Original Article

Serotype-specific clinical features and spatial distribution of dengue in northern Kerala, India

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Abstract

Background: Collection and compilation of spatial, meteorological, entomological, and virological data are critical in mitigating climate-sensitive emerging infections like dengue. This study was a holistic attempt to understand the dengue situation in the Kasaragod district of Kerala, India. **Methods:** This cross-sectional study was conducted in 13 health institutions from June to July 2021. Adult patients presenting with fever and testing positive for NS1 ELISA were subjected to Dengue RT-PCR and serotyping. The spatial and clinical features of the RT-PCR-positive patients, the district's meteorological data, and the vector indices were studied. **Results:** The pre-epidemic months were marked by intermittent rainfall, peak ambient temperature and high larval indices. Among the 136 dengue RT-PCR patients studied, 41.2% had DENV2 followed by DENV1 (22.8%), DENV3 (5.9%) and DENV4 (4.4%); with 25% mixed infections. DENV1 showed a higher risk of gastrointestinal manifestations (80.6%, p=0.019) and musculoskeletal symptoms (77.4%, p=0.026) compared with other serotypes. **Conclusions:** In the context of dengue hyperendemicity, the possibility of an emerging serotype's dominance coupled with the mixing up of strains should warn the health system regarding future outbreaks. Furthermore, the study emphasizes the importance of monitoring larval indices and the window of opportunity to intervene between environmental predictors and dengue outbreaks.

Keywords: Dengue, epidemiology, GIS, India, public health

Introduction

Dengue, the *Aedes* mosquito-borne infection threatens the world in the context of climate change.[1] More and more regions across the world are becoming conducive to the emergence of *Aedes* mosquitoes and related diseases.[2] Around four billion people are living in areas prone to infection and the numbers are likely to increase in the coming years. Age-standardized incidence rates have increased from 431 to 1371 per 100,000 population and the loss in Disability Adjusted Life Years has been estimated to be increased by five times from 1990 to 2017.[3] It is a known fact that the Dengue infection with a given serotype of Dengue could be complicated by the partial immunity imparted by a

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prior infection with a different serotype through a mechanism known as antibody-dependent enhancement.[4] Environmental factors like rainfall, temperature, and humidity play a major role in making an area hyperendemic to dengue.[5]

Lessons learned from current Dengue-endemic areas of the globe will be useful for other regions to tide over the future challenges from the emerging infection. Studies have reported the usefulness of understanding the Dengue serotypes in circulation and have highlighted that a serotype switch may lead to large outbreaks of severe Dengue.[6] Kerala, the southern state of India is hyper-endemic for Dengue and all four serotypes of Dengue virus (DENV) are being circulated.[7] Kerala reported a large outbreak of Dengue in 2017, supposed to be induced by the emergence of DENV1 over the endemic strain of DENV2. Recently, the state has reported a high incidence of mixed (an Real-Time Polymerase Chain Reaction (RT-PCR) evidence of more than one serotype of DENV) infections.[7,8] Some studies conducted in the state showed that Dengue epidemics could be predicted with moderate precision by incorporating spatial, meteorological, entomological, and virological data.[9] The Collection and compilation of data from different sources are critical to mitigating climate-sensitive vector-borne emerging infections like Dengue.[10]

In this paper, we report the incidence, geo-spatial distribution, and clinical features of Dengue infection due to various serotypes including that of mixed infection among the newly reported cases identified during monsoon in the Kasaragod district of Kerala, India. We also compared the differences in reported aedes larval indices and number of Dengue reported through the Integrated Disease Surveillance Programme (IDSP) during the premonsoon months (April and May) and monsoon months (June and July).

Methodology

The cross-sectional study was conducted in 13 health institutions (10 government hospitals, 2 private hospitals, and 1 co-operative hospital) in the Kasaragod district of Kerala from June to July 2021. These institutions were selected purposively ensuring geographical coverage from 41 government and 56 private hospitals from Kasaragod. Kasaragod is the northern district of Kerala, located in southwest India, sharing border with the State of Karnataka. Out of the 10 government hospitals, 7 are primary health centers (primary level hospitals), 2 are taluk headquarters hospitals (secondary level), and 1 is a general hospital (tertiary level). The co-operative and private hospitals included in the study provided secondary medical care. June-July months are the peak time for Dengue infection in southwest part of the Indian subcontinent because of the impact of the southwest monsoon. With the prevalence of mixed Dengue infection being reported as 42.9% in a meta-analysis of Dengue studies from India and 10% absolute precision, the sample size required was 93 individuals with DENV infection.[11] Consecutive sampling was done till sample size was attained.

The Institutional Human Ethics Committee, Central University of Kerala, approved the study proposal (Letter number CUK/IHEC/2021/02). Written informed consent was obtained from all study participants. Individuals with fever, aged 18 years or older attending the facilities were evaluated using the WHO case definition of dengue, and the probable dengue cases identified were subjected to the Dengue NS1 ELISA test. RT-PCR test was performed in the 166 samples that tested positive for dengue in Dengue NS1 ELISA, and individuals who tested positive for RT-PCR constituted the study population for the current analysis. The research team developed a semi-structured questionnaire to collect data from the eligible individuals. The tool was administered by a trained and qualified registered medical practitioner to the participant directly to obtain clinical, demographic, and geographic details. The questionnaire included items regarding co-morbidities of the patient, contact history, household factors favoring the occurrence of dengue infections, previous history of Dengue fever, and complications due to the present diagnosis of DENV infection. Patients were informed regarding their serotype positivity status while ensuring their privacy once the results were available.

We collected the temperature and rainfall data of the district for the year 2021 from the Indian Meteorological Department (IMD) and Weatherspark.[12] Monthly meteorological data for the district for the year 2021 till July were collected, which included mean day temperature, mean night temperature, mean of daily maximum temperature, mean of daily minimum temperature, and the number of rainy days. Data on the larval indices of *Aedes* were collected from the District Vector Control (DVC) unit. DVC is a group of trained people in medical entomology who continuously and randomly enumerate vector indices from different parts of the district. We used the data from DVC because of the expertise of the people collecting the data and its random nature. The data from DVC is likely to be free from selection bias and reporting bias as the team (DVC) is not a part of the management of the outbreak locally. Monthly mean House Index (percentage of houses or their premises positive for *Aedes* larvae), Breteau index (average number of breeding places per 100 houses or their premises), and Container index (percentage of water-holding containers with the presence of *Aedes* breeding) were recorded from DVC data for the previous two months (April and May 2021) of the study. Monthly Dengue cases reported by the routine surveillance system were obtained from the IDSP data which is the official data-capturing mechanism for communicable diseases in India.

RT-PCR

RNA isolation and RT-PCR were done at Central University of Kerala, Kasaragod. Standard hematological techniques were used for the extraction of serum from whole blood samples.[13] The total RNA extracted from serum samples was subjected to RT-PCR using RealStar® Dengue Type RT-PCR Kit 1.0 RUO (Altona Diagnostics, prod. No. 621003) following the manufacturers' instructions and serotyping was done.

Data analysis

The addresses corresponding to the dengue cases were cleaned and standardized to represent the village panchayat before geocoding. Geo-spatial locations of Dengue serotypes and Breteau index were mapped using QGIS version 3.28.4.[14]

Clinical features and adverse laboratory findings of the study participants were summarized using frequency and proportion. The Median (Inter-Quartile Range) of quantitative laboratory values is provided. DENV1, DENV2, and mixed infections were compared for differences in clinical features and laboratory parameters using the Chi-square test (categorical exposure factors) and Kruskal-Wallis test (quantitative exposure factors), and a *P* value less than 0.05 was considered statistically significant. Monotypic infections of DENV3 and DENV4 were not included in the comparative analysis because of low incidences. The statistical analysis was done using SPSS Statistics for Windows, licensed version 25.0 (IBM, Armonk, NY, USA).

Results

Among the 166 patients who were recruited from the out-patient departments for NS1 ELISA positive for DENV, 136 (82%) patients were found to possess DENV viral RNA and were considered for the current analysis. The median (Q_1, Q_3) age of the study participants was 39 (28,50) years with an almost equal proportion of men (n = 69) and women (n = 67). The median duration between the onset of symptoms to diagnosis was found to be 2 (1,3) days. Co-existing Diabetes mellitus, hypertension, and other long-term illnesses were found in 10 (7.35%), 12 (8.82%), and 6 (4.41%) individuals, respectively. Four (2.9%) patients reported a history of previous dengue infection.

The RT-PCR-based serotyping revealed the presence of all four serotypes of DENV (DENV1, DENV2, DENV3, and DENV4). Monotypic infection with DENV2 appeared to dominate other serotypes (n = 56, 41.2%), followed by mixed infections (n = 34, 25%) and monotypic DENV1 infection (n = 31, 22.8%). One-fourth (25.7%) of the patients had co-infection with more than one dengue serotype. Three different combinations of mixed infections were observed, of which DENV1 + DENV3 was the most common (32/34). Table 1 shows the proportions of various serotypes among the study participants.

Table 1: Proportion of various Dengue virus serotypes among the study participants (*n*=136)

Dengue Serotypes	Frequency (%)
DENV 1	31 (22.8)
DENV 2	56 (41.2)
DENV 3	9 (5.9)
DENV 4	6 (4.4)
DENV 1 and DENV 2	1 (0.7)
DENV 1 and DENV 3	32 (24.3)
DENV 1, DENV 2, and DENV 3	1 (0.7)

Among the monotypic infections, DENV2 was predominant in the southeast part of the district, comprising more rural and hilly terrain. DENV1 was more or less uniformly distributed, more toward the central part of the district predominantly comprising population-dense urban habitats and low terrains. However, there was no visual difference noted in the distribution of monotypic infections in comparison to the mixed infections [Figure 1].

The geospatial distribution of dengue cases was found to align with Breteau Index (BI). The blocks with high BI and their adjacent blocks reported a higher incidence of Dengue [Figure 2].

Apart from fever, which was universal, headache (n = 126, 92.7%), and body ache (n = 87, 64%) were the most common symptoms followed by nausea, retro-orbital pain, and vomiting. Twenty (14.7%) patients required hospitalization due to the illness, out of which only one patient reported a previous history of dengue fever. The various clinical features and the distribution of some laboratory parameters are summarized in Table 2. The laboratory parameters are available only for those patients in whom tests were prescribed as part of the treatment. No tests were conducted on patients exclusively for the purpose of the study. A quarter (28%) of the patients were anemic, and the majority had low WBC count (50.4%) and thrombocytopenia (58.7%). Out of 58 patients with available data on packed cell volume (PCV) at the time of reporting, seven reported a PCV of more than 45%.

Out of the 136 samples that tested positive as per the molecular analysis, 12 (8.82%) were enrolled from the co-operative hospital, 7 (5.15%) were from the two private hospitals, 7 (5.15%) were from the tertiary public hospital, and 33 (24.26%) were from the two secondary level public hospitals. The majority of cases (n = 77, 63.10%) were reported from the seven primary-level public hospitals in the study. Nine out of 31 (29.03%) DENV1 cases, 45 out of 56 (80.36%) DENV2, and 13 out of 34 (38.24%) mixed infections were reported from the primary-level health facilities. The reporting of DENV2 infections was significantly high and that of DENV1 and mixed infections were low in primary healthcare facilities (Chi-square statistic = 26.89, df = 2, $P < 0.001$).

The distribution of clinical features and laboratory findings across DENV1, DENV2, and mixed infections are given in Table 3. The clinical features showed no statistically significant difference across the serotypes but some significant patterns were found when the symptom complexes were considered.

Headache was the most common symptom apart from fever in all types of infections. Among the nine patients reporting rashes, none had mixed infection. Gastrointestinal manifestations (nausea/vomiting/abdominal pain/loose stools) were significantly ($P = 0.019$) more in DENV1 infection (80.6%) compared to DENV2 (58.9%) and mixed infections (47.1%). The proportion of musculoskeletal symptoms (body ache/arthralgia) was significantly ($P = 0.026$) less in DENV2 infection (53.6%)

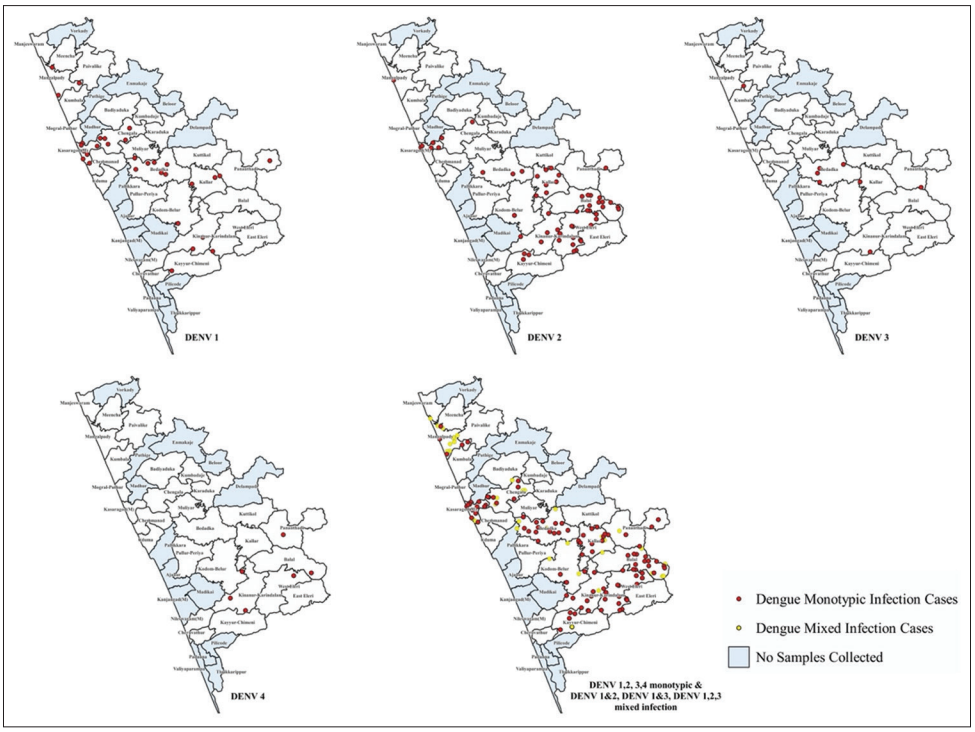

Figure 1: Geo-spatial distribution of DENV serotypes

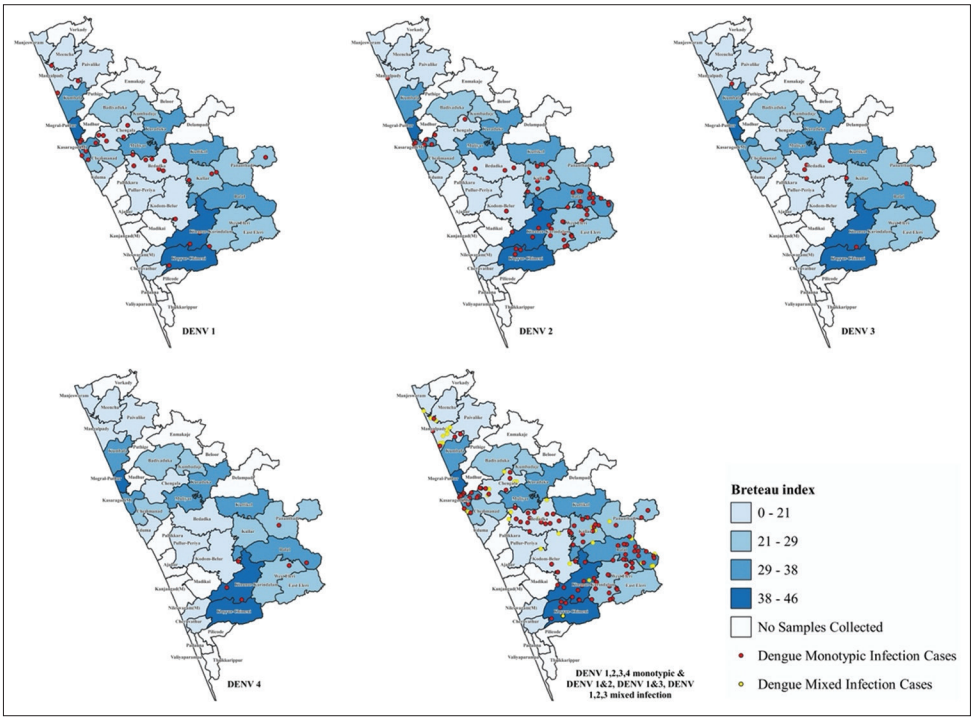

Figure 2: Geo-spatial distribution of DENV serotypes and Breteau Index

compared to DENV1 (77.4%) and mixed infections (76.5%). Among laboratory parameters, platelet count was found significantly lower in the DENV1 group ($P = 0.001$). One-fourth of the population with DENV1 were reported to have a platelet count of less than 59,000 per ml.

IDSP data showed that the incidence of dengue in the district peaked by June-July 2021 [Table 4]. Premonsoon months (April and May) were different from the monsoon months (June and July) in the highest recorded temperatures during the daytime (at 32°C–33°C v/s 28°C–29°C) and in the number of

Table 2: Clinical features and laboratory parameters of the study participants

Clinical features	Frequency (%)	Clinical features	Frequency (%)
Headache	126 (92.7)	Loose stools	17 (12.5)
Body ache	87 (64)	Cough	16 (11.8)
Nausea	51 (37.5)	Rashes	11 (8.1)
Retroorbital pain	44 (32.4)	Throat pain	9 (6.6)
Vomiting	40 (29.4)	Rhinitis	4 (3)
Arthralgia	38 (28)	Malena	1 (0.7)
Abdominal pain	31 (22.8)	Palmar erythema	1 (0.7)
Chills	27 (20)	Jaundice	1 (0.7)
Redness of eye	22 (16.2)	Altered consciousness	1 (0.7)
Lab Parameter	**Median (Q1, Q3)**	**Lab Parameter**	**Frequency (%)**
Hemoglobin (*n*=107)	13.1 (11.8, 14.1) gm%	Anemia (Hb <12 gm%)	30 (28)
Total WBC count per ml (*n*=127)	4.4 (3.2, 5.4)×10^3	WBC <4.5×10^3	64 (50.4)
Platelet count per ml (*n*=126)	1.4 (1.1, 1.8)×10^5	Platelet count <1.5×10^5	74 (58.7)
Packed cell volume (*n*=58)	38.9 (35.2, 43.4)	Increased PCV (>48%-male, >45%-female)	7 (12.1)

Table 3: Distribution of clinical features and laboratory finding across DENV1, DENV2, and mixed infections

Clinical features/Lab finding	DENV 1 *n* (%) 31 (100)	DENV 2 *n* (%) 56 (100)	Mixed infection *n* (%) 34 (100)	*P*
Headache	26 (83.9)	53 (94.6)	32 (94.1)	0.18
Retroorbital pain	10 (32.3)	21 (37.5)	6 (17.6)	0.13
Rashes	3 (9.7)	6 (10.7)	0 (0)	
Nausea	14 (45.2)	20 (35.7)	8 (23.5)	0.18
Vomiting	13 (41.9)	15 (26.8)	7 (20.6)	0.15
Body ache	21 (67.7)	29 (51.8)	24 (70.6)	0.142
Redness of eye	5 (16.1)	10 (17.9)	6 (17.6)	0.98
Cough	2 (6.5)	10 (17.9)	2 (5.9)	0.13
Chills	7 (22.6)	9 (16.1)	9 (26.5)	0.48
Arthralgia	12 (38.7)	13 (23.2)	8 (23.5)	0.25
Abdominal pain	10 (32.3)	7 (12.5)	9 (26.5)	0.07
Loose stools	4 (12.9)	4 (7.1)	3 (8.8)	0.81
Redness/Bleeding manifestations (Rashes/redness of eye/palmar erythema/malena)	7 (22.6)	14 (25.0)	6 (17.6)	0.72
Respiratory manifestations (Cough/throat pain)	5 (16.1)	13 (23.2)	3 (8.8)	0.21
Gastrointestinal manifestations (Nausea/vomiting/abdominal pain/loose stools)	25 (80.6)	33 (58.9)	16 (47.1)	0.02
Musculoskeletal manifestations (Body ache/arthralgia)	24 (77.4)	30 (53.6)	26 (76.5)	0.03
	n=27	*n*=53	*n*=32	
Median (IQR) WBC count in 10^3/ml	4.58 (3.50, 5.20)	4.30 (3.25, 5.40)	3.90 (2.85, 5.68)	0.79
	n=29	*n*=54	*n*=29	
Median (IQR) Platelet count in 10^5/ml	1.21 (0.59, 1.33)	1.45 (1.31, 1.75)	1.57 (1.13, 2.01)	0.001
	n=15	*n*=24	*n*=11	
Median (IQR) PCV in %	36.6 (34.6, 42.6)	39.3 (35.7, 44.4)	38.9 (35.1, 43.9)	0.53

Table 4: Temperature, rainfall, Aedes larval Indices, and reported Dengue cases during premonsoon and monsoon months

Season	Month	Mean day temperature (in degree Celsius)	Mean night temperature (in degree Celsius)	Mean daily maximum temperature (in degree Celsius)	Mean daily minimum temperature (in degree Celsius)	Number of Rainy days	Mean House Index	Mean Breteau Index	Mean Container Index	Dengue cases reported through IDSP
Pre-monsoon	April	33.3	25.8	34.4	25.9	4	22	28	25	191
	May	32.1	27.2	33.2	26.5	9	40	66	26	441
Monsoon	June	29.1	25.2	29.2	24.4	25	42	69	32	909
	July	27.8	24.6	28.1	23.7	28	33	41	23	555

rainy days (4–9 rainy days vs. 25–28 rainy days). The 2 months preceding the outbreak were marked by intermittent rainfall and a peaking of the ambient temperature.

Vector indices were obtained from the routine vector collection activity undertaken by the district vector control unit of Kasaragod district and were not undertaken separately as part of this study.

Vector indices are collected weekly and the highest values of each are available weekly. The monthly average of the highest weekly values for each index is shown in the supplementary file. All larval indices for *Aedes* showed an increasing trend during the period and they were very high just before the peak number of dengue cases was reported [Table 4]. *Aedes* mosquito breeding was reported in more than 40% of houses or their premises (House Index) with an average of more than 60 breeding sites in and around every 100 houses (Breteau Index).

Discussion

Dengue, as in the case of any other place in the Indian subcontinent peaked with monsoons. The intermittent rainfall and high temperature, along with the high breeding of *Aedes* mosquitoes were noted during the pre-monsoon months (April and May) and it might have contributed to the high Dengue incidence in Kerala.[9] Xavier LL, *et al.*[15] demonstrated that rainfall and temperature are the most important macro-environmental factors deciding Dengue outbreaks through influencing the *Aedes* abundance and this relationship can increase the burden of *Aedes*-borne diseases in the future because of climate change. The time gap between the experiences of the climatic factors and the outbreak that we noticed in our study is the window of opportunity where the public health intervention could mitigate the impact of the outbreak. From the geospatial pattern of the BI and the reported dengue fever, it can also be noted that areas with a BI of less than 20 in the pre-monsoon period were reporting much less incidence of dengue during the monsoons. The southeast part of the district with a very high index (around 40 breeding sources per 100 houses) contributed to the clustering of cases. A premonsoon BI of more than 20 appeared to be risky even if a dose-response relationship between the BI and the incidence of dengue cases is not apparently visualized in the plot. BI is considered to be a good predictor of dengue outbreaks with 75–80% sensitivity and 70–75% specificity in different settings if appropriate cut-off points are used.[16] A couple of analyses conducted in the Kalutara district of Sri Lanka highlighted the importance of environmental factors, vector dynamics, and their cross-linkages in the transmission of dengue.[17] Climate change is a global phenomenon, locally expressed in temperature and rainfall, which in turn determines vector activities.[18] The high larval burden can be considered an immediate determinant resulting from the climatic and environmental determinants, which can result in an outbreak in 1–2 months.[19]

The distribution of sociodemographic and clinical features may not be generalizable to the pattern of dengue infections in the region. As the inclusion criteria were restricted to patients with acute febrile illness (within 5 days), the clinical features manifesting later in the course of the infection might not be documented. However, our study gives a pattern of the clinical presentation of dengue fever. Headache was almost a universal symptom along with fever, followed by body aches, nausea, retroorbital pain, and vomiting. This finding endorses the utility of the WHO case definition of dengue and it is comparable to the symptom complex of dengue affecting south Kerala in 2017-2019.[7] But the presence of rash, which is part of the WHO case definition, was observed in less than 10% of patients only. It was reported that even if the rash is not common in dengue infections reported from this part of the land, the likelihood of dengue fever is very high in a febrile patient presenting with a rash. On the contrary, common symptoms like headaches may not have the discriminatory capacity to distinguish dengue from other viral fevers in the background.[20]

The study setting, the northern district of Kerala, India, which shares its border with the neighboring State of Karnataka was found to be hyperendemic for Dengue with all four serotypes being circulated.[8] The situation appears to be similar to other parts of the State and that of the neighboring States.[19,20] The spectrum of DENV serotypes in our study was dominated by DENV2 followed by DENV1 alone, or in combination with DENV3.

Three different co-infection combinations were observed namely, DENV1 + DENV2, DENV1 + DENV3, and DENV1 + 2 + 3. Even though DENV2 is the most common monotypic infection, mixed infections of DENV1 + DENV3 dominated mixed infections. The observation is comparable with the existing literature regarding mixed infections of DENV reported in northern Kerala during the outbreaks in 2013, 2014, and 2015.

Combinations of DENV1 + DENV3 were found to be the most common mixed infection.[8] Additionally, interference of signal in the detection of DENV1 and DENV3 during RT-PCR was ruled out in our study by setting two separate reactions of the same sample thereby almost nullifying the chance of false positivity. The large positivity of DENV1 + DENV3 can be due to the selection process through which the virus underwent to find a stable combination of infection. The selection process can occur in the midgut of mosquito vector harboring multiple DENV serotypes as was seen in laboratory experiments where mosquitoes were co-infected with DENV1 and DENV4.[21] The findings in our study could hence be due to the selective replicative advantage of DENV1 over DENV3 in the case of co-infected vectors.

As our study does not have a follow-up component and we included only the early features of the infection, we could not study the contribution of different serotypes in the clinical severity of the diseases. Musculoskeletal and gastrointestinal symptoms were found significantly more in DENV1 infections. The clinical profile showed higher symptomatic manifestations with DENV1 compared with DENV2. Moreover, the mean platelet count was significantly low in DENV1 infection. But contrary to our findings, low platelet counts and severity of infection were associated with DENV2 according to a hospital-based study from Srilanka.[22] DENV3 was found to have a three times higher risk of developing warning signs in a study conducted in the southern part of Kerala. The presence of more than one serotype from the same sample was identified as a severity indicator in many studies from other parts of India.[23–25] One-fourth of all infections in our study were found

to be due to mixed infections. N Sirisena PDN, *et al.*[26] in a review demonstrated that 2.5–30% (40–50% in exceptional situations) of all dengue infections in hyper-endemic geographical settings are due to mixed infections. The role of mixed infection in the clinical severity of dengue is still not clear. The above review demonstrates the presence of mixed infections both in the cohorts of milder infections and severe infections of Dengue. However, increased numbers of hepatic, renal, and respiratory complications were reported in mixed infections with DENV2 and DENV3 from Jakarta, Indonesia.[27] The current analysis did not yield any difference in the clinical presentation as we are reporting clinical presentation within five days of the onset of symptoms. The difference in symptomatology and severity profile may be related to the sequence of infection (primary/re-infection) and genotypic changes within the serotype.

Phylogenetic analysis showed that India could be the probable ancestral origin for certain genotypes of both DENV1 and DENV2. The cosmopolitan genotype of DENV1 circulating in India, Sri Lanka, west and east Africa, and eastern parts of Asia has India as its ancestral location. Whereas in DENV2, India can be a probable origin of the American genotype of the serotype. DENV2 might have started circulating in India by the early 20th century and DENV1 after three decades[28] A study conducted in the southern part of Kerala in 2017 documented the emergence of DENV1 over the endemicity by DENV2 as one of the major contributing factors to the large outbreak of Dengue in 2017.[29] A serial spatial analysis done in South Kerala documented that all four serotypes were in circulation in 2018 and 2019.[7] A massive outbreak of Dengue was reported in Nepal in 2022, where the epidemiological situation dominated by DENV2 and DENV1 was shifted to that of DENV1 and DENV3. The emergence of DENV3 might have contributed to the outbreak significantly.[30] DENV4 was found to be rare and the proportion of the serotype in circulation was found to be less than that of southern Kerala.

The spatial distribution of dengue serotypes showed some differences with DENV1 toward the northwest part and DENV2 toward the southeast part of the districts. Secondary and tertiary hospitals and co-operative and private hospitals are located more toward the western part of the district where the population density is higher and the area is more urbanized. Reporting of DENV1 was found to be more from such hospitals. However, the small primary hospitals located in the hilly terrain of the east reported more DENV2 infections. There is a possibility that DENV2 was the strain that dominated in the district like that of other parts of the State and the country, and a pattern of other emerging strains replacing DENV2 can be noted. The hyperendemicity resulted because of this mixing up may induce more severe dengue and it can challenge the health system.

The prompt health-seeking (a median of 2 days) noticed among the study participants should be viewed in the context of potential selection bias, as our study included patients with symptoms of less than five days only, to increase the yield of RT-PCR. As stated, we could not study the clinical severity of Dengue infection and other factors, including re-infections and the serotype variations contributing to it. Further, the study may lack the power for a comparison of clinical patterns between serotypes.

Conclusion

The study highlights the hyperendemicity of dengue fever with the cocirculation of four DENV serotypes in the northern district of Kerala. The geo-spatial clustering of DENV serotypes were also noted at different locations. DENV1 was found to manifest with more symptoms, both musculoskeletal and gastrointestinal when compared to DENV2 and mixed infections. With headache and body ache being the predominant symptoms in mono and mixed infections as is seen with most other causes of fever, differentiation of the type of infection at the clinical level is a tough challenge. DENV2 being the dominant endemic strain and the emergence of DENV1 should warn the health system of future severe outbreaks. Intermittent rainfall and rise in ambient temperature create favorable vector breeding conditions. The study thus underlines the importance of monitoring larval indices and the window of opportunity to act between the environmental predictors and the dengue outbreak.

Author contributions

RSV: Study design, Study implementation, Analysis and interpretation of Data, Major contribution to writing, Read and approved final version.

MJV: Study design, Study implementation, Analysis and interpretation of Data, Major contribution to writing, Read and approved final version.

AKS: Study design, Study implementation, Analysis and interpretation of Data, Major contribution to writing, Read and approved final version.

AJB: Analysis and interpretation of Data, Major contribution to writing, Read and approved final version.

PJ: Analysis and interpretation of Data, Major contribution to writing, Read and approved final version.

AR: Analysis and interpretation of Data, Major contribution to writing, Read and approved final version.

RP: Study design, Study implementation, Analysis and interpretation of Data, Major contribution to writing, Read and approved final version.

ATS: Study design, Analysis and interpretation of Data, Major contribution to writing, Read and approved final version.

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Ethical approval

The study is approved by Institutional Human Ethics Committee of Central University of Kerala (Letter number CUK/IHEC/2021/02)

Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author. Additional details are provided as supplementary file.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary file

Details of weekly vector survey in Kasaragod district

Month	Week	Highest HI	Highest BI	Highest CI	Highest PI	High risk areas	Aedes species
April	14	18	25	19	0	Erole, Ambalathigal, Manadukkam, Mykkayam, Chamudikunnu, Konnakad, Panathady.	Ae.albopictus
April	15	20	29	25.8	15.2	Manimoola, Acheri, Konnakkad, Kattipara, Adoor, Gardervalappu, Rajapuram.	Ae.albopictus
April	16	22	23.5	23	7.8	Konnakad, Edathode, Pathikara, Balal, Chamundi Kunnu, Vellachal, Avikkara.	Ae.albopictus
April	17	28	27.3	29	27.2	Parappa, Malakkallu, Chennikkara, Chonappallam, Rajapuram, Vellrikund.	Ae.albopictus
May	18	35	27.3	27.8	20	Parappa, Kathippara, Vaninagar, Nelkkala, Balal, Konnakad.	Ae.albopictus
May	19	32.2	61	17	18	Kushal Nagar, Kanhangad, Karivedakam, Malakkallu, Ennappara.	Ae.albopictus
May	20	34	66	22	17	Munnad, Choorithod, Marikappu, Mavugal, Padanekkad, Chenacode.	Ae.albopictus
May	21	44	78	29	16	Malakallu, Konnakkad, Nellikkunnu, Chittarical, Amey Colony-Kasaragod.	Ae.albopictus
May	22	51	96	39	18	Chinmaya Colony, Valiyapoyil, Muzhakkom, Mandampady, Malakkallu, Konnakkad, Edathod.	Ae.albopictus
June	23	42	84	23	37.5	Majeerpalla, Munnad, Payam, Vengat, Chandera.	Ae.albopictus
June	24	40	73.5	26	21.9	Majeerpalla, Munnad, Payam, Vengat, Chandera, Kasaragod, Vaninagr.	Ae.albopictus
June	25	43	64.7	46	17.7	Kallanchira, Kanhangad, Theruvath, Mogral, Bambrana, Chenacode, Edathode, Balal.	Ae.albopictus
June	26	41.5	58	37	22	Karattuvayal, Chithari, Kattukulangara, Pathikkal, Adoor, Beppu, Kanathur.	Ae.albopictus
June	27	45	65	28	23	Adukkam, Deenadukam, Mallikarjuna Area-Kasaragod, Porkalam, Manadukkam.	Ae.albopictus
July	28	42	68.6	31.8	19.3	Mundamed, Amey Colony, Echikovval, Mylatty, Kombanadukkam, Bare, Manimoola.	Ae.albopictus
July	29	37	25	17.6	25	Uppala, Kanhangad, Chittarical, Kuloor.	Ae.albopictus
July	30	35	55.6	28	18.5	Anandasramam, Ashok Nagar-Kasaragod, Ukrampadi, Kodibail, Majibail, Mullacheriyadukom, Athanadi.	Ae.albopictus
July	31	23.1	26	15.6	11.3	Thalipadappu-Kasaragod, Kannamkai, Kajampady, Mylatty.	Ae.albopictus
July	32	25	30.7	19.3	10.9	Nileshwar, Attadukkam, Kanhangad.	Ae.albopictus

Details of sample collection

Unique ID of Institution	Sample status				
	Received	Rejected	Processed	Positive	Negative
A	7	-	7	7	-
B	4	-	4	4	-
D	5	-	5	3	2
E	18	1	17	17	-
F	6	-	6	6	-
G	22	6	16	11	5
H	20	-	20	18	2
I	7	-	7	5	2
J	23	-	23	18	5
K	11	1	10	6	4
M	15	1	14	12	2
N	21	5	16	15	1
O	21	-	21	14	7
Total	180	14	166	136	30