



Short communication

## Outbreak of dengue virus type-3 in Malakand, Pakistan 2015; A laboratory perspective



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### ABSTRACT

An outbreak of dengue fever was reported in Malakand district, Khyber Pakhtunkhwa (KP) province of Pakistan during 2015. Detection of viral RNA by real-time PCR confirmed dengue virus serotype-3 (DENV-3) to be the causative agent causing the outbreak. Phylogenetic analysis based on partial E-NS1 gene sequences showed that the DENV-3 viruses belonged to genotype III with maximum homology with the dengue-3 strains previously reported from Pakistan and India. Our current report provides updated information on molecular epidemiology and phylogenetic analysis of dengue virus serotypes responsible for 2015 outbreak in KP.

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### 1. Introduction

Dengue fever is caused by the dengue virus (DENV) which belongs to the genus *flavivirus* within the family *flaviviridae* and has been classified into four serotypes (DENV-1-4). According to the World Health Organization (WHO), 2.5 billion people and 124 countries are at risk of dengue infection with over 100 million dengue cases and 30,000 estimated deaths per year. Over the last few years, the incidence rate of dengue has increased significantly became a major public health problem worldwide (World Health Organization, 2017a; World Health Organization, 2017b).

Pakistan is endemic to all four dengue serotypes circulating throughout the year with a peak incidence during the post-monsoon period i.e. between September–November. Recently, the transmission of dengue virus has intensified in the country evidenced by its increased incidence and geographic expansion. Aside from KP province, sporadic cases are reported round the year from other provinces like Punjab, Sindh Baluchistan and AJK (Paul et al., 1998).

During the last 30 years, multiple dengue virus outbreaks have been occurred in Pakistan. The first laboratory confirmed outbreak

of dengue fever was reported in 1994 (Humayoun et al., 2010). A severe outbreak of dengue type 1, 2 and 3 was reported in 1995 in Balochistan province (Rasheed et al., 2013). In 2003, DENV-2 was responsible for a severe outbreak causing 3500 dengue cases including 18 deaths in Haripur, Nowshera and Khushab districts (World Health Organization, 2017b). During 2004, a small number of cases were reported from Islamabad and Karachi with an expansion up to 500 cases and 13 deaths caused by co-infection of DENV-2 and 3 mainly in Karachi (Rasheed et al., 2013).

During 2006–2008, DENV-1, 2 and 3 help responsible for 9900 dengue cases and 79 dengue related deaths in multiple districts of Pakistan. In 2010 from Punjab, KP and Azad Jammu & Kashmir, 11024 cases and 40 deaths due to DENV-1 and 2 were recorded (Humayoun et al., 2010).

In 2011, Lahore, Punjab faced one of the world's worst ever reported dengue epidemic affecting approximately 252,935 suspected and 22000 laboratory confirmed cases leading to 375 deaths within 73 days of index case (Rasheed et al., 2013).

In 2013–2014, district Swat, adjacent to the district Malakand, was hit by a similar severity notifying more than 9310 laboratory confirmed cases due to DENV-1, 2 and 3 including 57 deaths (Government of Khyber Pakhtunkhwa, 2013).

Our study is based on molecular investigations of DENV outbreak in the District Malakand during 2015. Malakand district is a provincially administered tribal area and is situated at a strate-

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gically important location acting as a gateway to Swat, Dir, Chitral and Bajaur. Malakand District is bounded on the north by Lower Dir District, on the East by Swat District, on the south east and south west by Mardan and Charsadda districts respectively and on the west by Mohmand and Bajour Agencies (Fig S–1). It is surrounded by a series of mountains that are overgrown with a variety of vegetation. The area of Malakand is 952 km<sup>2</sup> with estimated population of 567,000 (Ahmed, 2014).

**2. Materials and methods**

From 15th August to 5 November 2015, a total of 27 blood samples along with epidemiological and clinical information were collected from suspected dengue patients hospitalized at Malakand district head quarter hospital. The proposed study plan was approved by the Internal Review board of National Institute of Health, Islamabad, Pakistan.

Blood samples were transported to the Department of Virology, National Institute of Health Islamabad Pakistan for confirmation and comprehensive laboratory analysis. After serum separation, samples were initially tested for NS1 antigen, (Platelia Biorad Laboratories, Marnes-la-Coquette,France) anti-IgM, and anti-IgG antibodies by ELISA (Panbio, Inverness Medical Innovations Australia Pty Ltd) according to the instructions provided by the manufacturer. Dengue viral RNA was detected by serotype specific real-time PCR established by the Centers for Disease Control and Prevention, USA (Johnson et al., 2005). RNA material was stored at –70 °C till further processing. Real-time PCR positive samples were further processed for sequencing of partial E-NS1 gene (Domingo. et al., 2011). Sequence of primers and probe used for the detection and sequencing of dengue virus RNA are given in Table S1 and S2.

Amplified genomic fragments were sequenced on ABI PRISM 3100 genetic analyzer (Applied Biosystems USA), using Big dye terminator v3.1 kit. The sequence reads were analyzed for closest matches using online BLAST tool. The nucleotide alignments were performed using CLUSTALW incorporated into MEGA v5.0. Phylogenetic analysis and trees were reconstructed by maximum likelihood method with 500 iterations.

**3. Results**

During the 2015 outbreak in malakand, a total of 398 suspected dengue cases were identified based on clinical signs and symptoms along with additional 17 cases reported from neighboring districtwide. Lower Dir and Upper Dir. Majority of the cases (66%; 263/398) were detected during October and 28% (113/398) were detected during September 2015, however a small proportion of cases (6%; 22/398) were detected during August and November 2015. Out of 398 total cases, 70% (n=276) were male and 30% (n=122) were female patients.

Data was analyzed to infer the age of dengue positive patients indicating 80% (n=314/398) cases from age group of 15–45 years, 10% (n=39/398) cases were aged above 45 years and a similar proportion (10%; 41/398) was identified among cases less than 15 years of age.

Out of the total 398 clinically suspected cases, blood samples were collected from 27 patients presented with signs and symptoms of acute dengue infection. All of these patients had fever for more than 05 days; other symptoms included headache (71% patients), rash (21% cases), body pain (37%) retro-orbital pain (17%) 17% and vomiting in 1% patients. Platelet count was found more than >100,000/μl in 84% and less than 100,000/μl in 15% of patients. Level of Hemoglobin was tested more than 12 g/dl found in 72% and less than 12 g/dl in 28% of the study patients (Table 1).

**Table 1** Epidemiological clinical and laboratory characteristics of dengue cases (n = 13).

S.No	Days after onset	Month of Onset	Age	Gender	Symptoms	Platelet count	Tlc count	Hb level	NS1 ELISA	IgM ELISA	IgG ELISA	real-time PCR	Serotype	RT-PCR, E/NS1	Type of infection
1	4	October	55	M	Fever,Headache,Rashes	92000mm <sup>3</sup>	7400mm <sup>3</sup>	11.2	+	+	-	+	DENV-3	+	Primary
2	2	October	25	M	Fever,Headache	104000	3000	15.3	+	+	-	+	DENV-3	+	Primary
3	1	October	27	M	Fever,body ache	130000	2650	14.7	+	-	-	+	DENV-3	+	Primary
4	3	October	43	M	Fever,Retro-orbital Pain	127000	5500	15.4	+	+	+	+	DENV-3	+	Secondary
5	2	October	16	M	Fever,Headache	140000	4800	13.2	+	+	-	+	DENV-3	+	Primary
6	3	October	8	F	Fever,Bodyache,vomiting	188000	4800	14.3	+	-	-	-	-	-	Primary
7	3	October	25	F	Fever,body ache, Rashes	146000	2700	16.2	+	+	-	-	-	-	Primary
8	1	October	29	F	Fever,Retro-orbital Pain	125000	3200	15.9	+	-	-	+	DENV-3	+	Primary
9	1	November	40	M	Fever,Headache,Rashes	123000	3100	13.2	+	-	-	+	DENV-3	+	Primary
10	1	October	40	M	Fever,Headache	80000	2400	9.1	+	-	-	+	DENV-3	+	Primary
11	1	October	25	M	Fever,body ache	131000	6300	12.3	+	-	-	+	DENV-3	+	Primary
12	5	November	37	M	Fever,Headache,Rashes	970000	2310	9.8	+	-	+	+	DENV-3	+	Secondary
13	4	November	47	M	Fever,Headache,Rashes	123000	2300	10.8	+	+	-	+	DENV-3	+	Primary

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