

REDESCRIPTION OF *Aedes aegypti* AND DETECTION OF DENGUE VIRUS FROM FIELD COLLECTED LARVAE FROM LAHORE, PAKISTAN

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Abstract Dengue virus is transmitted by female mosquito *Aedes aegypti* and it consists of the most common *flavivirus* infection. Pakistan is also at high risk of dengue epidemics. The present study focused on the redescription of *Aedes aegypti* and the detection of dengue virus from 685 field collected larvae of *A. aegypti* from different areas of Lahore. No morphological change, as compared to previous work, was observed. Temperature and humidity have direct effect on the distribution, population density and growth rate of *A. aegypti*. Population and growth rate increases during rainy season and decreases during dry season. High number of larvae (99) was found in July, 2012. Bhati Chowk area is more receptive to dengue transmission as compared to other areas because high percentage of larvae (23%) was found from there. A rapid identification of dengue virus from larvae of *A. aegypti* was done by RT-PCR. RNA extracted from each pool was tested by RT-PCR for detection of dengue virus. It was shown that dengue serotype 2 was prevalent in Lahore, Pakistan. DV-2 was found in 26 pools, DV-3 in 9 pools and DV-4 was present in 3 pools. DV-1 was not found in any pool.

Key words RT-PCR, serotype, morphology, population.

INTRODUCTION

In Pakistan *Aedes. aegypti* is considered as the most important vector related to the dengue outbreaks. Dengue fever and dengue hemorrhagic fever, commonly known as break-bone fever, is caused by dengue virus (DV). There are four different serotypes of the virus, belonging to genus *flavivirus*: DENV-1, DENV-2, DENV-3 and DENV-4 (Mitchell, et al., 1987), which are closely related to each other antigenically. The difference in the nucleotide sequences of all serotypes is about 24-36% (Fauquet et al., 2005). From all serotypes, DV-4 is the most different. As far as literature is concerned, no thorough study is available to describe the morphology of *A. aegypti* from Pakistan. The present study was carried out from July 2012 to September 2013 to redescibe *A. aegypti* and to know whether any change has occurred in it due to climatic conditions during past few years or correlate the abundance of *A. aegypti* with temperature and relative humidity. Another objective was to detect dengue virus from field collected larvae of *A. aegypti*.

MATERIALS AND METHODS

More than 600 larvae were collected from different localities of Lahore. *A. aegypti* larvae were redescibed by using key (Harrison, 2005). Seasonal population of *A. aegypti* in different localities, Sex ratio, and relation with environmental factors like temperature and relative humidity were noted during study also. Detection of Dengue virus (DV) was done by RT-PCR. Minimal infection rate and viral infection rate were also calculated.

RESULTS AND DISCUSSION

As described in materials and methods, 685 larvae of *A. aegypti* were collected from different areas of Lahore during July, 2012 to September, 2013. Larval and pupal skin was taken and studied to redescribe *A. aegypti*. As *A. aegypti* was not described morphologically from Pakistan so during this study, morphological features of *A. aegypti* were studied, hairs present on head, thorax and abdomen of larvae and pupae were also counted. Larvae of *A. aegypti* were studied previously by (Puri, 1931; Belkin, 1953; Christopher, 1960). Recently, Andrew and Bar (2013) described the morphology of *A. aegypti* adult mosquitoes from India. The presence of 12-15 hairs on the head region, 1-14 hairs on thorax region and 10-15 hairs on abdomen were observed. These studies also witnessed the presence of 8-12 Comb teeth present in a single row. The results of current study are identical to the previous work and no significant change has been observed. Pupae of *A. aegypti* were also studied 10-12 hairs on cephalothorax and 10-14 hairs on each segment of abdomen were counted (Belkin, 1953). A single hair on each pupal paddle was also observed (Christopher, 1960). Pupal body of *A. aegypti* consisted of a large and somewhat rounded cephalothorax with 10-12 hairs. Like larvae of *A. aegypti*, pupal abdomen is also having 8 segments with 10-14 hairs each segment. It was seen that 8th segment contains circular paddles which have stubbles on them.

Results also revealed that the highest percentage of *A. aegypti* larvae were found in the Bhati Chowk area (23%). Lowest percentage (4.5%) was found in the Raj Gerh area. It can be depicted from results that the area of Bhati Cowk is at high risk with the highest population (157) of *A. aegypti* larvae. Female larvae were found in great abundance (63.5%) as compared to male larvae (36.5%). As the ecology of *A. aegypti* is concerned, the number of collected larvae were correlated with the temperature and relative humidity (RH). The population of *A. aegypti* larvae were most abundant in the months of hot and humid weather. At the temperature of 32°C and 93% RH (July, 2012), maximum number of *A. aegypti* larvae was found. The minimum number of larvae were found at the temperature of 14°C and 86% RH in the month of January 2013. Previous studies also show that high population density of *A. aegypti* is mostly concentrated in densely populated areas (Chakravarti and Kumaria, 2005). Environment in densely populated areas predisposes large number of prone hosts to the bite of *A. aegypti* (Gibbons and Vaughan, 2002). *A. aegypti* is highly adapted to urban areas (Honorio, et al., 2003). Female *A. aegypti* lays eggs 1 km radius from their feeding sites, so they increase their boundaries (Chun et al., 2007; Weaver and Reisen, 2010). In addition to increase in population density, mass production of non-biodegradable plastic containers provide breeding sites for mosquitos (Kyle and Harris, 2008). A survey report (2009) of Lahore described that larvae of *A. aegypti* were prevalent in areas of Data Gunj Baksh and Samanabad as these areas were having artificial containers. The prevalence of *A. aegypti* was fewer in other areas that were not having artificial breeding sites.

Numerous environmental parameters like temperature and relative humidity were recorded during the study. All these factors have a strong association with mosquito abundance and development (Manimegalai, 2010; Devi and Jauhari, 2007; Leisnham et al., 2006). During extreme winters and summers, the larvae of *A. aegypti* do not survive. An average increase in daily temperature may have a major biological or morphological impact on *A. aegypti* (Tun-Lin et al., 2000; Mohammad and Chadee, 2011). Optimum temperature for larval growth is 32°C (Bar and Andrew, 2012) and optimum relative humidity is 80 ± 15% (Walker et al., 2011). The same results were found for mosquito abundance associated with temperature in the present study. Mosquitoes were found highly abundant in the summer season. *A. aegypti* larvae were plenty in number during the month of July and August of both years when the temperature was 32°C and humidity was between 80 to ≥ 90%.

About 300 larvae of *Aedes aegypti* were used for the detection of dengue virus. 30 pools of larvae were made to run the PCR. All serotypes (1-4) of dengue virus were detected. Out of 30 pools, Serotype 1 was not present in any pool. Agarose gel for serotype 2 with the band size 403 kb. 26 pools were positive with serotype 2. Serotype 3 was present in 9 pools. In serotype 3, bands (453 kb) were seen. Serotype 4 (401 kb) was also amplified. Out of 30 pools, serotype 4 was found in 3 pools. Viral Infection rate (VIR) and Minimal Infection Rate (MIR) were also calculated. Serotype 2 reveals the highest VIR and MIR i.e. 8.7 and 87, respectively. Species which transmit dengue virus transovarially or vertically in their next generations are *A. albopictus*, *A. aegypti*, *A. mediovittatus* (Freier and Rosen, 1988), *A. alcasidi*, *A. cooki* etc. (Rosen, 1988; Rohani et al., 2005). Dengue virus persists in several generations of *Aedes aegypti* through transovarial transmission (Joshi et al., 2002). Thenmozhi et al. (2007) studied the vertical transmission of dengue virus in *Aedes* mosquitoes in India. It was concluded that vertical transmission also occurs in nature. Vertical transmission via the transovarial route in female *Aedes* mosquitoes also occur in nature. (Mulyatno et al., 2012).

CONCLUSIONS

The established method in this study included two-step RT-PCR for the detection of dengue virus. This is a rapid and very sensitive technique to detect the viruses. Present study showed that dengue virus serotype 2 was dominant in the larvae of *Aedes aegypti*. The other serotypes present were serotype 3 and 4. Serotype 1 was absent. The current research work will have an intense influence on dengue control programs and further epidemiological studies. The molecular detection of dengue virus will help to have more precise virus surveillance in the areas where there is a large population of vectors. Detection or isolation of different serotypes of dengue virus from endemic areas will be easier by using molecular.

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