to its arid climate, which is characterized by a scarcity of water resources, storing water inside homes is a routine occurrence in this country. In general, water is collected from either sea or underground wells and stored in many types of artificial containers. Due to the fear of water supply shortage, many Saudi householders store water for long periods of time in large-sized containers. All surveyed houses had water storage tanks (HWST), used as major reserve. In most cases, these were found uncovered and placed in shaded areas within house yards. Such practices have been often associated with increase mosquito productivity. A striking example of this issue is the work from Arunachalam and colleagues[59].

In a study towards elucidating ecological, biological and social factors determinant to the densities of *Aedes* container-breeders, these authors performed a multivariate regression analysis. They observed a strong positive correlation between pupal number in household containers and the lack of use of stored water, the presence of shade over the container and the absence of complete cover of container. Despite the fact the pupal stage was not considered in the current survey, developing larvae were permanently present in Makkah City HWSTs. Water permanence in container habitats has been shown to highly influence mosquito abundance. The set up of plastic containers with small (2 L) and large (4 L) of water to mimic shallow and deep ponds resulted respectively in low and high prevalence of *Anopheles annulipes* larvae[60]. Such associations between mosquito abundance and water permanence have been also documented in dengue vectors. Tsuda and colleagues[61] found that larvae of a container-breeder mosquito are more abundant in "open type" tree holes than in "closed type". They attributed such a difference to the potential of the first type to collect more water than the second type.

In the present survey, HWSTs were found infested with larvae during the entire rainy season and during most of the dry season. Based on these statements, it is likely that HWSTs has accounted for much in the production and population maintenance of container-breeding mosquitoes of Makkah, as they provide regular supply of larval sites. Other container types that may have contributed more to the prevalence of container-breeding mosquitoes include cement basin (CB). Usually noticed in the basement of residences near elevators and underground parking lots, this container type, which has been also observed in some cases in farmhouse, receives water from damaged pipes and car washing.

In CBs, the four larval instars (1st, 2nd, 3rd, 4th) were always present at each visit over twelve months (April 2008 to March 2009). This is likely to suggest an increased oviposition activity. The continual abundance of larvae has been associated with increased oviposition in *Anopheles*[62]. This association may be more pronounced in *Anopheles* breeding in container habitats and may be true for *Aedes* mosquitoes. Actually, the oviposition behavior of mosquitoes is largely modulated by habitat features *i.e.*, quantity/quality of food resources, presence/absence of predators and abiotic factors[63]. In dengue vectors, evidence has been produced that females deposit their eggs in container habitats that enhance survival and development of their offspring[64,65]. Even though CBs was not assessed for nutrients, predators and physico-chemical conditions during the current survey, the continuous co-breeding of *Aedes*, *Culex*, and *Anopheles* leads to believe that their nutritional needs were met and that habitat quality was high.

Rainfall events either in the form of rain or precipitation had greater frequency and resulted in larger water volume in WS (21.36 mm) than DS (0.18 mm). There was also far higher relative humidity during the first period (WS: 71% vs. DS: 49%). Mean ambient temperature was about 35 ℃ in WS and ~ 42 ℃ in DS. A set of previous studies have explored the relationships between climatic factors and dengue incidence. In fact, temperature act on the replication of dengue viruses[66], oviposition activity[67], larval development[68,69] and density[70]. In general, high temperatures favor these processes, whereas low ones are detrimental[69]. According to Vezzani and his collaborators[67], ambient temperatures over 20.8 ℃ and rainfall higher than 150 mm are suitable for *Ae. aegypti* population increase. With reference to these reports and to the climatic conditions obtained in the present study, positive effects of rain, temperature and relative humidity are more likely to have occurred during the rainy season. It is also likely that the high temperatures that prevailed during the dry period (36 to 46 ℃) had adverse effects on the larval life and host as well as oviposition sites-seeking activities.

Furthermore many studies have considered larval indices as useful to diagnose dengue incidence risks[71,72]. In particular, there has been more focus on house and breteau indices, as they directly relate positive container habitats within human dwelling areas. BI predicted well dengue outbreaks in Southeast Asian[73]. In addition, this index has been recently successfully used to evaluate contemporary and future risks dengue risks in the other know endemic region, South America[74]. According to this author, a level of larval infestation comprised between 2% and 5% is enough to keep dengue endemic and to trigger small-scale outbreaks. In the present study, BI was 1.66 during DS and as high as 4.83 for the wet period, resulting in an average of 3.24. Referring to Vanzie[74-79], it appears clear that the annual and WS's BI obtained in Makkah City are within the range believed to require attention, as they indicate increased population maintenance and are adequate to cause outbreaks.

The most substantial information from this study is that the mosquito fauna of Makkah City contains mosquito genera in which species have been identified as medically important. *Culex* sp. and *Ae. aegypti* were the most abundant mosquitoes encountered and this pose threat to the public health.

Culex species are recognized as vectors of West Nile virus (WNV) of which infections are on the rise worldwide[80-82]. While WNV is a potential threat to Makkah City people, dengue is already an absolute threat. Results of our entomological survey illustrated that *Ae. aegypti* has increased breeding convenience inside homes; people permanently store water indoors in large-sized containers as a consequence of the natural scarce water resource. With the permanency of certain container habitats (*i.e.*, house water storage tanks, cement basin), the dengue vector is predicted to maintain some levels of population density, thereby potentially keeping up transmission risks. Also, climate conditions were likely conducive to the breeding of container-thriving mosquito vectors.

This, in combination with the high WS's BI, strongly suggests dengue endemicity in Makkah City. This study provide information on distribution and relative abundance of potential vector populations in Makkah City, which is an essential component of any mosquito-borne disease control, results of this research study advise more comprehensive mosquito surveillance with emphasis on *Culex* species and dengue vectors apace.

Conflict of interest statement

We declare that we have no conflict of interest.

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