Temporal variations on abundance, blood feeding activity of *Aedes aegypti* (Diptera: Culicidae) and dengue incidence in Makkah City, Saudi Arabia

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ABSTRACT

Objective: To determine the genera composition and mosquito abundance in Makkah City, Kingdom of Saudi Arabia (KSA) with emphasis on the seasonality, population structure and blood feeding activity of *Aedes aegypti* (*Ae. aegypti*) and dengue incidence. Methods: Residential areas throughout the city were inspected through wet and dry seasons (WS and DS, accordingly) from April 2008 to March 2009. Monthly visits were performed on a total of 9 323 houses, and adult mosquitoes were sampled with light-traps (LT) placed around houses and by pyrethrum sheet spraying (PSS). Dengue incidence data were provided by the appropriate KSA’s government bodies. Results: Three genera were identified with Culex and Anopheles being the most and least abundant, respectively. *Ae. aegypti*, the sole member of Aedes encountered, accounted for 3.9% of total adults collected, with abundance being greater when PSS was used. This mosquito was encountered throughout the survey period, but in greater numbers during WS. There were more females than *Ae. aegypti* males. Blood feeding activity of *Ae. aegypti* varied seasonally, with WS recording more fed females. Dengue virus was active in both seasons, but with more infection cases during WS. Conclusion: Besides providing some insights into the mosquito vectors’ fauna in Makkah City, this work also demonstrates a conjugated increase of both *Ae. aegypti* abundance and dengue incidence during WS. Thus, more focused efforts during this period can benefit dengue control in this country.

1. Introduction

The global prevalence of dengue has increased drastically over the past few years[1]. The disease is now present in more than one hundred countries[2] in Africa[3], the Americas[4], Southeast Asia and the Western Pacific[5] and the Middle East[6]. Besides imported cases occurrence in this region, recently, many cases of indigenous transmission of dengue virus have been reported in several countries including Saudi Arabia[7].

In this country, *Aedes aegypti* (*Ae. aegypti*) is established as a main vector of dengue[8]. This mosquito species is highly anthropophilic and mostly dwells in and around human habitation[9]. Past studies have shown that its females feed predominantly on humans in nature[10] and prefer to oviposit in domestic containers[11]. Since the first fatal case of dengue hemorrhagic fever (DHF) in Jeddah in 1993, major epidemics have been reported: a DEN–2 epidemic in 1994 with 469 cases of dengue, 23 cases of DHF, 2 cases of dengue shock syndrome (DSS) and 2 deaths; a DEN–1 epidemic in 2006 with 1 269 cases of dengue, 27 cases of DHF, 12 cases of DSS and 6 deaths; a DEN–3 epidemic in 2008 with 775 cases of dengue, 9 cases of DHF, 4 cases of DSS and 4 deaths. Since the beginning of 2010, 363 cases of dengue fever have been recorded in Jeddah[12].

Many factors come into play when the causes of dengue
spread are considered. Eminently, the abundance, survival, and behavior of the vectors; level of immunity to the circulating virus serotype in human populations; density, distribution and movement of humans are crucial. In addressing the factors determining dengue endemism, it is believed that the habits of people, in particular domestic water management play a key role.

Due to its arid climate, which is characterized by a scarcity of water resources, storing water inside homes is a routine occurrence in Saudi Arabia. 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. Such conditions are conducive to the occurrence and persistence of Aedes aegypti populations. The permanent presence of artificial containers inside homes of many Saudis, is likely to account for much in dengue occurrence, as this provides close vector–host contact, and thus increased blood feeding opportunities. Astonishingly, the role of water storage habit remains poorly studied as it affects the abundance of Aedes aegypti and its blood feeding activity. Here, we investigated the prevalence, population structure and blood feeding activity of this mosquito in Makkah City, taking seasonality into consideration.

2. Materials and methods

2.1. Study area and sampling sites

The study was conducted in Makkah City located in the Western part of KSA (Figure 1) from April 2008 to March 2009. The city lies at an elevation of 277 m above sea level. Its geographic coordinates are 21° 25′ 36″ N, 39° 49′ 34″ E and it has arid season and temperatures are high throughout the year. It is very hot from May to October with the daily temperatures varying between 30 °C and 40 °C. 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.

The urban and metropolitan areas of Makkah City have a total area of 850 km² and 1 200 km², respectively. The total population was 1 294 167 persons in 2004 and about 1 484 000 in 2009. There are 120 approved residential areas, 201 716 residential blocks and more than 7 800 blocks for repair shops and warehouses. The city is a pilgrimage point for Muslims worldwide due to the presence of the Kaaba, the most sacred site in Islam. Because of 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.

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

2.2. Sampling

To have more insights into disease transmission, we used light–trap method to target the host–seeking segment of mosquito populations, covering five residential areas used to survey larvae. To this end, we have used commercial Black Hole light traps (Black Hole, Light–trap Co., Seoul, Korea). Such traps have been successfully used for measuring the seasonal prevalence of Aedes aegypti in KSA. A Black Hole light trap consisted of an electric light trap that uses TiO₂ (titanium dioxide) and ultraviolet light to produce CO₂. Ten Black Hole light traps were set in each of the residential areas. The 50 traps were placed nearby homes at 1.5 m above ground and allowed to operate four times per month from dusk to dawn. Trapped adult mosquitoes were collected the following morning of each sampling time. They were killed by pyrethrum spraying prior to their transfer in labeled plastic vials (27 cm × 20 cm × 7 cm) and brought to the laboratory for further processing and identification.

We also used pyrethrum spray collection, which directly measures the number of resting mosquitoes and which has been reported as an efficient method to target Aedes aegypti with respect to its indoor habit. For this purpose, we have followed the procedure from Silver. A room was selected from each of the 9 329 houses where house owners accepted our request for entomological inspection. In each of these rooms, a white sheet (2 m × 2 m) made of fabric was laid over the floor after occupants were out and movable furniture transferred outside to avoid potential contacts with the product to be sprayed. All doors, windows and eaves were closed. In case these were infectious, careful efforts were taken to get the room to be treated air–tight. Once these routine procedures have been fulfilled, pyrethrin aerosol spray was applied for a time length ranging from 50 seconds to 1 minute depending on the size of the room. The treated room was left closed and undisturbed 10 minutes after spraying. Mosquitoes were then collected with the aid of forceps and transported to the laboratory for the same purpose and under the same conditions as those collected by light traps. Sampling of both host–seeking and resting mosquitoes was performed each month from April 2008 to March 2009.

2.3. Dengue incidence

In KSA, it is required to any medical practitioner to report all diagnosed and established dengue cases to Ministry of Health (MOH). It is also obligatory to any laboratory to declare all patients tested positive for dengue infection to MOH. These institutions define dengue and its hemorrhagic form as cases with confirmed laboratory blood tests of viral infection of any of the four serotypes. Based on this information, we collected monthly dengue data for the period of study from the Ministry of Health of Kingdom of Saudi Arabia through its Department of Health Affairs in Makkah.
2.4. Data collection and analysis

Field-sampled adult specimens (light-trap and pyrethrum spray collections) were identified to the genus level under a dissecting microscope (Olympus CX41; Olympus, Tokyo, Japan) using appropriate taxonomic keys[27]. Although this study was aimed at only Ae. aegypti, other encountered mosquito genera were also enumerated. Identified adults mosquitoes were counted, taking the technique of collection, season and month of collection into account, but in case of Ae. aegypti, sex was taken into consideration.

The blood feeding status of females of this mosquito species was examined based on WHO guidelines[28]. The resulting numbers were used to calculate relative abundance as follows: number of specimens belonging to a given genus or Ae. aegypti / (total number of specimens collected) × 100. Abundance patterns were determined based on percentages. Monthly and seasonal blood feeding rates of this species were calculated as the number of blood fed females divided by the total number of collected females.

Monthly numbers of confirmed dengue infection cases were aggregated to obtain seasonal cumulative numbers. These numbers were used to calculate relative incidence of dengue infections within each month or season as follows: (number of confirmed dengue infection cases / (total numbers of specimens collected)) × 100.

Where necessary, analysis of variance was performed using the statistical software package Systat v.11[29] (Systat Software, Inc., Richmond, CA, USA), with P<0.05 as indicator of statistical significance.

3. Results

3.1. Abundance variations in relation to genera and collection technique/location

A total of 29,095 mosquito individuals, representing three genera were collected. Since the survey was aimed at adults only, larvae and pupae were not collected. 47.5% (29,095/61,204) was at the adult stage. Of the collected adults, 3.9% (1,144/29,095) belonged to the genus Aedes, whereas, 94% (27,357/29,095) and 2% (594/29,095) were Culex and Anopheles, respectively (Table 1).

<table>
<thead>
<tr>
<th>Genera</th>
<th>Number of individuals</th>
<th>Adult</th>
<th>PSS</th>
<th>LT</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aedes</td>
<td>916</td>
<td>228</td>
<td>1,144</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culex</td>
<td>9,811</td>
<td>17,546</td>
<td>27,357</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anopheles</td>
<td>572</td>
<td>22</td>
<td>594</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11,299</td>
<td>17,796</td>
<td>29,095</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Culex adults were mostly collected genus outside homes by light traps (LT) (17,546/27,357: 64.14%), while the vast proportion of Anopheles adults (572/594: 96.29%) was collected indoors by the sheet spray (PSS) technique. A total of 80.06% (916/1,144) of the Aedes adults were collected inside homes (by PSS technique) and the remaining (228/1,144: 19.93%) came from outdoors LT collections (Table 1).

3.2. Seasonal variations of Ae. aegypti adult populations

There was significantly more Ae. aegypti adults collected by PSS than by LT (F = 16.455, df = 1, P < 0.001); more than 80% of the total collections resulted from the first method and less than 20% came from the second technique. 77.62% (711/916) of Ae. aegypti adults were collected by PSS, occurred during WS and 22.38% (205/916) during DS. No Ae. aegypti adult was obtained by LT during DS. The use of PSS during WS provided more than 75% of the total Ae. aegypti adult collections during the survey (Table 2).

<table>
<thead>
<tr>
<th>Collection technique</th>
<th>DS</th>
<th>WS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSS</td>
<td>205</td>
<td>711</td>
<td>916</td>
</tr>
<tr>
<td>LT</td>
<td>0</td>
<td>228</td>
<td>228</td>
</tr>
<tr>
<td>Total</td>
<td>205</td>
<td>939</td>
<td>1,144</td>
</tr>
</tbody>
</table>

3.3. Seasonal variations of Ae. aegypti adult populations in relation to sex

More than 50% of the total Ae. aegypti collections were females. The sex ratio within the total collected population of this mosquito was 0.84. This parameter was 0.77 during DS and 0.85 during WS. 87.86% (460/524) of the total males were collected during WS versus 12.21% (64/524) during DS. Of the total females collected during the survey, more than 85% were obtained during WS and less than 13% during DS (Table 3).

<table>
<thead>
<tr>
<th>Season</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS</td>
<td>64</td>
<td>83</td>
<td>147</td>
</tr>
<tr>
<td>WS</td>
<td>460</td>
<td>537</td>
<td>997</td>
</tr>
<tr>
<td>Total</td>
<td>524</td>
<td>620</td>
<td>1,144</td>
</tr>
</tbody>
</table>

3.4. Seasonal variations of the population size of PSS-collected female Ae. aegypti in relation to blood feeding status

Of the total of Ae. aegypti females collected inside houses, 45.82% (225/491) were blood fed. A total of 75.55% (170/225) of the blood fed females were collected during WS versus 24.46% (55/225) during the non rainy season. Of the total collected non–fed females, about 89% were obtained during WS. It is therefore likely that blood feeding activity of Ae. aegypti is high during the rainy season when compared to DS (Table 4).

3.5. Monthly variations of Ae. aegypti adult populations in...
relation to blood feeding

Table 4.
Seasonal variations of female *Ae. aegypti* populations in Makkah City during 2008 and the beginning of 2009.

<table>
<thead>
<tr>
<th>Season</th>
<th>Fed</th>
<th>Non-fed</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS</td>
<td>55</td>
<td>28</td>
<td>83</td>
</tr>
<tr>
<td>WS</td>
<td>170</td>
<td>238</td>
<td>408</td>
</tr>
<tr>
<td>Total</td>
<td>225</td>
<td>266</td>
<td>491</td>
</tr>
</tbody>
</table>

The mean numbers of blood-fed ($F = 8.617$, $df = 1$, $P = 0.005$) and un-fed ($F = 21.161$, $df = 1$, $P < 0.001$) females showed significant variations between months. From 10 to 15 individuals in April, the number of non-engorged females gradually decreased up to August. During this period, engorged females were captured, but in small numbers. The mean number of females found with a blood meal increased from October to November, attaining a peak, but dropped in the following month. The mean number of blood fed females rebounded in January but gradually deceased during the next two months. Between October to March, un-fed females were also highly present, but their monthly mean numbers were less than those of their blood fed counterparts, especially in November, January and February (Figure 2).

![Figure 1. The different residential areas of Makkah City where the entomological survey was carried out.](image)

Figure 1. The different residential areas of Makkah City where the entomological survey was carried out.

![Figure 2. Monthly variations of the population size of un-fed and fed *Ae. aegypti* females.](image)

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![Figure 3. Monthly variations of dengue infection confirmed cases in Makkah City during 2008 and the beginning of 2009.](image)

Figure 3. Monthly variations of dengue infection confirmed cases in Makkah City during 2008 and the beginning of 2009.

3.6. Dengue incidence and temporal variations

Dengue incidence showed considerable temporal changes during the study period. From 3 cases reported in April 2008, the number of dengue patients peaked (29 cases) in May 2008, but decreased the following month. In July 2008, the number of cases reached a second minor peak (15 cases) and sharply decreased thereafter. None of a single confirmed dengue infection case was reported in October 2008. This quiescence in dengue occurrence was followed by a gradual rise of infection cases from October 2008 to March 2009, the month that recorded the highest prevalence (300 cases) (Figure 3).

During the execution of the present study, there have been 505 confirmed dengue infection cases. Of this total, the vast majority of cases occurred during WS. The infection rate recorded during the rainy season was more than seven times higher than that DS (Table 5).

Table 5.
Seasonal variations of dengue infection confirmed cases in Makkah City during 2008 and the beginning of 2009.

<table>
<thead>
<tr>
<th>Season</th>
<th>Number of confirmed dengue infection cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS</td>
<td>59</td>
<td>11.68</td>
</tr>
<tr>
<td>WS</td>
<td>446</td>
<td>88.32</td>
</tr>
<tr>
<td>Total</td>
<td>505</td>
<td>100.00</td>
</tr>
</tbody>
</table>
4. Discussion

A total of 29,095 adult mosquitoes were collected during one year of survey in Makkah City. The genera recognized in this survey (Anopheles, Culex and Aedes), all have been described earlier in Saudi Arabia. Many Anopheles species have been described [(An. cinereus, An. multicolor, An. stephensi)\(^{[30]}\), An. flavititis, An. sergentii, An. tenebrosus\(^{[31]}\), Culex species have been described in the Kingdom [Cx. lactinectus\(^{[30]}\), Cx. molestus\(^{[32]}\) Cx. tritaeniorhynchus, Cx. univittatus, Cx. ochracea annulata]\(^{[31]}\). The most substantial information from our adult survey is that the mosquito fauna of Makkah City contains mosquito genera in which species have been identified as medically important. Anopheles sp., Culex sp. were the most abundant mosquitoes encountered and this pose threat to the public health. The presence of Anopheles, Culex and Ae. aegypti in Makkah City potentially exposes residents to biting nuisance and mosquito–borne diseases. In particular, Culex species are recognized as vectors of West Nile virus (WNV) of which infections are on the rise worldwide.

*Ae. aegypti* was present throughout the survey period, but more abundance during WS. Due to its increased sensitivity to climate factors, this mosquito commonly exhibits seasonal variations in population abundance\(^{[33]}\). In general, low densities occur during the dry and cool periods, whereas warm and wet periods trigger high densities\(^{[34]}\). In Makkah City, the weather is arid, but *Ae. aegypti* was encountered during the dry season, a period when no rain occurred. The existence of this species throughout the year is probably due to the behavior of local residents in water management. 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. 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 *et al.*\(^{[35]}\). 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. Water permanence in container habitats has been shown to highly influence mosquito abundance. The set up of plastic containers with small (2 liters) and large (4 liters) of water to mimic shallow and deep ponds resulted respectively in low and high prevalence of Anopheles annulipes larvae\(^{[36]}\) Such associations between mosquito abundance and water permanence have been also documented in dengue vectors. Tsuda *et al.*\(^{[37]}\) 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.

As in any un–autogenous species, blood–feeding in this species is essential for the production of viable eggs by *Ae. aegypti*\(^{[38]}\). Females of Aedes maintained on blood from live animals exhibit increased fecundity and longevity\(^{[39]}\). In fact, the completion of a gonotrophic cycle depends largely on both the hosts and breeding sites\(^{[40]}\). Female mosquitoes alternate between human hosts for blood feeding and aquatic habitats for oviposition. Although we did not investigate the oviposition activity of *Ae. aegypti* during the current survey, the persistence of adult population clearly suggests the availability of breeding sites and blood sources. The increased proportions of blood fed females among both DS– and WS–populations are likely to support our contention.

Makkah City was quite warm during the survey, with temperatures averaging 35 °C during WS. High temperature conditions will likely lead to increased mosquito adult abundance. The accelerated immature development known to result from such weather conditions\(^{[41]}\) will also bring on high cumulative adult emergence and increased population density, which is also likely to be associated with increased occurrence of disease. Clearly, in natural settings, high adult numbers will tend to result in increased human–vector contacts and stimulate biting activity. With increased blood feeding activities, females have increased chances of up–taking and transmitting dengue viruses. The increased human blood uptake will also favor aggravated longevity. A diet of human blood meal provides a proximate benefit in the synthesis of energy reserves and an ultimate advantage in mosquito fitness\(^{[42]}\). Many previous studies have established a relationship between adult abundance and dengue occurrence, Barrera *et al.*\(^{[43]}\), working with *Ae. aegypti* in Puerto Rico, reported a positive correlation between the number of dengue cases and the population level of females. Similarly, Turley *et al.*\(^{[44]}\) observed an increase in dengue occurrence four weeks after larval populations peaked.

It is interesting to note that *Ae. aegypti* was more collected inside the houses (by PSS technique) and in greater numbers during WS. This exposes the inhabitants to dengue fever. Laboratory blood tests revealed that the dengue virus was active during both seasons among Makkah City residents, with occurrence being greater during WS. The incidence of dengue has been commonly associated with the rainy season\(^{[45]}\). In India the wettest monsoons led to a spate of mosquito growth and dengue outbreak\(^{[46]}\). Most of vector borne diseases exhibit a distinctive seasonal pattern and climatic factors such as rainfall, temperature and other weather variables affect in many ways both the vector and the pathogen they transmit\(^{[47]}\). In fact, dengue viruses are transmitted to humans through the bites of infective female Aedes mosquitoes. Mosquitoes generally acquire the virus while feeding on the blood of an infected person.

It is important to mention that different collection techniques therefore provide much additional information and will yield samples of the population that more accurately represent the sex rate, age structure and physiological–engorged adult females which can be used to study host...
seeking[52]. In our present study LT trapping worked well during WS whereas it did not attract *Aedes aegypti* during DS. Generally, our estimates of *Ae. aegypti* population size during the period of this study has shown that a total of 80.06% (916/1 144) of the Aedes adults were collected inside homes (by PSS technique) and the remaining (228/1 444; 19.93%) came from outdoors LT collections. Using PSS and CDC light trap collection techniques, *Ae. aegypti* was only found in urban strata. In the three strata, mosquitoes were mainly found in high numbers during the wet season. Further, Anopheles gambiae, Culex quinquefasciatus, and *Aedes aegypti* mosquitoes were found occurring together inside the houses. This in turn exposes the inhabitants to the incursions of many strains and serotypes by the mosquito-borne disease control, results of this research come next.

In addition to provide information on the 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 work advise more comprehensive mosquito surveillance with emphasis on Culex species and dengue vectors space with a close surveillance of their susceptibility to the arsenal of insecticides currently used in control operations. This approach may also help to avoid or minimize the probability of the occurrence of secondary dengue infections, known as the greatest factor for severe disease, which may result from the incursions of many strains and serotypes by the increasingly massive arrival of pilgrims, among which, some maybe dengue virus carriers.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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