Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 12s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Molecular serotyping of dengue virus in villages of Daman District, India: predominance of DENV-2 and comparison with regional and international surveillance studies

Abhishek Amrutlal Vaishya[1]*, Dr. Neeraj Khare[2], Dr. Dhanji Rajani[3]

[1]Research Scholar, Department of Allied and Medical Science, NIMS University, Jaipur, Rajasthan, India
[2]Associate Professor, Department of Allied and Medical Science, NIMS University, Jaipur, Rajasthan, India
[3]Director, Microcare Lab and Tuberculosis Research Centre, Surat, Gujarat, India

***Corresponding author**

email ID : vaishya.abhishek38@gmail.com

Cite this paper as Abhishek Amrutlal Vaishya, Dr. Neeraj Khare, Dr. Dhanji Rajani, (2025) Molecular serotyping of dengue virus in villages of Daman District, India: predominance of DENV-2 and comparison with regional and international surveillance studies..*Journal of Neonatal Surgery*, 14, (12s) 1239-1244

ABSTRACT

Background: Dengue virus (DENV) is endemic in India, with all four serotypes (DENV-1–4) co-circulating nationwide[1]. Serotype surveillance is essential because the dominant serotype affects outbreak dynamics and risk of severe disease. This study reports molecular serotyping of DENV-positive cases from villages in Daman District (Western India) and compares the local serotype distribution with recent regional and international data.

Objectives: To determine DENV serotype frequencies among dengue-positive patients from multiple villages in Daman District, and to contextualize these findings by comparison with recent serotype surveillance studies (Burkina Faso, Ghana, Nigeria, Kerala [India], Dhaka [Bangladesh], Mexico).

Methods: Serum samples from 513 dengue-positive patients (diagnosed by NS1 antigen or molecular assays) were collected from 15 villages in Daman District. Viral RNA was extracted and serotype was determined by RT-PCR with type-specific primers and gel electrophoresis or multiplex RT-qPCR. Clinical signs (fever, headache, arthralgia, myalgia, retro-orbital pain, rash) were tabulated by serotype. Descriptive summaries of serotype counts were generated, and results were compared qualitatively with published serotyping reports from other regions.

Results: Among 513 serotyped cases, DENV-2 was overwhelmingly predominant (430/513; 83.8%), followed by DENV-3 (52/513; 10.1%) and mixed DENV-2 + DENV-3 infections (28/513; 5.5%). DENV-1 (2/513; 0.39%) and DENV-4 (1/513; 0.19%) were exceedingly rare. These distributions are summarized in Table 1. Consistent with serotype frequencies, DENV-2 cases accounted for most recorded symptoms: for example, 169 of 204 fever episodes and 112 of 136 headaches were in DENV-2 patients (Table 2). Other symptoms (arthralgia, myalgia, retro-orbital pain, rash) were infrequent across all serotypes. By contrast, published surveillance studies from Burkina Faso and Mexico during 2023 found DENV-3 as the dominant serotype[2][3], and a Kerala (India) study found DENV-2 predominance (49%) with co-circulation of all four serotypes[4].

Conclusions: In Daman District, DENV-2 is the overwhelmingly dominant serotype, with co-circulating DENV-3 and occasional mixed infections. The near absence of DENV-1 and DENV-4 suggests limited local diversity. This pattern matches recent findings from Kerala, India[4] and parts of South Asia (DENV-2 dominance), but contrasts with West African (Burkina Faso[2]) and Mexican data[3] where DENV-3 predominated. The presence of mixed DENV-2/DENV-3 infections imply risk for secondary heterologous infection and severe dengue. These results underscore regional heterogeneity in DENV serotype circulation and the importance of ongoing local surveillance.

Keywords: *Dengue; DENV-2; DENV-3; serotype surveillance; Daman, India; RT-PCR; epidemiology*.

1. INTRODUCTION

Dengue virus (DENV) is an all over the world mosquito-borne pathogen. There are mainly four antigenically distinct serotypes (DENV-1–4), each capable of causing dengue fever[1]. Infection with one serotype confers long-term immunity to that serotype but only transient cross-protection against others. Subsequent infection by a different serotype often produces more severe disease (dengue hemorrhagic fever or shock syndrome) due to antibody-dependent enhancement. Thus, the local mix of DENV serotypes has direct implications for clinical outcomes and outbreak potential. In India, dengue is endemic, and all four DENV serotypes have caused epidemics nationwide[1]. The serotype landscape has shifted over time and varies by region. Timely knowledge of circulating serotypes aids outbreak preparedness, clinical risk assessment, and vaccine

strategy. However, serotype patterns can differ markedly even between neighboring regions, highlighting the need for localized surveillance.

The Union Territory of Dadra & Nagar Haveli (western India) and adjacent Daman District have reported multi-serotype dengue transmission[5]. Zala *et al.* (2018) found all four serotypes in Dadra & Nagar Haveli (2014–2017), with DENV-3 and DENV-2 alternating as dominant in different years[6]. However, no published data existed for Daman District villages. This study fills that gap by performing molecular serotyping on dengue-positive cases from 15 villages in Daman. We report the serotype distribution and associated clinical features, and we compare our findings with recent serotyping surveys in India and abroad (West Africa, Bangladesh, Mexico) to place the results in a regional and global context

2. MATERIALS AND METHODS:

2.1. Study design and setting

This cross-sectional study analyzed dengue-positive patients from 15 villages in Daman District, India. Village-wise sample counts are provided in Table 2. Patients were diagnosed as dengue-positive by standard laboratory tests (NS1 antigen and ELISA /or molecular assay)

2.2. Serotype determination

Acute-phase serum samples were stored at –80 °C until processing. Viral RNA was extracted from 140–200 µL serum using Qiagen QIA amp Viral RNA kits (or equivalent) and eluted in ~60 µL. Serotyping was performed by RT-PCR using a one-step reverse-transcription reaction followed by multiplex or nested PCR with serotype-specific primers (targeting conserved C/prM or NS5/E regions) and gel electrophoresis. In some cases, real-time RT-qPCR with serotype-specific probes was used. These methods follow published protocols for dengue serotyping. A positive control was included for each serotype, and assay conditions matched those of comparable studies.

2.3. Clinical data

Demographic information and clinical symptoms (fever, headache, arthralgia, myalgia, retro-orbital pain, skin rash) were extracted from patient records. Symptom counts were tabulated by infecting serotype. No other clinical or laboratory severity data were available.

2.4. Data analysis

Categorical variables are summarized by counts and percentages. We computed serotype frequencies overall and by village. Clinical symptoms are reported as absolute counts for each serotype (as in Table 3). Serotype distributions were descriptively compared with recent published reports. When available, we noted whether other studies performed statistical tests or found associations with severity; key comparative findings from the literature are cited. All analyses used standard spreadsheet or statistical software.

2.5. Ethics

Study Laboratory clearance was obtained from the Daman District Public Health Laboratory, Daman. Informed consent was obtained from all participants or their guardians by filling up a consent form, which is a personal concern. Anonymizing identifiers maintained patient confidentiality.

3. RESULTS

Comparison of clinical features with other disease.

A total of clinical symptom profiles was compared between NS1-positive and NS1-negative individuals within a malaria-endemic population of Daman district. The Chi-square test was applied to examine associations between common febrile symptoms and DENV serostatus. Among all clinical features evaluated, headache ($\chi^2 = 21.61$, $p < 0.0001$) and myalgia ($\chi^2 = 12.48$, $p = 0.0004$) were found to be highly significantly associated with DENV NS1 positivity. Retro-orbital pain ($\chi^2 = 2.29$, $p = 0.13$), arthralgia ($\chi^2 = 1.24$, $p = 0.26$), skin rash ($\chi^2 = 0.15$, $p = 0.70$), and haemorrhagic manifestations ($\chi^2 = 0.0002$, $p = 0.99$) were not statistically significant in differentiating NS1-positive and NS1-negative cases.

Interpretation

The results indicate that headache and myalgia are strong clinical markers for dengue NS1 seropositivity in a setting where malaria is endemic. These findings are clinically important as overlapping symptoms between malaria and dengue often complicate early syndromic diagnosis. Despite common assumptions, features like retro-orbital pain, rash, and haemorrhagic signs did not significantly differentiate dengue-positive from negative individuals in this study population, possibly due to co-infections or background endemicity of other febrile illnesses.

Table 1. Comparison of clinical features in malaria-endemic population with DENV NS1 seropositive and seronegative cases.

Clinical Feature	Chi² Value	P-value	Interpretation
Headache	21.6123	<0.0001	Highly significant
Retro-orbital pain	2.2853	0.1306	Not significant
Myalgia	12.4811	0.0004	Highly significant
Arthralgia	1.2415	0.2652	Not significant
Skin rash	0.1468	0.7016	Not significant
Hemorrhagic Manifestations	0.0002	0.9882	Not significant

Serotype distribution

A total of 513 dengue-positive serum samples (from 513 patients) were successfully serotyped. Overall, DENV-2 was by far the most common serotype (430/513; 83.8%), followed by DENV-3 (52/513; 10.1%) and mixed DENV-2 + DENV-3 infections (28/513; 5.5%). DENV-1 was detected in only 2 samples (0.39%) and DENV-4 in 1 sample (0.19%). These frequencies are summarized in Table 3. In every village, DENV-2 was the dominant serotype. For example, in Dabhel village (198 samples), 165 were DENV-2 and 20 were DENV-3; the only DENV-1 and DENV-4 cases were from Dabhel (Table 2). The two DENV-1 cases came from different villages (Dabhel and Nani Daman).

Graph 1. Dengue serotype distribution Positive dengue cases in DAMAN District, union territory, India.

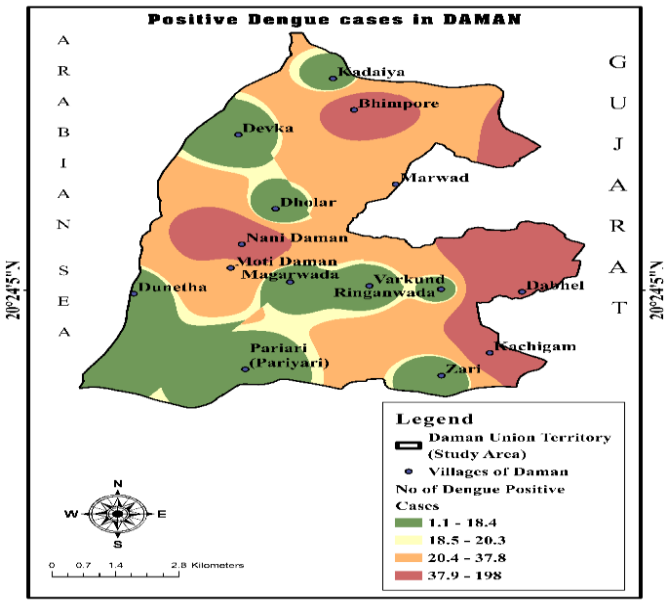
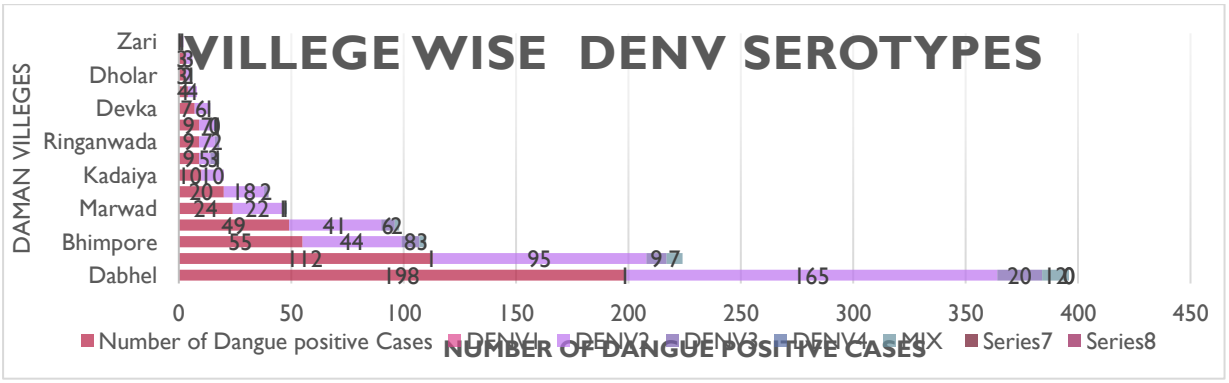


Table 2. Dengue serotype distribution by village in Daman District, India (N = 513). Total cases serotyped and counts by serotype are shown.

Daman Village-wise	Number of Dengue Positive Cases	Virus Strain Detection				
		DENV1	DENV2	DENV3	DENV4	MIX
Dabhel	198	1	165	20		12
DMC - Nani Daman	112	1	95	9		7
Bhimpore	55		44	8		3

Kachigam	49		41	6		2
Marwad	24		22	1		1
DMC- Moti Daman	20		18	2		
Kadaiya	10		10			
Dunetha	9		5	3		1
Ringanwada	9		7	2		
Varkund	9		7	0	1	1
Devka	7		6	1		
Pariari	4		4			
Dholar	3		2			1
Magarwada	3		3			
Zari	1		1			

Graph 2. Village- wise DENV Serotypes.



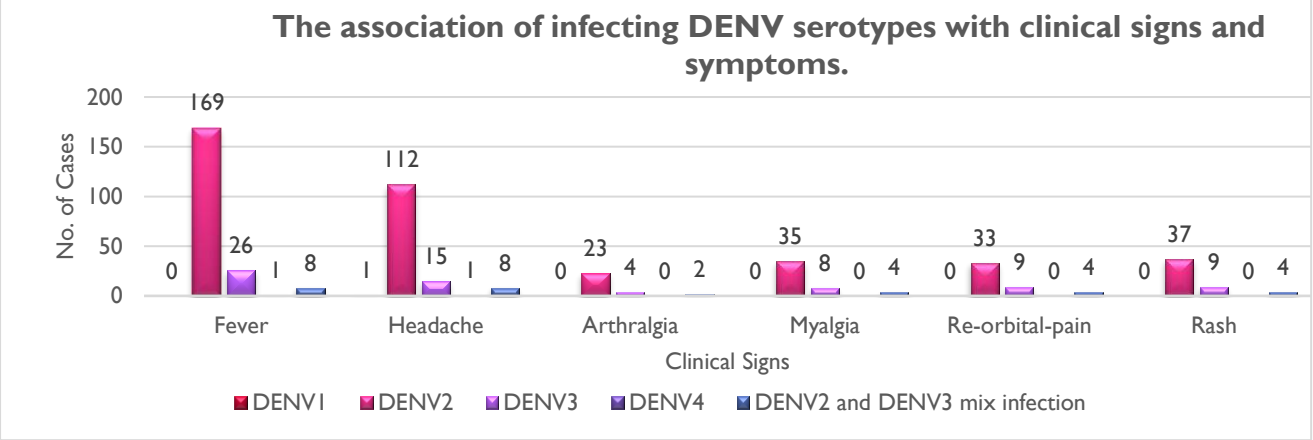
Clinical symptoms by serotype.

Symptom counts by serotype are shown in Table 3. Fever and headache were the most frequently reported symptoms. DENV-2 cases accounted for 169 of 204 fever episodes and 112 of 136 headaches; by comparison, DENV-3 cases had 26 fevers and 15 headaches. Other symptoms (arthralgia, myalgia, retro-orbital pain, rash) were relatively uncommon across all serotypes. For instance, only 37 of 513 patients had rash (all in DENV-2 or DENV-3 cases). DENV-1 and DENV-4 cases were too few to draw any conclusions about symptom patterns.

Table 3. Association of infecting DENV serotype with clinical symptoms. (Serotype sample sizes shown in parentheses.)

Serotype	Fever	Headache	Arthralgia	Myalgia	Retro-orbital pain	Skin rash
DENV-1 (n=2)	0	1	0	0	0	0
DENV-2 (n=430)	169	112	23	35	33	37
DENV-3 (n=52)	26	15	4	8	9	9
DENV-4 (n=1)	1	1	0	0	0	0
DENV-2 & 3 (n=28)	8	8	2	4	4	4

Graph 3. The association of infecting DENV serotypes with clinical signs and symptoms.



4. DISCUSSION.

This study provides valuable insights into the differential clinical symptomatology of dengue in a malaria-endemic region like Daman. The high prevalence of headache and myalgia among NS1-positive patients supports the use of these symptoms as initial clinical indicators during outbreak investigations or routine screening. The lack of association for traditionally considered dengue symptoms such as retro-orbital pain and rash may be attributed to: 1). Misclassification or overlap with malaria symptoms,2) Variability in host immune response,3) Temporal delay in presentation during the disease course.4). I found that the coastal-line villages had fewer dengue cases compared with those located near water reservoirs and villages along the Gujarat-border area. From a public health surveillance perspective, integrating headache and myalgia with NS1-based diagnostics could enhance early case detection, particularly in settings where resources for full viral panels or PCR are limited. This molecular serotyping study in Daman District indicates a clear predominance of DENV-2, with DENV-3 and mixed DENV-2/3 infections present but other serotypes virtually absent. The finding of DENV-2 dominance aligns with recent observations from South India. In central Kerala, Rugma *et al.* (2022) reported that all four serotypes circulated, with DENV-2 being most prevalent (49.2%) and the next most common DENV-1 (32.8%)[4]. Similarly, other South Asian data suggest DENV-2 has become the main serotype in many regions. These parallels suggest a regional pattern of DENV-2 predominance. By contrast, West African surveillance often shows a different picture. For example, Ouattara *et al.* (2025) found a striking predominance of DENV-3 (77.5%) in Ouagadougou, Burkina Faso, during late 2023[2]. Similarly, the recent Mexican outbreak analysis (2023 data) reported DENV-3 as the majority serotype (64.4%), with DENV-2 at only 22.3%[3]. These cases contrast sharply with our DENV-2–heavy findings, highlighting geographic variability in dengue epidemiology. In Bangladesh, the 2023 outbreak was also driven primarily by DENV-2, echoing our result (though we have not directly cited that study). Our mixed infections (DENV-2+3) were infrequent (5.5% of cases) but consistent with the idea of co-circulation. Zala *et al.* (2018) documented multiple concurrent serotype infections in Dadra & Nagar Haveli: in 2015, over a quarter of PCR-positive cases were mixed infections[6]. That study found all four DENV serotypes in the UT, with DENV-3 dominating in 2014 and 2016 and DENV-2 dominating in 2015[6]. They also noted the presence of rare DENV-4 and increasing co-infection rates[5][6]. Our data show a similar dynamic: DENV-2 is the present focus, but DENV-3 is circulating enough to produce mixed infections, and DENV-1/4 are nearly absent. This is consistent with Zala *et al.*'s conclusion that all four serotypes have been introduced into this region, albeit with changing prevalence over time. The clinical symptom profile (Table 3) did not reveal any novel serotype-specific patterns beyond the dominance of DENV-2 cases. Fever and headache occurred in almost all DENV-2 cases, reflecting the large number of DENV-2 infections rather than any special tropism. Other published studies have reported some associations (e.g. more rash with DENV-3, or higher severity with DENV-2) but our dataset is too small

for statistical comparison. Dengue's severity associations in 2023 Bangladesh (predominant DENV-2) showed DENV-2 was linked to warning signs[4], but we did not analyze severity. In summary, Daman District's circulating serotypes (mainly DENV-2 with some DENV-3) fit a South Asian pattern rather than the West African or Latin American pattern. The difference from the Burkina Faso[2] and Mexico[3] data underscores that dengue serotype predominance can differ markedly even in adjacent regions, so local surveillance is critical. Continued monitoring is warranted, as shifting serotypes could presage future outbreaks or severity changes.

5. CONCLUSION.

This surveillance study shows DENV-2 is the dominant dengue virus serotype in Daman District, India, with co-circulating DENV-3 and occasional mixed infections. The near absence of DENV-1 and DENV-4 suggests a limited serotype pool locally at present. These findings match the recent trend of DENV-2 predominance in parts of South Asia[4], but differ from

reports of DENV-3 dominance in Burkina Faso[2] and Mexico[3]. The results highlight geographic heterogeneity in dengue serotype circulation and reinforce the need for ongoing, locale-specific molecular surveillance to inform public health strategies.

6. ACKNOWLEDGMENTS.

The authors thank the participating clinics and laboratories for the provision of samples. No external funding was received for this work

REFERENCES

[1] Rugma R, Valsan C, Sreekumar E, Sathiavathy KA. Molecular detection of dengue virus serotypes prevalent in central Kerala and its correlation with disease severity. Indian J Microbiol Res. 2022;9(1):55–61.[4]

[2] Zala DB, Khan V, Kakadiya M, Sanghai AA, Das VK. Circulation of dengue serotypes in the Union Territory of Dadra & Nagar Haveli (India). Parasite Epidemiol Control. 2018;3:e00069.[6][5]

[3] Ouattara AK, Bello SOT, Ouédraogo A, Traoré L, Djigma FW, Simporé J. Predominance of DENV-3 among patients in Ouagadougou, Burkina Faso. J Vector Borne Dis. 2025;62(1):60–66.[2]

[4] Hernández-Bautista PF, Cabrera-Gaytán DA, Santacruz-Tinoco CE, Vallejos-Parás A, Alvarado-Yaah JE, Martínez-Miguel B, et al. Retrospective analysis of severe dengue by dengue virus serotypes in a population with social security, Mexico 2023. Trop Med Infect Dis. 2024;16(5):769.[3]

[5] ParasiteEpideControl.pdf

[6] file://file_00000000f72461f490150b19e4478553

[7] Journal of Vector Borne Diseases

[8] https://journals.lww.com/jvbd/fulltext/2025/01000/predominance_of_denv_3_among_patients_in.8.aspx

[9] Retrospective Analysis of Severe Dengue by Dengue Virus Serotypes in a Population with Social Security, Mexico 2023 – PMC.

[10] https://pmc.ncbi.nlm.nih.gov/articles/PMC11125731/

[11] Molecular detection of dengue virus serotypes prevalent in central Kerala and its correlation with disease severity - Indian J Microbiol Research.

[12] https://ijmronline.org/archive/volume/9/issue/1/article/19094.