

Circulation of dengue serotypes in the Union Territory of Dadra & Nagar Haveli (India)

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ABSTRACT

The presence of chronological infection by the multi serotype of dengue virus (DENV) is a major contributing factor for the induction of Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). The results indicate that all serotype of dengue along with concurrent infection were reported from the third smallest Union Territory of India furthermore, rare serotype DENV 4, gradually spreads in new areas of India. Therefore, a need to understand the epidemiology of locally circulating serotype and accordingly the control strategy can be implemented. The study was conducted during 2014–2017 in the Union Territory of Dadra & Nagar Haveli (India).

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1. Introduction

Dengue is a major public health problem worldwide, especially in the tropical and subtropical areas with around 2.5 billion people living in areas at risk. Dengue virus (genus *Flavivirus*, family *Flaviviridae*) is mosquito borne and the principal vector (*Aedes aegypti*) is a day-biting domestic mosquito of public importance that breeds in natural or artificial water (Liu-Helmersson et al., 2014). Dengue illnesses are caused by any of the four serologically related viruses designated as DENV-1 to DENV-4 (Moi et al., 2016). In Indian scenario, the first case of dengue was reported from Kolkata in 1945 and now endemic in both urban and semi urban areas. All four dengue virus serotypes circulate and cause epidemics in throughout in the India (Kalappanvar et al., 2013; Mishra et al., 2015., Mutheneni et al., 2017). In recent years co-circulation of multiple dengue serotypes is being increased with concurrent infections (Reddy et al., 2017). Therefore, it is necessary to determine the DENV serotypes in various geographical locations to understand the epidemiology of local circulating serotypes. It should be an integral part of control strategy. Hence, the present study was conducted in order to specifically identify the serotypes of dengue virus and to assess the current epidemiology in the UT of Dadra & Nagar Haveli (Western part of India), during four consecutive years (2014–2017).

2. Methods

The Union Territory of Dadra & Nagar Haveli is located at latitude - 20° 54' 41" N to 20° 21' 36" N, Longitude - 72° 54' 41" N to 73° 13' 13" N in the Western Ghats of India. Silvassa is the capital of Dadra & Nagar Haveli. In recent years this has under gone a large scale development through urbanization and industrialization. The 487 km² area is hilly, forested, occupied by mainly tribes (population 3.42 lakh) in 72 villages and one town. Due to owing to subsidiary in taxes to industries approximately 2.5 lakh skilled and unskilled workforce comes to Dadra and Nagar Haveli from different states of India. The health services to natives and migrant populations are provided through Seventy one Sub Centers (SC), Nine Primary Health Centers (PHC), Two Community Health Centers (CHC), One Sub District Hospital in rural area and one 316 bedded District Hospital (Shri Vinoba Bhawe Civil

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Hospital) with multi-specialty at district headquarters (Silvassa). In addition, in private sector, there are ten private hospitals and fifty medical practitioners provide health services to the public of Dadra & Nagar Haveli.

Acute phase blood samples were collected from the suspected dengue patients and tested by ELISA (Panbio, Australia) at District Public Health Laboratory of Shri Vinoba Bhave Civil Hospital Silvassa (Latitude $-20^{\circ}16'14.635$ N Longitude $-73^{\circ}0'27.301$ E) from 2014 to 2016. Those serum samples positive for NS1 antigen were stored at -80°C . For molecular serotyping of DENV the Nucleic acid extraction was performed by using the QIAamp Viral RNA Mini Kit (Qiagen; Valencia, CA). All extractions were carried out as per the manufacturer's recommendations and guidelines. Extractions were performed by using $140\ \mu\text{L}$ of patient serum, eluted into $60\ \mu\text{L}$ of buffer AVE on QIAcube (Qiagen; Valencia, CA). The DENV multiplex RT-PCR was performed using the SuperScript III Platinum One-Step qRT-PCR kit (Invitrogen; Carlsbad, CA) as per the CDC protocols (2013). The study was carried out as a part of routine surveillance and outbreak investigation; hence Ethical Committee approval were not required.

3. Results and discussion

During the last four years (2014 to 2017), the annual attendance of outdoor patients in this Civil Hospital has increased from 0.66 million to 1.1 million. A total of 4924, 13,618, 22,201 and 11,906 suspected Dengue Fever (DF) patients were subjected to sero-diagnosis in 2014, 2015, 2016 and 2017 respectively. Out of this 924 in 2014, 2431 in 2015, 4498 in 2016 and 2064 in 2017 were found seropositive with NS1 ELISA. The sero-positivity were ranged between 17.85 and 20.26% during the corresponding period. In general, dengue cases were reported throughout the year in varying proportion, but the majority of the cases were reported from the month of August to December (monsoon and the post monsoon season). Fig 1. Based on the number of total dengue cases reported, the villages were grouped into three categories: low endemic areas with dengue cases between 0 and 25 cases per ten thousand populations, moderate endemic areas with 26 to 50 cases per ten thousand population and high endemic areas with dengue cases more than 50 per ten thousand populations (Fig. 2). The Fig. 2 shows that the dengue was constantly endemic in the urban and semi urban/industrial zones of Dadra & Nagar Haveli.

Among the seropositive samples, 106 NS1 ELISA positive samples were tested by RT-PCR (30 samples in 2014, 30 sample in 2015 and 46 samples in 2016). All these samples were from the UT of Dadra & Nagar Haveli. Among them 88.7% sample were found positive for dengue viral RNA (24 samples (80%) in 2014, 29 samples (96.6%) in 2015 and 41 (89.1%) sample in 2016). In the year 2014, the DENV 3 (79.2%) was found as most dominant serotype, following DENV 2 (12.5%) and Co-infection with more than one serotype (8.3%). But in 2015, the scenario was changed DENV 2 (55.2%) was the most dominant serotype reported, following DENV 3 (13.7%), DENV 1 (3.4%) and Co-infection with more than one serotype (27.6%). In 2016, DENV 3 were re dominated and reached up 56%, following DENV 1 (22%), DENV 2 (9.8%), Co-infection with more than one serotype (9.8%) and DENV 4 (2.4%) (Table 1). The distribution of dengue serotype in different age groups is given in Table 2. This shows that the infection of all four serotypes of DENV and co infection (infection with more than one serotype) were reported in 19–35 age group. The age group of 16–18 years and 36 to 50 years also shows the positivity of DENV-1, DENV-2, DENV-3 and co-infection (infection with more than one serotypes). In the Union Territory of Dadra & Nagar Haveli, the infection of all four serotypes of DENV and co-infection (Infection with more than one serotype) were reported from the urban areas. The circulation of DENV serotype 1,2,3 and co-infection (Infection with more than on serotype) were observed in the semi urban/ industrial zones, and the presence of DENV serotype 3 and co-infection (infection with more than one serotype) were noted from the tribal/rural areas. (Table 3).

The presence of all the four serotypes of DENV in the community is the most challenging public health concern. However, the immunity produced by the person against the infected dengue serovar is lifelong, but it will be protective only for 3 to 4 months for another serovars. After this period the person can be infected with another serovar. Day by day. The magnitude of the disease

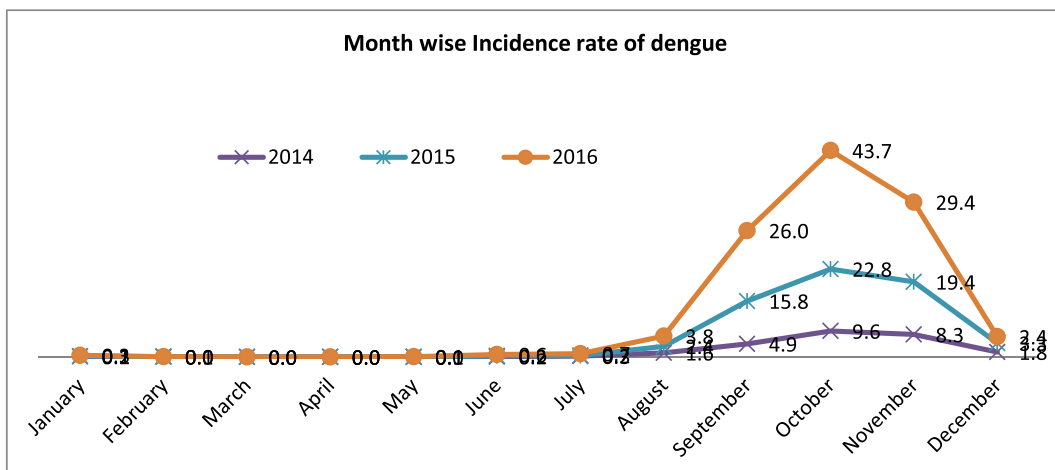
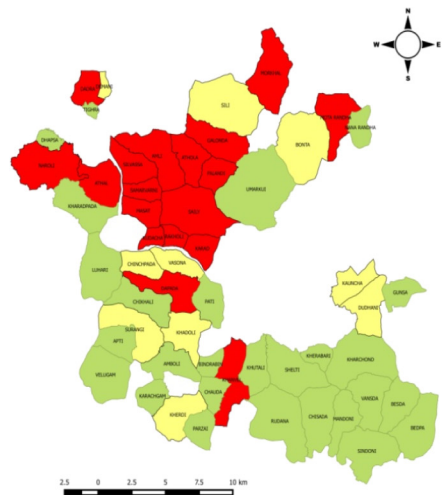


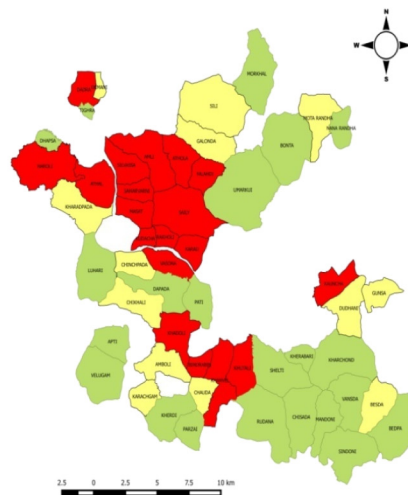
Fig. 1. Showing incidence of dengue cases in the UT of Dadra & Nagar Haveli.

Distribution of dengue cases in Union Territory of Dadra & Nagar Haveli for the year 2015



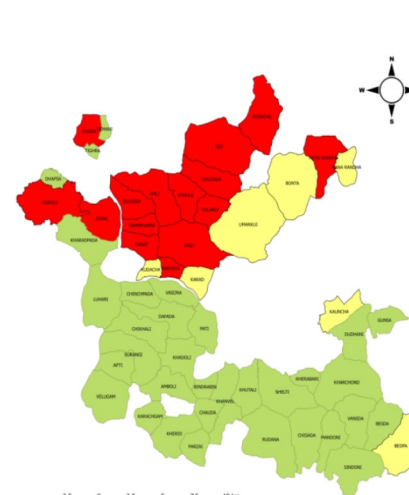
Legend
Village_Boundary_DNH_P
0-25
26-50
50 +

Distribution of dengue cases in Union Territory of Dadra & Nagar Haveli for the year 2016



Legend
Village_Boundary_DNH_P
0-25
26-50
50 +

Distribution of dengue cases in Union Territory of Dadra & Nagar Haveli for the year 2017



Legend
Village_Boundary_DNH_P
0-25
26-50
50 +

Fig. 2. Showing spatial distribution of dengue cases in Union Territory of Dadra & Nagar Haveli from 2015 to 2017.

Table 1

Showing analysis of dengue infected sample in the UT of Dadra & Nagar Haveli.

S.N.	Sample description	2014	2015	2016
1	Total no of sample tested for serotype	30	30	46
2	Ns1 positive	30	30	46
3	RNA positive No (%)	24 (80%)	29(96.6%)	41(89%)
4	Co-infection with more than one serotype No (%)	2(8.3%)	8(27.6%)	4(9.8%)
5	DENV 1 infection no (%)	0(0%)	1(3.4%)	9(22%)
6	DENV 2 infection no (%)	3(12.5%)	16(55.2%)	4(9.8%)
7	DENV 3 infection no (%)	19(79.2%)	4(13.7%)	23(56%)
8	DENV 4 infection no (%)	0(0%)	0(0%)	1(2.4%)

Table 2

Showing age wise distribution of dengue serotype in the UT of Dadra & Nagar Haveli.

Age	DENV-1 n(%)	DENV-2 n(%)	DENV-3 n(%)	DENV-4 n(%)	Co-infection n(%)
0-5	0 (0)	0 (0)	1 (1.1)	0(0)	0(0)
6-18	2 (2.1)	1(1.1)	9(9.6)	0(0)	1(1.1)
19-35	5(5.3)	16(17.0)	18(19.1)	1(1.1)	5(5.3)
36-50	3(3.2)	2(2.1)	11(11.7)	0(0)	7(7.4)
50+	0(0)	4(4.3)	7(7.4)	0(0)	1(1.1)

Table 3

Showing location wise distributions of dengue serotypes in the UT of Dadra & Nagar Haveli.

Location	DENV-1 n(%)	DENV-2 n(%)	DENV-3 n(%)	DENV-4 n(%)	Co-infection n(%)
Urban	6 (6.4)	15(16)	32(34)	1(1.1)	8(8.5)
Semi urban/industrial zone	4(4.3)	8(8.5)	11(11.7)	0(0)	5(5.3)
Tribal/rural	0(0)	0(0)	3(3.2)	0(0)	1(1.1)

is increasing and affecting the newer areas and new populations. The unavailability of vaccines and inadequate strategies of vector control results in severe upsurge of dengue incidences. The present investigation leads to develop an overview about the dengue infection (serotypes) prevalent in the UT of Dadra & Nagar Haveli. The results shows that all the four serotypes of dengue which are identified in different states of India and south Asia are present in Dadra & Nagar Haveli (third smallest union territory of India) is alarming.

Table 4

Showing circulating dengue serotype in different regions of India & South Asia.

Place	Year	Total number positive sample	DEN -1	DEN -2	DEN -3	DEN -4	Concurrent infection	Reference
Cebu city Philippines	2017	13	10	2	1	0	0	Alera et al. (2016)
Myanmar	2014	30	14	5	2	9	0	Myat et al. (2016)
Singapore	2013	1270	770	332	133	35	0	Hapuarachchi et al. (2016)
Singapore	2014	1531	1150	334	43	4	0	Hapuarachchi et al. (2016)
Pakhtunkhwa Pakistan	2013	196	24	45	64	1	62	Suleman et al. (2017)
Pakhtunkhwa Pakistan	2014	140	13	59	68	0	0	Suleman et al. (2017)
Pakhtunkhwa Pakistan	2015	326	0	115	179	0	32	Suleman et al. (2017)
Yunnan China	2013	23	5	6	12	0	0	Song Hu et al. (2017)
Yunnan China	2014	49	45	4	0	0	0	Song Hu et al. (2017)
Yunnan China	2015	93	39	47	0	7	0	Song Hu et al. (2017)
Delhi	2015	113	69	36	0	8	0	Changal et al. (2016)
Delhi	2015	17	1	7	3	2	3(1&2), 1(2&4)	Islam et al. (2016)
Arunachal Pradesh	2014	35	0	0	27	0	7 (1&3), 1(2&3)	Khan et al. (2014)
Portblair	2014	7	0	0	7	0	0	Muruganandam et al. (2014)
Hyderabad	2014	80	1	57	20	2	0	Neeraja et al. (2014)
Mangalore	2013-14	35	6	17	12	0	0	Damodar et al. (2017)
Odisha	2014	5		2		1	2(2&3)	Mishra et al. (2017)
Present study								
Dadra & Nagar Haveli	2014	24	0	3	19	0	2	Present study
Dadra & Nagar Haveli	2015	28	1	16	4	0	8	Present study
Dadra & Nagar Haveli	2016	41	9	4	23	1	4	Present study

The presence of rare serotype DENV 4, was also found in Dadra & Nagar Haveli, which is reported only in the few cities of India viz. Delhi, Pune and Hyderabad (Damodar et al., 2017). Various reports from pan India (Changal et al., 2016; Damodar et al., 2017; Islam et al., 2016; Khan et al., 2014; Mishra et al., 2017; Muruganandam et al., 2014 and Neeraja et al., 2014) and south Asia (Alera et al., 2016; Hapuarachchi et al., 2016; Myat et al., 2016; Song et al., 2017 and Suleman et al., 2017) shows that the co-infections (infection with more than one serotype), is frequently reported (Table 4). In Dadra & Nagar Haveli the co-infections (infection with more than one serotype) rate is from 7.3% to 17.2% during study period (2014–2016). The co-circulation of DENV serotypes suggests that the UT of Dadra Nagar Haveli has become a hyperendemic state from an endemic one.

4. Conclusion

In Dadra & Nagar Haveli (third smallest union territory of India), all four serotype of DENV and co-infection (infection with more than one serotype) were reported and day by day the magnitude of the disease is increasing and affecting the newer areas and new populations. This indicates that Dadra and Nagar Haveli is prone for dengue infection. We recommend that a vigilant sero surveillance of DENV at different geographic locations of India, to understand the transmission dynamics of the disease.

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