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Short Communication

Aedes aegypti is the Major Vector for Transmission of Dengue Virus in Lahore, Pakistan

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Authors' Contributions
FM and IA conceived and designed the study. HR and FM collected samples and performed experimental work. MS, IA and SA analyzed the data. MI and TRS wrote the article.

Key words
Parasitism, Aquatic Mites, Mosquitoes, *Culex pipiens.*

ABSTRACT

The present study focused on a rapid identification of dengue virus from larvae of *Aedes aegypti* (L) and *Aedes albopictus* (Skuse) by using PCR. During this study, larvae of *A. aegypti* and *A. albopictus* were collected from different localities of Lahore during May to September of 2012 to 2014. RNA extracted from each mosquito larvae pool was tested by nested PCR for detection of dengue virus. Out of total 50 pools of *Aedes aegypti* larvae, DV-2 was the most prevalent serotype. DENV-1 was not found in any pool. While all pools of *Aedes albopictus* were negative for dengue virus. Our study showed that the main cause of spreading of dengue virus was *Aedes* aegypti. Serotype DENV-2 was dominant in the field collected larvae of *Aedes aegypti* in Lahore and Sheikhupura, Pakistan.

Dengue infection is an arboviral infection caused by dengue virus that is taxonomically classified in the Family Flaviviridae and genus *Flavivirus* (Fatima *et al*., 2011). The important vector of dengue virus are the members of *Aedes* genus, *i.e., Aedes aegypti* and *Aedes albopictus* (Sabila *et al*., 2015). There are four different serotypes of the virus, DENV-1 to DENV-4, which are closely related to each other antigenically (Fauquet *et al*., 2005). Dengue virus can either cause dengue fever, dengue hemorrhagic fever or dengue shock syndrome due to the antibody-dependent enhancement (Chew *et al*., 2012). Infecton with a certain serotype gives life time immunity from that particular serotype but other serotypes may cause infection.

Over the last few years, Pakistan is emerging as a region of endemic dengue activity (Ahsan, 2008). It was reported for the first time in Pakistan as an undifferentiated fever in 1985. The first epidemic was reported in 1994 in Karachi. Serotype 1 and 2 of dengue virus were present in serum of children (Akram *et al*.,1998). The spread of serotype 1 and 2 was the reason for dengue fever in Balochistan (Paul *et al*.,1998).With the passage of time, cases of dengue were increased in 2003. Dengue serotype 2, 3 and 4 were reported from Lahore in 2008. In 2011, Pakistan was on worst attack of dengue due to the co-circulation of DENV-2 and DENV-3 (Chan *et al*.,1995). More recently, the incidence of dengue infection in 2013 with the presence of serotype 1-3 was reported (Khan and Khan, 2015).

The current study was conducted to detect the serotype of dengue virus isolated from the larvae of *Aedes aegypti* and *Aedes albopictus* from Lahore, Pakistan.

Materials and methods

Two species of mosquitoes *Aedes aegypti* and *Aedes albopictus,* were collected from different localities of Lahore (Aziz Bhatti Town, Wahga Town, Shalimar Town, Ferozwala Town and Gulberg Town), where there was high population of *Aedes* larvae. In the laboratory, mosquitoes were fed on cow liver powder and newly emerged larvae were fed on 10% sucrose solution. Fifty pools of *A. aegypti* and 50 pools of *A. albopictus* larvae were established, each containing one to 10 larvae in each vial. All pools were stored in freezer at -70°C.

Viral RNA was extracted from larvae using Viral Nucleic Acid extraction Kit (Favorgen Biotech Corporation, Australia). cDNA was synthesized from RNA of dengue virus in 30 cycles using 10 µl of RNA with a reaction mixture of 10 µl containing 4 µl 5X first strand buffer (FSB), 0.5 µl 0.1 M Dithiothreitol (DTT), 2 µl 10 mM dNTPs, 1 µl 20 pM antisense primers, 1.3 µl dH_2O,

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Table I.- Oligonucleotide sequences used to amplify C-prM gene junction of dengue virus.

Primer name	5´-3´ sequence	Size of amplified product in base pairs	Use in PCR round
D1-D	TCAATATGCTGAAACGCGWGAGAAACCG	511 bp	1st round
D2-D	TTGCACCARCARTCWATGTCTTCWGGYTC		
TS1-F	AGGACCCATGAAATTGGTGA	411 bp	2nd round
TS1-R	ACGTCATCTGGTTCCGTCTC		
TS2-F	AGAGAAACCGCGTGTCAACT	403 bp	2nd round
TS2-R	ATGGCCATGAGGGTACACAT		
TS3-F	ACCGTGTGTCAACTGGATCA	453 bp	2nd round
TS3-R	CAGTAATGAGGGGGCATTTG		
TS4-F	CCTCAAGGGTTTGGTGAAGAG	401 bp	2nd round
TS4-R	CCTCACACATTTCACCCAAGT		

Table II.- Detection of all four serotypes of Dengue virus in positive pools of larvae in *A. aegypti and A. albopictus.*

Species	No. of pools assayed	Total no. of larvae	No.(%) of positive pools	No. (%) of dengue virus serotype detected from positive pools			
				DEN1	DEN2	DEN3	DEN4
A. aegypti	50	1038	38(76%)	0(0%)	26(68.4%)	9(23.7%)	3(7.9%)
A. albopictus	50	979	0 (0%)	0(0%)	0(0%)	0(0%)	0(0%)

0.2 µl RNA inhibitor (RMI) and 1 µl of Moloney Murine Leukiemia Virus (MMLV) Reverse Transcriptase (RTse) (Invitrogen Biotechnology, USA). The 20 µl reaction mixture was incubated at 37°C for 50 min. followed by 2 min. heat inactivation of M-MLV at 95°C. The samples were then incubated for 2 min. at 22°C.

Dengue serotype specific degenerate primers designed according to the primer sequences targeting C-prM gene junction (Lanciotti et al., 1992) were used. The size of products was 411 bp for DENV-1, 403 bp for DENV-2, 453 bp for DENV-3 and 401 bp for DENV-4 (Table I).

PCR was used to detect the serotype of dengue virus from each sample. For amplification of cDNA, 2 µl of cDNA was used with 8 µl of master mix. PCR mix was made by mixing 1 µl 10X PCR buffer (with ammonium sulphate), 2.4 µl $MgCl_2$, 1 µl 500 µM dNTPs, 1 µl pM forward and reverse primer each, 2.4 µl d H_2O and 0.2 µl of 5 U of Taq DNA polymerase (Invitrogen Biotechnology, USA). The thermal profile for first round using outer sense D1-D and antisense D2-D was initial denaturation at 94ºC for 2 min followed by 30 cycles of denaturation at 94ºC for 45 sec, annealing at 52ºC for 45 sec, extension at 72ºC for 2 min and final extension at 72ºC for 10 min. Same thermal profile was used for second round using type specific primers. Only the annealing was done at 54ºC for 45 sec in 30 cycles. Isolated DENV- PK/45/2009 was used as positive control as it gave good results for each serotype 2 and 3 while HCV was used as a negative control.The PCR product was visualized by 2% agarose gel, stained with ethidium bromide, visualized under UV light.

Results and discussion

During the year 2012 to 2014 from the months of May to September each year, a total of 2017 larvaes were collected, out of which 1038 (51.5%) were *A. aegypti* and 979 (48.5%) were *A. albopictus* (Table II).

From the pools of *A. aegypti*, dengue serotypes 2, 3 and 4 were detected and serotype 1 was not present in any pool. Out of 50 pools, 36 were positive for serotype 2, 9 pools for serotype 3 and 3 for serotype 4. On the other hand, all pools of *A. albopictus* were 100% negative for any serotype. Figure 1 shows the positive samples of DENV 2 and DENV 3.

In the absence of a safe and effective vaccine for mass immunization, the prevention and control of dengue outbreaks are dependent upon the control of the vector mosquitoes *A. aegypti* and *A. albopictus* (Knudsen and Sloof, 1992; Gubler and Clarke, 1994). The current study

suggests that *A. aegypti* is the main transmitting agent of dengue (WHO, 2011) and not the *Aedes albopictus*.

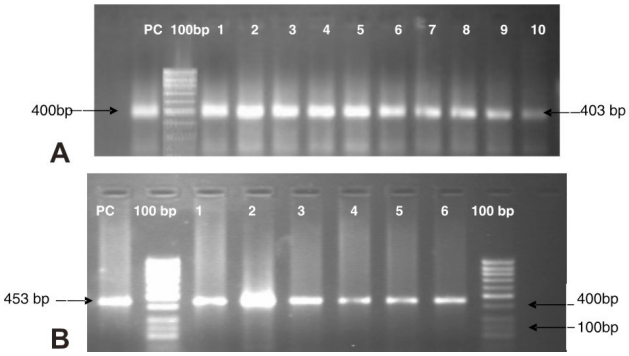

Fig. 1. Amplification of dengue serotype 2 (DENG 2) (A), and serotype 3 (DENG 3 (B). PC, positive control; 100 bp, DNA size marker; Lanes 1-10 in A show 403 bp DENG 2 product, while lanes 1-6 in B shows 453 bp DENG 3 production.

No doubt all the four distinct serotypes, DENV-1, DENV-2, DENV-3, and DENV-4 of dengue virus have been reported as the cause of dengue infection; however, serotypes 2 and 3 remained the major cause of infection in humans worldwide which is also supported by this study.

Conclusions

Based on the results of current study, it is concluded that *Aedes aegypti* is main source for the spread of dengue virus but not *Aedes albopictus* and the predominant serotypes of dengue virus in Pakistan are 2 and 3.

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Statement of conflict of interest

We declare that we do not have conflict of interest.

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